

**Ultra-low-dose decitabine for patients with poor graft function post-allogenic
hematopoietic stem cell transplantation: a multicenter, open-label, randomized,
phase 3 study**

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Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) serves as an effective therapeutic modality for multiple hematological disorders. Prior to transplantation, patients receive conditioning therapy, followed by infusion of donor-derived stem cells to reconstitute the recipient's hematopoietic and immune systems. Typically, initial hematopoietic recovery occurs within two weeks post-transplantation. However, a subset of patients presents with hypocellular proliferation in the granulocytic, erythroid, or megakaryocytic lineages despite adequate donor chimerism, defined as poor graft function (PGF), which represents one of the major factors adversely affecting post-transplant prognosis.

PGF is defined as cytopenia in two or more peripheral blood lineages beyond day +14 after transplantation, specifically hemoglobin ≤ 85 g/L, neutrophils $\leq 1.5 \times 10^9$ /L, and platelets $\leq 30 \times 10^9$ /L, with bone marrow examination confirming disease remission, hypocellularity in two or more lineages, donor chimerism $\geq 95\%$, absence of grade III–VI graft-versus-host disease (GVHD) and active cytomegalovirus (CMV) infection, persisting for more than 14 days¹. The incidence of PGF ranges from 5% to 27% and is frequently associated with multiple post-transplant complications, including GVHD, infections, and medication effects²⁻⁴.

The pathogenesis of PGF is complex. Current evidence suggests it is closely linked to factors encompassing stem cell dose ("seed"), abnormal bone marrow microenvironment ("soil"), and dysregulated immune modulation ("climate")⁵. Therapeutic strategies largely rely on institutional experience, lacking robust evidence-based support. Previous studies reported that infusion of selected CD34⁺ cells yielded response rates of 36%–83%; however, most were single-arm, small-sample retrospective studies, limiting reliable efficacy assessment⁶⁻⁹. Prophylactic use of N-acetylcysteine in high-risk patients with reduced vascular endothelial cell mass may partially reduce PGF incidence¹⁰. Mesenchymal stem cell therapy for post-transplant PGF, administered at 1×10^6 /kg per infusion for 1–3 cycles, showed response rates of 33.3%–85.0%, though limited case numbers necessitate validation through large-scale

clinical trials¹¹⁻¹².

Decitabine (5-aza-2'-deoxycytidine), a 2'-deoxycytidine analog, was approved by the U.S. Food and Drug Administration in 2006 for the treatment of myelodysplastic syndromes¹³. Previous studies showed that decitabine promotes megakaryocyte maturation and platelet production. In a multicenter clinical trial conducted at our center, low-dose decitabine was administered to patients with refractory severe thrombocytopenia post-allo-HSCT, achieving a response rate of 70% (versus 19.4% in controls), with significant increases in total megakaryocytes and mature platelet-producing megakaryocytes. No significant drug-related adverse events were observed. Post-treatment improvements were also noted in bone marrow endothelial progenitor cells, endothelial cells, and cytokines reflecting endothelial function¹⁴⁻¹⁵. Whether bone marrow microenvironment improvement represents a direct mechanistic pathway or a concomitant phenomenon of decitabine action remains unclear. Beyond megakaryopoiesis, prior studies also demonstrated decitabine's efficacy in enhancing erythroid proliferation and differentiation.

Based on this evidence, we propose a prospective, randomized, open-label clinical trial to evaluate the efficacy and safety of ultra-low-dose subcutaneous decitabine for the treatment of PGF following allo-HSCT.

Objective of the Study

This study aims to conduct a prospective, randomized, open-label clinical trial to evaluate the efficacy and safety of ultra-low-dose decitabine administered via subcutaneous injection for the treatment of PGF following allo-HSCT.

Study Protocol

➤ Study Endpoints

◆ Primary Endpoint

Treatment response rate at 4 weeks post-intervention and the 1-year OS rate

◆ Secondary Endpoints

Bone marrow recovery at 4 weeks post-treatment;

Incidence of disease relapse and GVHD at 3 months post-treatment;

Disease-free survival at 1 year post-treatment.

◆ Safety Endpoints

Incidence of new-onset infections and bleeding events at day +28 post-treatment;

Incidence of adverse events (AEs) and serious adverse events (SAEs).

