Version number: GZXY.RhD-HPT-V0.1-CTP
Version Date: September 5, 2016

A multi-center, single-arm clinical trial for exploring the safety and effectiveness of RhD+ blood transfusion in Chinese "Asia type" DEL patients

Institute in charge of research: Guangzhou Blood Center
Address: 31 Luyuan Road, Yuexiu District, Guangzhou, China

Statistical analysis Institute:
*School of Public Health, Southern Medical University
Address: 1023 Shatai South Road, Baiyun District, Guangzhou, China
*Guangdong Provincial Institute of Biological Products and Materia Medica
Address: No. 200, Xipengling Road, Jiahe Hebian, Baiyun District, Guangzhou, China

Summary of previous version changes

Version number	Chapter with change	Reason for revision	Revised version number

Declaration

This trial will be carried out in accordance with the *Drug Clinical Trial Quality Management Standards* and *Guidelines for Quality Management of Vaccine Clinical Trials (tentative standard)* (Administrative Order No. 28).

The investigators who participating the clinical trials and the staffs who are responsible for performing, managing, and supervising the clinical trials, need to receive the training about the protocol.

The trial protocol, informed consent for blood transfusion treatment and all relevant files need to be submitted to the ethics committees for approval. All amendments of the plan must be reviewed and approved by the ethics committee before implementation. Before the subjects are selected, the review approval of protocol ethics needs to be obtained.

Privacy statement

This research protocol is confidential, and it is provided to the medical experts related to this research, the researchers participating in this research and other research-related staffs, and the medical institutions, the ethics committees, and other cooperated research organizations that undertake this research. Without the prior written consent of the sponsor, no part of this research protocol could be disclosed to the third parties. In addition, publishing of part or all results of this clinical study need to be approved by the sponsor.

Ethical statement

& The documents need to be followed:

The trial must comply with all SOPs related to the clinical trial established by the Guangzhou Blood Center, and follow the design and regulations of this protocol. The trial protocol was designed to follow the documents below:

- ✧ *Management Practices for the Quality Control of Drug Clinical Trials*
- ✧ *The Helsinki Declaration*
- ✧ *Technical Specifications for Clinical Blood Transfusion*
- ✧ *Technical Guidelines for Vaccine Clinical Trials*
- ✧ *Guiding Principles for Quality Management of Vaccine Clinical Trials (tentative standard)*
- ✧ *Regulations on the Management of Serious Adverse Events in Vaccine Clinical Trials (tentative standard)*

& Institutional Review Board and Ethics Committee

The trial protocol needs to be reviewed and approved by the Guangzhou Blood Center Clinical Trial Ethics Committee before implementation. During the review, the sponsor and researcher should provide the ethics committee with the documents required, such as the clinical trial protocol, informed consent for blood transfusion treatment, and case report form and so on. This clinical trial must comply with *the Helsinki Declaration*, *Management Practices for the Quality Control of Drug Clinical Trials* issued by the National Medical Products Administration (NMPA) and related regulations in China. Before the trial begins, this study must be approved by the clinical trial ethics committee of the Guangzhou Blood Center. Any changes of the protocol must be approved by the ethics committee. The researchers are responsible for submitting the trial report regularly in accordance with the relevant requirements of the ethics committee.

& The informed consent of transfusion therapy

Before each possible subject participates in any activities related to the trial, the investigator must explain to the subject in details about the nature, purpose, relevant procedures, expected time, potential risks and benefit and any discomfort that may arise. Each subject must know that participation in the trial is voluntary and that he/she can withdraw from the trial and withdraw informed consent for blood transfusion treatment at any time without affecting his/her subsequent treatment or relationship with the responsible physician.

After explaining the major content of the trial, the investigator has been convinced that each subject who will participate in the trial has knowledge of the trial and each subject who will participate in the trial should be required to sign the informed consent form for the transfusion, date, and contact information. Subjects should read and consider carefully before signing the informed consent of blood transfusion treatment.

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Abbreviations Table

Acronyms	Full name
AE	Adverse Event
ALP	alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BLD	Urine occult blood
BUN	Blood urea nitrogen
Cr	Creatinine
CRF	Case Report Form
DBIL	Direct bilirubin
ECG	Electrocardiogram
GLU	Fasting blood glucose
Hb	Hemoglobin
Hct	Hematokrit
LEU	leukocyte
NEUT%	Neutrophil ratio
NMPA	National Medical Products Administration
PLT	Platelet
PRO	Proteinuria
RBC	Red Blood Cell
SS	Safety Data Set
SAE	Serious Adverse Reaction
TBIL	Total bilirubin
TP	Total protein
WBC	White Blood Cell
γ-GT	γ-glutamyl transpeptidase

