Study Protocol

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Project Title:

A Single-Arm, Single-Center Exploratory Study of Amimestrocel Injection for the Prevention of Gastrointestinal Mucositis Induced by Conditioning Regimens Containing TBI and/or Melphalan

Sponsor:

Department of Hematology, The First Affiliated Hospital of Soochow University

Responsible Department:

Department of Hematology

Principal Investigator(s):

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Investigator Statement and Protocol Signature Page

As the principal investigator of this research project, I will adhere to the ethical principles of the Ministry of Health's "Measures for Ethical Review of Biomedical Research Involving Humans" (2016), the WMA's "Declaration of Helsinki" (2013), CIOMS's "International Ethical Guidelines for Biomedical Research Involving Human Subjects" (2002), and GCP. Under the guidance of Good Clinical Practice, I will conduct the research using the protocol approved by the Ethics Committee, in accordance with the requirements of this protocol, to ensure the scientific validity of the research and protect the health and rights of the subjects.

Protocol Synopsis

Study Objective	To preliminarily evaluate the safety and efficacy of prophylactic intravenous infusion of Amimestrocel injection in

	reducing the incidence of severe (Grade 3-4) oral mucositis in patients undergoing hematopoietic stem cell transplantation with conditioning regimens containing TBI and/or melphalan.
Sample Size	22 cases (considering a 10% dropout rate).
Study Population	Patients aged 18-65 years planned to undergo myeloablative allogeneic hematopoietic stem cell transplantation containing TBI and/or melphalan.
Study Design	Single-arm, single-center, open-label, exploratory interventional study.
Inclusion Criteria	1. Age 18-65 years. 2. Planned to undergo myeloablative allogeneic hematopoietic stem cell transplantation with conditioning containing TBI and/or melphalan. 3. ECOG performance status 0-1. 4. Basically normal cardiac, hepatic, and renal function. 5. Voluntarily sign the informed consent form.
Exclusion Criteria	1. History of allergy to mesenchymal cells or any component of the preparation. 2. Presence of active, uncontrolled infection. 3. History of other malignancies within the past 5 years (except for cured specific types). 4. Pregnant or lactating women. 5. Previous receipt of any cell therapy product. 6. Severe psychiatric or cognitive impairment. 7. Any other condition deemed by the investigator as unsuitable for participation.
Intervention	Single infusion of Amimestrocel injection (6.0×10^7 cells/150ml), completed within 24-48 hours after the last cyclophosphamide administration.
Primary Endpoint	Incidence of Grade 3-4 oral mucositis within 28 days post-transplantation.
Secondary Endpoints	1. Overall severity and duration of mucositis. 2. Severity and duration of gastrointestinal mucositis (assessed via diarrhea). 3. Oral pain score and opioid usage. 4. Time to neutrophil engraftment. 5. Safety and incidence of adverse events (up to day +100 post-transplantation).
Study Schedule	Screening period (Day -14 to -7 pre-transplant) → Intervention

	infusion → Intensive observation period (Day 0 to +28 post-transplant) → Safety follow-up period (up to day +100 post-transplant) → Data analysis and publication.
Statistical Analysis	Descriptive statistical analysis will be used. The incidence rate of the primary endpoint and its 95% confidence interval will be calculated. Secondary endpoints will be summarized descriptively.
Publication Plan	Planned for publication as a peer-reviewed journal article. Results will also be presented at academic conferences. The study protocol will be registered and made publicly available.

1. Study Background

Conditioning regimens containing total body irradiation (TBI) and/or high-dose melphalan are commonly used intensive chemotherapy protocols in hematopoietic stem cell transplantation (HSCT). However, their severe dose-limiting toxicity—gastrointestinal mucositis—significantly impacts patient safety and quality of life. Gastrointestinal mucositis can lead to severe pain, dysphagia, serious diarrhea, impaired nutrient absorption, and secondary infections, prolonging hospitalization and increasing healthcare costs[1].

