A clinical study of ultra-transplantation for the treatment of thalassemia major scheme

Project Title: Clinical study of ultra-transplantation for the treatment of thalassemia major

Project undertaker: The First People's Hospital of Yunnan Province

Project Leader/Department:XXX

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1. Introduction to the study

(1) The purpose and significance of the study

Thalassemia (thalassemia, referred to as thalassemia), the third most common among all birth defects, is a major public health problem worldwide. Yunnan is located in the subtropics, adjacent to Southeast Asia, is one of the high incidence areas of hemoglobinosis in China, its abnormal hemoglobinopathy and thalassemia carry rate is high, with obvious uniqueness and diversity, the detection rate of thalassemia carriers in the province is about 10.71%, and Dehong Prefecture, Xishuangbanna Prefecture and Wenshan Prefecture are as high as more than 20.1%, according to which it is estimated that the number of patients with moderate and severe thalassemia in Yunnan is about 97,000. Common treatments for thalassemia patients include blood transfusions and hematopoietic stem cell transplantation. The cost of hematopoietic stem cell transplantation for each child with thalassemia is about 25 to 300,000 yuan. The financial burden of blood transfusion therapy in children with thalassemia is greater and increases with age. A 1-year-old child with thalassemia needs about 20,000 milliliters of blood per year, and the annual treatment cost is about 100,000 yuan. Until the patient survives the age of 40, the total amount required is about RMB 4 million. With such a high cost of treatment, it is difficult for the average family to afford. Hematopoietic stem cell transplantation (HSCT) is the only clear means to cure thalassemia, and the development of gene therapy clinical research and basic research has led to a success rate of about 70%. Both HSCT and gene therapy require radiotherapy, chemotherapy and immunosuppression. Complications of HSCT include multi-organ dysfunction caused by conditioning drugs, poor stem cell implantation, acute and chronic GVHD, some special complications (capillary leak syndrome (CLS), hepatic sinusoidal occlusion syndrome (SOS), interstitial pneumonia, thrombotic microangiopathy (TMA), post-transplant lymphoproliferative disorder (PTLD), hemorrhagic cystitis, central nervous system complications, etc.). It seriously affects the prognosis and quality of life of patients, and causes varying degrees of damage to children's reproductive development. The screening of HSCT donors is also an urgent problem to be solved. The 5-year survival rate of transplantation in MSD donors is 95-97%, but the complete compatibility rate between siblings is 25%; The 5-year survival rate of MUD is 90-95%, and our province is multi-ethnic, and the polymorphism of HLA is significantly different from that of other Han populations, and the success rate of non-blood hologeneous matching type is very low. The 5-year survival rate for haplotype donors is 80-87%, and the complication rate is significantly increased. In China, patients with thalassemia have long-term blood transfusion, high bone marrow load hematopoiesis, irregular iron removal treatment, and the incidence of abnormal liver iron is 89%, and the incidence is higher with age. Factors such as heart damage and hepatic iron deposition caused by long-term anemia make the success rate and long-term productivity of HSCT significantly lower in ⩾-year-old

patients with severe thalassemia than in <-year-old patients, and most of the patients at the age of > 19 years lose the opportunity of HSCT. The economic and social development of our province has been in a backward state for a long time, and there are many patients with severe thalassemia who are not eligible for transplantation due to the non-standard treatment of thalassemia and the lack of MUD donors. Therefore, finding a safer, more effective and low-cost treatment strategy for such patients with severe thalassemia who are not eligible for transplantation is a key problem in the prevention and treatment of thalassemia in our province and even in the whole country and the world.

