# Clinical study protocol

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| **Title:** | Analysis of Immune Reconstitution Characteristics and Related Influencing Factors in Patients After Umbilical Cord Blood Transplantation |
| **Portocol number:** | SKIRT-CB-OB-001 |
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## Declaration of Secrecy

This document is confidential information of Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College (Tianjin, China) and is only used for the purpose of this clinical study. It shall not be disclosed to anyone other than the participating researchers and members of the institutional review board. This information cannot be used for any purpose other than the evaluation or implementation of clinical studies without the prior written consent of Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College.

## 1. Research background

At present, there are about 40,000 new allogeneic hematopoietic stem cell transplantation (HSCT) cases every year in the world, among which about a quarter occur in China[1,2]. Allo-HSCT has become the primary treatment method for some hematological diseases such as refractory and relapsed acute leukemia and bone marrow failure, while changes in immune patterns after transplantation are directly related to patient survival, making this a major focus and challenge in the field.

Restoration of the individual components of the immune system occurs with different dynamics in which innate immunity (neutrophils, monocytes and natural killer cells) typically precedes adaptive immunity (T- and B-lymphocytes)[3]. Moreover, both the stem cell source and the conditioning regimen could influence the speed of immune reconstitution[4,5].

After transplantation, patients undergo a prolonged process of immune reconstitution lasting more than two years, whereas past research in this area has been relatively weak compared to other fields of immunology[6]. Previous studies usually did not conduct an independent validation of their major results and in addition were often limited by small population size (less than 200 patients) [7-11], short duration of immune profiling (1 year or less)[4,12,13], a single patient age group (only adults or only children), a single source of hematopoietic stem cells (bone marrow, peripheral blood, or cord blood), and the lack of integration of multiple immune variables post-transplantation[14].

**Previous foundation of research**

The researchers have 111,50 post-transplant immune profiles of 1945 patients who underwent HSCT at the Institute of Hematology, Chinese Academy of Medical Sciences (IHCAMS) and the First Affiliated Hospital of University of Science and

Technology of China between 2012 and 2020. In the first two months after transplantation, the recovery of cell numbers is mainly driven by $CD8^+$ T cells, followed by a broad recovery of NK cells, $CD8^+$ T cells, $CD4^+$ T cells, and B cells. Individual heterogeneity in immune reconstitution reaches its maximum approximately 60 days after transplantation. At the population level, $CD8^+$ T cell count rose slower in umbilical cord blood allo-HSCT than in the other types of HSCT. On the other hand, NK, $CD4^+$ T, and B cell counts eventually reached higher levels in umbilical cord blood transplantation (UCBT) than in the other types of transplantation. NK cell count rose slower in auto-HSCT than in the other types of transplant.


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## 2. Study design

This is a prospective observational study.

## 3. Study population

### 3.1 Inclusion criteria

1) umbilical cord blood transplant;

2) Patients have to sign an informed consent form before the start of the research procedure.

### 3.2 Exclusion criteria

1) Patients who are ineligible for the study due to other factors, or will bear great risk if participating in the study.

### 3.3 Drop-out and withdrawal criteria

A patient may withdraw from the study if he/she does not wish to continue participating in the study, and the date and reason for withdrawal shall be recorded. The investigator may also decide to discontinue a patient from the clinical study if there is an unacceptable risk.

## 4. Trial Protocol

For adult patients, complete blood count, lymphocyte subset, T-cell receptor (TCR) repertoire analysis, immunoglobulins blood test, and bone marrow assessment of measurable residual disease (MRD) will be performed on post-transplant days 30 ± 3, 60 ± 3, 90 ± 3, 120 ± 3, 180 ± 7, 270 ± 7, and 360 ± 7.

For pediatric patients, complete blood count, lymphocyte subset, TCR repertoire analysis, immunoglobulins blood test, and bone marrow assessment of MRD will be performed on post-transplant days 30 ± 3, 60 ± 3, 90 ± 3, 180 ± 7, 270 ± 7, and 360 ± 7.

## 5. Follow-up

Subjects will be followed up to collect prognostic information within one year after transplantation, including engraftment syndrome, infections, relapse, non-relapse mortality, graft-versus-host disease, and survival.

## 6. Study endpoints

### 6.1 Primary endpoint

The quantity and diversity of immune cells such as T cells, B cells, and NK cells within one year after transplantation in the overall patients, adult patients, and pediatric patients.

### 6.2 Secondary endpoints

Incidence of engraftment syndrome, infections, relapse, non-relapse mortality, graft-versus-host disease, and survival within one year after transplantation in the overall patients, adult patients, and pediatric patients.

## 7 Sample size calculation

≥ 200 adult patients and ≥ 80 pediatric patients.

## 8 Statistical analysis

R statistical analysis software will be used to conduct the statistical analysis tailored to data properties. All comparison tests will be two-sided, and statistical significance will be defined as $P < 0.05$ in all the analyses.

## 9 Ethical review

The study will be conducted in accordance with the Declaration of Helsinki (2024), relevant regulations issued by the government of the People's Republic of China, and additional precautions required by the ethics review committee at the IHCAMS.

Before the study, the investigator will obtain approvals from the Ethics Review

committee at the IHCAMS regarding the study protocol data sheet, informed consent form, subject enrollment form, and other relevant information to be provided to the subject before enrollment. During the study, if there is any amendment to the study protocol data sheet, informed consent form, subject enrollment form, or other relevant information provided to subjects, renewed approvals shall be obtained from the IHCAMS Ethics Review committee before continuation of the study.

## 10 Preservation of research data

All data of this study will be stored at the IHCAMS. Data sharing among the researchers will abide by the regulations of the People's Republic of China to safeguard data security and subject privacy.