Protocol Abstract

Name of the trial	**A multi-center, single-arm clinical trial for exploring the safety and effectiveness of RhD+ blood transfusion in Chinese "Asia type" DEL patients**
Sponsor	Guangzhou Blood Center
Purpose of the trial	To explore the safety and effectiveness of RhD+ blood transfusion in Chinese “Asia type” DEL patients to lay the foundation for establishing a new blood transfusion strategy for “Asia type" DEL patients.
Design of the trial	A multi-center and single-arm trial
Target indication	Patients with “Asia type” DEL blood group who need transfusion therapy in clinic
Sample size	“Asia type” DEL is one of rare blood groups with a distribution frequency of 0.75-1‰. So, to trying to collect as many samples as possible according to the inclusion criteria in this study.
Study population	**Inclusion criteria:** 1. Patients identified as "Asia type" DEL blood group; 2. Male patients, or female patients ≥49 years of age, or female patients with severe illness and no further pregnancy plan; 3. Patients with need for blood transfusion in line with the guidelines for internal or surgical and traumatic blood transfusion: * In line with the guidelines for internal blood transfusion of *the Technical Specifications for Clinical Blood Transfusion*, red blood cell transfusion is considered for the patients with chronic anemia caused by excessive destruction of red blood cells, loss or production disorders with hypoxic symptoms; hemoglobin <60g / L or hematocrit <0.2; * In line with the guidelines for surgical and traumatic blood transfusion of *the Technical Specifications for Clinical Blood Transfusion*, the red blood cell transfusion is considered for the patients who need to increase blood oxygen carrying capacity with normal or corrected blood volume; hemoglobin <70g/L; 4. Signing the informed consent voluntarily for blood transfusion treatment before the trial. Patients and/or their legal representatives have ability to fully understand the trial content, process and possible adverse reactions, and enable patients to comply with the visiting and inspections specified in the protocol. **Exclusion criteria:** 1. Patients who had a transfusion reverse reaction in a previous transfusion; 2. Patients had allergies, immunodeficiency, and immunosuppression treatment; 3. Patients needed blood transfusion for acute blood loss such as trauma;

	4. Female patients had positive result of pregnancy test; 5. Patients had conscious dysfunction and severe mental illness; 6. Patients were judged unsuitable for the study by the investigator.
Intervention method	When the transfusion departments of the participating hospitals encounter the patients with the rare primary RhD negative blood group who needed to apply for blood transfusion in non-emergency situations, the application form and blood samples of the patients were sent to the reference lab of Guangzhou Blood Center for "Asia type" DEL blood group typing. The patients identified as "Asia type" DEL blood group and meeting the inclusion criteria will be provided the compatible RhD+ blood with the same ABO type after cross-matching test by Guangzhou Blood Center. RhD+ blood transfusion for the patients with "Asia type" DEL blood group was performed in the cooperated hospitals in line with *the Technical Specifications for Clinical Blood Transfusion*.
Evaluation index for effectiveness	Hemoglobin concentration (Hb); Hematocrit (Hct) etc.
Evaluation index for safety	1. Incidence of anti-D alloimmunization; 2. Incidence of adverse blood transfusion reactions; 3. Observation of vital signs: temperature, heart rate, breathing, blood pressure etc.; 4. Laboratory testing; 5. Incidence and severity of other adverse events.
Visiting time	Phase I of Visiting: -7~ 0 days before transfusion; Phase II of Visiting：Blood transfusion treatment period（0~1 days）; Phase III of Visiting：1 day to 1 month after blood transfusion; Phase IV of Visiting：1 month to 12 months after blood transfusion (based on the actual return visit in the hospital or follow-up at home) ; Phase V of Visiting：Over 12 months after blood transfusion.
Statistical Analysis	As the “Asia type” DEL is a rare blood group with a frequency of around 0.75-1‰ in East and Southeast Asian population, it is not feasible to collect the enough samples to conduct the common statistical analysis. So, the descriptive statistics was regarded as the principal analysis method in this study. Continuous variables are described by number of cases, mean, standard deviation, median, minimum, and maximum; categorical variables are summarized by frequency and percentage. Statistical analysis is conducted using SAS9.4 statistical software.
Number of research centers	A total of 9 hospitals participate the trial.
Progress of the trial	The trial is expected to be completed within 48 months after beginning.

Protocol · Text

I. Research Background

Alloanti-D immunization against RhD blood group antigens in RhD-negative (D–) individuals can cause severe adverse transfusion reactions and fetal and neonatal hemolytic disease (HDFN). Therefore, routine RhD blood group testing and RhD matched blood transfusion strategies have been adopted in the most countries of the world.

The D– phenotype is more common in white (15-17%) and African (3-5%) populations, but is rare in the Asian population (0.3-0.4%), which led to supply shortage of D– blood in Asian countries. In recent years, a series of studies have shown that 9–30% of the population with serologically apparent D– phenotype actually belong to a variant blood type called DEL (RhD elution), not the true D– phenotype in East and Southeast Asia, including China [1-7], South Korea [8, 9], Japan [10], and Thai [11], which is very rare among white and black races. DEL is a very weak form of expression of RhD antigen, that is, there is a very small amount of RhD blood group antigen expressing on the surface of red blood cells (RBC). It will not cause an effective agglutination reaction in conventional RhD blood group typing, so it will be detected as the rare D– blood group. Among the traditional serological methods, only adsorption/elution experiments can detect the DEL phenotype, but this method is time-consuming and reagent-intensive, and has high technical requirements for the operators, so it is not be applied as a routine clinical testing. In East Asia and Southeast Asia including China, the patients with the DEL phenotype are always clinically detected as the rare D– patients and receive the rare D– blood transfusion to prevent alloanti-D immunization.

