**Clinical Trial Protocol**

**Allogeneic umbilical cord-derived mesenchymal stem cells (UC-MSCs) for patients with severe liver failure and receiving ABO incompatible liver transplantation**

**Study title**

Application of allogeneic MSCs in ABO-incompatible liver transplantation for severe hepatic failure: a phase I/II randomized, open-labeled, rituximab -controlled trial

|  |  |
| --- | --- |
| **Protocol Version** | 2.0 |
| **Principal Investigator** | Yang yangDepartment of Hepatic Surgery and Liver Transplantation Center of the Third Affiliated Hospital,& Organ Transplantation InstituteSun Yat-sen University |
| **Clinical phase** | Phase I/II |

|  |
| --- |
| **Confidentiality**This study regimen is confidential. The information contained in this document is provided to you as an investigator, potential investigator, or consultant, and may be reviewed by you, your employees, and applicable institutional review boards or independent ethics committees. This information is only used for authorized clinical studies of the study drug described in the protocol. You may not disclose any information to others without the written authorization of the researcher, unless you have obtained the informed consent of the person who may provide the drug. |

**Protocol Synopsis**

|  |  |
| --- | --- |
| Study Title | Safety and efficacy of allogeneic umbilical cord-derived mesenchymal stem cells in patients with severe liver failure and receiving ABO incompatible liver transplantation: a phase I/II randomized, open-labeled, rituximab-controlled trial |
| Trial sites andinvestigators | Professor Yang Yang,Dept. Hepatic Surgery and Liver Transplantation Center of the Third Affiliated Hospital, Organ Transplantation Institute, Sun Yat-sen University, ChinaProfessor Yingcai ZhangDept. Hepatic Surgery and Liver Transplantation Center of the Third Affiliated Hospital, Organ Transplantation Institute, Sun Yat-sen University, ChinaProfessor Shuhong Yi,Dept. Hepatic Surgery and Liver Transplantation Center of the Third Affiliated Hospital, Organ Transplantation Institute, Sun Yat-sen University, China |
| Contractresearchorganization | / |
| Clinical phase | Phase I/II |
| Study design | Monocentric, prospective, open label, randomized controlled |
| Number ofSubjectsplanned | 22 subjects in total11 subjects in each treatment arm |
| Targetpopulation | The target population involved adults with severe hepatic failure (SHF), who were hospitalized for an emergency ABO-incompatible liver transplantation (ABO-i LT). |
| Objectives | The aim of this study is to evaluate the safety and efficacy of multi-doses allogeneic UC-MSCs for 6 months versus rituximab treatment in severe hepatic failure patients who received ABO-i LT and prone to develop antibody-mediated rejection (AMR).To complete 12 months of randomized, parallel comparisons of UC-MSCs therapy versus rituximab treatment, the primary outcomes, including the assessments of MSC-related adverse events (fever, headache, rash, vomiting, diarrhea and carcinogenesis) and the incidence of allograft rejection (AR) [including antibody-mediated rejection (AMR) and acute cellular rejection (ACR)], and the secondary outcomes (containing: 1. the evaluation of graft and recipient survivals at month 12; 2. the causes of death; 3. the changes of graft function; 4. the incidence of postoperative complications [including biliary complications and specific infections]; 5. the changes of intrahepatic immune cell populations) were investigated. |
| Duration ofstudy planned | From the institutional review broad (IRB) approval date to 20, September,2019(enrollment period: 24 months, treatment period: 6 months, Follow-up and data analysis period: 12 months) |
| Study object | Allogeneic umbilical cord-derived mesenchymal stem cells. |
| Study endpoints | 1) Primary endpoint- To evaluate the safety and feasibility of multi-doses UC-MSCs administration in study subjects with the assessments of MSC-related adverse events (including fever, rash, diarrhea, lung embolism and carcinogenesis et al.) at day 7, 14, 28, 56, 84, 112, 140, 168 and 365 since the first dose transfusion.- To evaluate effects on the incidence of postoperative AR (including AMR and ACR) measured by serological and histopathological diagnosis.2) Secondary endpoints- To evaluate effects on graft and recipient survivals at transplantation of 12 months.- The cause of death.- To evaluate effects on the changes of graft function (ALT, AST, ALB, TIBL, ALP and GGT).- To evaluate effects on the incidence of postoperative complications (including biliary complications, acute rejection (AR) and specific infections).- To evaluate effect on intrahepatic immune cell populations following graft biopsy. |
| EligibilityCriteria (Inclusion/Exclusion) | 1) Inclusion criteriaPatients must meet all of the following criteria:1. Male or female, 18 to 65 years of age.2. Meld score >25.3. Eligibility for ABO-incompatibility liver transplantation for severe liver failure according to center standard.4. Undergoing ABO incompatibility liver transplantation for severe liver failure.5. Capable of understanding the purpose and risk of the study.6. Patients or proxy must give written informed consent before any assessment is performed.2) Exclusion criteriaPatients will be excluded from the study for any of the following reasons:1. Age <18 years old.2. Undergoing multi-organ transplantations.3. Receiving any form of solid organ transplantation in the past.4.ABO blood group antibodies (IgM and IgG) before surgery >1:64.5. Pregnant or breastfeeding women.6. Hepato-biliary malignancies or history of any extrahepatic malignancy.7. History of pulmonary embolism.8. Patients with active autoimmune disease.9. HIV seropositive or HTLV seropositive.10. Patients with thrombophilia.11. Pre-existent thrombosis of portal vein.12. Known abuse for drugs or alcohol.13. Specific contraindication to UC-MSCs infusion.14. Severe infection before transplantation (including bacterial, fungus, virus and parasite).15. Any clinical-relevant condition that might affect study participation and/or study results.16. Participation in any other intervention trial. 17. Unwillingness or inability to following the study protocol in the investigator’s opinion. |
| **Statistical Analyses** | Sample Size JustificationThis study is a clinical trial of two groups of subjects. The main observation index is objective effectiveness (ORR). Following the principle of randomized control study, it is designed as a non-inferiority test. The Primary endpoint is the safety of UC-MSCs administration and the incidence of postoperative AMR. With reference to the previous research results, both the expected effective frequency of the case group and the control group are 95% (α risk 0.05, power 0.8). The design of the experiment adopted unilateral test, and the sample size of 10 cases for each group was required according to the statistical formula. Considering the representative clinical trial and dropout rate, we decided to recruit 11 patients in each group.Randomization and MaskingPatients were randomly assigned to a standard immunosuppressive strategy and rituximab group（375mg/m2）(The control group) or a standard immunosuppressive strategy and 9 doses of UC-MSCs infusion group（1.0×106/kg）(The case group) in a 1:1 ratio by computer-generated random sequence. The assignment information is stored in an opaque sealed envelope. The statisticians who generated the random sequence and assigned information did not participate in the rest of the study. Prior to the assignment, the research assistant at the clinic did not know the group assignment of the participants. This is an open-label study; both participants and the research teams were unblinded to treatment allocation.Statistical analysis As appropriate, summary data with continuous variables were presented as the descriptive statistics, including n, mean ± standard deviation (SD), median/interquartile or maximum/minimum, whereas the categorical variables were summarized using frequency and percentages. The frequency comparison was performed by chi-square test, and the quantitative data was compared using Student's *t*-test (when values were normally distributed) or non-parametric Mann-Whitney U test. Survival rates were calculated. As mentioned earlier, we used liver function changes to assess the severity of liver disease. All data were processed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA) and a value of P < 0.05 was considered statistically significant. The study was registered at chictr.org.cn, number ChiCTR2000037732. |

