Clinical study protocol

Title:	A prospective, single-arm clinical trial of prevention of severe acute graft-versus-host disease after adult patients receiving allogeneic hematopoietic stem cell transplantation using a daGOAT model
Portocol number:	daGOAT-adult-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. Abstract

Title	A prospective, single-arm clinical trial of prevention of severe acute graft-versus-host disease after adult patients receiving allogeneic hematopoietic stem cell transplantation using the daGOAT model
Study objective	To evaluate the efficacy and safety of ruxolitinib for prophylactic therapy of adult patients who are predicted to have a high risk for developing severe acute graft-versus-host disease (aGVHD) by the daGOAT model.
Study design	This is a prospective single-arm historical-control clinical trial. Adult patients receiving human leukocyte antigen (HLA)-mismatched allogeneic hematopoietic stem cell transplantation (allo-HSCT) will be enrolled after October 1, 2022. The propensity score matching method will be used to select controls at a ratio of 1:3 from the historical cases.
Study population	Adult patients receiving HLA-mismatched allo-HSCT.
Inclusion criteria	1. Patients must be > 16 years of age; 2. Patients receiving HLA-mismatched and non-cord blood allo-HSCT; 3. Patients who can take oral medication; 4. Patients have to sign an informed consent form before the start of the research procedure.
Exclusion criteria	1. Tandem transplantation or multiple transplantations; 2. Patients who are allergic to or cannot tolerate ruxolitinib; 3. Mental or other medical conditions that make the patients unable to comply with the research treatment and monitoring requirements; 4. Patients who are pregnant or cannot take appropriate contraceptive measures during treatment; 5. Patients who are ineligible for the study due to other factors or will bear great risk if participating in the study.
Sample size	The incidence rate of severe aGVHD within 100 days of previous adult transplant patients at the Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College (IHCAMS) was 18% during 1 December

	2020–31 December 2021. The incidence rate after using the daGOAT model will fall to 8% per the researchers' estimation. Comparison will be performed using a propensity score matching method to select controls at a ratio of 1:3 from the historical cases. To attain a 0.05 significance level and a 0.8 power at a presumed 5% dropout rate, ≥ 102 participants will need to be enrolled, and ≥ 306 historical controls will need to be matched.
Dosage regimen	➢ Model-predicted high-risk patients: ruxolitinib 5mg bid po until at least day 60 post-transplant and 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 as appropriate, and can continue to be used after hematology recovery. ➢ Model-predicted moderate-risk patients: ruxolitinib 2.5mg bid p po until at least day 60 post-transplant and 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 as appropriate, and can continue to be used after hematology recovery. ➢ Model-predicted low risk: regular aGVHD prophylactic regimens.
Study endpoints	Primary endpoint: incidence of severe aGVHD after transplantation within 100 days. Secondary endpoints: incidence of aGVHD (any grade) in various target organs, overall survival after transplantation, relapse-free survival rate, relapse rate, incidence of severe infections, safety of treatment, and total cost of treatment.
Safety evaluation	Encompassing physical examination, vital signs, adverse events, concomitant therapy, and abnormal results of laboratory tests.
Statistical analysis	SAS and R statistical analysis software will be used to conduct the statistical analysis tailored to data properties. The propensity score matching method will be used to select controls at a ratio of 1:3 from the historical cases. Efficacy and safety variables will be compared between the two groups of the matched patients. All comparison tests will be two-sided, and statistical significance will be defined as $P < 0.05$ in all the analyses.

2. Research background

At present, there are about 40,000 new allogeneic hematopoietic stem cell transplantation (allo-HSCT) cases every year in the world [1, 2], among which about a quarter occur in China. Allo-HSCT has become the primary treatment method for some hematological diseases such as refractory and relapsed acute leukemia and bone marrow failure. The individuals are affected by dynamic and static multi-dimensional parameters as well as post-transplant immune reconstitution. The use of machine learning to integrate big data for predicting severe aGVHD (grade III–IV) may provide a new path towards understanding immune complications after transplantation.

