

STUDY PROTOCOL

Safety and Feasibility of Selective Fecal Microbiota Transplantation (FMT) for Immune Reconstitution in Immunodeficient Patients after Hematopoietic Stem Cell Transplantation (HSCT)

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INVESTIGATOR'S STATEMENT AND SIGNATURE PAGE

As the Principal Investigator of this study, I hereby affirm that I will conduct this research in accordance with the ethical principles of the Ministry of Health's "Measures for Ethical Review of Biomedical Research Involving Humans" (2016), the WMA Declaration of Helsinki (2013), CIOMS International Ethical Guidelines for Biomedical Research Involving Humans (2002), and GCP guidelines. I will use the protocol approved by the Ethics Committee and conduct the study according to this protocol to ensure the scientific integrity of the research and protect the health and rights of the subjects.

PROTOCOL SUMMARY

Study Objectives	Composite Endpoint of Safety and Feasibility of Selective FMT in Post-Allo-HSCT Patients
Sample Size	Phase I: 50–80 allo-HSCT patients; Phase II: 6 patients in the immunodeficiency group. Total not exceeding 86 patients.
Study Population	Adult patients undergoing first allo-HSCT. Phase II includes patients with severe humoral immunodeficiency at +6 months post-transplant (serum IgA < 0.07 g/L, with IgG and IgM not recovered).
Study Design	Phase I: Prospective observational cohort; Phase II: Single-arm, open-label, Phase I/IIa proof-of-concept and safety exploratory trial.
Inclusion Criteria	① Age ≥18 years; ② Undergoing first allo-HSCT (myeloablative or

	reduced-intensity conditioning); ③ Able to complete scheduled follow-up visits and sample collection; ④ Signed informed consent.
Exclusion Criteria	① Early post-transplant death or loss to follow-up; ② Active grade III–IV acute GVHD; ③ Ongoing high-dose immunosuppressant use (prednisone equivalent >1 mg/kg/d); ④ Refusal to participate.
Intervention	Selective FMT via oral administration of healthy donor-derived gut microbiota capsules, $\geq 2 \times 10^{11}$ organisms/g. Administered for 3 consecutive days, 20 capsules per day (divided oral doses), total 60 capsules per course.
Primary Endpoint	Fecal sIgA increase $\geq 50\%$ from baseline or return to normal (≥ 50 $\mu\text{g/g}$ feces) at 3 months post-FMT.
Secondary Endpoints	Change in fecal propionate concentration at 2 weeks post-FMT; changes in serum IgA and IgM at 3 months post-FMT; changes in butyrate-producing and propionate-producing bacterial abundance; adverse events (CTCAE v5.0).
Study Timeline	Phase I: Enrollment and 6-month follow-up completed within 1 year; Phase II: 6–12 months; Total study duration approximately 2 years.
Statistical Methods	Phase I: Linear mixed-effects models, extreme phenotype analysis (deficiency vs. normal groups only), LEfSe differential analysis, random forest/LASSO modeling. Phase II: Wilcoxon signed-rank test, Spearman correlation analysis.
Dissemination	SCI journal publications; oral presentations or poster sessions at domestic hematology conferences.

1. BACKGROUND

Humoral immune reconstitution mediated by B cells following allogeneic hematopoietic stem cell transplantation (allo-HSCT) is extremely slow and often incomplete. IgM typically recovers at 2–6 months, IgG gradually recovers within 3–18 months, and IgA recovers the latest, potentially delayed up to 3 years, with full functional humoral immunity often requiring more than 5 years [1]. Abedi et al. (1990) monitored 131 allo-BMT patients for 24 months and found that patients with grade II–III acute GVHD had persistently lower IgA levels from 3 months to 2 years post-transplant compared to those without GVHD; chronic GVHD was also a significant cause of IgA deficiency [2].

The conditioning regimen severely disrupts the gut microbiome, destroying the intricate bidirectional regulatory network between the microbiota and host immunity.

Goguyer-Deschaumes et al. (2023) emphasized in their review that the interplay between microbial metabolites (e.g., short-chain fatty acids) and secretory immunoglobulin A (sIgA) is central to maintaining intestinal homeostasis [3].