The DEL phenotype has many different molecular genetic backgrounds with strong genetic heterogeneity. So far, more than 40 alleles have been reported to relate to the DEL phenotype, but *RHD*1227A* (*RHD*01EL.01*) is the predominant allele (>99%) distributed in the DEL individuals among East and Southeast Asian populations [1, 3, 6, 8-12]. Therefore, DEL with this allele is called the "Asia type" DEL blood group [13]. The DEL blood group can be divided into two main types according to whether the epitopes are intact and whether anti-D alloimmunization could occur [14]. One type is the "partial DEL" phenotype with missing D epitopes and anti-D alloimmunization can occur under the stimulation of D+ red blood cells. The "partial DEL" patients should be treated as D– rare patient in clinic to receive D– blood transfusion. The other is the “complete DEL"

phenotype with complete D antigenic determinants (with the same D antigen structure comparing with normal D+ antigen), but with low number of antigen expression. It will not cause anti-D alloimmunization under the stimulation of intact D+ antigen, so it is not necessary to treat it as the rare D– blood patients in clinic.

In recent years, more and more experimental and clinical data show that the most common "Asia type" DEL in Asian populations has the characteristics of "complete DEL". First, in several independent studies, the adsorption/elution experiments did not detect the absence of D antigen determinant in "Asia type" individuals [2, 14]. In addition, the synonymous mutation in exon 9 of the *RHD* gene (c.1227G> A, p.K409K) defined the "Asia type" DEL allele have been shown to cause abnormal mRNA splicing in molecular mechanisms studies. It resulted in a series of abnormal mRNA transcripts lacking exon 9, but further Mini Splicing function analysis in vitro found that the "Asia type" DEL allele express extremely low abundance transcripts including complete exon 9. This transcript may express a very low density of normal D antigen, thus forming the "Asia type" DEL phenotype [15]. Most importantly, several prospective and retrospective studies on observation of "Asia type" DEL pregnant women have shown that none of them developed alloanti-D. These studies involved the data from at least a thousand of "Asia type" DEL pregnant women (including more than 600 cases studied in our study and hundreds of samples included in several other studies) [6, 13, 16, 17] in China, which covering more than one million of people. In contrast, in the true D– Chinese population excluding “Asia type" DEL, the frequency of alloanti-D immunity ranges from 8.5% to 40% [13, 17]. Therefore, clinical data show that in the case of “Asia type" DEL Chinese pregnant women with high probability of having a normal RhD-positive fetus (>95%), alloanti-D will not be produced by D+ red blood cells from the fetus and newborn during pregnancy and childbirth.

Based on the results of previous research, it is extremely urgent to conduct a clinical trial of D+ blood transfusion in “Asia type" DEL patients, and to follow up in detail to obtain the anti-D immunization data in transfusion patients. This study is essential to set up a new blood transfusion strategy for “Asia type" DEL patient. In South Korea, two cases of “Asia type" DEL patients have been reported to receiving D+ blood transfusion, but still lacking of systematic clinical trial data to make the conclusion [18]. Therefore, the Korean National Blood Transfusion Guidelines (KNGBT) concludes that now there is not enough direct evidence to prove the safety of “Asia type" DEL

patients receive D+ blood transfusion, and D+ blood transfusion is only allowed in the condition of emergency or lacking of D– blood supply for “Asia type" DEL patients [18]. In summary, this clinical study has important practical significance and can provide basic evidence whether anti-D alloimmunization will occur in “Asia type" DEL patients with ordinary D+ blood transfusion. If a new strategy of D+ blood transfusion in “Asia type" DEL patients can be established in the future, that is, the routine “Asia type" DEL testing in patients with a primary D– phenotype could be performed, and then to treat the “Asia type" DEL patients as ordinary D+ patients, rather than the rare D– patients in clinic. It will save at least 25% of rare D– blood in East and Southeast Asian countries, and it will also prevent “Asia type" DEL pregnant women receive anti-D immunoglobulin injection and other medical intervention/monitoring to prevent anti-D associated fetal neonatal hemolytic disease, which also could save the precious blood product anti-D immunoglobulin that derived from the sera from the D– volunteers who are sensitized.

II. Purpose of the trial

To explore the safety and effectiveness of RhD+ blood transfusion in Chinese “Asia type” DEL patients to lay the foundation for establishing a new blood transfusion strategy for “Asia type" DEL patients.

III. Design of the trial

The aim of this study is to analyze whether anti-D alloimmunization will occur in “Asia type" DEL patients after D+ blood transfusion, and then to compare the results with the data from the true D– patients received D+ blood transfusion to determine the “Asia type" DEL patients should be treated as D+ or D– patients in the future. The ideal study control group is the incidence of anti-D alloimmunization of true D– patients receiving D+ blood transfusion. But for ethical reasons (according to the requirements of *Technical Specifications for Clinical Blood Transfusion*, true D– patients can only be transfused with D+ blood in urgent situation or lacking of D– blood supply), and it is not appropriate to set up the control group (to transfuse D+ blood in true D– patients in non-emergency situations). Although the summarized rate of alloanti-D occurrence (22.9%, 53/231) [19-22] in true D– patients transfused with D+ blood in previous reported studies could be used as the control for the statistical analysis including sample size calculation, our study could not satisfy

the demands as the “Asia type” DEL transfusion satisfied the inclusion criteria belong to a rare event because of the rare distribution of “Asia type” DEL in our population (0.75-1‰). In theory, more than 2000 “Asia type” cases receiving D+ blood transfusion, which covering around fifty millions population, are necessary to compare the difference of alloanti-D occurrence between the “Asia type” DEL patients group in our study and the true D– control patients group with similar disease categories receiving D+ RBCs transfusion. It is not feasible in practice for this study.