Mesenchymal stem cells (MSCs) have attracted considerable attention due to their potent immunomodulatory and tissue repair functions. Their mechanism of action involves not direct differentiation into tissue cells, but rather the paracrine secretion of various anti-inflammatory factors (such as PGE2, IDO, IL-10) and growth factors (such as KGF, EGF, HGF), which suppress local excessive inflammatory responses and promote epithelial cell proliferation and repair. This has demonstrated potential for preventing and treating mucositis in preclinical and clinical studies[2-4].

Amimestrocel injection (trade name: Ruibo Sheng) is an allogeneic umbilical cord-derived mesenchymal stem cell finished injection product. This study aims to preliminarily explore the efficacy and safety of the prophylactic application of this product in reducing the risk of severe gastrointestinal mucositis in patients receiving conditioning regimens containing TBI and/or melphalan.

2. Study Objectives

Primary Objective:

To preliminarily evaluate the efficacy of prophylactic intravenous infusion of Amimestrocel injection in reducing the incidence of severe (Grade 3-4) oral mucositis in patients undergoing HSCT with conditioning regimens containing TBI and/or melphalan.

Secondary Objectives:

1. To assess the impact of Amimestrocel injection on the overall severity (highest grade) and duration of gastrointestinal mucositis.

2. To assess the impact of Amimestrocel injection on the severity and duration of gastrointestinal mucositis (assessed via diarrhea).

3. To assess the impact of Amimestrocel injection on patients' oral pain and the need for opioid medication.

4. To observe the impact of Amimestrocel injection on the time to neutrophil engraftment.

5. To evaluate the safety and incidence of adverse events/serious adverse events associated with prophylactic infusion of Amimestrocel injection.

3. Study Rationale

1. **Scientific Basis:** Based on the well-established immunomodulatory and tissue repair functions of MSCs and preclinical/clinical evidence in graft-versus-host disease (GVHD) and mucositis repair, there is theoretical rationale for conducting this exploratory study.

2. **Subject Selection:** The study precisely targets HSCT patients receiving TBI and/or melphalan-containing regimens, who have the highest expected historical mucositis incidence (up to 71%) and thus the greatest potential benefit. Strict criteria control confounding factors and risks.

3. **Dose and Administration:** A single fixed dose (6.0×10^7 cells) is used. The timing of administration (24-48 hours after the last chemotherapy) aims for prevention before injury initiation. The regimen is based on experience with similar products, prioritizing safety.

4. **Endpoint Selection:** The primary endpoint is the reduction in the incidence of Grade 3-4 oral mucositis, the most critical clinical outcome. It comprehensively assesses pain, nutrition, engraftment, and safety, with exploratory indicators providing mechanistic clues.

5. **Risk-Benefit Assessment:** The potential benefit (prevention of severe complications) is clear and significant. For all known risks (infusion reactions, infection, engraftment, etc.), rigorous full-cycle monitoring and contingency plans have been established, making risks controllable.

4. Study Design

1. Sample Size Calculation

Basis and Parameters: Based on literature (Nakagaki et al., 2022), the historical incidence (p_0) of severe (Grade 3-4) oral mucositis in patients receiving similar TBI-containing conditioning regimens is 71%.

Expected Target: It is hypothesized that Amimestrocel injection can reduce this incidence by 30 percentage points, i.e., the expected incidence in the trial group (p_1) is 41%.

Statistical Assumptions: Significance level (α) is set at 0.05 (two-sided), with a test power (1-β) of 80%.

Calculation and Adjustment: The calculated sample size required to meet these statistical criteria is 19 cases. Considering an approximate 10% dropout rate, the final sample size is determined to be 22 cases.

2. Selection of Study Population (Including Inclusion, Exclusion, Withdrawal, and Termination Criteria)

Main Inclusion Criteria

1. Aged 18-65 years, any gender.

2. Planned to undergo myeloablative allogeneic hematopoietic stem cell transplantation.

3. Conditioning regimen is a myeloablative regimen containing total body irradiation (TBI) and/or melphalan.

4. Eastern Cooperative Oncology Group (ECOG) performance status of 0-1.

5. Basically normal cardiac, hepatic, and renal function. Left ventricular ejection fraction ≥ 50%; serum total bilirubin ≤ 1.5 × upper limit of normal (ULN); serum creatinine ≤ 1.5 × ULN or creatinine clearance ≥ 60 mL/min.