**Time & Event Table**

|  |  |  |  |
| --- | --- | --- | --- |
| **Assessment / Procedure** | **Screening** | **Baseline** | **On-Treatment** |
|  | W0 | D1 | W1 | W2 | W3 | 1M | 2M | 3M | 4M | 5M | 6M | 7M | 8M | 9M | 10M | 11M | 1Y |
| Informed consent  | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Treatment History | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Inclusion/Exclusion Criteria  | √ | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Physical Examination1 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Randomization |  | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Vital signs2 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Hematology3 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Chemistry4 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Renal function5 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| HBV DNA | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HBsAg | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HBsAb/ HBeAg/HBeAg | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Anti HCV / Anti-HIV | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Infection-related index6 | √ | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |  |  |  |  |  |
| Metabolism-related index7 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Hemagglutinin titer | √ | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |  |  |  |  |  |
| Tacrolimus blood concentration |  |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Peripheral blood and serum for storage |  | √ |  | √ | √ |  | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |
| CEUS or CT or MRI | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Liver biopsy8 |  |  |  |  | √ |  |  |  |  |  |  | √ |  |  |  |  |  |  |
| MSCs infusion |  |  | √ | √ | √ |  | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |
| Safety evaluation |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Adverse Events |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Compliance check |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Concomitant Medications |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |

1. Mainly assess liver, abdomen, and lung signs during physical examination.
2. The vital signs include blood pressure, heart rate body temperature and body weight.
3. Haematology: Hemoglobin, red blood cell (RBC), white blood cell (WBC), neutrophilic granulocyte percentage (NEUT), lymphocyte percentage and platelet count.
4. Chemistry: AST, ALT, ALB, GGT, ALP, TBIL, DBIL, BUN, CREAT, total protein, Na＋, K＋.
5. Renal function: BUN, creatinine
6. Infection-related index: PCT, (1,3)-β-D-glucan detection (G experimental), CRP, ESR, Pathogen examination.
7. Metabolism-related index: GLU, CHOL, TG, HDL, LDL.
8. Liver biopsy: Liver biopsy at the second and sixth months after surgery for pathological diagnosis.

