Clinical study protocol

Title:	Using the Composite Immune Risk Score to assess and modulate the patient's immune reconstitution after allogeneic hematopoietic stem cell transplantation, a prospective, multicenter, randomized controlled study.
Portocol number:	SKIRT-001
Research unit:	Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College.
Address:	Nanjing Road, Heping district, Tianjin, China
Version:	2.0
Version date:	Mar 21, 2024

Declaration of Secrecy

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. 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) [3-8]. Moreover, aside from some exceptions, researchers often did not evaluate multiple immune variables holistically when assessing a patient's immune reconstitution [9-10]. 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 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 (FAHUSTC) between 2012 and 2020. 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 [11].

[1] Gratwohl A, Pasquini M C, Aljurf M, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. Lancet Haematol, 2015, 2(3): e91-100.

[2] Xu L P, Wu D P, Han M Z, et al. A review of hematopoietic cell transplantation in China: data and trends during 2008-2016. Bone Marrow Transplant, 2017, 52(11): 1512-1518.

[3] 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.

[4] 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.

[5] 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.

[6] 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.

[7] 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.

[8] 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.

[9] 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.

[10] 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.

[11] 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

To assess the effectiveness of interventions including health monitoring and regular return follow-up reminders for patients with a high CIRS.

3. Study design

This is a prospective multi-center randomized-controlled clinical trial.

4. Study population

Adult patients receiving allo-HSCT.

4.1 Inclusion criteria

1) Patients must be ≥ 16 years of age;
2) Patients receiving haploidentical allo-HSCT;
3) Patients have to sign an informed consent form before the start of the research procedure.

4.2 Exclusion criteria

Patients who meet any of the following criteria will not be enrolled in the study:

1) Tandem transplantation or multiple transplantations;
2) Mental or other medical conditions that make the patients unable to comply with the research treatment and monitoring requirements;
3) Patients who are ineligible for the study due to other factors, or will bear great risk if participating in the study.

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

5. Study regimen

5.1 Risk prediction

The patients were randomly divided into intervention group and control group (the probability of entering the intervention group is 0.5). Physicians assessed the risk level of patients in the intervention group based on their immune status on days 91-180 using the CIRS.

- Scored as high risk: health monitoring & reminders for blood tests weekly & reminders for follow-up visits until 1 year post-transplant.
- Scored as intermediate risk: health monitoring & reminders for blood tests monthly & reminders for follow-up visits until 1 year post-transplant.
- Scored as low risk: reminders for follow-up visits until 1 year post-transplant.

5.2 Health surveillance

Things as follows will be recorded by patients every day using the app:

1) Body temperature
2) Body weight
3) White blood cell count, platelet, hemoglobin
4) Total bilirubin, alanine aminotransferase, glutamine aminotransferase, creatinine
5) Any discomfort? (Difficulty in breathing, cough, sputum, abdominal pain, diarrhea, vomiting, palpitations, chest pain, Frequent, urgent or painful urination, edema of eyelids or lower extremities, dry eyes or mouth, new rash, bleeding from nose or gums or visible bleeding spots on skin, drowsiness or impaired consciousness)

5.3 Early warning and suggestion

When there is any abnormality in the patient's daily health monitoring information, the app will suggest the patient to consult the doctor in time or send a message to remind the doctor to pay attention to the patient's condition.

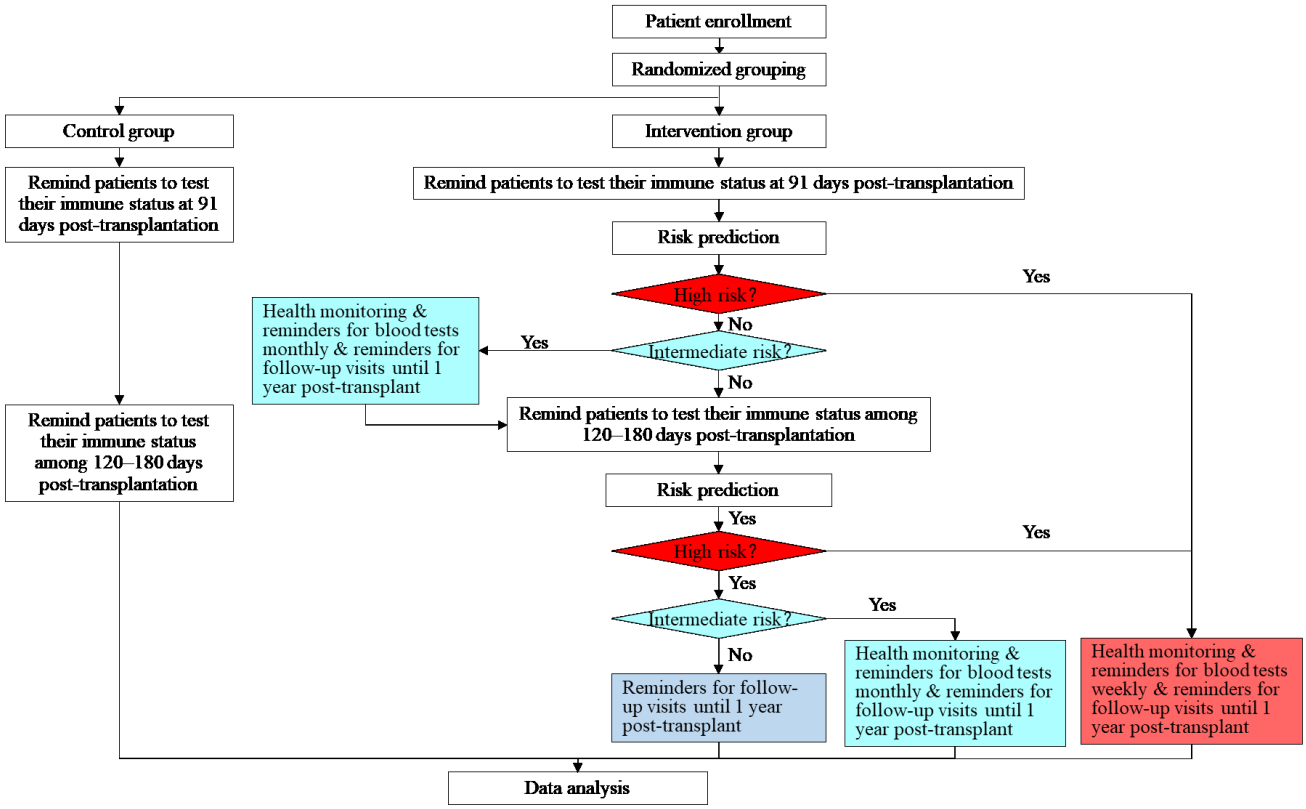

Figure 1. Study flowchart

6. Study assessment and follow-up

Patients will be regularly followed up after transplantation. Data on infection, relapse and survival will be collected.

7. Study endpoints

- Primary endpoint: survival after transplantation.
- Secondary endpoints: non-relapse mortality rate, relapse rate, incidence of infections and immune cells count.

8. Sample size calculation

456 transplant patients are expected to be enrolled.

The 1.5-year survival rates of previous adult patients at the IHCAMS was 86% during 1 January 2019 ¬ 31 December 2021. The 1.5-year survival rates of intervention group will improve to 92% per the researchers' estimation. To attain a 0.05 significance level and a 0.8 power at a presumed 10% dropout rate, ≥ 456 participants will need to be enrolled.

9. Statistical analysis

SAS and 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.

10. 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.

11. 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.