Clinical study protocol

Title:	A cohort study on the association between the autonomic nervous system function and acute GvHD after patients receiving allogeneic hematopoietic stem cell transplantation
Portocol number:	vagaGOAT-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
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Declaration of Secrecy

1. Research background

At present, there are about 40,000 new allogeneic hematopoietic stem cell transplantation (allo-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.

Evidences indicate a bidirectional interaction between the autonomic nervous system (ANS) and the immune system, including inflammatory responses, which are closely related to transplantation outcomes.[3, 4] However, the role of the ANS in acute graft-versus-host disease (acute GvHD) in patients remains incompletely unclear and requires further study.

Previously we have developed a dynamic forecasting model for severe acute GvHD, termed the 'daGOAT model', was constructed, achieving an AUROC score of more than 0.78.[5] We subsequently conducted a prospective study of deploying daGOAT as a conditional autonomous artificial intelligence (AI) agent to prescribe a drug to prevent severe acute GvHD following human leukocyte antigen (HLA)-mismatched haematopoietic cell transplantation.[6] We plan to utilize this model as a tool for early classification and interrogate ANS function of transplant recipients.

1. Gratwohl A, Pasquini MC, Aljurf M, et al. One million haemopoietic stem-cell transplants: a retrospective observational study. Lancet Haematol 2015;2(3):e91-100.
2. Xu LP, Wu DP, Han MZ, et al. A review of hematopoietic cell transplantation in China: data and trends during 2008-2016. *Bone Marrow Transplant* 2017;52(11):1512-18.
3. Katayama Y, Battista M, Kao WM, et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell 2006;124(2):407-21.
4. Hwa YL, Shi Q, Kumar SK, et al. Beta-blockers improve survival outcomes in patients with multiple myeloma: a retrospective evaluation. *Am J Hematol* 2017;92(1):50-55.

5. Liu X, Cao Y, Guo Y, et al. Dynamic forecasting of severe acute graft-versus-host disease after transplantation. *Nat Comput Sci* 2022;2(3):153-59.
6. Chen J, Cao Y, Feng Y, et al. Autonomous artificial intelligence prescribing a drug to prevent severe acute graft-versus-host disease in HLA-haploidentical transplants. *Nat Commun* 2025;16:8391.

2. Study design

This is a prospective observational study.

3. Study population

Adult patients receiving HLA-haploidentical transplants at the Institute of Hematology, Chinese Academy of Medical Sciences (IHCAMS).

3.1 Inclusion criteria

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

3.2 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 requirements;
3) 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

During days 17 to 23 posttransplant, the daGOAT model will be used for dynamic risk prediction and stratification. Autonomic nervous system functional tests including heart rate response to standing, piloerection, and dynamic

electrocardiography will be performed on patients before transplantation and during 17 to 25 days after transplantation.

5. Sample collection and omics analysis

Blood and bone marrow samples will be collected from patients for analyses with the aim to further improve the daGOAT predictive model and facilitate molecular biological investigations.

6. Study assessment and follow-up

7.1 Medical history

Medical history (previous treatments, including chemotherapy).

7.2 Physical examination

Physical examination before transplantation, including body height, body weight, body surface area, and the Eastern Cooperative Oncology Group score.

7.3 Laboratory tests

Laboratory tests performed at diagnosis and before and after transplantation.

7.4 Follow-up.

Patients will be followed up after transplantation; data on infection, relapse, survival, and quality of life will be collected.

7. Study endpoints

8.1 Primary endpoint

Autonomous nervous system functional test results.

8.2 Secondary endpoints

Incidence of severe acute GvHD after transplantation within 100 days and incidences of acute GvHD (any grade) in various target organs.

8. Sample size calculation

60 adult patients receiving allo-HSCT.

9. 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.

10. 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.

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 to safeguard data security and subject privacy.