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# 1. Study Title and Phase

## 1.1. Study Title

Application of allogeneic MSCs in ABO-incompatible liver transplantation for severe hepatic failure: a phase I/II randomized, open-labeled, rituximab -controlled trial

## 1.2. Study Phase

Phase I/II

# 2. Study Sites and Investigators

|  |  |  |
| --- | --- | --- |
|  | Investigator | Adress |
| The Third Affiliated Hospital & OrganTransplantation Institute of Sun Yat-sen University | Yang Yang | No. 600, Tianhe District, Guangzhou, Guangdong, China |
| The Third Affiliated Hospital & OrganTransplantation Institute of Sun Yat-sen University | Yingcai Zhang | No. 600, Tianhe District, Guangzhou, Guangdong, China |
| The Third Affiliated Hospital & OrganTransplantation Institute of Sun Yat-sen University | Shuhong Yi | No. 600, Tianhe District, Guangzhou, Guangdong, China |

# 3. Study Objectives and Background

## 3.1. Study Objectives

The aim of this study is to evaluate the safety and efficacy of multi-doses allogeneic UC-MSCs for 6 months versus rituximab treatment in severe hepatic failure patients who received ABO-i LT and prone to develop antibody-mediated rejection (AMR).

To complete 12 months of randomized, parallel comparisons of UC-MSCs therapy versus rituximab treatment, the primary outcomes, including the assessments of MSC-related adverse events (fever, headache, rash, vomiting, diarrhea and carcinogenesis) and the incidence of allograft rejection (AR) [including antibody-mediated rejection (AMR) and acute cellular rejection (ACR)], and the secondary outcomes (containing: 1. the evaluation of graft and recipient survivals at month 12; 2. the causes of death; 3. the changes of graft function; 4. the incidence of postoperative complications [including biliary complications and specific infections]; 5. the changes of intrahepatic immune cell populations) were investigated.

**3.1.1. Primary Endpoints**

- To evaluate the safety and feasibility of multi-doses UC-MSCs administration in study subjects with the assessments of MSC-related adverse events (including fever, rash, diarrhea, lung embolism and carcinogenesis et al.) at day 7, 14, 28, 56, 84, 112, 140, 168 and 365 since the first dose transfusion.

- To evaluate effects on the incidence of postoperative AR (including AMR and ACR) measured by serological and histopathological diagnosis.

**3.1.2. Secondary Endpoints**

Secondary endpoints

- To evaluate effects on graft and recipient survivals at transplantation of 12 months.

- The cause of death.

- To evaluate effects on the changes of graft function (ALT, AST, ALB, TIBL, ALP and GGT).

- To evaluate effects on the incidence of postoperative complications (including biliary complications, acute rejection (AR) and specific infections).

- To evaluate effect on intrahepatic immune cell populations following graft biopsy.

## 3.2. Background

Severe hepatic failure (SHF) is a life-threatening illness with high mortality and morbidity, which is pathologically characterized by sudden and severe hepatocellular necrosis and clinically characterized by coagulopathy, jaundice and hepatic encephalopathy.[1] Emergency liver transplantation (LT) is still identified as the only durable effective therapeutic approach for SHF.[2] Although the effect of LT today is satisfaction, the severe shortage of donors remains a critical factor to affect the waitlist survival.[3, 4] Breaching of the barrier of ABO blood type is a potential approach to increase donor access, and ABO-incompatible LT (ABO-i LT) increasingly becomes a life-saving therapeutic approach for SHF.[5] In 1979, Thomas E. Starzl et al. firstly reported on their eleven cases of ABO-i LT.[6] At that time, eight of these eleven patients survived more than two months, and only two cases were suspected of graft loss caused by ABO incompatibility. As a result, ABO-i LT was considered as an effective approach in the 1970s and early 1980s.[7] However, the next researches revealed that ABO-i LT prone to occur higher risks of hepatic artery thrombosis, bile duct complications, antibody-mediated rejection (AMR), infection and poor graft and recipient survival, and concluded that using conventional immunosuppression was clearly associated with inferior graft survival after ABO-i LT. In order to overcome these disadvantages, various novel therapeutic strategies have been introduced over the past two decades, comprising CD20 monoclonal antibody (rituximab), intravenous immunoglobulin (IVIG), splenectomy, immunoadsorption and plasma exchange.[8-12] Of all, rituximab is an immune-chimeric monoclonal antibody that specially points to the transmembrane protein CD20 molecule to deplete B cells.[13] Since Usuda et al firstly reported the beneficial effect of rituximab in ABO-i LT in 2005, rituximab is widely used and has become the critical component of the protocol for ABO-i LT.[14] Previous studies demonstrated that a higher incidence of postoperative complications and worse prognostic outcomes were observed before the rituximab era, and the encouraging results by the administration of rituximab have been shown that the graft and recipient after ABO-i LT were comparable to those in the ABO-c LT group.[15-18] The further multivariate analysis demonstrated that the absence of rituximab administration was an independent risk factor for AMR.[19] However, the large sample-size retrospective research also reported that the incidence of infection and bile duct injury still high after treated with rituximab.[15]

