Protocol Synopsis

Protocol number:

I. Protocol title：

Fecal Microbiota Transplantation for the Treatment of Steroid Refractory Acute Gastrointestinal Graft-Versus-Host Disease in Patients After Allogeneic Hematopoietic Stem Cell Transplantation

II. Objectives：

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for various hematologic diseases. However, one of the major challenges of allo-HSCT is the occurrence of graft-versus-host disease (GvHD), particularly acute gastrointestinal GvHD (GI-GvHD). GvHD occurs when donor T cells recognize the recipient's tissue as foreign and mount an immune attack against it. Acute GI-GvHD is a common complication following allo-HSCT and a significant cause of mortality. If the initial steroid treatment for acute GvHD fails, mortality rates can reach as high as 81%.

Recent studies have shown a strong association between reduced gut microbiota diversity and high mortality in patients with acute GI-GvHD, highlighting the critical role of the gut microbiome in regulating immune responses and maintaining intestinal homeostasis. Consequently, fecal microbiota transplantation (FMT) has emerged as a potential therapeutic strategy aimed at restoring a healthy gut microbiome and improving clinical outcomes in patients with acute GI-GvHD.

This study aims to evaluate the efficacy and safety of FMT in patients with steroid-refractory or steroid-resistant acute GI-GvHD. The findings of this research will contribute to establishing FMT as a potential and effective treatment option for managing severe acute GI-GvHD, thereby improving patient outcomes and reducing transplant-related mortality.

III. Test drug/investigating product

1. Name: Fecal microbiota transplantation, FMT
2. Dosage form: 250cc microbiota fluid (approximately 60 cm^3 of stool materials; $6x10^{13}$ bacteria)
3. Strength: not applicable
4. Dosage and administration: 250cc microbiota fluid administered into the terminal ileum or cecum via ileocolonoscopy or into terminal duodenum via panendoscopy
5. Mechanism of action (if known): gut microbiome regulates immune responses and maintaining intestinal homeostasis.
6. Pharmacological category: Other specified medical technologies

IV. **Development Phase：**

☐First in human ☐I ■ II ☐III ☐IV ☐others

V. Study Design：

1. ☐Control: ☐placebo

☐active (please specify name and dosage)

☐other

■ Uncontrolled

2. Blinding: ■ open-label □evaluator blind □single blind □double blind
□double dummy □other

3. Randomized: □yes ■ no

4. □Parallel □Cross-over ■ Other

5. Duration of treatment: 2 months

6. Titration: □forced □optional ■ none

7. □Multi-national □Multi-center(Taiwan) ■ Single center

VI. Endpoints

1. Primary endpoint:
   a. Event-free survival
   b. Overall response rate of acute GI-GvHD on day 28 after first FMT.
   c. Complete response rate of acute GI-GvHD on day 28 after first FMT.
2. Secondary endpoints:
   a. Overall response rate of acute GI-GvHD on day 56 after first FMT
   b. Complete response rate of acute GI-GvHD on day 56 after first FMT
   c. Time to response and best response
   d. Relapse rate of acute GI-GvHD
   e. Response rates of skin and liver acute GvHD in the presence of any skin or liver GvHD.
   f. Steroid dose reduction rate
   g. Overall survival
   h. Non-relapse mortality
   i. Incidence of adverse events
      - microbial bloodstream infection
      - other adverse effects: e.g. severe cytopenia, viral reactivation, gastrointestinal bleeding, bowel rupture.

VII. Selection Criteria：

1. Main inclusion criteria：

(1) Stage II to IV steroid refractory acute GI-GvHD in allo-HSCT recipients
   a. Stage II to IV acute GI-GVHD subjects, having >1000 mL stool per day, diarrhea > 5 times/day, or abdominal cramping, bleeding or ileus, AND
   b. Resistant to a first-line therapy with corticosteroids (CS)
      a) Lack of improvement after 5 days of treatment with CS at 2 mg/kg/d methylprednisolone or other CS with equivalent dose,
      b) Progression after 3 days of treatment with CS at 2 mg/kg/d methylprednisolone or other CS with equivalent dose.

