Informed Consent Form for the Study of the Efficacy and Safety of Umbilical Cord Blood Transplantation (UCBT) with a Total Marrow Irradiation (TMI)-Based Conditioning Regimen for the Treatment of Refractory/Relapsed Aplastic Anemia (AA) in Adults

Respected Participant:

Given your diagnosis of severe/very severe aplastic anemia at our hospital, you are being invited to participate in a clinical research study titled "A Phase II Study on the Efficacy and Safety of Umbilical Cord Blood Transplantation (UCBT) with a Total Marrow Irradiation (TMI)-Based Conditioning Regimen for the Treatment of Refractory/Relapsed Aplastic Anemia (AA) in Adults." This study will be conducted at the Umbilical Cord Blood Transplant Center of the Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Hematology. The study has been reviewed and approved by the Ethics Committee of the Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Hematology and filed with the relevant national authorities.

This informed consent form is designed to help you decide whether to participate in this study. It provides information on the background and objectives of the study, the procedures involved, potential risks and benefits, alternative treatment options, how your personal information will be used, and your right to withdraw from the study at any time.

Before you decide whether to participate in this study, please read this document carefully and ensure that you fully understand it. If you have any questions, please feel free to consult the study physician or researchers to obtain satisfactory answers.

If you agree to participate in this study, you will receive a copy of the signed informed consent form.

Section I: General Information for Participants

■Purpose of the Study:

To evaluate the efficacy of a Total Marrow Irradiation (TMI)-based conditioning regimen followed by single-unit unrelated cord blood transplantation (UCBT) for the treatment of refractory/relapsed aplastic anemia (AA) in adults.

■Background of the Study:

The study involves a TMI-based conditioning regimen followed by UCBT.

Section II: Study Design and Population

Study Design:

This is a single-arm clinical study. The study will enroll 10 adult patients with refractory/relapsed severe aplastic anemia (V/SAA) to assess the efficacy and safety of the TMI-based conditioning regimen followed by UCBT. The study duration is from June 2025 to June 2028.

Inclusion Criteria:

Participants eligible for this study must meet all of the following criteria:

1) Age ≥18 years and <50 years, regardless of gender.

2) Diagnosis of aplastic anemia according to the guidelines of the British Committee for Standards in Haematology; hypocellular bone marrow without infiltration of abnormal cells or bone marrow fibrosis; peripheral blood must meet at least two of the following three criteria: absolute neutrophil count (ANC) <1.5 × 10^9/L; platelet count <50 × 10^9/L; hemoglobin <100 g/L.

- Diagnostic Criteria for Severe Aplastic Anemia (SAA):

  - Peripheral Blood Criteria: Meet at least two of the following three criteria: ANC <0.5 × 10^9/L; reticulocyte count <20 × 10^9/L; platelet count <20 × 10^9/L.

  - Bone Marrow Criteria: Bone marrow cellularity <25% of normal; if ≥ 25% but <50% of normal, then the proportion of residual hematopoietic cells should be <30%. Bone marrow aspiration shows hypocellular or severely hypocellular marrow with a marked reduction in hematopoietic cells and an increase in non-hematopoietic cells such as lymphocytes and reticular cells.

  - Other: Exclude other diseases causing pancytopenia, such as myelodysplastic syndrome (MDS), paroxysmal nocturnal hemoglobinuria (PNH), and congenital bone marrow failure syndromes. If ANC <0.2 × 10^9/L, the diagnosis is very severe aplastic anemia (VSAA); those who do not meet the SAA criteria are classified as non-severe aplastic anemia (NSAA).

3) Meet the criteria for refractory aplastic anemia:

  - For SAA: Ineffective after 6 months of first-line ATG + CSA treatment.

  - For NSAA: Meet one of the following criteria: (1) Persistent transfusion dependence in two blood cell lines; (2) Ineffective after 12 months of immunosuppressive therapy with cyclosporine or other agents, or ineffective after 6 months of treatment with androgens and/or TPO receptor agonists, or progression to SAA.

4) Karnofsky Performance Status≥60points, ECOG score≤2, Hematopoietic Cell Transplantation - Comorbidity Index (HCT-CI) ≤2.

5) No HLA-identical sibling donor or no HLA-identical unrelated donor available.

6) Understand the study procedures and voluntarily provide written informed consent.

Exclusion Criteria:

Participants with any of the following conditions are not eligible for this study:

1) Other causes of pancytopenia and hypocellular bone marrow diseases (including PNH, etc.).

2) Use of medium-to-high doses of cyclophosphamide (≥20 mg/kg/day) for immunosuppressive therapy within 3 months prior to enrollment.

3) Previous history of hematopoietic stem cell transplantation.

4) Known or suspected contraindications or allergies to fludarabine, melphalan, or other drugs.

5) Uncontrolled bleeding and/or infection after standard treatment prior to screening.

6) Active viral hepatitis (hepatitis B, hepatitis C, etc.), HIV infection, or syphilis at screening or prior to treatment.