With the properties of immunomodulation and regeneration, mesenchymal stem cell (MSC) is emerging as a promising approach for many diseases, including acute-on-chronic hepatic failure (ACLF), rheumatoid arthritis and inflammatory bowel disease (IBD).[20-23] Of all, as the important roles in modulating the function of macrophage, natural killer (NK), T and B cells as well as further inducing the translation of Treg and Breg cells, MSC is believed to prevent post-operative complications after transplantation and reduce the side effects of pharmacologic immunosuppression.[24-26] In previous trial, Tan et al. showed that autologous MSC administration exhibited lower incidence of acute rejection, lower risk of opportunistic infection, better renal function restores after kidney transplantation, compared to anti-IL-2 receptor monoclonal antibody treatment.[27] Detry was the first man who reported the use of MSC treatment for liver transplant recipients and showed that there were no toxic and severe side-effects after a single-dose MSC transfusion.[28] Wang et al. showed that without any side effects, umbilical cords-derived MSC (UC-MSC) transfusion is feasible for inhibiting acute graft rejection after LT via increasing the percentage of Treg cells and the Treg/Th17 ratio.[29] Our previous study also revealed the beneficial effect of UC-MSC on attenuating ischemia-type biliary lesions (ITLBs) after LT.[30] However, to our knowledge, clinical research of UC-MSC administration in SHF patients undergoing ABO-i LT have not been conducted.

# 4. Study Design

Monocentric, prospective, open label, randomized controlled

# 5. Projected Duration of the Study

From the IRB approval date to 20, September,2019

Enrollment period: 24 months

Treatment period: 6 months

Follow-up and data analysis period: 12 months

The study could be extended according to the scientific merit.

# 6. Target Disease

Severe hepatic failure (SHF) which is need for an emergency liver transplantation (ABO-i LT).

# 7. Study Subjects Criteria (Inclusion/Exclusion)

## 7.1. Inclusion criteria

Patients must meet all of the following criteria:

1. Male or female, 18 to 65 years of age.

2. Meld score >25.

3. Eligibility for ABO-incompatibility liver transplantation for severe liver failure according to center standard.

4. Undergoing ABO incompatibility liver transplantation for severe liver failure.

5. Capable of understanding the purpose and risk of the study.

6. Patients or proxy must give written informed consent before any assessment is performed.

## 7.2. Exclusion criteria

Patients will be excluded from the study for any of the following reasons:

1. Age <18 years old.

2. Undergoing multi-organ transplantations.

3. Receiving any form of solid organ transplantation in the past.

4.ABO blood group antibodies (IgM and IgG) before surgery >1:64.

5. Pregnant or breastfeeding women.

6. Hepato-biliary malignancies or history of any extrahepatic malignancy.

7. History of pulmonary embolism.

8. Patients with active autoimmune disease.

9. HIV seropositive or HTLV seropositive.

10. Patients with thrombophilia.

11. Pre-existent thrombosis of portal vein.

12. Known abuse for drugs or alcohol.

13. Specific contraindication to UC-MSCs infusion.

14. Severe infection before transplantation (including bacterial, fungus, virus and parasite).

15. Any clinical-relevant condition that might affect study participation and/or study results.

16. Participation in any other intervention trial.

17. Unwillingness or inability to following the study protocol in the investigator’s opinion.

# 8.Study Procedures and Methods

## 8.1. The Control and Case Groups

The control group is treated with standard immunosuppressive strategy and rituximab（375mg/m2）, and the case group is treated with standard immunosuppressive strategy and 9 doses of UC-MSCs infusion（1.0×106/kg）.

## 8.2. Method of Assigning Patients to Treatment Groups and Randomisation

1. The control group: Standard immunosuppressive strategy and rituximab（375mg/m2）
2. The case group: Standard immunosuppressive strategy and 9 doses of UC-MSCs infusion（1.0×106/kg）
3. Assignment (The randomization process)

We randomly allocated patients in a 1:1 ratio to receive the standard immunosuppressive strategy and rituximab (The control group) or standard immunosuppressive strategy and 9 doses of UC-MSCs infusion (The case group). Patients were randomly allocated with a computer-generated randomisation sequence. The statistician who generated the randomisation sequence was not involved in the rest of the study. This is an open-label study. Both participants and the study team were unmasked to treatment allocation.

