Clinical study protocol

Title:	A multicenter randomized controlled trial of daGOAT model-guided prevention of severe acute graft-versus-host disease in patients undergoing allogeneic hematopoietic stem cell transplantation
Protocol number:	daGOAT-RCT-001
Research unit:	Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College.
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Declaration of Secrecy

1. Research background

Acute graft-versus-host disease (GvHD) is one of the most common complications and causes of death after allogeneic hematopoietic stem cell transplantation (allo-HSCT) [1]. Literature reports indicate that the incidence of severe (grade III–IV) acute GvHD is approximately 14% [2, 3]. The 6-month non-relapse mortality (NRM) rate of patients with severe acute GvHD who have poor response to treatment is as high as 44% [4]. Among deaths within 100 days after allo-HSCT, 24% are caused by acute GvHD, second only to cancer recurrence (29%) and infection (28%) [5]. If severe acute GvHD can be predicted, it will be possible to strengthen preventive treatment for high-risk patients, instead of blindly reducing the immunity of all transplant patients and increasing the infection rate [6].

The researchers have collected and analyzed the clinical data of the patients who received HLA-mismatched allo-HSCT at the Institute of Hematology, Chinese Academy of Medical Sciences (IHCAMS) from 2012 to 2021. A dynamic forecasting model for severe acute GvHD, termed the 'daGOAT model', was constructed, achieving an AUROC score of more than 0.78. This result has been published in *Nature Computational Science* [7].

Subsequently, the researchers deployed the daGOAT as a conditional autonomous AI in the hospital information system, which automatically extracted subjects' data and performed risk prediction. Physicians conducted clinical interventions on intermediate- and high-risk patients. With the use of daGOAT, the incidence of severe acute GvHD decreased from 16% in the historical cohort to 5.5%. This research result has been published in *Nature Communications* [8].

The researchers will conduct a prospective, multicenter randomized controlled trial aimed at using the daGOAT model to provide early warning and prescribe a drug for the prevention of severe acute GvHD after transplantation.

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5. Styczynski J, Tridello G, Koster L, et al. Death after hematopoietic stem cell transplantation: changes over calendar year time, infections and associated factors. Bone Marrow Transplant 2020;55(1):126-36. doi: 10.1038/s41409-019-0624-z

6. Paczesny S. Biomarkers for posttransplantation outcomes. Blood 2018;131(20):2193-204. doi: 10.1182/blood-2018-02-7915093.

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8. 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(1):8391. doi: 10.1038/s41467-025-62926-0 [published Online First: 2025/09/26]

2. Study objective

This study aims to evaluate the efficacy of prophylactic ruxolitinib in adult patients predicted to be at intermediate-to-high risk of severe acute GvHD using the daGOAT model.

3. Study design

This is a prospective, multicenter, randomized controlled trial.

4. Study population

4.1 Inclusion criteria

1) Age ＞ 16 years old.
2) HLA-haploidentical transplant.
3) Able to take oral medications.
4) 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 have undergone tandem transplantation or multiple transplantations.
2) Patients who are allergic to or cannot tolerate ruxolitinib.
3) Patients with mental or other medical conditions that make them unable to comply with the study treatment and monitoring requirements.
4) Patients who are ineligible for the study due to other factors, or who will bear great risk if they participate 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 investigators may also decide to discontinue a patient from the study if there is an unacceptable risk.

5. Dosage regimen

Patients will be randomly assigned to the intervention group or the control group at a 1:1 allocation ratio. For patients in the intervention group, the daGOAT model will be used to predict the occurrence of severe acute GvHD from day 17 to day 23 after transplantation. Each subject will be stratified into low-, intermediate-, and high-risk groups, and corresponding preventive interventions were implemented according to

their risk levels.

5.1 The group of daGOAT model prevention:

1) Model-predicted high-risk patients: will receive standard prophylaxis plus ruxolitinib 5mg twice daily (bid) orally until at least day 60 post-transplantion and will be terminated after day 100. If severe hematological signs occur such as when there is severe neutropenia ($<0.1\times10^9$/L), ruxolitinib can be used at half dose or discontinued until recovery.
2) Model-predicted moderate-risk patients: will receive standard prophylaxis plus ruxolitinib 5mg once daily (qd) orally until at least day 60 post-transplantion and will be terminated after day 100. If severe hematological signs occur such as when there is severe neutropenia ($<0.1\times10^9$/L), ruxolitinib can be used at half dose or discontinued until recovery.
3) Model-predicted low-risk patients: will receive standard prophylaxis without additional GvHD prophylactic agents, including mesenchymal cell infusion, anti-CD25 monoclonal antibodies, and ruxolitinib outside the scope specified in the study protocol.

5.2 Control group:

Will receive standard prophylaxis without additional GvHD prophylactic agents, including mesenchymal cell infusion, anti-CD25 monoclonal antibodies, and ruxolitinib outside the scope specified in the study protocol.

6. Study assessment and follow-up

6.1 Medical history

Medical history (previous treatments, including chemotherapy).

6.2 Physical examination

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

6.3 Laboratory tests

Laboratory tests performed at diagnosis and before and after transplantation at various time points.

1) Blood routine: Red blood cell count, hemoglobin, platelet count, white blood cell count, white blood cell classification count, etc.
2) Urine routine: specific gravity, pH, urine sugar, urine protein, urobilinogen, ketone bodies, microscopic red blood cells, urine white blood cells, bacteria, crystals, tube type, etc.
3) Coagulation function tests: activated partial thromboplastin time, plasma prothrombin time, international normalized ratio of prothrombin time, thrombin time, plasma fibrinogen, and plasma D-dimer.
4) Bone marrow:
   - Smear or biopsy analysis;
   - Immunophenotype analysis by flow cytometry;
   - Chromosome karyotype;
   - Gene mutation analysis.
5) Blood biochemistry.
   - Electrolyte includes sodium, potassium, chlorine, calcium, phosphorus, etc.
   - Liver function tests include alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase, lactate dehydrogenase, total protein, albumin, total bilirubin, direct bilirubin, total cholesterol, triglyceride, etc.
   - Renal function tests include blood urea nitrogen, uric acid, creatinine, etc.

6) Immune cell subset analysis by flow cytometry.

6.4 Other examinations.

1) 12-lead electrocardiogram;
2) Chest computed tomography;
3) Abdominal ultrasound and abdominal CT when necessary;
4) Echocardiography when necessary.
5) Other examinations that the investigators consider appropriate.

7. Follow-up.

Patients will be followed up regularly after transplantation, and data on GvHD, relapse, survival, and other relevant outcomes will be collected.

8 Study endpoints

8.1 Primary endpoint

Incidence of severe acute GvHD after transplantation within 100 days.

8.2 Secondary endpoints

1) Incidence of severe acute GvHD after transplantation within 180 days.
2) Incidence of acute GvHD (any grade) in various target organs.
3) Overall survival, relapse-free survival rate, and relapse rate.

9. Adverse event and severe adverse event

Starting from the date of signing the informed consent form until the last visit, all adverse events will be evaluated and recorded according to the Common Terminology Criteria for Adverse Events version 5.0.

10. Sample size calculation

Assuming the incidence of severe acute GvHD is 13% in the control group and 5.5% in the intervention group and an enrollment ratio of 1:1 between the two groups, to

attain a 0.05 significance level and a 0.8 power at a presumed 5% dropout rate, ⩾ 438 patients need to be enrolled.

11. 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.

12. 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.

13. 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.