Theoretical Framework: From "Butyrate-Producing Bacteria" to the "Propionate/Butyrate Dual-Pathway Synergy" Hypothesis

Classical views hold that butyrate-producing bacteria (e.g., *Blautia*, *Faecalibacterium*, *Roseburia*) promote B-cell differentiation through short-chain fatty acids (SCFAs). Kim et al. (2016) demonstrated that SCFAs upregulate key genes in plasma cell differentiation, providing energy for antibody production [4]. Qu et al. (2023) systematically summarized the full-process regulatory role of SCFAs in B-cell differentiation and antibody formation [5].

However, our team's preliminary clinical data revealed that 100% (5/5) of immunodeficient patients post-allo-HSCT exhibited specific propionate deficiency, while gastroenterology patients with dysbiosis predominantly showed butyrate deficiency. This suggests that in the extreme post-transplant environment, the propionate synthesis pathway, primarily driven by *Bacteroidetes*,

is more profoundly destroyed. Propionate is a key metabolite for inducing and maintaining intestinal regulatory T cell (Treg) differentiation [11,12]. The "normal" or "elevated" butyrate levels in transplant patients represent a compensatory bloom of a few surviving strains, while the critical multifunctional butyrate producers (e.g., *Faecalibacterium prausnitzii*) have long disappeared—a fragile state incapable of supporting complete immune reconstitution. Based on the ecological "keystone species" theory, propionate-producing bacteria such as *Bacteroides* have been identified as highly connected nodes in the gut microbial network; their loss leads to profound changes in microbiome structure and function [13,14]. Animal studies show that removal of *Bacteroides caecimuris* (a propionate-producer) significantly alters community composition and host inflammatory responses [15]. Cross-feeding relationships exist between propionate- and butyrate-producing bacteria, where the former degrades complex carbohydrates to provide growth substrates for the latter [16].

Accordingly, we propose a "propionate first, butyrate second" two-stage synergistic reconstitution hypothesis: **Stage I (Microenvironment Preparation):** Preferential restoration of propionate-producing bacteria elevates intestinal propionate levels, induces Treg cells, and creates an immune-permissive environment. **Stage II (Effector Activation):** Butyrate-producing bacteria successfully colonize in the improved microenvironment, restoring butyrate synthesis and driving B-cell differentiation and sIgA secretion. Only when both propionate and butyrate pathways are synergistically restored can efficient and complete humoral immune reconstitution be achieved. Uzzan et al. (2020) revealed a T-cell-independent IgA production pathway in the gut that depends on the TACI receptor on B cells, which is particularly important when T-cell reconstitution is delayed [6]. sIgA deficiency can lead to pathogen overgrowth, disruption of the intestinal mucosal barrier, and inhibition of beneficial bacterial colonization, forming a vicious cycle [2,18].

The general pattern of gut microbiota recovery after HSCT is: diversity follows a "decrease then increase" trajectory; *Firmicutes* proportion decreases, while *Proteobacteria* abundance increases significantly; butyrate-producing bacteria are generally reduced or absent; and community structure shows phased succession [19-21]. However, the bridging role of propionate-producing bacteria in ecological reconstitution remains an unexplored research gap.

Rashidi et al. (2023) demonstrated in a Phase II trial that oral FMT is safe and feasible in allo-HSCT patients, can reduce GVHD risk, and that patients with greater microbiota disruption derive greater benefit [9]. Based on this novel hypothesis, we propose an innovative selective FMT strategy: based on the patient's "microbiota–metabolite deficiency profile," precisely match a "super-donor" simultaneously enriched in high-abundance propionate- and butyrate-producing bacteria with high fecal propionate and butyrate concentrations.

2. STUDY OBJECTIVES

2.1 Primary Objectives

- To characterize the dynamic changes in humoral immunity, lymphocyte subsets, and intestinal metabolites (propionate, butyrate) before and at 1, 3, and 6 months post-allo-HSCT, and to analyze metabolite differences between immunodeficient and normal recovery groups.
- To identify gut microbiota and metabolite (especially propionate and butyrate) differences between severe humoral immunodeficiency and normal recovery groups at 6 months post-transplant.
- To perform selective FMT based on the "propionate + butyrate dual-high-yield bacteria" matching strategy in immunodeficient patients, assess its safety and feasibility, and observe preliminary biological signals of fecal sIgA recovery.

2.2 Secondary Objectives

- To validate the "propionate first" hypothesis by analyzing the temporal association between early (2-week) propionate changes and late (3-month) fecal sIgA and serum IgA recovery post-FMT.
- To explore the association between propionate-/butyrate-producing bacterial reconstitution trajectories and immune recovery after FMT.
- To compare NK cell and T-cell immune development differences between the two patient groups.