Therefore, a multi-center, single-arm clinical trial was performed in this study. When any hospital in Guangzhou city applies for D– blood supply in a non-emergency situation, routine “Asia type" DEL typing, antibody screening and identification, and cross-matching test for “Asia type" DEL patients were conducted in the reference lab of Guangzhou Blood Center. The “Asia type" DEL patients meeting the inclusion criteria were recruited in each cooperative hospital. After the informed consent for D+ blood transfusion was obtained from the patient or family members, cross-matched D+ blood was provided by Guangzhou Blood Center. D+ blood transfusion was performed at the corresponding hospital according to *Technical Specifications for Clinical Blood Transfusion* [23] and *Technical Guidelines for Vaccine Clinical Trials* [24].

IV. Selection of Subjects

(I) Inclusion criteria (same to the Protocol Abstract)

(II) Exclusion criteria (same to the Protocol Abstract)

(III) Rejection criteria

1. Cases that did not meet the inclusion criteria or belong to one of the exclusion criteria after enrollment;
2. Cases who did not receive blood transfusion finally in clinic after enrollment.

(IV) Dropout criteria

1. All patients, who signed the informed consent of blood transfusion, meet the inclusion criteria and enrolled, but failed to complete the follow-up observation in accordance with the protocol requirements;
2. The subject requested to withdraw from the trial;

3. In the trial, if the subject has an adverse event that prevents him/her from completing the clinical trial, the investigator should record the process and outcome of the adverse event in detail, and make a judgment on the cause and effect of the trial. Cases dropout due to adverse events should be included in the statistical analysis of adverse events, but were not included in the evaluation of efficacy;

4. The subject get Lost for follow-up after receiving transfusion treatment;

5. The data of the CRF table is incomplete or cannot be traced to the source, which affects the evaluation of efficacy and safety.

(V) Criteria for termination of the clinical trial

Study termination means that the clinical study has not been completed as planned, and all studies have been stopped halfway. The purpose of the study termination is to protect the rights and interests of the subjects, ensure the quality of the trial, and avoid unnecessary financial losses.

1. Serious safety problems occurred during the study, and the study should be suspended timely;

2. It is found that there are major mistakes in the clinical protocol in the study, and it is difficult to evaluate the treatment effect;

3. The research was stopped due to the requirements for funding and management;

4. The administrative department revokes the test, etc.;

When this occurs, the investigator needs to inform the subjects before stopping the trial.

V. Experimental intervention

When any hospital in Guangzhou city applies for D– blood supply in a non-emergency situation, the application and the patient's blood sample will be sent to the Reference laboratory of Guangzhou Center for “Asia type" DEL routine testing. For the “Asia type" DEL patients identified, the patients who meet the inclusion criteria will be enrolled in the hospital. After the patient or family members signed the informed consent for blood transfusion treatment, the matching D+ blood will be provided by Guangzhou Blood Center. According to the clinical blood transfusion guidelines, the blood transfusion for “Asia type" DEL patients was conducted in the corresponding hospital. According to *the Technical Specifications for Clinical Blood Transfusion*, the registration of

blood out-put and in-put, checking, and issuing should be carefully done in the blood transfusion departments or blood banks of all participating hospitals and the blood should be stored and transported in the temperature of 4 ± 2 ° C.

1. Before blood transfusion, two medical staffs should check the cross-matching report, the label of the blood bag, and check whether the blood bag is damaged or leaking and whether the blood color is normal. When all of these have been confirmed to be normal, the transfusion could start.
2. During the blood transfusion, two medical staffs will bring the medical records to the patient's bed to check the patient's name, gender, age, blood type, medical record number, outpatient/ward room, bed number, etc., and confirm that the blood matches the cross-matching report. After checking the blood again, the blood transfusion begins using a transfusion device that meets the standards.
3. The blood should be transfused as soon as possible after taken back and not be stored. Before transfusion, to gently mix the ingredients in the blood bag and avoid violent shaking. No other drugs are permitted be added into the blood. If the dilution is needed, only saline for intravenous injection can be used.
4. Flushing the vessel with the intravenous saline before and after blood transfusion. When the patient receives continuous transfusion with the blood from different donors, after the previous bag of blood has been transfused, the transfusion set should be flushed with the intravenous saline, and then the next bag of blood could be continuously transfused.
5. During the process of blood transfusion, the speed of transfusion should be slow in the beginning and then faster, adjust the infusion rate according to the condition and age of the patient. It should be closely observed the recipients for adverse transfusion reactions. If abnormal conditions occur, the patient should be treated in time.

VI. Observation index

(I) Demographics

To collecting the ID information, date of birth, gender, and telephone number of the patient.

(II) Subject characteristics

The source of the patient, case number, chief complaint, current medical history, clinical diagnosis, blood type, pregnancy-labor history, history of infectious diseases, blood transfusion history,

allergy history, and family history.

(III) Safety observation indicators

During the screening period, the laboratory test results within 3 days before the informed consent is signed are acceptable, and the method for laboratory tests are not limited and the results can be traced. For any abnormal symptoms, signs, laboratory test results (except pregnancy test results) in clinical research, no matter the degree of severity or the relation to treatment and the authorized investigator should determine that a repeated test is necessary or not. If necessary, the patients should be asked for regular visits, and the characters and treatment history should be recorded in detail. The follow-up should be continuing until the patient recover to the normal or baseline levels, or the investigator could reasonably explain the abnormality and do not think the follow-up is necessary.