➤ Study Design

This is a prospective, multicenter, open-label, Phase III clinical trial investigating ultra-low-dose decitabine subcutaneous injection for PGF after allo-HSCT. Eligible patients diagnosed with PGF post-allo-HSCT will be enrolled and follow a standardized screening and treatment protocol. Participants meeting inclusion/exclusion criteria will be randomly assigned to the experimental or control group to receive corresponding interventions until treatment completion, intolerable toxicity, death, withdrawal of informed consent, sponsor termination, or other protocol-specified reasons (whichever occurs first).

Definition of Study Completion: A participant is considered to have completed the study upon finishing follow-up or withdrawal. The entire study concludes after the last participant completes follow-up.

Criteria for Study Suspension or Termination: The study may be temporarily suspended or terminated early with written notification to participants, investigators, sponsors, and regulatory authorities, documenting reasons. Circumstances include but are not limited to: unexpected significant risks to participants; non-compliance with protocol; participant withdrawal of consent; evidence indicating termination is warranted; investigator judgment favoring withdrawal; achievement of primary endpoint.

Early Termination Criteria: Inaccurate/incomplete data; poor protocol adherence; unacceptable AE frequency/severity suggesting participant risk; protocol modifications.

➤ Study Population

◆ Inclusion Criteria

(1) Signed informed consent and ability to comply with the protocol;

- (2) Being diagnosed with PGF ≥ 28 days post-allo-HSCT for hematologic malignancy: cytopenia in ≥ 2 blood lineages (hemoglobin ≤ 85 g/L, neutrophils $\leq 1.5 \times 10^9$ /L, platelets $\leq 30 \times 10^9$ /L); bone marrow showing disease remission with hypocellularity in ≥ 2 lineages, donor chimerism $\geq 95\%$, absence of grade III–VI GVHD and active CMV infection, persisting >14 days;
- (3) ECOG performance status 0–2;
- (4) Expected survival >1 month.

◆ Exclusion Criteria

- (1) Hypersensitivity to decitabine or any excipient;
- (2) Liver cirrhosis (Child-Pugh score ≥ 5);
- (3) Severe active infection, grade IV GVHD, or transplant-related thrombotic complications;
- (4) Hypercoagulable disorder or recent thrombotic event;
- (5) Severe dysfunction of heart, brain, liver, lung, or kidney;
- (6) Disease relapse;
- (7) Decitabine treatment within 1 month prior to enrollment;
- (8) Pregnancy or lactation.

◆ Withdrawal Criteria

- (1) Participant or legal representative requests discontinuation;
- (2) Occurrence of severe AEs or death requiring treatment cessation;
- (3) Development of comorbidities hindering further treatment;
- (4) Disease relapse (hematologic or molecular) during the trial;
- (5) Incomplete critical study data;
- (6) Investigator determines withdrawal is in the participant's best interest;
- (7) Inability to adhere to treatment or follow-up per protocol.

➤ Sample Size Estimation

The necessary sample size was calculated based on the response rate of decitabine in patients with refractory prolonged thrombocytopenia post allo-HSCT in previous study¹⁴⁻¹⁵. Assuming an alpha of 0.05 and a statistical power of 0.95, considering a randomization ratio of 2:1 and an expulsion rate of 20%, the sample size for Arm A and

Arm B was at least 48 and 24, respectively.

➤ Enrollment Procedure

Investigators will enroll subjects following these steps:

- ◆ Obtain signed informed consent from the subject or their legally authorized representative prior to any study-related procedures.
- ◆ The principal investigator or designated trained personnel will review eligibility/ineligibility criteria to formally confirm subject enrollment qualification.
- ◆ Subjects not meeting study criteria may undergo re-screening once. For re-screening, the subject must provide a new signed informed consent and be assigned a new subject identification number.
- ◆ Treatment Protocol and Investigational Products

Eligible subjects will be randomly assigned to Arm A or B and sequentially numbered.

Arm A: Decitabine 6 mg/m² (Days 1–3), once daily, subcutaneous injection.

Arm B: Supportive care with growth factors (granulocyte colony-stimulating factor, erythropoietin, TPO receptor agonists) and component blood transfusions.