2.3 Exploratory Objectives

- To preliminarily construct a model for predicting FMT efficacy based on baseline microbiota–metabolite characteristics.
- To explore the relationship between changes in the propionate/butyrate ratio post-FMT and GVHD risk.

3. STUDY RATIONALE

3.1 Preclinical and Literature Basis

(1) Preliminary observational data: Our team's comparative analysis of 5 post-transplant immunodeficient patients versus non-transplant gastroenterology patients found that 100% of post-transplant patients exhibited propionate deficiency, providing the direct source for our hypothesis.

(2) SCFAs and B-cell regulation: Kim et al. (2016) and Qu et al. (2023) elucidated the molecular mechanisms by which SCFAs regulate B-cell differentiation and antibody production [4,5].

(3) TACI pathway: Uzzan et al. (2020) revealed that the gut T-cell-independent IgA pathway depends on the TACI receptor [6].

(4) Ecological keystone species theory: Banerjee et al. (2018), Shin et al. (2024), and Weiss et al. (2023) confirmed in theoretical definitions, reviews, and animal experiments that propionate-producing bacteria (especially *Bacteroides* spp.) are gut keystone species [13-15].

(5) FMT in HSCT: Rashidi et al. (2023) demonstrated safety, feasibility, and GVHD reduction in a randomized controlled trial, providing essential safety and dosing references [9].

3.2 Subject Selection Rationale

Phase I selection of allo-HSCT patients is based on the slow humoral immune reconstitution in this population, making it the optimal clinical model for validating our hypothesis. Phase II selection of the most severely immunodeficient patients (IgA < 0.07 g/L) is based on evidence that patients with greater microbiota disruption may derive stronger immune-protective benefits from FMT, and these patients have the most urgent clinical need [9].

3.3 Dose Selection/Administration/Dose Adjustment Rationale

Oral FMT capsule dosing follows the Phase II clinical trial protocol by Rashidi et al. (2023) published in *J Clin Oncol*: 3 consecutive days, 20 capsules per day, total 60 capsules per course. This regimen has been proven safe and effective for engraftment [9]. Each capsule contains $\geq 2 \times 10^{11}$ organisms/g. No dose adjustments are made in this study.

3.4 Endpoint Selection Rationale

Fecal sIgA is a direct indicator of intestinal humoral immune status. Given the small sample size (N=6) in Phase II, this endpoint is exploratory, intended to observe biological signals rather than formal statistical hypothesis testing. The 2-week post-FMT propionate change is used to validate the "propionate first" hypothesis. Safety endpoints are based on CTCAE v5.0 criteria.

3.5 Risk-Benefit Assessment

(1) Risks: Transient gastrointestinal symptoms, rare infections, and GVHD exacerbation.

Mitigation measures: Rigorous donor screening; exclusion of active GVHD; close monitoring; immediate suspension and emergency management for $\geq$ Grade 3 FMT-related adverse events.

(2) Equipoise: Immunodeficient patients currently lack effective reconstitution strategies. This FMT intervention, based on the safety data from Rashidi et al. (2023), has the potential to promote humoral immune reconstitution and reduce infection risk.

(3) Social benefit: Identifying propionate deficiency markers and validating the selective FMT strategy will advance the field of post-transplant immune reconstitution toward "precision microbiome intervention."

4. STUDY DESIGN

4.1 Sample Size Calculation

Phase I is an observational cohort. The incidence of persistent low IgA at 6 months post-transplant is approximately 15–30% [2]. We plan to enroll all allogeneic transplant patients at our center over a 6-month period to screen approximately 20 patients for the deficiency group, sufficient for LEfSe differential analysis [10]. Phase II includes 6 patients as a proof-of-concept safety lead-in study [9].

4.2 Study Population Selection

Phase I

Inclusion Criteria: ① Age ≥ 18 years; ② Undergoing first allo-HSCT (myeloablative or reduced-intensity conditioning); ③ Able to complete follow-up visits and sample collection at +1 month, +3 months, and +6 months post-transplant; ④ Signed informed consent.

Exclusion Criteria: ① Early post-transplant death or loss to follow-up; ② Active grade III–IV acute GVHD; ③ Ongoing high-dose immunosuppressant use (prednisone equivalent >1 mg/kg/d); ④ Refusal to participate.