Previous foundation of research

The researchers have compiled the postoperative clinical data of the patients who received HLA-mismatched allogeneic hematopoietic stem cell transplantation (allo-HSCT) at the IHCAMS from 2012 to 2021—named the 'aGOAT dataset', which contained 194 dynamic variables of 584 adult patients and 159 dynamic variables of 45 pediatric patients, including complete blood counts, blood biochemistry, electrolytes, cytokines, flow cytometry, antibody, fluid inflow/outflow, and vital signs. Severe aGVHD occurred within 100 days in 16% of the adult patients and 24% of the pediatric patients. A dynamic forecasting model for severe aGVHD, termed the 'daGOAT model', was constructed, achieving an AUROC score of more than 0.78. This result has been published in *Nature Computational Science* in 2022.[3]

This study aims to prospectively evaluate the use of the daGOAT model in real-world clinical settings at the IHCAMS.

1. *Gratwohl, A., et al., One million haemopoietic stem-cell transplants: a retrospective observational study. Lancet Haematol, 2015. 2(3): p. e91-100.*

2. *Xu, L.P., et al., A review of hematopoietic cell transplantation in China: data and trends during 2008-2016. Bone Marrow Transplant, 2017. 52(11): p. 1512-1518.*
3. *Liu, X., et al. Dynamic forecasting of severe acute graft-versus-host disease after transplantation. Nat Comput Sci , 2022.2: p. 153-159.*

3. Study objective

To evaluate the efficacy and safety of ruxolitinib for prophylactic therapy of adult patients who are predicted to have a high risk for developing severe aGVHD according to the daGOAT model.

4. Study design

This is a prospective single-arm historical-control clinical trial. Adult patients receiving HLA-mismatched allogeneic hematopoietic stem cell transplantation (allo-HSCT) at the IHCAMS will be enrolled after October 1, 2022. The propensity score matching method will be used to select controls at a ratio of 1:3 from the historical cases.

The daGOAT model will dynamically predict the risk for severe aGVHD daily from day 17 to day 23 after transplantation, and medication will be adjusted according to the predicted risk.

The study flowchart is shown in Figure 1 (LR, low risk; MR, moderate risk; HR, high risk).