As we all know, Professor Ai's team pioneered non-myeloablative transplantation (NST) in Asia and China in the late 90s of the last century and led the Chinese NCT Collaborative Group. Since then, in 2011, they have initiated microtransplantation internationally and organized and led the International Microtransplantation Interest Research Group. Micrografting is simple, effective, and very safe, with almost no GVHD; Microtransplantation has been widely supported and replicated at home and abroad. On this basis, after more than ten years of research and discussion, Professor Ai's team has innovatively proposed and carried out new theories and models of hypertransplantation. Hypertransplantation does not require any pretreatment of the recipient, nor does it use any cytotoxic drugs, chemoradiotherapy, immunosuppressants, etc., and finally forms a completely stable donor implantation (FDC) through the immune response of the donor and recipient. The results of animal experiments have shown that even without any GVHD prophylaxis, successful transplant recipients did not develop GVHD. At present, in the pioneering study of super-transplantation by Professor Ai's team, the super-transplantation experiment of C57BL/6 male mice as donor mice and CB6F1β654 female mice as transplant recipients has been completed in animal experiments. Experimental study of hypertransplantation in H2-hasynchesic leukemia mice and thalassemia recipient mice. In particular, in the supertransplantation treatment of thalassemia recipients, all the rats did not do any GVHD pretreatment and GVHD prophylaxis, but all the rats could form a stable complete donor implantation, and the thalassemia genes were reduced to a very low level close to 0. Hemoglobin, red blood cell count, white blood cell and platelet count were all restored to the normal mouse level. Peripheral blood lymphocytes and thymus were significantly restored and immune reconstitution was completed; Moreover, acute and chronic GVHD was not observed in the rats. In addition, the gonads of the women in the super-transplantation group were intact after transplantation, and they could conceive, give birth and suckle the mice normally. The good results of hypertransplantation in animal experiments with thalassemia is a major breakthrough in the treatment of thalassemia transplantation, and it also lays a very good foundation for our upcoming clinical research on thalassemia hypertransplantation.

The center is the earliest unit in our province to carry out HSCT treatment of hematological diseases, and the technical level of HSCT is in the leading position in the province, and it has a solid clinical application conditions and foundation. We are willing to accept and adopt the new concept and treatment of hypertransplantation in Professor Ai Huisheng's team, and carry out clinical research on the treatment of thalassemia major under the guidance and help of Professor Ai, verify its "safety and efficacy", cure patients and promote the continuous development of science, and create a safe and effective new treatment model for the treatment of thalassemia major without pretreatment, reproductive damage, and post-transplant complications.

2. Research objectives

It is planned to recruit 3-5 patients with thalassemia major without HSCT indication and unable to undergo thalassemia gene therapy from June 01, 2024 to June 2025, and use the super transplantation treatment plan of Professor Ai's team to observe the cell reinfusion response, hematopoietic remodeling, hemoglobin level and blood transfusion, thalassemia clonal clearance, immune reconstitution, endocrine function recovery, gastrointestinal function recovery, infection and other complication rates. To verify the safety and efficacy of super transplantation in the treatment of thalassemia major.

3. Research design and methodology

(1) Research Design:

1. This study is a single-center, single-arm, prospective clinical study. There is no control and no blinding.

2. Subject recruitment and management

1) Patient recruitment: The center publishes recruitment information to screen patients who meet the inclusion criteria;

2) Patients were enrolled after signing the informed consent form;

3) Check the inclusion criteria: Subjects are included in strict accordance with the inclusion criteria, and if there is any situation described in the exclusion criteria, the subjects will be excluded in a timely manner, and a special person will be responsible for checking and recording relevant information.

4) Collect medical history and complete the personal information and medical history part of the study record form.

3. Lifestyle requirements

Patients should be on a hyperbaric sterile diet from the beginning of the super graft

process, and dietary hygiene should be observed. Different stages of the period have different dietary requirements, and subjects and their families should follow the arrangements of medical staff.

4. Withdrawal criteria for subjects

The study physician and the sponsor may also request to stop or withdraw from this study for the following reasons:

(1). Serious safety problems occur during the test;

(2). There are serious complications that the investigator deems necessary to terminate the trial treatment;

(3). From the perspective of the patient's interests, the best choice for the investigator is to terminate the trial treatment;

(4). The subject withdraws the informed consent or actively asks to withdraw;

(5) The subject has progressed and is no longer suitable to participate in this study;

(6). The subject had poor compliance during the trial, refused to cooperate with the treatment and follow-up, and could not continue the study.