7) Creatinine clearance rate <60 ml/min or serum creatinine >140 μ mol/L prior to treatment.

8) Pregnant or breastfeeding women.

Section III: Study Procedures

1. Study Protocol:

1) Conditioning Regimen:

Flu 30 mg/m^2 × 5 days (Days -6, -5, -4, -3, -2);

TMI 4 Gy qd (Day -2);

Mel 100 mg/m^2 qd (Day -1).

2) GVHD Prophylaxis:

Cyclosporine (CSA) in combination with Mycophenolate Mofetil (MMF).

- CSA: 2.5 mg/kg/day continuous intravenous infusion starting on Day -1. After myeloid recovery and gastrointestinal function restoration, switch to oral administration at twice the intravenous dose, maintaining trough levels of 150-200 ng/mL until 2 months post-transplant. CSA will be tapered gradually based on GVHD status, infection, and disease relapse risk, and discontinued around 5-6 months post-transplant. (Alternative: FK506 0.03 mg/kg continuous 24-hour intravenous infusion or oral administration, maintaining levels of 8-10 ng/ml starting on Day -1. The oral dose of FK506 is twice the intravenous dose, divided into two doses per day.)
- MMF: 30 mg/kg/day, intravenous infusion (or oral), divided into two doses per day, starting on Day +1, with gradual tapering upon myeloid recovery and discontinuation around Day +60.

3) Cord Blood Selection:

- HLA Matching: High-resolution HLA typing of 12 loci for cord blood, selecting units with ≥4/6, 5/8, 6/10 compatibility.
- Cord Blood Cell Count: Frozen cord blood TNC ≥ 1.5-2 × 10^7/kg (patient's body weight), CD34+ cells ≥ 1.0 × 10^5/kg (patient's body weight), and post-thaw CD34+ cells ≥ 0.83 × 10^5/kg (patient's body weight).
- DSA: Avoid using DSA-positive cord blood.

2. Tests and Examinations:
Peripheral blood tests: Complete blood count + reticulocyte count + C-reactive protein, coagulation panel, liver and kidney function + anemia panel + electrolytes + glucose + hormone panel + thyroid function, ENA antibody profile + antinuclear antibody, immunoglobulin quantification + rheumatoid panel, direct antiglobulin test (transfusion department), PNH clone detection (within 3 months), infectious markers (14 items), viral panel (antibody detection), EBV/CMV/HBV-DNA quantification, T-SPOT testing, ABO blood type + Rh blood type (RhD + other Rh antigens) + difficult blood type identification + blood type antibody titer testing (antibody testing twice, labeled anti-A, anti-B), DSA (HLA typing + Class I and II-specific antibodies), chimerism testing (STR), lymphocyte subsets (B/T/NK, Th1/Th2, T-cell immunity), cytokine detection by flow cytometry.
Bone marrow tests: Iliac crest bone marrow aspiration and/or sternum bone marrow aspiration, pathological examination of iliac crest bone marrow biopsy, chromosomal karyotype, flow cytometry (MDS/MPN), panel of immunohistochemistry (CD41), hematologic gene mutation screening (age >40 years) and/or congenital bone marrow failure gene testing (age <40 years).
Imaging: Head MRI; liver, pancreas, and heart MRI T2 (for iron overload patients to assess iron load), chest CT, thyroid, liver, gallbladder, pancreas, spleen, urinary system, testes (male)/gynecological (female), pelvic ultrasound, echocardiography, Holter monitoring, electrocardiogram, pulmonary function test.

3. Biological Samples to be Collected:
Peripheral blood, bone marrow.

Section IV: Your Responsibilities

If you decide to participate in this study, you are expected to:
1) Follow the guidance of the study physician, comply with the follow-up schedule, and cooperate with the collection of biological samples.
2) During your participation in this study, if you develop graft-versus-host disease (GVHD), you may receive supportive treatments such as corticosteroids or ruxolitinib, but ATG is not permitted.
3) Inform your study physician immediately if you experience any issues or side effects.

Section V: Potential Risks and Discomforts

1) Acute/chronic graft-versus-host disease (a/cGVHD).
2) Toxic side effects related to radiotherapy/chemotherapy: nausea, vomiting, rash, fever, cytopenia, infection, bleeding, liver damage, myocardial injury, gonadal damage, etc.

During the study, researchers will monitor adverse reactions to the drugs. If you experience any discomfort, it is crucial that you report it to the researchers immediately. Researchers may

control the side effects of the study drugs through other medications or treatment methods. If you or your researchers believe that you cannot tolerate these side effects, the study drugs may be reduced, suspended, or completely discontinued, and you may be withdrawn from the study.

The treatment risks you face may also exist if you do not participate in this clinical study and receive conventional treatment. If you decide to withdraw from the study, you can consult your study physician about other treatment options.