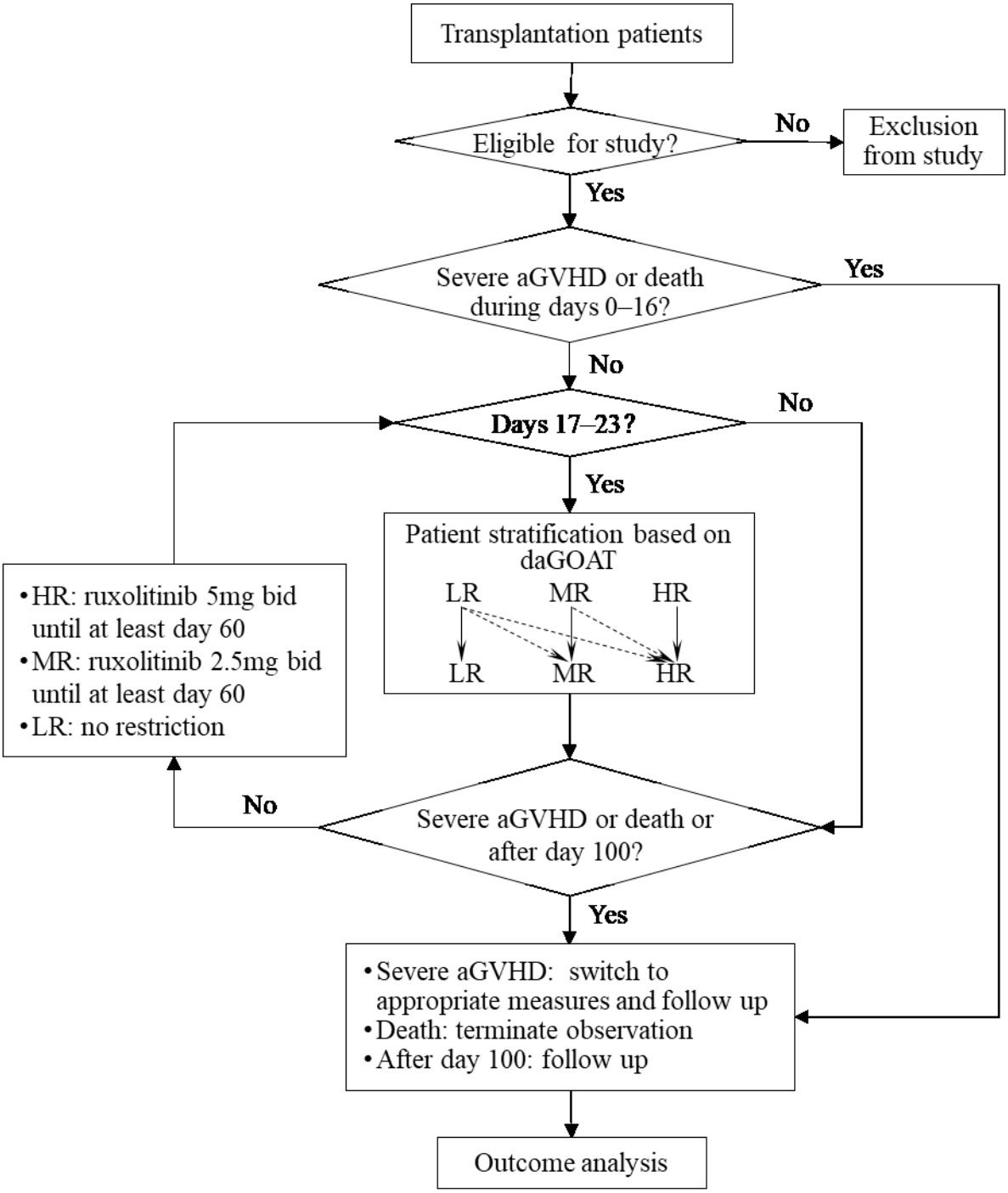

Figure 1. Study flowchart

5. Study population

Adult patients receiving HLA-mismatched allo-HSCT from the IHCAMS.

5.1 Inclusion criteria

1) Patients must be > 16 years of age;
2) Patients receiving HLA-mismatched and non-cord blood allo-HSCT;
3) Patients who can take oral medication;
4) Patients have to sign an informed consent form before the start of the research procedure.

5.2 Exclusion criteria

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

1) Tandem transplantation or multiple transplantations;
2) Patients who are allergic to or cannot tolerate ruxolitinib;
3) Mental or other medical conditions that make the patients unable to comply with the research treatment and monitoring requirements;
4) Patients who are pregnant or cannot take appropriate contraceptive measures during treatment;
5) Patients who are ineligible for the study due to other factors, or will bear great risk if participating in the study.

5.3 Drop-out and withdrawal criteria

1) Failure of neutrophil engraftment within 30 days of transplantation;
2) 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.

6. Dosage regimen

1) Model-predicted high-risk patients: ruxolitinib 5mg bid po until at least day 60 post-transplant and 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: ruxolitinib 2.5mg bid. po until at least day 60 post-transplant and 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: regular aGVHD prophylactic regiments.

7. 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 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 and 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 and creatinine, etc.
6) Immune cell subset analysis by flow cytometry.

7.4. 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.

7.5. Follow-up.

Patients will be followed up at days 14, 28, 42, 60, 90, 180, 270, 360 and 540 after transplantation; data on infection, relapse, survival, and quality of life will be collected.

8. Study endpoints

8.1 Primary endpoint

Incidence of severe aGVHD after transplantation within 100 days.

8.2 Secondary endpoints

Incidence of aGVHD (any grade) in various target organs, overall survival after transplantation, relapse-free survival rate, relapse rate, incidence of severe infections, safety of treatment, and total cost of treatment.

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

The incidence rate of severe aGVHD within 100 days of previous adult transplant patients at the IHCAMS was 18% during 1 December 2020 ¬ 31 December 2021. The incidence rate after using the daGOAT model will fall to 8% per the researchers' estimation. Comparison will be performed using a propensity score matching method to select controls at a ratio of 1:3 from the historical cases. To attain a 0.05 significance level and a 0.8 power at a presumed 5% dropout rate, $\geqslant$ 102 participants will need to be enrolled, and $\geqslant$ 306 historical controls will need to be matched.

11. Statistical analysis

SAS and R statistical analysis software will be used to conduct the statistical analysis tailored to data properties. The propensity score matching method will be used to select controls at a ratio of 1:3 from the historical cases. Efficacy and safety variables will be compared between the two groups of the matched patients. All comparison tests will be two-sided, and 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.