Phase II

Inclusion Criteria: ① Serum IgA < 0.07 g/L at +6 months, with IgG and IgM not recovered; ② No active severe GVHD; ③ Off antibiotics for ≥ 2 weeks; ④ Willing to receive FMT and sign dedicated informed consent.

Exclusion Criteria: ① Gastrointestinal obstruction, perforation, or severe intestinal dysfunction; ② Severe cardiac, hepatic, or renal insufficiency; ③ Known allergy to FMT preparation components.

Withdrawal Criteria: ① Subject withdraws informed consent; ② Occurrence of $\geq$ Grade 3 FMT-related adverse event; ③ Investigator determines termination is in the subject's best interest.

Termination Criteria: Occurrence of definite FMT-related subject death; Ethics Committee request for termination.

4.3 Specific Study Content

Phase I: Blood and fecal samples collected pre-transplant and at +1, +3, and +6 months for serum IgG/IgA/IgM, fecal sIgA, TBNK lymphocyte subsets, fecal 16S rRNA, and SCFA quantitative concentrations. Based on +6-month data, patients are stratified by serum IgA levels into three groups:

- **Immunodeficiency group:** IgA < 0.07 g/L with IgG/IgM not recovered;
- **Delayed recovery group (intermediate):** $0.07 \text{ g/L} \leq \text{IgA} \leq 0.8 \text{ g/L}$, or IgA < 0.07 g/L but IgG/IgM recovered;
- **Normal recovery group:** IgA > 0.8 g/L with IgG/IgM normal.

Data analysis strategy: For microbiota differential comparison (LEfSe) and metabolite comparisons, only the two extreme groups (immunodeficiency vs. normal recovery) are used for extreme phenotype analysis to maximize statistical power and avoid dilution effects from the intermediate group. Intermediate group data are used only for longitudinal trend description and exploratory correlation analyses.

Phase II: Baseline comprehensive assessment for deficiency group patients, generation of "microbiota–metabolite deficiency map," donor matching, and FMT administration via oral capsules for 3 consecutive days, followed by 3 months of follow-up.

4.4 Subject Grouping

Phase I has no grouping—single-arm longitudinal observation. Phase II is a single-arm, open-label FMT intervention.

Intervention: Oral human-derived gut microbiota capsules. This FMT preparation is an investigator-initiated experimental therapy, not an off-label use of an approved drug. The informed consent form will clearly state the investigational nature of FMT.

Dose Selection/Adjustment: Fixed dose, no adjustment. 3 consecutive days, 20 capsules per day (divided oral doses). Each capsule $\geq 2 \times 10^{11}$ organisms/g.

Administration Timing: Fixed daily oral administration.

Blinding/Unblinding: Open-label.

Concomitant Medications: Standard anti-rejection and anti-infection prophylaxis are permitted. Probiotics/prebiotics and non-essential broad-spectrum antibiotics are prohibited from 1 day before to 1 week after FMT.

Rescue Medications and Supportive Care: For confirmed post-FMT infections, anti-infective treatment based on pathogen identification. For GVHD exacerbation, immunosuppressants are added or adjusted per standard clinical practice.

4.5 Study Flowchart

Phase I: Pre-transplant (baseline) → +1 month (serum Ig, fecal sIgA, TBNK, 16S, SCFAs, dietary assessment) → +3 months (comprehensive follow-up) → +6 months (comprehensive follow-up, group assignment)

Phase II: Pre-FMT (baseline comprehensive) → FMT (3 consecutive days) → +2 weeks (immunology + microbiota + SCFAs + diet) → +1 month (immunology + microbiota + SCFAs + diet) → +2 months (immunology + microbiota + SCFAs + diet) → +3 months (immunology + microbiota + SCFAs + diet)

5. STUDY PROCEDURES

5.1 Subject Management

5.1.1 Recruitment Methods: Screening by attending physicians during routine post-transplant follow-up; after initial physician introduction, researchers provide detailed information. Eligible subjects sign informed consent for formal enrollment.

5.1.2 Informed Consent Process: Conducted in hospital wards or outpatient clinics; researchers describe the protocol in detail; written informed consent provided; subjects given at least 24 hours for review and questions; voluntarily signed in duplicate; one copy given to the subject.

5.1.3 Eligibility Verification: Investigators verify inclusion/exclusion criteria item by item.

5.1.4 Medical History and Concomitant Medication Documentation: Detailed recording of transplant type, conditioning regimen, GVHD history, infection history, and antibiotic use in the past 2 weeks.