1. Allocated patients

Number of total populations–22(control: 11, case: 11)

1. Masking method:

As an open-label trial, all the doctors and patients know assigned drugs

1. Treatment – The Drugs, method of administration and dosage.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Group | Number of patients | Drug and dosage | Frequency of administration | Route of administration |
| Control | 11 | Standard immunosuppressive strategy and rituximab（375mg/m2） | Once after surgery | Intravenous drip |
| Case | 11 | Standard immunosuppressive strategy and 9 doses of UC-MSCs infusion（1.0×106/kg） | The first time to treated with 10% UC-MSCs through portal vein after graft reperfusion and 90% transfused through peripheral vein during LT; and the subsequent 8 times (week 1, 2, 4, 8, 12, 16, 20, 24 after operation) which were intravenously transfused via the vein of forearm. | Intravenous drip |

## 8.3. Treatment Assessments

**8.3.1. Time & event table**

|  |  |  |  |
| --- | --- | --- | --- |
| **Assessment / Procedure** | **Screening** | **Baseline** | **On-Treatment** |
|  | W0 | D1 | W1 | W2 | W3 | 1M | 2M | 3M | 4M | 5M | 6M | 7M | 8M | 9M | 10M | 11M | 1Y |
| Informed consent  | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Treatment History | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Inclusion/Exclusion Criteria  | √ | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Physical Examination1 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Randomization |  | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Vital signs2 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Hematology3 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Chemistry4 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Renal function5 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| HBV DNA | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HBsAg | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HBsAb/ HBeAg/HBeAg | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Anti HCV / Anti-HIV | √ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Infection-related index6 | √ | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |  |  |  |  |  |
| Metabolism-related index7 | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Hemagglutinin titer | √ | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |  |  |  |  |  |
| Tacrolimus blood concentration |  |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Peripheral blood and serum for storage |  | √ |  | √ | √ |  | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |
| CEUS or CT or MRI | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Liver biopsy8 |  |  |  |  | √ |  |  |  |  |  |  | √ |  |  |  |  |  |  |
| MSCs infusion |  |  | √ | √ | √ |  | √ | √ | √ | √ | √ | √ |  |  |  |  |  |  |
| Safety evaluation |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Adverse Events |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Compliance check |  |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| Concomitant Medications |  | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |

1. Mainly assess liver, abdomen, and lung signs during physical examination.
2. The vital signs include blood pressure, heart rate body temperature and body weight.
3. Hematology: Hemoglobin, red blood cell (RBC), white blood cell (WBC), neutrophilic granulocyte percentage (NEUT), lymphocyte percentage and platelet count.
4. Chemistry: AST, ALT, ALB, GGT, ALP, TBIL, DBIL, BUN, CREAT, total protein, Na＋, K＋.
5. Renal function: BUN, CREAT
6. Infection-related index: PCT, (1,3)-β-D-glucan detection (G experimental), CRP, ESR, Pathogen examination.
7. Metabolism-related index: GLU, CHOL, TG, HDL, LDL.
8. Liver biopsy: Liver biopsy at the second and sixth months after surgery for pathological diagnosis.

**8.3.2. Study Procedures**

**1) Screening visit**

* + - Explain the study for the subjects and Obtain written informed consent Review of inclusion/exclusion criteria
		- Basic information, Medical history, including hepatitis history and surgical treatment history
		- Complete physical examination and vital signs
		- Evaluate concomitant medications
		- Laboratory assessments (Hematology, Chemistry, Prothrombin Time, Renal function, Infection-related index, Metabolism-related index, HBsAg, Anti-HCV/anti-HIV, HBsAb/HBeAg/HBeAb, HBV DNA level, Hemagglutinin titer). If there are results of same laboratory tests performed in 28 days, it is applicable.
		- Abdominal US, CT or MRI
	1. **Baseline Visit**
		+ - Review of inclusion/exclusion criteria
			- Complete physical examination and vital signs
			- Randomization and therapeutic regimen dispensing
			- Laboratory assessments (Hematology, Chemistry, Prothrombin Time, Renal function, Infection-related index, Metabolism-related index, HBsAg, Anti-HCV/anti-HIV, HBsAb/HBeAg/HBeAb, HBV DNA level, Hemagglutinin titer, Peripheral blood and serum for storage). If there are results of same laboratory tests performed in 28 days, it is applicable.
			- Perform the CEUS or CT or MRI.
			- Evaluate concomitant medications
			- Assessment of adverse events
	2. **All visits**
		+ - Complete physical examination and vital signs
			- Laboratory assessments (Hematology, Chemistry, Renal function, Metabolism-related index, Tacrolimus blood concentration)
			- Perform the CEUS or CT or MRI
			- Drugs dispensing and assessment of compliance
			- Evaluate concomitant medications
			- Safety evaluation and assessment of adverse events
	3. **Week 1, 2, 3, 4.**
		+ - Complete physical examination and vital signs
			- Laboratory assessments (Hematology, Chemistry, Renal function, Infection-related index, Metabolism-related index, Hemagglutinin titer, Tacrolimus blood concentration)
			- Perform the CEUS or CT or MRI
			- Drugs dispensing and assessment of compliance
			- Evaluate concomitant medications
			- Safety evaluation and assessment of adverse events
	4. **Week 1, 2, 4, 8, 12, 16, 20, 24**
		+ - Complete physical examination and vital signs
			- Laboratory assessments (Hematology, Chemistry, Renal function, Metabolism-related index, Tacrolimus blood concentration, Peripheral blood and serum for storage)
			- Perform the CEUS or CT or MRI
			- UC-MSCs infusion and assessment of compliance
			- Drugs dispensing and assessment of compliance
			- Evaluate concomitant medications
			- Safety evaluation and assessment of adverse events
	5. **Week 2, 24**
		+ - Complete physical examination and vital signs
			- Laboratory assessments (Hematology, Chemistry, Renal function, Metabolism-related index, Tacrolimus blood concentration)
			- Perform the CEUS or CT or MRI
			- Perform the liver biopsy
			- Drugs dispensing and assessment of compliance
			- Evaluate concomitant medications
			- Safety evaluation and assessment of adverse events

# 9. Study regimen

The investigator and the pharmacists in each study site take the responsibility for management of the study regimen during the clinical trial.