(2) Age ≥ 18 years old.

(3) Allo-HSCT with any type of donor, stem cell source, GvHD prophylaxis or conditioning regimen.

(4) Allow vancomycin-resistant enterococcus (VRE) colonization and asymptomatic cytomegalovirus

(CMV) viremia, which is defined as a detectable CMV viral load in plasma but without tissue-invasive disease.

(5) Patients able to have a minimum of 12 hours discontinuation of systemic antibiotics in order to perform the allogeneic FMT (antiviral and antifungal agents are allowed)

(6) Signature of informed and written consent by the subject or by the subject's legally acceptable representative for patients under guardianship or trusteeship. Subject must understand and voluntarily sign an informed consent form prior to any study-related assessments/procedures being conducted.

2. Main exclusion Criteria：

(1) Absolute neutrophil count < 500 cells/uL.

(2) Absolute platelet count < 30000 /uL which is not correctable by transfusion

(3) Hemodynamically unstable status with the following conditions: systolic blood pressure < 90 mm Hg, pulse oximeter oxygen saturation (SpO2) < 90%, PaO2 < 60 mm Hg, or respiratory rate > 22/minute.

(4) Uncontrolled and active infection from bacteria, virus, or fungus as determined by the investigators.

VIII. Study Procedures：

1. Study design: a prospective single arm open label clinical trial.
2. Patients' recruitment: a total of 35 patients will be enrolled. Evaluable number is estimated as 35 patients.
3. Screening: Patients will be recruited from hematopoietic stem cell transplantation center in CGMH. Informed consent will be obtained if patients agree to participate in this study.
4. Study time: 2 years plan; **2024/12/19-2028/02/28 (estimated first case enrolment since 2026/03/01)**
5. Clinical evaluations
   a. All patients will be hospitalized throughout the study period for evaluation of the grading and severity of acute GI-GvHD. The grading and severity of acute GvHD is according to standardized clinical scoring systems-MAGIC criteria.
   b. The screening periods will be two weeks before first FMT.
   c. Participants will be instructed to report all the adverse events (AE) and severe adverse event (SAE) to the investigators immediately. During the study period whenever an AE or SAE is reported, it will be recorded regardless it's related to FMT or not. They will be recorded as: not related, unlikely related, possibly related, probably related and definitely related to FMT.
   d. During the post-FMT follow-up, the same evaluations will be repeated. The post-FMT follow-up includes daily records of abdominal symptoms, input and output amount, stool character, frequency and volume, weekly blood culture, and any adverse events.
6. Specimen process:

Blood and stool samples will be obtained at screening, before each FMT, on day-28 and day-56 after first FMT. Liver echo with fibroscan will be performed before first FMT. Biopsy of gastrointestinal

tissues will be performed during ieliocolonoscopy or panendoscopy if clinically indicated.

a. Fecal samples: Stool samples will be sent for stool routine, bacterial cultures, CMV shell virus culture, Clostridium difficile toxin, calprotectin, and microbiota analysis in the department of laboratory medicine and microbiota treatment center at CGMH.

b. Blood samples: Blood samples will be sent for complete blood counts, coagulation profiles, T/B/NK/Treg cell subsets analysis, inflammatory cytokines, renal and liver panels, electrolytes, glycohemoglobin, insulin, lipid profile, high-sensitive CRP, ANA, CMV/EBV PCR, HBV, and HCV titers during the screening periods, before each FMT, on day +28 and day +56 after FMT in the department of laboratory medicine at CGMH.

c. Gastrointestinal mucosa biopsy is not mandatory but will be performed if the investigator consider it is indicated to differentiate acute GI-GvHD, viral enterocolitis, fungal infection, and malignancy after balancing the risk of post-procedure bleeding. The tissue specimen will be subjected for Hematoxylin & Eosin staining for GI-GvHD pathological grading, and fungal chemical staining or CMV immunostaining if indicated.