5.1.5 Screening Number Assignment: Unique study numbers assigned sequentially upon enrollment.

5.1.6 Treatment/Randomization Number Assignment: Phase II subjects assigned numbers sequentially by enrollment; no randomization.

5.1.7 Compliance Management: Researchers establish effective communication with subjects; medication intake recorded; compliance assessed at each visit with return of empty medication boxes.

5.2 Safety Evaluation Procedures

CTCAE v5.0 criteria for assessment. Routine safety evaluations at each visit; SAEs trigger immediate specialized assessment. Regular safety reports submitted to the Ethics Committee.

5.3 Risk Control and Management Procedures

Pre-event: Rigorous donor screening; strict inclusion/exclusion criteria; emergency preparedness planning.

During-event: For $\geq$ Grade 3 FMT-related adverse events: immediate emergency medical care; report to PI and Ethics Committee within 24 hours; suspend subsequent enrollment and FMT administration until safety review completion.

Post-event: Summarize cause; revise protocol or terminate study if necessary.

5.4 Efficacy Measurement Procedures

Performed by uniformly trained laboratory/flow cytometry personnel following standard operating procedures.

5.5 Discontinuation/Withdrawal Procedures

(As described in withdrawal/termination criteria above)

5.6 Blinding/Unblinding Procedures

Open-label study; no blinding.

5.7 Visit Requirements

5.7.1 Screening Period (Phase I pre-transplant): Serum Ig panel, fecal sIgA, TBNK, fecal 16S, SCFAs

5.7.2 Treatment Period (Phase II D1-D3): Daily oral FMT capsules

5.7.3 Post-Treatment Visits

Time Point	Assessment Content
+1 month, +3 months, +6 months (Phase I)	Serum Ig + sIgA + fecal 16S + SCFAs + TBNK + dietary assessment (3-day 24-hour recall prior to fecal sampling)
+2 weeks (Phase II)	Fecal 16S + SCFA quantification + dietary assessment
+1 month (Phase II)	Serum Ig + sIgA + fecal 16S + SCFAs + dietary assessment
+2 months (Phase II)	Serum Ig + sIgA + fecal 16S + dietary assessment
+3 months (Phase II, endpoint)	Comprehensive (same as baseline) + dietary assessment

Implementation Notes: Dietary assessment is conducted by research nurses or nutritionists using a structured 24-hour dietary recall questionnaire, focusing on intake of staple foods, vegetables, fruits, and meat in the 3 days prior to fecal sampling, with calculation of total daily dietary fiber (g/d). These data will be included as covariates in multivariate analyses to adjust for potential confounding effects of dietary intake on SCFA levels.

6. SAFETY MONITORING AND ADVERSE EVENT MANAGEMENT

Safety Evaluation Procedures: CTCAE v5.0 criteria for assessment. Routine safety evaluations at each visit; SAEs trigger immediate specialized assessment. Regular safety reports submitted to the Ethics Committee.

Risk Control and Management Procedures:

Pre-event: Rigorous donor screening; strict inclusion/exclusion criteria; emergency preparedness planning.

During-event: For $\geq$ Grade 3 FMT-related adverse events: immediate emergency medical care; report to PI and Ethics Committee within 24 hours; suspend subsequent enrollment and FMT administration until safety review completion.

Post-event: Summarize cause; revise protocol or terminate study if necessary.

7. DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data Management

Case report forms (CRFs) and study records ensure accurate, complete, and timely data collection. Data entry using double data entry by two independent personnel.

All data stored on encrypted electronic devices; accessible only to authorized personnel.

Statistical Analysis

Phase I: Linear mixed-effects models for longitudinal trend analysis; LEfSe for differential microbiota screening between groups; random forest/LASSO for IgA prediction modeling.

Phase II: Primary endpoint expressed as counts (proportions) with exact confidence intervals; secondary endpoints analyzed using Wilcoxon signed-rank test; Spearman correlation for temporal associations. Missing data handled by last observation carried forward (LOCF). Two-sided tests with significance level of 0.05. Software: R 4.x and others.

8. DISSEMINATION OF RESULTS

Upon study completion, results will be submitted for publication in SCI-indexed journals. Findings will also be presented as oral presentations or posters at domestic hematology conferences.

9. REFERENCES

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