<u>The main safety observation indicators shown below:</u>

1. The incidence of anti-D alloimmunization;

2. The Incidence of adverse blood transfusion reactions;

It is necessary to monitor the whole process of blood transfusion, and record the specific manifestations of adverse reactions of blood transfusion in detail.

3. Observation of vital signs

To monitoring the heart rate, breathing, blood pressure, and temperature. To measure blood pressure while resting.

4. Laboratory testing

1) Blood routine: white blood cells (WBC), neutrophils (NEUT), hemoglobin (Hb), hematocrit (Hct), platelets (PLT);

2) Four items for infections detection;

3) Liver function testing: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), total plasma protein (TP), plasma albumin (ALB);

4) Renal function testing: Urea, creatinine (Cr);

5) Electrolyte inspection: potassium (K^+), sodium (Na^+), calcium (Ca^{2+}), phosphorus (P^{3+}), magnesium (Mg^{2+});

6) Examination of coagulation function: prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB);

7) Urine routine;

8) Stool routine, occult blood test.

5. Pregnancy test: The women patients of childbearing age who are judged to need a pregnancy test based on the actual situation need to obtain a negative result to rule out pregnancy before enrollment. The women patients who reach menopause, or are undergoing menstruation at the time of the test, or have undergone birth control surgery do not need to have a pregnancy test, but need to record the reason for not doing so.

6. Observation and recording of the adverse events

It is necessary to monitor and record the occurrence of the adverse events and its development in detail throughout the clinical trial.

(IV) Observation index of curative effects

To record the changes in hemoglobin concentration and hematocrit before and after blood transfusion.

(V) Concomitant treatment

During the study, all concomitant treatments or combined medications (from the beginning of the signed informed consent to the end of the trial) must be recorded in detail in the original medical record, including the intervention name, intervention purpose, dosage form, single dose, dosage unit, frequency of use, route of administration, start date, end date, and whether it is still being used.

For the combined treatment in the clinical research process, the corresponding analysis and recording should be done, especially the combined treatment in the event of adverse events, recorded and reported in a timely manner.

VII. Evaluation Index

(I) Main indicators for evaluation of the effectiveness

Hemoglobin concentration and hematocrit.

(II) The indicators for evaluation of the safety of the trial

1. Incidence of anti-D alloimmunization;

2. Transfusion r adverse reaction;

3. Vital signs: temperature, heart rate, breathing, blood pressure;

4. Blood routine (WBC, NEUT, Hb, Hct, PLT), renal function (Urea, Cr), liver function (ALT, AST, TBIL, DBIL, TP, ALB), electrolytes (K^+, Na^+, Ca^{2+}, P^{3+}, Mg^{2+}) before and after transfusion;

5. Incidence and severity of adverse events.

VIII. Steps of Clinical trial

(I) Phase I of Visiting: -7 ~ 0 days before transfusion

1. To explain the research purpose, methods, and requirements to the subjects, and to sign the informed consent for blood transfusion treatment by the two parties jointly;

2. To Collecting the demographics, characteristics, past/concomitant medical history of the subjects, and to conduct detailed physical examinations, and measure vital signs (including heart rate, breathing, blood pressure, body temperature; to measure blood pressure in a resting state);

3. Laboratory testing:

1) "Asia type" DEL typing;

2) Cross-matching test (only for the "Asia type" DEL patients);

3) Blood routine: white blood cells (WBC), neutrophils (NEUT), hemoglobin (Hb), hematocrit (Hct), platelets (PLT);

4) Four items for infections detection;

5) Liver function testing: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), total plasma protein (TP), plasma albumin (ALB);

6) Renal function testing: Urea, creatinine (Cr);

7) Examination of coagulation function: prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen (FIB);

8) Urine routine;

9) Stool routine, occult blood test;

10) Pregnancy test: The women patients who reach menopause, or are undergoing menstruation at the time of the test, or have undergone birth control surgery do not need to have a pregnancy test, but need to record the reason for not doing so.

11) Electrocardiogram.

4. Combined treatment/medication;

5. Evaluation of the patients for participation of this study based on inclusion and exclusion

criteria.

(II) Phase II of Visiting：Blood transfusion treatment period（0~1 days）

1. To record the detail of transfusion, including the time of transfusion start and end, the type of transfusion, the volume of transfusion, and to observe the adverse reaction during the transfusion treatment;

2. Observation of vital signs: temperature, heart rate, breathing, blood pressure;

3. Observation and recording of adverse events;

4. Combined treatment/medication.

(III) Phase III of Visiting：1 day to 1 month after blood transfusion

1. Physical examination, observation of vital signs (including heart rate, breathing, blood pressure, body temperature and to measure blood pressure in a resting state);

2. Laboratory testing:

1) Detection of anti-D immune response (using antibody screening test);

2) Blood routine: including white blood cells (WBC), neutrophils (NEUT), hemoglobin (Hb), hematocrit (Hct), platelets (PLT);

3. Observation and recording of adverse events;

4. Combined treatment/medication.

(IV) Phase IV of Visiting：1 month to 12 months after blood transfusion

1. Laboratory testing:

1) Detection of anti-D immune response (using antibody screening test);

2) "Asia type" DEL blood group typing.