7. Pre-FMT preparation:
   - Blood and stool sample collection.
   - Maintain an absolute neutrophil count > 500 cells/uL
   - Patients must have platelet count > 30000/uL.
   - Discontinue antibacterial antibiotics at a minimal of 12 hours before FMT.
   - Discontinue sulfamethoxazole/trimethoprim 24 hours before FMT.
   - Hold anti-platelet and anti-coagulants medications.
   - Prophylactic antiviral and antifungal agents are allowed.
   - Combination treatments with other immunosuppressant agents for acute GvHD is allowed.
   - Start low-fiber diet 3 days before FMT if via ileocolonoscopy
   - Start liquid-only diet 24 hours before FMT if via ileocolonoscopy
   - Start split doses of laxatives the night before FMT if via ileocolonoscopy
   - Nothing per mouth 2 hours before FMT.
8. FMT is performed by administration of 250 cc microbiota fluid (containing about 6 x 10^{13} bacteria) from three random donors into the terminal ileum or cecum via ileocolonoscopy or into terminal duodenum via panendoscopy.
9. The second FMT will be performed 7-21 days after the first FMT. The third FMT is optional and will be performed depends the response.
10. FMT should preferentially be performed via ileocolonoscopy, rather than panendoscopy, unless specific contraindications exist, such as a markedly increased risk of colonic perforation or intolerance to bowel preparation.
11. Upon completion of the study procedures, if acute GI-GvHD has not achieved complete remission, or if the disease relapses after an initial successful response, participants may choose whether to receive an additional course of FMT, which will be provided free of charge.

12. The storage of residual participants' specimen: The residual participants' specimen will be storage in -80°C in the microbiota center until two years after this study is closed. After the date, the specimen will be destroyed.

IX. Concomitant Treatments：

1. **Permitted:** The patients will keep any medications for acute GvHD treatment as clinical judgment. Medications for acute GvHD include but are not limited to steroids, cyclosporin, antithyroglobulin, ruxolitinib, tacrolimus, mycophenolate mofetil, etanercept, basiliximab, or vedolizumab.
2. **Prohibited:** A minimal 12-hour discontinuation of systemic anti-bacteria antibiotics is required to perform FMT. Consider avoiding antibiotics that may impact gut microbiota after FMT, but antibiotics after FMT is not contraindicated if clinically use is indicated.