Patients with neutropenia (neutrophil count lower than $1.5 \times 10^9/\text{L}$) received granulocyte colony-stimulating factor (5-10 ug/kg.d, subcutaneously). Treatment to stimulate platelet production included recombinant human thrombopoietin (TPO) and thrombopoietin receptor agonists (TPO-RAs) (e.g., eltrombopag, romiplostim, avatrombopag, or herombopag). Platelet transfusion was administered for those with a platelet count lower than $10 \times 10^9/\text{L}$. Patients would receive red blood cell transfusions when their hemoglobin levels were lower than 60 g/L. Other therapies to promote erythroid proliferation, including erythropoietin, luspatercept, roxadustat, etc. For patients with HLA antibodies, plasma exchange, CD20 antibody, or immunoabsorption therapy was adopted.

Decitabine administration is suspended upon occurrence of any of the following non-hematologic toxicities until resolution: (1) serum creatinine ≥ 2.0 mg/dL; (2) SGPT or total bilirubin $\geq 2 \times$ ULN; (3) active/uncontrolled infection; (4) uncontrolled bleeding.

Discontinuation criteria: cardiac/respiratory arrest, intracranial hemorrhage, severe infection, or other life-threatening complications.

Permitted concomitant medications: Anti-GVHD agents (calcineurin inhibitors, corticosteroids, CD25 monoclonal antibody, TNF- α inhibitors, ruxolitinib, etc.); anti-infectives; targeted therapies (TKIs, FLT3 inhibitors); VOD prophylaxis (heparin, ursodeoxycholic acid, alprostadil); nutritional support; chronic disease medications (e.g., for hypertension, diabetes); other drugs deemed not to interfere with efficacy assessment by the investigator.

Prohibited medications: Other hypomethylating agents (azacitidine); other immunotherapies or cellular therapies; drugs potentially affecting efficacy assessment per investigator judgment.

➤ Study Procedures and Assessments

◆ Screening Period (3 days): Eligibility criteria verification; demographic data (age, sex, ethnicity, contact); medical history, allergies, primary disease status, transplant type, conditioning regimen, cell dose, post-transplant timeline, complications, prior cytopenia treatments; physical examination (height, weight, BP, temperature, pulse, BSA, cardiopulmonary/neurological exams, hepatosplenomegaly, ECOG score); laboratory tests (CBC, biochemistry, virology, T-cell subsets, NK cell function, macrophages); bone marrow assessment (morphology, megakaryocyte count, fibrosis, immunophenotyping); chest CT.

◆ Treatment Period (28 days): Protocol-directed administration; daily CBC monitoring; weekly biochemistry, infection markers, and peripheral immune function; vital signs recording; adverse event documentation and management per protocol.

◆ Efficacy Assessment Period: Repeat physical examination and laboratory/bone marrow assessments as in screening; chest CT.

◆ Follow-up Period (1 year): Monthly CBC; documentation of post-transplant complications; survival status until study conclusion or death.

➤ Study Evaluation

◆ Efficacy Assessment

Hematological response (HR) was defined as recovery in all cell lineages (neutrophils $\geq 1.5 \times 10^9/L$, platelets $\geq 30 \times 10^9/L$ and hemoglobin $\geq 85 \text{ g/L}$) without the need for transfusion or growth factor support for consecutive 7 days. Hematological

improvement (HI) was defined as recovery of at least 1 lineage. No response (NR) refers to those who failed to reach HR or HI. Bone marrow recovery was assessed using quantitative morphometric analysis to determine marrow cellularity, the myeloid-to-erythroid ratio, and megakaryocyte counts. Diagnosis and grading of GVHD was performed according to NCCN Clinical Practice Guidelines, 2020¹⁶. Severity of infection was evaluated based on a grading system for allo-HSCT recipients¹⁷. Bleeding events were assessed according to the previous study by Nevo et al¹⁸.

Efficacy assessment performed at Day +28 post-treatment, with documentation of blood product transfusion volumes (platelets, RBCs).

Blood count recovery and transfusion status recorded at Days +45, +60, and +90.

Peripheral blood immune function monitored at Days +7, +14, +21, +28, +45, +60, and +90.

◆ Safety Evaluation

(1) Symptoms and Signs: Assessment for new-onset symptoms post-treatment (excluding infection-related causes): fever, nausea, cough, somnolence, depression, constipation, diarrhea, petechiae, edema, pharyngitis, pulmonary edema, atrial fibrillation, tachycardia, hyperglycemia, elevated bilirubin, elevated creatinine, etc.