## 9.1. Management and Record

The umbilical cord derived mesenchymal stem cells (UC-MSCs) needed for the clinical trial will be provided by the Cell-gene Therapy Translational Medicine Research Center of The Third Affiliated Hospital of Sun Yat-sen University. Clinical trial pharmacists are responsible for ensuring full accountability for all used and unused research UC-MSCs. In addition, patients will be injected with UC-MSCs according to the procedures specified in the clinical trial protocol, while side effects will be observed and evaluated. All used and unused study UC-MSCs distributed to subjects must be returned to the site. UC-MSCs left after study should be disposed of in an appropriate way or returned to the sponsor.

Side effects of UC-MSCs provided below could occur rarely. Since mesenchymal stem cells have been tested in experimental clinical studies around the world and the clinical safety data has been accumulated, it could be very safe.

## 9.2. Adverse events

UC-MSCs:

* + - Systemic: fever
		- Gastrointestinal: vomiting, diarrhea
		- Neurologic: headache
		- Dermatologic: rash

## 9.3. Concomitant Medications

All concomitant medications administrated from the point of obtaining informed consent to the end of the study must be recorded.

## 9.4. Rules for Stopping The clinical trial

* + - * UC-MSCs treatment was found to be ineffective.
			* Death or severe disability caused by UC-MSCs treatment.
			* The shedding rate of enrolled patients was more than 10%.
			* When serious ethical issues arised during treatment.

# 10. Safety Evaluation

Our center's preclinical and clinical studies on umbilical cord mesenchymal stem cells, as well as the current clinical studies around the world, have proved that UC-MSCs has a good safety in clinical application. Therefor investigators will individually evaluate the well-known side effect, common side effects and unpredictable side effects that have not been reported yet.

## 10.1. Definition of Adverse Events

Adverse events (AE) is any untoward medical occurrence in a patient or a subject under clinical investigation that are time-related to the use of a medicinal product, but not necessarily causal to the treatment. All adverse events will be assessed by the investigator or qualified designee and recorded on the AE CRF page.

## 10.2. Assessment of AEs

All AEs and SAEs occurring after initiation of treatment and until the end of follow-up/final visit should be recorded in the CRF. The occurrence time, severity, duration, action taken, and outcome of adverse events would be recorded.

## 10.3. Severe Adverse Events (SAEs)

A serious adverse event is any type of experience that occurs that has a significant hazard to the subject and/or affects continued treatment. The following events are included in clinical work:

* Results in death or life-threatening
* Requiring hospitalization or prolongation of existing hospitalization
* Persistent or significant disability/incapacity
* Development of fetal anomalies
* Medically significant or clinically required medical intervention to prevent the occurrence of one or other of these outcomes

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

## 10.4. Reporting of Adverse Events

For all the adverse events, the following must be assessed and documented in the adverse event record section of the case report form: severity, relationship to the experimental drug/stem cell, actions taken adverse reactions following the use of experimental drugs/stem cells, and the outcomes.

Reporting serious adverse events is a statutory requirement. All serious adverse events must also be recorded on the serious adverse events page of the case report form.

All serious adverse events must be reported to the principal investigator, the discipline committee, and the IRB within 24 hours

## 10.5. Grading of AE

|  |  |  |
| --- | --- | --- |
| Grade | Score |  |
| Mild | 1 | Symptoms causing no or minimal interference with usual social &functional activities. |
| Moderate | 2 | Symptoms causing greater than minimal interference to reduceusual social & functional activities. |
| Severe | 3 | Symptoms causing inability to perform usual social & functionalActivities. |
| Life-threatening | 4 | Symptoms causing inability to perform basic self-care functions ORMedical OR operative intervention indicated to prevent permanentimpairment, persistent disability, or death. |

## 10.6 Causal Relationship of AE

The following categories and definitions of causal relationship to study drug should be used for AEs:

1. Definitely related
	* Event or laboratory test abnormality, with plausible time relationship to UC-MSCs infusion
	* Cannot be explained by concomitant diseases or other drugs
	* Response to withdrawal of UC-MSCs (pharmacologically, phenomenologically, pathologically)
	* Rechallenge satisfactory
	* Event definitive pharmacologically or clinically (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon)
2. Probably related
	* Event or laboratory test abnormality, with reasonable time relationship to UC-MSCs infusion
	* Unlikely to be attributed to concomitant diseases or other drugs
	* Response to withdrawal clinically reasonable
3. Possibly related
	* Could also be explained by concomitant diseases or other drugs
	* Response to withdrawal clinically reasonable
4. Probably not related
	* Event or laboratory test abnormality, could be explained by concomitant diseases or other drugs than UC-MSCs infusion
	* Response to withdrawal unsatisfactory or vague
	* Rechallenge unsatisfactory or vague
5. Unknown
	* Cannot be judged because information is insufficient or contradictory
	* Data cannot be supplemented or verified
6. Definitely not related
	* Event or laboratory test abnormality, with a time to UC-MSCs infusion that makes a relationship improbable (but not possible)
	* Disease or other drugs provide plausible explanations

# 11. Statistical Considerations

## 11.1. Sample Size Justification

This study is a clinical trial of two groups of subjects. The main observation index is objective effectiveness (ORR). Following the principle of randomized control study, it is designed as a non-inferiority test. The Primary endpoint is the safety of UC-MSCs administration and the incidence of postoperative AR. With reference to the previous research results, both the expected effective frequency of the case group and the control group are 95% (α risk 0.05, power 0.8). The design of the experiment adopted unilateral test, and the sample size of 10 cases for each group was required according to the statistical formula. Considering the representative clinical trial and dropout rate, we decided to recruit 11 patients in each group.

## 11.2. Randomization and Masking

Patients were randomly assigned to a standard immunosuppressive strategy and rituximab group（375mg/m2）(The control group) or a standard immunosuppressive strategy and 9 doses of UC-MSCs infusion group（1.0×106/kg）(The case group) in a 1:1 ratio by computer-generated random sequence. The assignment information is stored in an opaque sealed envelope. The statisticians who generated the random sequence and assigned information did not participate in the rest of the study. Prior to the assignment, the research assistant at the clinic did not know the group assignment of the participants. This is an open-label study; both participants and the research teams were unblinded to treatment allocation.

## 11.3. Statistical analysis

As appropriate, summary data with continuous variables were presented as the descriptive statistics, including n, mean ± standard deviation (SD), median/interquartile or maximum/minimum, whereas the categorical variables were summarized using frequency and percentages. The frequency comparison was performed by chi-square test, and the quantitative data was compared using Student's *t*-test (when values were normally distributed) or non-parametric Mann-Whitney U test. Survival rates were calculated. As mentioned earlier, we used liver function changes to assess the severity of liver disease. All data were processed using SPSS 23.0 software (SPSS Inc., Chicago, IL, USA) and a value of P < 0.05 was considered statistically significant. The study was registered at chictr.org.cn, number ChiCTR2000037732.

# 12. Measurement of Adherence

If the actual dosage is less than 80% of the theoretical amount, it is considered to be poor compliance. The importance of compliance with the treatment regimen will be emphasized at each visit. A record of this reconciliation must be maintained using the accountability forms, and any issues of non-compliance discussed with the subject. If there are still those who cannot comply, they are advised to withdraw from the study.

# 13. Discontinuation and Withdrawal

Subjects may be withdrawn from the study at the investigator’s discretion in any of the following instances:

* The subjects withdraw the agreement of participation of the trial
* Development of a toxicity or serious adverse event which warrants UC-MSCs discontinuation
* Vital violations of the clinical trial protocol
* The subjects refuse the administration of the study regimen or safety tests

The treatment after discontinuation or withdrawal will be determined by the investigator. In case of discontinuation or withdrawal due to adverse events or safety issue, subjects should be followed until recovery and the events should be recorded in CRFs

# 14. Efficacy Evaluation

**(1) Primary endpoint**

- To evaluate the safety and feasibility of multi-doses UC-MSCs administration in study subjects with the assessments of MSC-related adverse events (including fever, rash, diarrhea, lung embolism and carcinogenesis et al.) at day 7, 14, 28, 56, 84, 112, 140, 168 and 365 since the first dose transfusion.

- To evaluate effects on the incidence of postoperative AR (including AMR and ACR) measured by serological and histopathological diagnosis.

**(2) Secondary endpoint**

- To evaluate effects on graft and recipient survivals at transplantation of 12 months.

- The cause of death.

- To evaluate effects on the changes of graft function (ALT, AST, ALB, TIBL, ALP and GGT).

- To evaluate effects on the incidence of postoperative complications (including biliary complications, acute rejection (AR) and specific infections).

- To evaluate effect on intrahepatic immune cell populations following graft biopsy.

# 15. Protection of the Study Subjects

The institution ensures that it will provide the necessary personnel and facilities to conduct the study appropriately based on clinical trial protocol, and do its utmost to ensure safety. The sub-investors should be fully aware of the adverse events and precautions documented in the protocol. In addition, if serious adverse events occur during the trial, study treatment should be stopped immediately, and the IRB should be notified after appropriate treatment has been taken.