X. Statistics：

1. Primary hypothesis: ☐superiority ■non-inferiority
☐equivalence ☐other

2. Sample size: enrolled 35
Evaluable 35

3. Efficacy population: ☐ITT ■PP ☐other
Safety population: ☐ITT ■PP ☐other

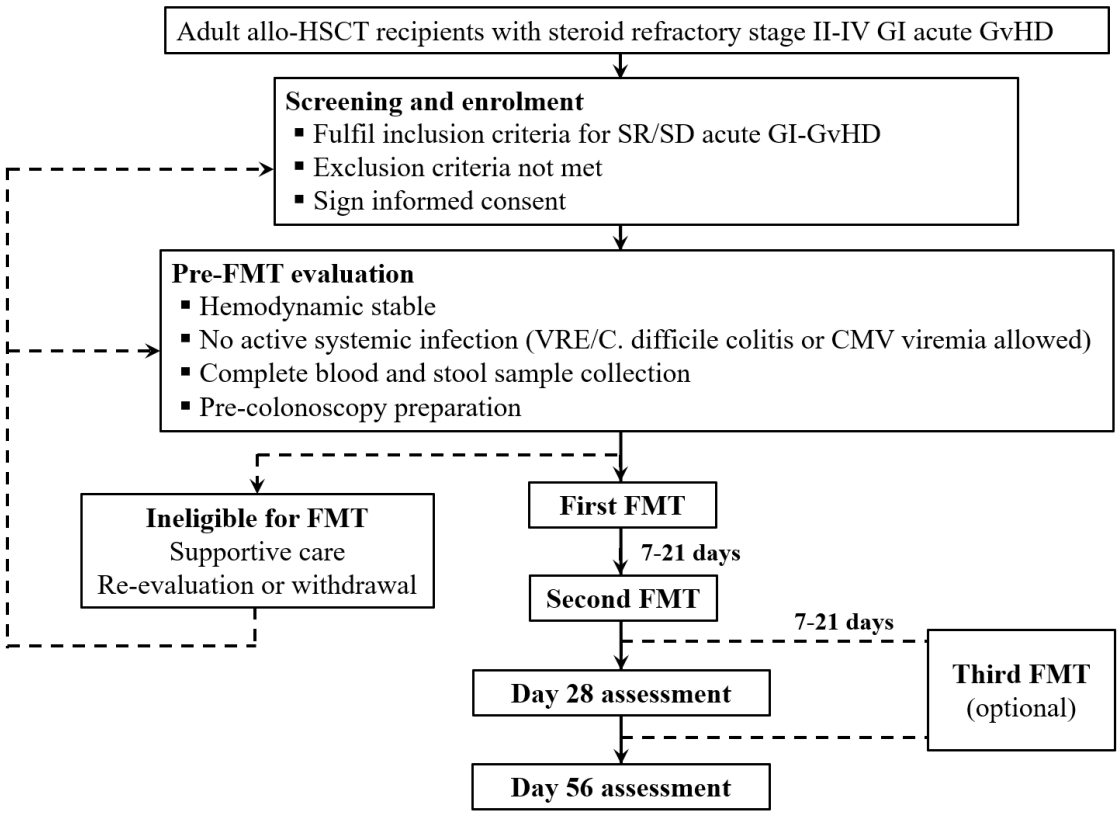
4. Statistical method(s):

The average number of patients who develop severe acute GI-GvHD after allo-HSCT is about 15 per year. Previous studies showed an overall response rate of FMT for acute GI-GvHD is about 50%-80%. We aim to test the non-inferiority of overall response rate in this study. A sample size of 35 achieves 80.0% power to detect a non-inferiority difference (margin) of -0.15 (H0: $P-P0 \leq \delta$ vs H1: $P-P0 > \delta$) using a one-sided, one-sample exact test with a significance level (alpha) of 0.05. These results assume a baseline proportion (P0) of 0.7 and the actual difference (P1-P0) is 0.05. The sample size was computed using PASS 2024, version 24.0.3. Descriptive statistics for overall response rate, rates of adverse reactions with CTCAE 5.0 grading, and survival analysis will be performed. Quantitative variables will be presented as mean ± standard deviation (SD) and categorical variables will be presented as frequency with proportion.

5. Planned interim analysis: ■yes ☐no

XI. Please attach flow chart and/or assessment schedule, if available.

1. Flow chart



2. Assessment schedule

Hospitalization of all participants, 1st FMT as day 0	**Screening, day -14~ -1**	**1st FMT Day 0**	**2nd FMT Day 7-21**	**3rd FMT (optional)**	**Follow-up Day 28 ± 3#**	**Follow-up Day 56 ± 3#**
History, physical exam	X	X	X	X	X	X
I/O, stool volume and characters	Daily	Daily (day 0 ~ +7)	Daily (day 0 ~ +7)	Daily (day 0 ~ +7)	Weekly	Weekly
Informed consent	X					
Inclusion/exclusion criteria	X					
Stool routine/ cultures/CD toxin	X		X	X	X	X
Fecal calprotectin		X (-1)	X	X	X	X
Fecal microbiota analysis		X (-1)	X	X	X	X
Liver echo + Fibroscan	X					
CBC/DC, Biochemistry, virus	X	X	X	X	X	X
PT, APTT	X	X	X	X		
GVHD biomarkers		X (-1)			X	X
Ileocolonoscopy or panendoscopy		X	X	X		
GI mucosa biopsy (optional)		X	X	X		
Blood culture weekly	X	X	X	X		
GvHD grading	Daily	Daily (day 0 ~ +7)	Daily (day 0 ~ +7)	Daily (day 0 ~ +7)	Weekly	Weekly