6. Voluntarily join the study and sign the informed consent form.

Main Exclusion Criteria

1. History of allergy to mesenchymal cells or any component of the preparation.

2. Presence of active, uncontrolled bacterial, fungal, or viral infection.

3. History of other malignancies (except target disease) within the past 5 years (cured basal cell skin carcinoma, cervical carcinoma in situ, etc., are excluded).

4. Pregnant or lactating women, or those planning pregnancy during the study period.

5. Previous receipt of any cell therapy product.

6. Suffering from severe psychiatric illness or cognitive impairment, unable to cooperate with study assessments.

7. Any other condition deemed by the investigator as unsuitable for participation in this study.

Withdrawal Criteria:

1. The subject voluntarily requests to withdraw informed consent and exit the study.

2. The investigator judges that continued participation may be detrimental to the subject.

3. The subject becomes pregnant during the study period.

Termination Criteria:

1. Occurrence of a serious adverse event related to the investigational product during the study, deemed by the Ethics Committee to require study termination.

2. Discovery of a major flaw in the study protocol that prevents achieving the study objectives.

3. Specific Study Content

This is a clinical study aiming to preliminarily explore the efficacy and safety of the prophylactic application of Amimestrocel injection in reducing the risk of severe gastrointestinal mucositis in patients undergoing HSCT with conditioning regimens containing TBI and/or melphalan.

Main activities include: a single infusion of the investigational product for eligible subjects, followed by a 28-day intensive efficacy observation period (daily assessment of mucositis, pain, etc.) and a 100-day safety follow-up period.

4. Subject Grouping

Grouping: Single-arm design. All eligible and enrolled subjects receive the same intervention. No control group.

Intervention (Off-label Use Justification):

Nature: This study involves off-label use of the investigational product. Amimestrocel injection (allogeneic umbilical cord mesenchymal stem cells) is used in this study for the prevention of HSCT-related mucositis, representing an exploration of a new therapeutic use.

Safety Basis:

- **Relatively Clear Mechanism:** MSCs primarily exert immunomodulatory and tissue repair effects through paracrine mechanisms rather than unlimited proliferation, theoretically posing low tumor risk.
- **Existing Safety Data:** Similar MSC products have accumulated some clinical safety data in indications like GVHD (see references).
- **Rigorous Monitoring:** The protocol includes a comprehensive 100-day safety monitoring plan and clear adverse event management procedures.

Necessity Basis:

- **Unmet Clinical Need:** Severe mucositis induced by TBI and/or melphalan-containing conditioning has a high incidence and serious consequences, and effective preventive measures are currently lacking.
- **Matching Potential Mechanism:** The anti-inflammatory and pro-repair properties of MSCs highly align with the pathophysiological process of preventing mucosal injury.

Informed Consent: The informed consent form must clearly state that this study involves off-label use, detailing the study purpose, potential risks and benefits, and obtain the subject's written consent.

Dose Selection/Adjustment:

Dose: Fixed dose, single infusion of one bag (12ml) containing 6.0×10^7 cells.

Adjustment: No dose adjustment based on body weight. The protocol does not include dose escalation or adjustment schemes.

Timing of Administration: Single infusion administered within 24-48 hours before stem cell infusion.

Blinding/Unblinding:

Open-label study, not blinded. Both investigators and subjects are aware of the treatment received.

Criteria for Concomitant Medications:

All patients will receive the center's standard HSCT procedures and supportive care (including oral care, pain management, nutritional support, etc.). Medications required for treating other comorbidities are permitted but must be recorded in detail.

Rescue Medications and Supportive Therapy:

For potential infusion-related reactions, graded management measures are established: observation for mild reactions; pause infusion and provide symptomatic treatment for moderate reactions; immediate cessation and emergency resuscitation per medical routine (e.g., epinephrine, corticosteroids, antihistamines) for severe allergic reactions.