2. Observation and recording of adverse events.

(V) Phase V of Visiting：Over 12 months after blood transfusion

1. Laboratory testing:

1) Anti-D immune response test (using antibody screening test);

2) "Asia type" DEL blood group typing.

2. Observation and recording of adverse events.

IX. Adverse events, serious adverse events

The clinical trials of blood transfusion during the study period shall strictly abide *the*

Management Practices for the Quality Control of Drug Clinical Trials (GCP) [25], and *the Guiding Principles for Quality Management of Vaccine Clinical Trials (tentative standard)* [26] and comply with relevant regulatory requirements. Adverse events that occur in clinical trials need to be monitored by relevant regulatory authorities and relevant parties (registered applicants, ethics committees, and researchers), and to be reported in accordance with *the Regulations on the Management of Serious Adverse Events in Vaccine Clinical Trials (tentative standard)*. The researchers should strictly comply with the GCP principles and closely observe the occurrence of adverse events in each subject. All adverse events should be fully recorded in the CRF form, including symptoms, time of occurrence, severity (mild, moderate, severe), duration time, measures taken and condition of recovery. The researchers make a judgement about the relationship between the adverse events and research methods according to prescribed standards. The researcher should inform the subject or his legal guardian that he or she should promptly notify the doctor of any health problems so that the adverse events can be recorded or handled in a timely manner.

(I) Definition

Adverse Event (AE): An adverse medical event that occurs after a clinical trial subject received a blood transfusion treatment, but it does not necessarily have a causal relationship with the treatment.

Serious adverse event (SAE) refers to the medical events that require hospitalization or prolonged hospital stay, disability, affecting work ability, threatening the life or death, and resulting in congenital malformations during the clinical trial. These include the following unexpected medical events:

- ✧ Events leading to death;
- ✧ Life-threatening event (defined as the subject has risk of death at the time of the event);
- ✧ Events requiring hospitalization or extension of hospitalization;
- ✧ Events that can cause permanent or severe disability/dysfunction;
- ✧ Congenital anomalies or birth defects;
- ✧ Causing other medical events and have the conditions listed above in the condition of lacking of treatment.

(II) Grading criteria for the adverse events

When filling out the adverse event form, the researchers will use "mild, moderate, and severe" to describe the extent of the adverse event. To harmonize the standards, the levels of incidents are classified as follows:

- ✧ Level 1: Mild, short-term (less than 48 hours) or minor discomfort, no need for treatment;
- ✧ Level 2: Moderate, Mild or moderately restricted movement, may require medical attention, no or only need for minor treatment;
- ✧ Level 3: Severe, movement is significantly limited, requiring medical attention and treatment, and may require hospitalization;
- ✧ Level 4: Severe or potential life threatening, severely restricted movement, requiring treatment, emergency or hospitalization.

The grading of the clinical observation indicators (local manifestation, vital signs, and systemic manifestation), laboratory testing indicators (blood biochemistry, blood routine, and urine routine), the clinical abnormalities, and the adverse events in the trial can refer to *the Guideline of the Grading Standard for the Adverse Events in Preventive Vaccine Clinical Trials*.

(III) Evaluation of the correlation between adverse events and research interventions

The researcher should conduct a comprehensive analysis according to the specific circumstance of adverse events of the subject and the subject's previous medical history, concomitant disease, and concomitant medication, to determine the relationship between the adverse event and the drug (product or treatment) with reference to *the Manual of Adverse Drug Reaction Reporting and Monitoring* issued by the State Drug Administration in November 2012 and *the Administrative Measures for Adverse Drug Reaction Reporting and Monitoring* implemented on July 01, 2011 (Order of the Ministry of Health No. 81). As far as possible, it should try to determine the relationship between the adverse events (including serious adverse events) and the drug (product or treatment). If it is determined to be related, no matter the conclusion is to be positively related, likely to be related, or may be related, all these will be regarded as adverse reactions caused by the intervention, and the incidence of adverse reactions will be counted.

The five principles for evaluating the correlation between the intervention methods and adverse reactions: First, is there a reasonable time relationship between the intervention methods and the occurrence of adverse reactions/events? Second, does the response conform to the type of adverse reactions known to the intervention method? Third, does the reaction disappear or alleviate after the intervention method is stopped? Four, does the same reaction/event occur again when the method is used again? Five, can the adverse reaction/event be explained by the effect of

the combined medication/treatment, the progress of the patient's condition, and the impact of other treatments?

According to the above five principles, the relevance evaluation is classified into 6 levels of positive, very likely, possible, possibly irrelevant, to be evaluated, and unable to be evaluated.

Principles / Results	①	②	③	④	⑤
Positive	+	+	+	+	—
Very likely	+	+	+	?	—
Possible	+	±	±?	?	±?
Possibly irrelevant	—	—	±?	?	±?
To be evaluated	With a need to supplement evidence to evaluate				
Unable to be evaluated	The necessary materials for evaluation are not available				

Note: '+' means positive; '—' means negative; '±' means difficult to affirm or negative; '？' means unknown

(IV) Adverse events management

Any adverse event, such as discomfort and abnormal laboratory tests of the subject, should be treated seriously, analyzed carefully, and immediate measures should be taken to protect the safety of the subjects.