Subjects will still be provided with appropriate treatment options after withdrawing from the pilot study, and subjects can withdraw from the study without discrimination or retaliation after notifying the investigator at any stage of the trial, and any medical treatment and rights will not be affected as a result. After withdrawing from the study, the subjects will still be informed in detail of the appropriate medical plan for continuation, and the subjects will still be regularly followed up and guided to guide the follow-up treatment of the patients.

5. Description of the study intervention

This study is a prospective, single-center, single-arm clinical study, and it is planned to include 3-5 patients with thalassemia major who have no HSCT indication and cannot receive thalassemia gene therapy. The diagnosis of thalassemia major was determined by the thalassemia genotype and the clinical manifestations of the patients, and the patients who met the inclusion criteria and did not meet the exclusion criteria were screened and enrolled and received super transplantation therapy. Hypertransplantation is an innovative treatment plan of Professor Ai Huisheng's team, which uses hematopoietic stem cell transplantation from healthy donors with the same haplotype as the patient, and observes the patient's cell reinfusion response, hematopoietic reconstitution, hemoglobin level and blood transfusion, thalassemia clonal clearance, immune

reconstitution, endocrine function recovery, gastrointestinal function recovery, as well as the incidence of graft-versus-host disease, infection and other complications after transplantation.

The duration of follow-up was 24 months. Follow-up visits include:

1) Blood routine: transplant 0d-1m at least 3 times a week; 1m-3m at least 1 time per week; 3m-6m at least 1 time per month; ⩾6m at least once every 2 months. If the condition changes, the number of tests can be increased as appropriate.

2) DNA mosaicism rate of peripheral blood/bone marrow donors: +4, +7, +14, +30d, +90; 1 time per month for 1 year after transplantation; Within 3 years after transplantation. 1 time from March to June.

3) Thalassemia gene: 1 time before transplantation and 1 year after transplantation, once a month.

4) Liver and kidney function and serum ferritin: transplant 0d-1m at least once a week; 1m-3m at least 2 times a month; 3m-6m at least 1 time per month; ⩾6m at least once every 2 months. If the condition changes, the number of tests can be increased as appropriate.

5) Cardiac color ultrasound: 1 time before transplantation, 1 time every 3 months within 1 year after transplantation.

6) Heart and liver MRI iron test: once before transplantation, once every 6 months within 1 year after transplantation.

7) Lymphocyte subsets: 1 time before transplantation, 1 time per month within 1 year after transplantation.

8) Cytokines: 1 time before transplantation, 1 time per month within 1 year after transplantation.

9) Thyroid function, ACTH and gonadal function: once before transplantation, once every 6 months within 1 year after transplantation. 10) Other items that need to be studied: thalassemia gene level, DNA chimerism status and child growth indicators.

6. Relevant provisions on concomitant medications, permitted drugs, cautionary drugs, prohibited drugs and concomitant treatment

Concomitant medications:

Permitted medications and concomitant treatments:

(1) Other therapeutic drugs and treatments that the investigator believes do not have an

impact on the efficacy and safety evaluation of the research subjects are allowed.

(2) Any concomitant medication and concomitant therapy need to be approved by the investigator.

7. Research steps

One week before transplantation, the center evaluated the patients, and those who met the inclusion criteria were screened and enrolled. The study doctor will inform the benefits and risks of participating in this study in detail, and sign the informed consent form with full understanding of this study.

Transplantation protocol and procedure:

1) G-CSF mobilization and donor collection: the same as the conventional peripheral blood mononuclear cell (PBMC) protocol.

2) Transplantation plan and treatment principles:

(1) Pretreatment: None.

(2) GVHD prophylaxis: 1/2 dose of conventional transplant GVHD prophylaxis, and the use time is half a year, see separate article for details.

(3) Donor cell infusion, etc., are detailed in a separate article.

(4) Prevention and treatment of complications: fungal, pulmonary prevention programs and antiviral treatment.

