Highlight Sheet Study CTNPT 038 Dr Jean-Pierre Routy (SI) / Dr Stephane Isnard (Post-doctoral fellow) 22May2024

Title: Fecal microbiota transplantation to reduce immune activation in ART-treated people living with HIV with low CD4/CD8 ratio: The Gutsy study

Primary Objective: To evaluate changes in plasma levels of the marker of gut permeability REG3α before and after FMT compared to placebo

Secondary Objectives: To assess, before and after FMT, compared to the placebo arm:

- Safety and tolerability of FMT
- Changes in plasma levels of gut damage markers
- Changes in plasma levels of pro-inflammatory markers and anti-inflammatory markers
- Changes in T and myeloid cell activation in blood

Study Design: Single-blind, randomized, placebo-controlled, interventional study

Population: Male or female, ≥18 years of age; Diagnosed HIV infection on ART for at least 3 years, Viral load suppressed to <50 copies/mL for at least 3 years (viral blips below 200 copies/ml are allowed) CD4 count >200 and CD4/CD8 ratio <1

Target N = 20

Sites: Chronic Viral Illness Service (CVIS) at McGill University Health Centre;

St. Joseph's Health Care hospital, London, Ontario (Recruiting stool donors only)

Intervention: FMT capsules containing 80-100g of healthy donor stool processed, freeze dried and encapsulated in gelatin capsules

Randomization: 1:1 Ratio FMT capsules: Placebo capsules

Study Duration: Participants on study for 12 weeks from Baseline 2 (first treatment visit)

Enrolment Period: Jul 2024 – April 2025

CTN In-kind Services: Database/data management, Statistical analysis, Communications and KT, Regulatory Support (to a maximum of 2 protocol amendments, DSMC)

Visit Schedule/Procedures:

Visit Type	**Screening**	**Study visits**					
		Baseline 1	**Bowel cleanse**	**Baseline 2, Treatment 1**	**Treatment 2**	**Follow-up**	
Visit Window **Procedures:**	**Visit 1 Day -56 to -7 days**	**Visit 2 Day -14 (±7 days)**	**Day -1**	**Visit 3 Day 0**	**Visit 4 Day 22 (±7 days)**	**Visit 5 Week 6** ***(±7 days)***	**Visit 6 Week 12 (±7 days)**
Visit No.	1	2		3	4	5	6
Informed Consent	X						
Eligibility Assessment	X	X		X			
Concomitant Medication	X	X		X	X	X	X
Medical History	X						
Complete Physical Exam and Vital Signs	X						
Targeted Physical Exam and Vital Signs		X		X	X	X	X
Adverse Event Assessment				X	X	X	X
Serum Pregnancy Test	X	X		X	X	X	X
Hematology*	X	X†		X	X	X	X

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Serum Chemistry**	X	X†		X	X	X	X
Serology***	X			X			
Serology - HIV-1 Viral Load***	X	X†		X	X	X	X
Randomization	X††						
Immune activation markers/cytokines (ELISA)****		X		X	X	X	X
Monocyte and T cell activation markers+		X		X	X	X	X
Markers of gut barrier integrity, inflammation and microbial translocation++		X		X	X	X	X
Size of HIV reservoir in Latently Infected CD4 T cells+++		X		X	X	X	X
Stool sample collection and microbiota composition++++		X		X	X	X	X
Bristol Chart		X		X	X	X	X
Alcohol use (AUDIT-Full), Appendix 2	X						
Alcohol use (AUDIT-C), Appendix 3		X		X	X	X	X
Bowel cleanse			X†††				
Study Product Dispensation (FMT or placebo)				X	X		
Colon mucosal biopsies#				X			X

*CBC, CD4 and CD8 T cell counts, erythrocyte sedimentation rate (ESR).

**Alkaline phosphatase, ALT, Amylase, AST, Bilirubin (total), Creatine kinase, Creatinine, D-dimer, fasting blood glucose, HbA1c, high sensitivity C-reactive protein (hsCRP), Lipase, lipid profile (total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), Triglycerides), serum phosphate, Urea

***Serology measurements include: Cytomegalovirus (CMV), Hepatitis B virus (HBV), Hepatitis C virus (HCV) and HIV viral load. Since HIV viral load will be measured at each visit, it was put as a separate line item.

****Immune activation markers/cytokines include: soluble CD14, pro-inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α) and anti-inflammatory cytokine IL-10. Measured in plasma by ELISA.

+Monocyte and T cell activation markers include: HLA-DR and CD38. T cell exhaustion marker: PD-1. Measured by staining and flow cytometry.

++Markers of gut barrier integrity, microbial translocation and inflammation: lipopolysaccharide, soluble ST2, I-FABP (measured in plasma by ELISA).

+++PBMCs will be isolated and then latent CD4 T cells will be isolated by flow cytometry. HIV viral reservoir in the latent CD4 T cell population will be measured by nested qPCR. More specific TILDA analysis will be performed on Baseline Week 0 and End-treatment Week 12 samples to assess the HIV viral reservoir (Exploratory analysis).

++++qPCR of A. muciniphila, 16S and 18S rDNA sequencing for other members of the microbiota.

Optional sub-study procedure.

† Not required when the same tests have been performed at the screening visit within the past 14 days, with the exception of CBC, CD4, CD8 (and serum pregnancy test)

†† *upon validation of eligibility.*

††† Bowel cleanse the day before Visit 3 (Baseline 2).