(2) Laboratory Tests: Complete blood count, blood biochemistry, virology markers, electrocardiogram, etc.

(3) AEs: Any untoward medical occurrence in a subject from the time of informed consent signing until the end of post-treatment follow-up, regardless of relationship to investigational product. AE records must document onset time, severity, duration, interventions, and outcome.

(4) AE Severity Grading:

Mild: Transient, no interference with daily activities.

Moderate: Noticeable discomfort, interferes with daily activities.

Severe: Inability to perform normal daily activities.

(5) Causality Assessment (based on temporal relationship, known drug profile, alternative explanations, dechallenge/rechallenge):

Definitely related: Meets criteria A–E.

Probably related: Meets criteria A–D.

Possibly related: Meets criteria A and B.

Possibly unrelated: Meets criterion A only.

Definitely unrelated: Symptoms fully explained by other causes.

(6) SAE: Any event resulting in death, life-threatening condition, hospitalization/prolonged hospitalization, persistent/significant disability, congenital anomaly, or other medically significant condition requiring intervention to prevent serious outcomes.

(7) Handling Procedures: In the event of a SAE, the investigator shall immediately implement necessary measures prioritizing the protection of participant safety. All AEs shall be tracked, investigated, and documented in detail regarding management process and outcomes, with signatures affixed.

(8) AEs Reporting: Investigators shall inform participants to truthfully report changes in condition following drug administration. Regardless of whether an adverse reaction or event is related to the investigational product, detailed records must be maintained, including onset time, symptoms, signs, severity, duration, laboratory findings, management methods and outcomes, course of events, follow-up timing, and concomitant medications to assess causality with the investigational drug. All entries require signature and date.

(9) Contact: Department of Hematology, The First Affiliated Hospital of Soochow University – Han Yue, Tang Yaqiong (Tel: 0512-67781856)

Statistical Analysis

Continuous variables meeting normality assumptions are described as mean $\pm$ SD; otherwise, median (interquartile range) is used. Categorical variables are summarized as frequency (percentage). For between-group comparisons of continuous variables (e.g., by sex or age group), t-test or ANOVA is applied if normality and homogeneity of variance are satisfied; otherwise, rank-sum test is used. For categorical variables, chi-square test or Fisher's exact test is applied as appropriate. All tests are two-sided with $\alpha = 0.05$.

Safety analysis is performed using descriptive statistics. Summaries include treatment-emergent AEs (TEAEs), SAEs, laboratory parameters, and vital signs. Group comparisons (if conducted) employ chi-square test or Fisher's exact test.

Data Preservation and Documentation

Investigators must retain original source documents for each participant (typically in medical records), ensuring all Case Report Form (CRF) entries are traceable. Source documents include signed informed consent forms (with trial number and title), laboratory data, ECGs, etc. The clinical trial sponsor shall preserve essential documents for at least 5 years post-trial per national regulations, including:

- ◆ Ethics committee approvals for protocol and amendments
- ◆ All source documents and CRFs
- ◆ Informed consent forms
- ◆ Other trial-related files
- ◆ Documentation of compliance with GCP and the Declaration of Helsinki.

Ethical Considerations

The protocol and informed consent form must be reviewed and approved by the institutional ethics committee prior to trial initiation. Any protocol amendments or SAEs occurring during the study must be promptly reported to the committee and documented. Before enrollment, investigators shall explain the trial's purpose, procedures, potential benefits, and risks to participants or their legal representatives, clarifying that refusal to participate will not affect medical care or rights. Participants may withdraw at any stage. After thorough explanation, participants sign and date the informed consent form.

Study Monitoring

Protocol modifications require written amendments. Changes significantly impacting participant safety, study scope, or scientific validity (e.g., dose escalation, extended duration, major design alterations, increased invasive procedures, addition/removal of safety assessments) require prior ethics committee re-approval.

Publication Policy

All data generated belong to the principal investigator as confidential information. The investigator retains the right to publish results. All personnel must maintain strict confidentiality regarding trial data and recognize the scientific value of findings to the principal investigator. Unauthorized disclosure is prohibited.

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