3. Follow-up period: All subjects started on the first day after transplantation and were followed up to 24 months after transplantation. The patient's cell reinfusion reaction, hematopoietic reconstitution, hemoglobin level and blood transfusion, thalassemia clonal clearance, immune reconstitution, endocrine function recovery, gastrointestinal function recovery, infection and other complications were recorded.

8. Evaluation

1) Efficacy evaluation:

Main indicator: Donor cell engraftment rate, time: 3 months after transplantation.

Secondary indicators: thalassemia gene carriage, growth and development assessment, time: 1 year.

2) Safety evaluation

Main indicator: transplant-related death, time: 3 months after transplantation.

Secondary indicators: GVHD, infectious complications, time: 1 year.

Any adverse events that occur are recorded during the study period and treated in a timely manner, and the patient's vital signs, physical examination, laboratory tests, etc. are recorded in detail. In this trial, corresponding management and treatment measures have been formulated for potential major risk events such as allergic reactions and infections.

1) Physical examination: examination of all systems of the whole body;

2) Vital signs: body temperature, respiration, heart rate, blood pressure;

3) Laboratory tests: blood routine, urine routine, blood biochemistry, electrolytes, coagulation function;

4) 12-lead ECG;

5) Detection of infectious markers;

6) Adverse events and serious adverse events. The severity of adverse events was based on the CTCAE version 5.0 classification criteria.

9. Statistical analysis and statistical methods

(1) General method

In this study, descriptive statistics and multivariate regression analysis were used, absolute numbers were used for categorical data, and median data were used for continuous data, and chi-square test and nonparametric test were mainly used, $p<0.05$ was statistically significant.

and (2) analysis of primary and secondary study endpoints

(3) Safety analysis

AEs were coded and calculated, which could be expressed in terms of severity, frequency, and association with the intervention. Adverse events leading to discontinuation of study intervention and serious AEs resulting from treatment should be listed individually.

(4) Baseline descriptive analysis

Descriptive statistics were used to compare demographic characteristics and laboratory indicators at baseline between groups.

(5) Subgroup analysis

The study endpoints could be analyzed according to the transplantation conditioning regimen, pre-transplantation remission status, age, neutrophil and platelet engraftment

time, etc., and the association between the primary and secondary study endpoints and different factors could be evaluated by COX regression.

10. Quality control and quality assurance

In order to ensure that the trial can be carried out in strict accordance with the clinical research protocol, in the process of clinical trial, the clinical investigator, the sponsor and the clinical supervisor should operate in accordance with the requirements of the clinical trial quality management practice, and must ensure that the test procedures are standardized, the experimental data are accurate, the experimental data are traceable, and the research conclusions are reliable.

IV. Inclusion and exclusion guidelines

(1) Patient enrollment criteria:

Patient inclusion criteria (the following 4 criteria must be fully met):

1) Confirmed diagnosis of thalassemia major, regardless of α and β type;

2) Age between 7-12 years old, male or female; Weight< 40kg

3) Patients with or without HLA-matched or haploidentical donors, but unconditionally transplanted or refused to undergo blood stem cell transplantation; Patients who are unconditional or refuse to undergo thalassemia gene therapy;

4) There are HLA fully compatible or incompatible donors, and the physical examination meets the donor conditions;

5) The patient and his or her family agree to receive ultra-transplantation therapy and sign a written informed consent form before the transplantation trial.

(2) Patient exclusion criteria:

1) Psychopaths;

2) Those who have participated in clinical trials of other drugs in the past 1 month;

3) There are no suitable HLA incompatible donors.

4) Other investigators who judge that they are not suitable to participate in this study.

(3) Donor selection criteria:

1) The HLA match is consistent with the patient's haplotype

2) KIR matching

3) NIMA

4) DSA negative

5) Routine physical examination

6) Thalassemia gene screening carriers, mild or non-thalassemia carriers

7) Sign the informed consent form.

5. Subject protection measures (reasons for the selection of research subjects, recruitment plans and procedures, explanations of the process of obtaining informed consent; measures to protect the privacy of subjects and keep confidential information of subjects; plans for reasonable compensation for research subjects; and plans for adverse event reporting). Where applicable, it should also include data and safety monitoring plans, plans for the use and storage of biological samples, etc.