All adverse events will receive necessary symptomatic and supportive treatment as per clinical routine.

5. Study Flow Chart

> Screening Period (Day -14 to -7) ↓ Eligible after Screening (n=?) ↓ Sign Informed Consent Form ↓ Start Standard Conditioning Regimen ↓ Intervention: Infuse Amimestrocel injection (within 24-48h before stem cell infusion) ↓ Infuse Hematopoietic Stem Cells (Day 0) ↓ Intensive Follow-up Period (Day 0 to +28) - Daily assessment: mucositis, pain, diarrhea - Record supportive care ↓ Safety Follow-up Period (until Day +100) - Record AE/SAE, GVHD, relapse, survival ↓ Study End, Data Statistical Analysis

5. Study Procedures

1. Subject Management

1) Subject Recruitment Methods

Channels: Primarily through the HSCT outpatient clinic or wards of the research center (hospital). Research physicians will identify and recruit from patients planned to receive TBI and/or melphalan-containing conditioning.

Materials: Recruitment materials (brief introduction) must be submitted to the Ethics Committee for approval beforehand. Content must be truthful, not exaggerated, clearly stating the study nature, potential risks, and benefits.

Process: The investigator or designated coordinator gives a preliminary introduction to the study to potential subjects, assessing their basic willingness and preliminary eligibility.

2) Informed Consent Process

Timing and Setting: Conducted during the screening period in a private, undisturbed space.

Executor: Conducted by the principal investigator or an authorized research physician designated by them.

Key Content:

- Detailed explanation of study purpose, nature, procedures, expected duration.
- Clear statement: The use of Amimestrocel injection for mucositis prevention constitutes "off-label use," explaining its exploratory nature.
- Clear disclosure of all possible risks, discomforts, and potential benefits.
- Explanation of data confidentiality, insurance, voluntary participation, and the right to withdraw unconditionally at any time.

Signing: Ensure the subject fully understands before the subject (or their legal representative) and the investigator executing the consent process jointly sign and date the form. Provide a copy to the subject.

3) Verification of Inclusion/Exclusion Criteria

Procedure: After signing the ICF, the investigator verifies the subject's medical records, test results, and inquiry information item by item against all protocol-specified inclusion and exclusion criteria.

Recording: Complete recording of the verification result for each criterion in the Case Report Form (CRF).

Quality Control: A double-check mechanism is recommended to ensure accuracy.

4) Review of Medical History and Concomitant Medication Records

Content: Comprehensive review of medical records to record relevant past medical history, surgical history, allergy history. Detailed recording of all concomitant medications (including prescription, over-the-counter, herbal medicines) during screening and the study period: name, dose, administration method, start/stop dates, and reason for use.

Purpose: To assess baseline health status, determine if there are protocol-prohibited comorbidities or medications, and provide background for subsequent safety analysis.

5) Assignment of Screening Number

A unique screening number is assigned to each subject who signs the ICF and undergoes screening. This number identifies all screening data and documents, retained even if screening fails, for traceability.

6) Assignment of Treatment/Randomization Number

As this is a single-arm study, all successfully enrolled subjects receive the same treatment, so randomization is not required. But a unique subject identification number (or enrollment number/sequence number) should be assigned to each enrolled subject, serving as the "grouping number" to identify all data, biological samples, and investigational product during the study.

7) Trial Compliance Management

Education and Communication: Explain study procedures, importance of visits, self-recording requirements (e.g., pain diary) to subjects in detail.

Visit Reminders: Establish visit reminder mechanisms (phone, SMS, etc.) to ensure timely completion of visits and examinations.

Medication Compliance: Record actual infusion time and dose of the investigational product to ensure compliance with protocol requirements.

Recording: Detail any protocol deviations and perform root cause analysis.

2. Safety Evaluation Procedures (Assessment, Detection, and Reporting of Adverse Events)

Assessment and Detection:

Continuous Monitoring: Actively inquire about and monitor all adverse events from first administration until study end (day +100 post-transplant).