# 16. Informed Consent, Agreement of Compensation, Post-Study Treatment

## 16.1 Patient Information and Informed Consent

The investigator is responsible for obtaining written informed consent from each individual participating in the study, prior to fully demonstrating the purpose, method, objectives, and potential risks of the study. Handle any formalities related to learning. The investigator must use the IRB approved consent form to record the written informed consent. Each informed consent form will be duly signed and dated by the legally authorized representative of the subject or subject and the person obtaining the consent. A copy of the signed informed consent and any other patient information must be provided to each patient or patient's legal representative. If the subject or representative cannot read, an impartial witness is required.

The process of obtaining informed consent should be recorded in the subject’s original document.

## 16.2. Compensation Available to the Patients in the Event of Trial Related Injury

In this study, subjects have been insured for clinical trials, and they can get timely medical care and corresponding compensation for damages caused by adverse reactions related to this study.

## 16.3. Treatment of the Subjects after the End of the Clinical Trial

Subjects who completed the study will receive standard immunotherapy after liver transplantation. Subjects who discontinue the study or who do not respond to UC-MSCs should receive other appropriate treatments. The effect of UC-MSCs is now non-deducible but, before the end of this study, it is expected to be changed into deducible. After the study is over, the treatment will be determined by the subject's clinical status and the clinician.

# 17. Predicted Study Result and Implications

The current study is aimed to evaluate the safety and efficacy of multi-doses of UC-MSCs versus rituximab in the SHF patients receiving ABO-i LT. Thus, the results of this study could suggest the standard therapeutic strategy to prevent postoperative complications after ABO-i LT which has currently been controversy. We might clear whether UC-MSCs therapy is sufficient for preventing severe infections and biliary complications after ABO-i LT, which could not inevitable after utilized rituximab. This would be a novel and viable option for preventing complications of SHF patients after ABO-i LT that lead to graft loss and even recipient mortality. Especially, if there is no significant difference in the incidence of AMR between UC-MSCs and rituximab, it could be concluded that multi-doses administration of UC-MSCs not only reduce the rate of graft loss and the risk of recipients’ mortality, but also improve the recipients’ life qualities after ABO-i LT.

# 18. Additional Considerations for the Study

## 18.1. Compliance and modification of the clinical trial protocol

The study must be conducted in accordance with a clinical trial protocol, including written informed consent approved by the IRB. All protocol modifications should be discussed between the sponsor and the principal investigator. All modifications to the Protocol, except those intended to reduce the risk of immediate impact to subject, should be submitted to the IRB in accordance with local requirements. Approval must be approved before the change is implemented. If you apply for a modification before approval by the IRB to prevent immediate damage to the subject, you should report it to the IRB as soon as possible.

## 18.2. Quality control and monitoring

The researcher is responsible for operating and maintaining the quality assurance and quality control system in accordance with the SOP, ensuring that the test implementation and data generation, documentation and reporting are in accordance with the protocol, SOP, GCP and applicable regulatory requirements. The responsibility for the accuracy, completeness, and reliability of the collected data lies with the investigator who collects the data.

The researcher or clinical research institution may arrange quality control for the clinical research center itself as part of the quality assurance implementation. Quality control is independent of routine inspections and may include on-site review of regulatory documents, case report forms, and original documents. Researchers must agree to allow quality control personnel to access these documents directly.

According to the GCP regulations, the researcher/research institution should retain the evidence that the collection of data is derived from the full medical report and source data of each subject, as well as all the research documents specified in the GCP, as well as the requirements of the current regulatory requirements. All research documents. Researchers/research institutes should take steps to prevent accidental or premature damage to these documents.

## 18.3. Storage of the Trial Related Documents and Data

Investigators must maintain sufficient and accurate records to allow the study to be fully documented and the study data subsequently validated. The investigator is responsible for maintaining and providing basic documentation of clinical trials. The basic clinical trial documents are those that allow you to assess the performance of clinical trials, as well as data obtained individually or collectively from clinical trials. The basic clinical trial documents will include protocol/amendments, CRF and query forms, IRB approval with correspondence, informed consent, drug records, and monitoring records and other appropriate documents and correspondence.

Subject clinical source files contain all observation dates, clinical trial activity records, and all reports and records used to evaluate and reconstruct clinical trials. Therefore, the subject's clinical source document should include a record of all procedures performed in accordance with the clinical trial protocol.

All clinical research documents must be retained by the investigator until at least 3 years after the end of the study.

## 18.4. Confidentiality of the Data and Records of the Subjects

The investigator should keep the information and data collected during the trial confidential. The investigator must ensure that the subject's anonymity is strictly maintained. You should only use the subject initials or identification codes when accessing. Their identities must be protected from unauthorized people. However, only the Clinical research center, the investigator, the person conducting the inspection, the IRB, and the CFDA Director can review the data of the research object within the scope of relevant regulations without violating the confidentiality of the research object to verify the reliability of the research and the research process. All persons involved in this study are subject to this confidentiality clause.

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