1. Handling procedure

The adverse events occurred during the trial should be recorded in the CRF forms in terms of type, degree, occurrence time, duration, treatment measures, treatment process and outcome, etc. And to evaluate the relevance of the trial interventions with the reverse events based on the comprehensive consideration of comorbidities and combined medications and then recorded in detail by the physician. The investigator should follow up the adverse event until the symptoms disappear or stabilize. For the more serious adverse events, it should be followed up for a longer period, and the follow-up ways can be inpatient, outpatient, telephone, etc. The adverse events happened at the end of the study need to be followed up within one month after the study end. It should follow up the cases withdrawn due to adverse events and adverse reactions, and to record the continuity, outcome, and disappearance in detail.

If the testing results for safety detection (blood routine, urine routine, stool routine, ECG, liver

function, kidney function) are abnormal after treatment, they must be retested at an appropriate time, truthfully recorded, and comprehensively analyzed to determine whether they are related to the intervention.

2. Handling of the serious adverse events

When there is a serious adverse event in a clinical trial, the researcher should immediately take appropriate treatment measures for the subject and report it to the clinical trial management department of the institution and then to notify the sponsor with a writing report referring to *the Manual of Adverse Drug Reaction Reporting and Monitoring* issued in November 2012 and *the Administrative Measures for Adverse Drug Reaction Reporting and Monitoring* implemented on July 01, 2011 (Order of the Ministry of Health No. 81), and *The Regulations on the Management of Serious Adverse Events in Vaccine Clinical Trials (tentative standard)* issued on the January 17, 2014. The clinical trial management department shall report to the corresponding ethics committee and the food and drug supervision and administration department and the health authority of the province, autonomous region, and municipality where the clinical trial institution is located within 24 hours. For the death case, clinical trial agencies and researchers should provide all the information they need to the ethics committee and sponsors.

The researcher should record all adverse events and method defects found during the clinical trial, and work with the sponsor to analyze the cause of the event, to form a written analysis report, and to put forward opinions on continuing, suspending or terminating the trial. Then, to submit the report to the ethics committee for review by the clinical trial management department of the clinical trial institution.

For the serious adverse events and deficiencies in treatment methods that may lead to serious clinical consequences, the sponsor should report to the clinical trial supervision and management department and the health authority within 5 working days of being informed, and should also report at the same time to other clinical trial participating institution and the investigator. It shall also promptly notify the ethics committee of the clinical trial institution through their medical clinical trial management department.

In this trial, please report the serious adverse event report to the National Medical Products Administration, the National Health and Family Planning Commission in a timely manner according to the following contact information, and notify the research institution regularly.

The investigator must complete a Serious Adverse Event Report Form, and should record the time, the way, and to whom to report the serious adverse events in the original data. The sponsor guarantees it to satisfy all reporting requirements of laws and regulations.

The institute to report	Contact department /person	Telephone
National Medical Products Administration（NMPA）	Registration department	010-88330732/010-88330742
Institute of clinical blood transfusion, Guangzhou Blood Center	Yongshui Fu	020-83595049

In case of any serious adverse event unrelated to the trial method, the researchers shall withdraw from the clinical trial when they think it is necessary or the subject or his legal guardian requests to withdraw from the trial, and the withdrawal case shall complete the last laboratory examination.

（V） Follow-up of the adverse events

All adverse events should be followed up to the normal or baseline level, or the investigator can reasonably explain the abnormality and think that no follow-up is necessary to ensure the safety of the subjects. According to the type and severity of the adverse reactions, the follow-up methods can be selected as inpatient, outpatient, home visiting, and telephone visiting and other ways.

X. Quality control of the clinical trial

（I） Quality control measures

Both the sponsor and the investigator should adopt Standard Operating Procedure (SOP) to ensure the quality control and the implementation of quality assurance system of the clinical research. All the observation results and findings in the clinical research should be verified to ensure the reliability of the data and the conclusions in the clinical research origin from the original data. Quality control must be adopted at every stage of data processing to ensure that all data are reliable and processed correctly.

（II） Training of researchers

Before the formal starting of clinical research, the responsibility and attitude of the researchers participating in the clinical research must be emphasized firstly. The person in charge of the trial shall train the researchers on the experimental scheme, unify the understanding, be familiar with the collection methods and procedures, and understand the special requirements of the research project, so as to improve the internal observation consistency and inter observer consistency of the clinical research data collectors and ensure the reliability of the research conclusion. At the same time, the researchers are required to strictly implement SOP in the whole process of clinical research to ensure the implementation of quality control measures in clinical research and improve the quality of clinical research and case report form.

（III） The measures to improve patients' compliance

1. The investigator should carefully implement the informed consent of transfusion treatment to make the patient or his legal guardian fully understand the requirements of the trial and cooperate well. The sponsor provides free trial intervention and related laboratory tests.

2. To monitor the compliance of the patient during the trial.

（IV） The monitoring of clinical research

The sponsor authorizes Guangzhou Haibote Pharmaceutical Technology Co., Ltd. to appoint qualified supervisors to conduct regular on-site monitoring visits in the experimental hospitals to ensure that all contents of the research plan are strictly observed and the original data are checked to ensure that they are consistent with the contents on the CRF. The appointed supervisor shall carry out the audit according to the requirements of GCP, and write the audit report, which includes:

1. Whether the clinical trial institute has appropriate conditions;

2. The patient's informed process complies with the declaration and regulations of Helsinki;

3. The implementation of the trial plan;

4. The protection measures for patients during the trial;

5. To confirm that all data records and reports are correct and complete;

6. To confirm that all adverse events are recorded and serious adverse events are reported and recorded within the specified time;

7. To verify that the test method is carried out in accordance with the relevant laws and regulations, and have the corresponding records;

8. Including the supervision date, time, the name of the supervisor, the problems found during the supervision and handling methods, etc.