Systematic Recording: Record the occurrence time, severity, duration, actions taken, relationship to the investigational product, and outcome of all AEs/SAEs.

Regular Examinations: Conduct assessments of vital signs, physical examinations, blood tests, liver/kidney function, electrolytes, infection, and GVHD at time points specified in the protocol.

Reporting:

SAE Reporting: Any SAE must be reported in writing to the local Ethics Committee and the Sponsor within 24 hours of the investigator's awareness. Reporting must follow GCP and specific Ethics Committee requirements.

Periodic Summary Reporting: Submit periodic safety update reports to the Ethics Committee as required (e.g., semi-annually or annually).

3. Risk Control and Management Procedures

Potential Risk Identification:

- Infusion-related reactions (allergy, fever, etc.).

- Increased risk of infection.
- Potential impact on hematopoietic engraftment.
- Potential tumor promotion or disease relapse risk (theoretical).
- Other unknown risks.

Control Measures:

- **Strict Inclusion/Exclusion Criteria:** Screen for low-risk patients who can tolerate the procedure, excluding high-risk individuals.
- **Standardized Infusion Procedures:** Standardized operation, equipped with emergency equipment and medications.
- **Comprehensive Monitoring Plan:** Intensive clinical and laboratory monitoring for early detection of abnormalities.
- **Clear AE/SAE Management Plan:** Establish graded management procedures.
- **Data and Safety Monitoring:** Regular review of safety data by investigators and sponsor.
- **Ethical Oversight:** All major decisions (e.g., study termination) require Ethics Committee approval.
- **Emergency Response Plan.**

4. Efficacy Measurement Procedures

Primary Efficacy Indicator:

Incidence of Grade 3-4 oral mucositis.

Tool: Grading using WHO or NCI-CTCAE criteria.

Assessor: Daily assessment by trained, designated research physicians or nurses.

Recording: Record the highest daily grade until it decreases to ≤ Grade 1 or until day +28 post-transplant.

Secondary Efficacy Indicators:

Duration of mucositis, diarrhea, oral pain (NRS score), opioid usage, days of parenteral nutrition, time to neutrophil engraftment, etc.

Assessments and recordings must be performed strictly according to the time points and methods specified in the protocol to ensure data consistency and comparability.

Data Collection:

Use electronic Case Report Forms (eCRF) to ensure real-time, accurate data entry.

5. Discontinuation/Withdrawal Procedures

Subject-Initiated Withdrawal:

Respect their decision. Record the specific reason and date of withdrawal. Complete safety assessments at withdrawal (e.g., physical exam, key lab tests) as much as

possible. Continue to collect information on any SAEs that have occurred and are related to the study (if the subject agrees).

Investigator-Initiated Discontinuation:

When a subject meets protocol-defined withdrawal criteria (e.g., continued participation may be detrimental) or experiences an AE requiring treatment termination, the research physician should discontinue the subject's study treatment. Record the detailed reason and complete safety assessments at the discontinuation visit. The subject's data are typically still included in the safety analysis set.

Overall Study Termination:

When protocol-defined termination criteria are met (e.g., major safety issue or protocol flaw), the principal investigator, in consultation with the sponsor and upon approval by the Ethics Committee, will announce early termination of the study.

6. Blinding/Unblinding Procedures

Blinding Status: This is an open-label (non-blinded) study. Both investigators and subjects are aware of the treatment.

Unblinding: Therefore, conventional emergency unblinding procedures are not involved. However, if future data analysis requires the statistician to remain blinded, the sponsor or independent data manager will provide group identifiers after database lock. In this single-arm study, this process is simpler.

7. Visit Requirements

1) Screening Period: Day -14 to -7 pre-transplant.

Content: Sign ICF, comprehensive medical history, physical exam, ECOG score, lab tests (CBC, liver/kidney function, electrolytes, infection screening, etc.), pregnancy test (if applicable), confirm all inclusion/exclusion criteria.