1. Subject recruitment and management

1) Patient recruitment: The center publishes recruitment information to screen patients who meet the inclusion criteria;

2) Patients were enrolled after signing the informed consent form;

3) Check the inclusion criteria: Subjects are included in strict accordance with the inclusion criteria, and if there is any situation described in the exclusion criteria, the subjects will be excluded in a timely manner, and a special person will be responsible for checking and recording relevant information.

4) Collect medical history and complete the personal information and medical history part of the study record form.

2. Informed consent process

Informed consent should be completed prior to the study subject's consent to participate in the study and continue throughout the study. Informed consent form is agreed by the ethics committee, and the study subject should read the informed consent form. The researcher will explain the research process and answer questions from the research subjects; and inform the research subjects of the possible risks and their rights. Study subjects may discuss with family members or guardians before agreeing to participate. The investigator must inform the study subject that participation in the study is voluntary and that they can withdraw from the study at any time in the study. A copy of the informed consent form may be provided to the study subject for safekeeping. The rights and benefits of study subjects will be protected, and it is emphasized that the quality of their medical care will not be compromised by refusal to participate in the study.

3. Privacy protection

In all the documents submitted by the investigator, the identity of the subject is indicated by the clinical identification code, and the patient's name, ID number, hospitalization number and other private information do not appear. The investigator strictly keeps the record of the subject's detailed information and does not submit it to the sponsor or other representatives.

4. Collection and use of specimens and materials

The specimen information involved in the study is tested in our hospital, and no third-party testing or storage is involved. After the end of the study, the laboratory examination results of the specimens were kept in the clinical records of the research center. The data were collected based on follow-up and laboratory results, which may be used in other relevant studies after the end of the study.

5. Medical treatment and protection of subjects

(1) Medical treatment and protection of subjects during the study

Provide medical monitoring, psychological and health counseling; Subjects have the right to voluntarily withdraw from the study at any time during the course of the trial; Subjects will still be provided with appropriate treatment options after withdrawing from the pilot study, and subjects can withdraw from the study without discrimination or retaliation after notifying the investigator at any stage of the trial, and any medical treatment and rights will not be affected as a result. The cost of treatment in this trial is paid by the participants.

(1) Medical treatment and protection of subjects after the research process

After the research process, the subjects will still be informed in detail of the continued medical plan, and the subjects will still be regularly followed up and guided to treat, and the follow-up treatment of the patients will be guided in detail.

6. Adverse events and serious adverse events

It is the responsibility of the investigator to ensure that all AEs reported by the subject after the investigator's observation have been reported in the eCRF during the post-study safety follow-up period since the subject started study treatment, and at any time during the safety follow-up after the end of study treatment, the investigator should report any AEs that the investigator believes may be relevant to study dosing, and during the safety follow-up period, the investigator should report all SAEs.

Any serious adverse events that occur in the trial, regardless of whether they are related to the trial intervention, should be promptly rescued by the investigator, and the Serious

Adverse Event (SAE) Report Form provided by the sponsor should be filled in and dated within 24 hours after being informed, and reported to the sponsor immediately. SAEs should detail a description of symptoms, severity, time of onset, time of treatment, measures taken, timing and manner of follow-up, and prognosis. Serious adverse event reports and follow-up reports should indicate the identification code of the subject in the clinical trial, rather than the identity information such as the subject's real name, ID number and address.

7. Benefits and risks

7.1 Benefits:

1) Obtain a chance of thalassemia major cure;

2) Free of charge: donor stem cell mobilization and collection, stem cell infusion pretreatment, lymphocyte subsets and cytokine detection 12 times, thalassemia gene detection 13 times, donor DNA mosaic rate monitoring 12 times. The total monitoring cost is about 40,000 yuan.

4) Insurance 200 yuan/person, insurance amount 200,000.

7.2 Risks:

During the treatment, there is a risk of adverse reactions such as donor implantation failure, cytokine release syndrome, graft-versus-host disease, drug side effects, infection, etc., which are associated with transplant therapy and cannot be completely avoided.