（V） The inspection of clinical research

The clinical trial supervision and management department and the sponsor could authorize the auditors to carry out systematic inspection on the clinical study to determine whether the implementation of the trial is consistent with the trial scheme and whether the reported data is consistent with the records of all clinical participating institutes, that is, whether the data reported or recorded in the case report form is the same with the medical record or other original records. The audit shall be performed by the person not directly involved in the clinical study.

XI. Data management

（I） Data entry and modification

The leading institute of the trial is responsible for the data entry and management. For the questions in the case report form, the data manager will send the questions to the researcher through the supervisor, and the researcher shall answer and return them as soon as possible. The data manager will modify, confirm and input the data according to the researcher's answers, and send the DRQ again if necessary.

（II） Data locking

After all doubts are solved and the established database is confirmed to be correct, the data manager should write the data management audit report and lock the audited data. The locked data file and statistical plan will not be changed.

XII. Statistical analysis

（I） The population for statistical analysis

The population for analysis includes safety analysis set (SS) and full analysis set (FAS).

1. The Safety analysis population: the subjects receiving at least one time of blood transfusion.

2. Full analysis population.

（II） Statistical analysis method

1. Demographic data and baseline analysis

* Analysis according to FAS;

* Descriptive demographic data and other baseline characteristic values;

* To calculate the number of cases, mean value, standard deviation, median, minimum and maximum for the continuous variables;

* To calculate the frequency for the count and grade data.

2. Safety analysis

The SS population was used for safety analysis, and safety indexes included adverse events, laboratory examination, vital signs, etc. To summarize the data by stages and units. The adverse events will be aggregated.

* Adverse events

All adverse events will be coded with MedDRA.

According to NCI CTCAE V5.0, the incidence (frequency) and severity distribution of AE, TEAE, SAE and AESI were summarized according to SOC and PT in MedDRA code.

The subjects who terminated the treatment due to the adverse events, the subjects who had SAE and the dead subjects will be listed (at least including the start date, end date, severity, relationship with trial intervention, measures taken, outcome of AE).

* Laboratory examination

Blood routine and biochemical indexes were used to describe the measurement value and change value before and after treatment with mean ± standard deviation, maximum value, minimum value and median. And the normal and abnormal changes before and after treatment were described with cross classification table.

Urine routine: use cross classification table to describe the normal and abnormal changes before and after treatment.

The proportion of the subjects with abnormal and clinically significant changes will be described, in which whether the abnormal has clinical significance is judged by the researchers.

The list of subjects with abnormal changes (with or without clinical significance) after treatment.

* ECG examination

Descriptive statistical ECG indicators compared with the baseline changes, using a cross classification table to describe the normal and abnormal changes before and after treatment, and a list of data will be provided.

* Vital signs, physical examination and other safety related examinations

The examination results of vital signs and changes compared with the baseline will be described statistically.

The subjects with abnormal changes in physical examination compared with baseline were described in the form of list.

（III） The software for analysis

SAS9.4 is used for the statistics analysis of the general data.

XIII. Preservation of the original data

The original data are kept in each clinical trial institution. The data kept by the researchers include the trial protocol and its amendment, the informed consent (form), the approval document of ethics committee, the member form of ethics committee, the original outpatient or inpatient medical records, the case report form and the research medical record (filled in, signed and dated), and the original serious adverse event report sent by the researcher to the sponsor. The researchers keep all research data, including confirmation of all patients that can effectively check different records, such as the original records of the hospital, all original informed consent signed by the patients, all case observation forms, research medical records, detailed records of transfusion treatment, etc. The researchers should keep the clinical trial data for at least 5 years after the termination of clinical trial.

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XV. The flow chart of the Clinical research

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Visiting time	-7~ 0 Days before transfusion	Blood transfusion treatment period （0~1days）	1 day to 1 month after blood transfusion	1 month to 12 months after blood transfusion	Over 12 months after blood transfusion
Informed consent signed	●				
Basic information collection	●				
Physical examination	●		●	●	●
Vital signs	●	●	●		
Blood routine	●		●		
Urine routine	●				
Stool routine + Occult Blood [ab]	●				
Liver function [a]	●				
Renal function [a]	●				
Coagulation function [a]	●				
Four items for infections detection	●				
Electrocardiogram [a]	●				
HCG test [b]	●				
Genotyping of “Asia type” DEL	●			●	●
Detection of alloanti-D	●		●	●	●
Cross-matching test [c]	●				
Review of the inclusion and exclusion criteria	●				
Recording of adverse events		●	●	●	●

Recording of combined treatment	●	●	●	●	●
CRF reviewed by the person in charge					●
CRF reviewed by the inspector					●

Note：[a] In case of any abnormality of the patients during the follow-up, the authorized investigator shall determine whether re-examination is necessary. If necessary, the investigator shall be instructed to make a timely return visit, record in detail, and follow-up to the normal or baseline level, or the investigator can reasonably explain the abnormality and think that no follow-up is necessary;

[b] Optional, depending on the clinical situation;

[c] “Asia type” DEL patients will be involved.