Clinical study protocol

Title:	A prospective study on immune reconstitution and clinical prognosis in patients after umbilical cord blood transplantation
Protocol number:	SKIRT-CB-RCT-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

Allo-HSCT has become the primary treatment method for some hematological diseases such as refractory and relapsed acute leukemia and bone marrow failure. Immune reconstitution after HSCT is highly variable among patients, and this variability influences prognosis.

Due to the high heterogeneity of patients and overall small sample sizes, however, it is difficult to summarize all the published literature regarding post-transplant immune reconstitution. 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) or short duration of immune profiling (1 year or less) [1-6]. Moreover, aside from some exceptions, researchers often did not evaluate multiple immune variables holistically when assessing a patient's immune reconstitution [7-8]. In other words, there has been no consensus on how to assign a score to a patient's multi-time-point and multivariate immune data.

Previous foundation of research

The researchers retrospectively analyzed 111,50 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 (FAHUSTC) between 2012 and 2020, including 561 umbilical cord blood transplantation (UCBT) cases.

The previous work identified the Composite Immune Risk Score (CIRS) that integrates eight immune factors for predicting early mortality after HSCT, and validated the CIRS in two independent subsets of the patients from two medical centers. In multivariate analysis, a CIRS during days 91–180 remained an

independent risk factor for early mortality after allogeneic HSCT (HR, 1.80; 95% C.I., 1.28–2.55; p = .00085). This result has been published in *American Journal of Hematology* in 2022 [9].

UCBT is characterized by rapid innate immune recovery, delayed reconstitution of adaptive immunity (especially T-cells), higher risk of early infection, and the capacity to develop a broad TCR repertoire at later time points. Immune reconstitution after UCBT is one of the key factors affecting treatment outcomes and prognosis of recipients.

[1] Storek J, Gooley T, Witherspoon R P, et al. Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. Am J Hematol, 1997, 54(2): 131-8.

[2] Fedele R, Martino M, Garreffa C, et al. The impact of early CD4+ lymphocyte recovery on the outcome of patients who undergo allogeneic bone marrow or peripheral blood stem cell transplantation. Blood Transfus, 2012, 10(2): 174-80.

[3] Kim S Y, Lee H, Han M S, et al. Post-Transplantation Natural Killer Cell Count: A Predictor of Acute Graft-Versus-Host Disease and Survival Outcomes After Allogeneic Hematopoietic Stem Cell Transplantation. Clin Lymphoma Myeloma Leuk, 2016, 16(9): 527-535 e2.

[4] Rocha V, Labopin M, Sanz G, et al. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia. N Engl J Med, 2004, 351(22): 2276-85.

[5] Storek J, Dawson M A, Storer B, et al. Immune reconstitution after allogeneic marrow transplantation compared with blood stem cell transplantation. Blood, 2001, 97(11): 3380-9.

[6] Petersen S L, Ryder L P, Bjork P, et al. A comparison of T-, B- and NK-cell reconstitution following conventional or nonmyeloablative conditioning and transplantation with bone marrow or peripheral blood stem cells from human leucocyte antigen identical sibling donors. Bone Marrow Transplant, 2003, 32(1): 65-72.

[7] Waller EK, Logan BR, Fei M, et al. Kinetics of immune cell reconstitution predict survival in allogeneic bone marrow and G-CSF-mobilized stem cell transplantation. Blood Adv. 2019;3:2250-2263.

[8] van der Maas NG, von Asmuth EGJ, Berghuis D, et al. Modeling influencing factors in B-cell reconstitution after hematopoietic stem cell transplantation in children. Front Immunol. 2021;12:684147.

[9] Cao Y, Gong X, Feng Y, et al. The Composite Immune Risk Score predicts overall survival after allogeneic hematopoietic stem cell transplantation: A retrospective analysis of 1838 cases. Am J Hematol, 2023, 98(2): 309-321.

2. Study objective

In this prospective randomized controlled trial of umbilical cord blood hematopoietic stem cell transplant recipients, post-transplant immune data will be collected from two groups. A composite immune risk score will be applied to evaluate immune reconstitution and perform risk stratification. We will assess its effects on infection monitoring frequency, medication adjustments aimed at boosting immune reconstitution or infection prophylaxis, outpatient visit counts, as well as prognostic outcomes including immune reconstitution, infection rate and chronic GVHD incidence.

3. Study design

This is a prospective randomized controlled trial.

4. Study population

4.1 Inclusion criteria

1) Age ⩾ 14 years old.
2) Umbilical cord blood transplant
3) Patients must provide written informed consent before the start of the study procedures.

4.2 Exclusion criteria

Patients who meet any of the following criteria will be excluded from the study:

1) Patients who are ineligible for the study due to other factors, or who will bear great risk if they participate in the study.
2) Death or loss to follow-up of the patient prior to immune reconstitution stratification

4.3 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 investigators may also decide to discontinue a patient from the study if there is an unacceptable risk.

5. Trial Protocol

Patients will be randomly assigned to the intervention group or the control group with a 0.5 probability of allocation to the intervention group. At 90 ± 3 days post-transplantation, the composite immune risk score will be used to perform immune reconstitution risk stratification. Attending physicians will be notified of the stratification results of intervention-group patients. In contrast, no stratification information of patients in the control group will be provided to physicians.

6. Softudy assessment and follow-up

6.1 Primary endpoints

- **Frequency of infection monitoring (6 months post-transplant)**
- **Number of medication adjustments intended to accelerate immune reconstitution or enhance infection prophylaxis (6 months post-transplant)**, including immunosuppressants (e.g., cyclosporine, ruxolitinib, mycophenolate mofetil, glucocorticoids), immunostimulatory agents (e.g., ubenimex, interferon, human immunoglobulin), and prophylactic anti-infective drugs (antifungal prophylaxis, antiviral prophylaxis, *Pneumocystis jirovecii* prophylaxis, etc.)
- **Number of return visits (6 months post-transplant)**

6.2 Secondary endpoints

- **Frequency of infection monitoring (1 year post-transplant)**
- **Number of medication adjustments intended to accelerate immune reconstitution or enhance infection prophylaxis (1 year post-transplant)**, including immunosuppressants (e.g., cyclosporine, ruxolitinib, mycophenolate mofetil, glucocorticoids), immunostimulatory agents (e.g., ubenimex, interferon,

human immunoglobulin), and prophylactic anti-infective drugs (antifungal prophylaxis, antiviral prophylaxis, *Pneumocystis jirovecii* prophylaxis, etc.)

- **Number of return visits (1 year post-transplant)**
- **Immune reconstitution status (at 4, 6, 9 and 12 months post-transplant)**
- **Infection rate, incidence of chronic GVHD, relapse rate, and survival (at 6, 9 and 12 months post-transplant)**
- **Hospitalization rate and outpatient rate (at 6, 9 and 12 months post-transplant)**

7. Sample size calculation

Assuming the mean frequency of infection monitoring is 18 times in the intervention group and 15 times in the control group during days 91–180 after transplantation. With $\alpha = 0.05$, power $(1 - \beta) = 0.8$, an expected loss-to-follow-up rate of 5%, and a 1:1 allocation ratio between groups, $\geqslant$ 200 patients need to be enrolled.

8. Statistical analysis

SAS and R statistical analysis software will be used to conduct the statistical analysis tailored to data properties. Efficacy variables will be compared between the two groups. 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 (2013), 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, and other relevant information to be provided to the subject before enrollment, 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 regarding desensitization.