Section VI: Withdrawal from the Study

The study physician may withdraw you from the study or terminate study treatment for any important reason and will discuss alternative treatment options with you. These reasons may include:

You have the right to withdraw consent and discontinue participation at any time without penalty or loss of benefits to which you are otherwise entitled. Your study records and previously collected biological samples will be retained by the researchers.

Section VII: Study Termination

The study may be suspended or terminated early if any of the following occurs:
1) Serious safety issues are identified during the study.
2) The treatment with TMI, fludarabine, melphalan, cyclosporine, tacrolimus, mycophenolate mofetil, methylprednisolone, etc., is found to be too ineffective or clinically worthless.
3) Significant errors in the study protocol or serious deviations during implementation are identified.
4) The investigator believes that the participant is no longer benefiting from the study.
5) The study is ordered to be terminated by the regulatory authorities or the ethics committee for any reason.

Section VIII: Potential Benefits

Participating in this clinical study may offer therapeutic benefits for your condition, but it may also not improve your disease. We hope that this study will provide valuable information for future patients with similar conditions. We sincerely thank you for your positive contribution.

Section IX: Alternative Treatment Options

If you do not participate in this study, you may still receive alternative treatments such as

haploidentical hematopoietic stem cell transplantation.

Section X: Costs Associated with the Study

Participation in this study is not free of charge. You will be responsible for all treatment and examination costs associated with the study.

You will not receive financial compensation for participating in this study. The study physician will not provide financial compensation for any medical treatment costs resulting from the study treatment being ineffective or disease progression.

Section XI: Insurance and Compensation

During the study, if you experience any discomfort or adverse events, regardless of whether they are related to the study drugs, please inform the study physician immediately. They will make a judgment and provide appropriate medical treatment.

If you suffer any harm related to the study, such as adverse reactions or serious adverse events, you will receive active treatment at the hospital. The study will cover reasonable treatment costs and compensation related to any study-related damages.

Section XII: Privacy and Confidentiality of Your Information

During the study, researchers will collect your personal information, including but not limited to your name, date of birth, gender, ethnicity, physical health or mental health status, etc. This information will be securely stored at the hospital and kept strictly confidential. Within the limits allowed by law, researchers, ethics committee members, or representatives of regulatory authorities may review your study records.

Your personal identity will never be disclosed. We will make every effort to protect your privacy within the limits allowed by law. If study results are published, your identity will not be revealed.

If you withdraw from the study, no follow-up data will be collected unless your consent is information on your health, disease status, and treatment received by other means.

Section XIII: Your Rights as a Participant

Your participation in this clinical trial is entirely voluntary. You have the right to refuse to participate. Your decision to participate is free from interference by any individual or

organization. You also have the right to withdraw from the study at any stage without facing discrimination or retaliation, and it will not affect your medical treatment or rights. If you decide to discontinue participation, we hope you will inform your study physician in a timely manner. The study physician can provide suggestions and guidance on your health status. Additionally, if you do not comply with the relevant regulations during the clinical study or if your study physician believes that you are no longer suitable to continue participating in the study, the physician has the right to decide whether you should continue to participate in the study to protect your interests.

During the study, new important information may be obtained that could affect your willingness to participate in the study. If this occurs, we will inform you in a timely manner.

If the study is terminated early, we will also notify you in a timely manner, and the study physician will provide recommendations for your next treatment plan based on your health status.

Section XIV: Obtaining More Information

You may obtain information related to this study at any time, or if you have any questions related to the study, please contact your study physician in a timely manner.

Study Physician:_____

Phone:_____

If you have any questions regarding the rights of participants in this study, or if you wish to report difficulties, dissatisfaction, or concerns encountered during participation, or if you would like to provide feedback or suggestions related to the study, you may contact the Ethics Committee of our hospital by phone.

Ethics Committee Contact:_____

Phone:_____

Part II: Informed Consent Form - Consent Signature Page

Declaration of the Study Physician

As the study physician, I, _____, have explained the details of this study to the participant, including the purpose, procedures, potential benefits and risks, principles of

participation, and data confidentiality. I have informed the participant that they may withdraw from the study at any time without affecting future treatment and have provided them with a copy of the signed informed consent form.

Study Physician Signature:_____

Date:_____（YY/MM/DD）

Declaration of the Participant

I have read the above information about this study and have had the opportunity to discuss it with the physician and ask questions. All my questions have been answered to my satisfaction.

I understand the potential risks and benefits of participating in this study and voluntarily agree to participate. I confirm that I have had ample time to consider this and understand the following:

- I can obtain more information from the study physician at any time.

- I can withdraw from this study at any time without facing discrimination or retaliation, and my medical treatment and rights will not be affected.

- If I need to take any other treatment options due to changes in my condition, I will consult the doctor in advance or inform the doctor truthfully afterward.

- I agree that researchers, ethics committee members, or regulatory authorities may review my study records.

- I will receive a copy of the signed and dated informed consent form.

Finally, I，_____， decide to participate in this study.

Participant Signature:_____

Date:_____（YY/MM/DD）

Participant Contact Information:_____