2) Treatment Period (Intervention & Intensive Observation):

Intervention Day: 24-48 hours before stem cell infusion after completion of conditioning. Administer investigational product infusion, closely monitor for infusion-related reactions.

Hematopoietic Stem Cell Infusion Day: Defined as Study Day 0.

Day 0 to +28: Daily visits/assessments. Core content includes: oral mucositis grading, diarrhea record, oral pain score, analgesic and nutritional support record, temperature monitoring, necessary physical exams. Perform scheduled laboratory tests.

3) Post-Treatment Visits:

Safety Follow-up Visit: Day +100 post-transplant. Primarily conducted via outpatient clinic or phone. Assessment includes: survival status, GVHD occurrence, infection, disease relapse, recording of any SAEs and concomitant medications during the period.

Survival Follow-up (not specifically mandated beyond +100 in this protocol): The protocol mainly follows up to day +100. Long-term survival data may be suggested to be obtained through tumor registry systems or routine clinical follow-up, but this should be explained in the ICF.

6. Study Safety Monitoring and Adverse Event Management Plan

Safety Monitoring: All adverse events must be recorded regarding their occurrence time, severity, duration, relationship to the investigational product, and outcome.

AE Management:

- **Mild AE:** Close observation, usually no need to stop infusion.
- **Moderate AE:** Pause infusion, provide symptomatic treatment, reassess whether to continue infusion after symptoms alleviate.
- **Severe AE/Severe Allergic Reaction:** Immediately stop infusion and perform emergency resuscitation per medical routine (e.g., use epinephrine, corticosteroids, antihistamines, etc.). The investigator must report to the Ethics Committee and Sponsor within 24 hours.

SAE Reporting: All SAEs must be reported in writing to the local Ethics Committee within 24 hours of awareness.

7. Study Data Management and Statistical Analysis

Data Management: Use electronic Case Report Forms (eCRF) for data collection to ensure authenticity, accuracy, and completeness. Establish a data query mechanism to issue queries for questionable data.

Statistical Analysis:

- **Analysis Sets:** Full Analysis Set (FAS) and Safety Analysis Set (SAF).
- **Statistical Methods:** Descriptive statistical analysis will be employed.
    - Continuous variables described using mean ± standard deviation or median (interquartile range).
    - Categorical variables described using frequency and percentage (%).
- **Primary Endpoint Analysis:** Calculate the incidence rate of Grade 3-4 oral mucositis and its 95% confidence interval. A non-formal descriptive comparison may be made with historical data from the center (e.g., similar patients not receiving MSCs).
- **Secondary Endpoint Analysis:** Descriptive summaries will be provided for each secondary endpoint indicator.

8. Form of Research Results Publication

As an exploratory clinical study, its core findings should be formally published as a complete, methodologically transparent, peer-reviewed journal article. Simultaneously, academic exchanges via conference abstracts will be pursued, and open science principles will be practiced through protocol registration and publication. All publication activities must be based on data authenticity and ethical compliance, objectively presenting the study's findings and limitations.

9. References

[1] Sonis S T. The pathobiology of mucositis[J]. Nat Rev Cancer, 2004, 4(4):277-284.

[2] Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study[J]. Lancet, 2008, 371(9624):1579-1586.

[3] Lv K, Gao B, Ye W, et al. Promotion of Epithelial Healing in Oral Mucositis by hESC-derived Mesenchymal Stem Cells via the PI3K/AKT Pathway[J]. Curr Stem Cell Res Ther, 2025, 20(7):810-823.

[4] Kebriaei P, Hayes J, Daly A, et al. A Phase 3 Randomized Study of Remestemcel-L versus Placebo Added to Second-Line Therapy in Patients with Steroid-Refractory Acute Graft-versus-Host Disease[J]. Biol Blood Marrow Transplant, 2020, 26(5):835-844.

[5] Nakagaki M, Kennedy G A, Gavin N C, et al. The incidence of severe oral mucositis in patients undergoing different conditioning regimens in haematopoietic stem cell transplantation[J]. Support Care Cancer, 2022, 30(11):9141-9149.