

Daily, Oral FMT for Long-Term Maintenance Therapy in Ulcerative Colitis: Results of a Single-Center, Prospective, Randomized Pilot Study.

Jessica Wood Crothers (✉ Jessica.Crothers@uvmhealth.org)
University of Vermont <https://orcid.org/0000-0003-4594-5051>

Nathaniel D. Chu
Massachusetts Institute of Technology School of Engineering

Le Thanh Tu Nguyen
Massachusetts Institute of Technology School of Engineering

Magen Phillips
University of Vermont College of Medicine

Cheryl Collins
University of Vermont College of Medicine

Karen Fortner
University of Vermont College of Medicine

Roxana del Rio-Geurra
University of Vermont College of Medicine

Brigitte Lavoie
University of Vermont

Peter Callas
University of Vermont

Mario Velez
University of Vermont College of Medicine

Aaron Cohn
University of Vermont Medical Center

Ryan J. Elliott
OpenBiome

Wing Fei Wong
OpenBiome

Elaine Vo
Finch Therapeutics

Rebecca Wilcox
University of Vermont College of Medicine

Mark Smith
Finch Therapeutics

Zain Kassam
Finch Therapeutics

Ralph Budd
University of Vermont College of Medicine

Eric J. Alm
Massachusetts Institute of Technology School of Engineering

Gary M. Mawe
University of Vermont

Peter L. Moses
University of Vermont College of Medicine

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Abstract

This randomized study was designed to assess the safety and feasibility of long-term fecal microbiota transplantation (FMT) in subjects with mild to moderate UC using frozen, encapsulated oral FMT (cFMT). FMT induction was implemented by colonoscopy, followed by 12 weeks of daily oral administration of frozen encapsulated cFMT. Ribosomal 16S bacterial sequencing was used to assess donor-induced changes in the gut microbiota. Changes in T regulatory (Treg) and mucosal associated invariant T (MAIT) cell populations were evaluated as an exploratory endpoint. Twelve subjects with active UC were randomized: 6 subjects completed the full 12-week course of FMT plus cFMT, and 6 subjects received sham treatment by colonic installation and longitudinal oral treatment. Chronic administration of cFMT was found to be safe and well-tolerated. Protocol adherence was high, and none of the study subjects experienced FMT-associated treatment emergent adverse events. While n-values were too small to draw firm conclusions, 3/6 subjects that received cFMT exhibited multiple signs of clinical improvement. These pilot data suggest that daily encapsulated cFMT may extend the durability of index FMT-induced changes in gut bacterial community structure and that an association between MAIT cell cytokine production and clinical response to FMT may exist in UC populations. Oral frozen encapsulated cFMT is a promising FMT delivery system and may be preferred for long-term treatment strategies in UC and other chronic diseases but further evaluations will have to address home storage concerns. Larger trials should be done to explore the benefits of cFMT and to determine its long-term impacts on the colonic microbiome. Trial registration: ClinicalTrials.gov (NCT02390726). Registered 17 March 2015, <https://clinicaltrials.gov/ct2/show/NCT02390726?term=NCT02390726&draw=2&rank=1>.

Introduction

FMT is highly efficacious for treating recurrent and refractory *Clostridium difficile* infections, often requiring only a single administration of alternative microbes and resulting in significant and sustained microbial changes in the gut microbiota [8–10]. In four recently published randomized controlled trials that include a total of 277 patients, FMT has demonstrated early clinical promise as a safe, cost effective treatment strategy in a subset of UC patients [11–14]. However, all of these studies included multiple endoscopic or enema-based administrations, raising concerns about the generalizability and long-term feasibility of these approaches.

Recently, oral FMT formulations have shown promise for the treatment of recurrent *C. difficile* infection, demonstrating equivalent clinical efficacy as well as the ability to induce sustained microbial changes in the gut [15–18]. These novel oral cFMT formulations may offer a path forward in the development and widespread applicability of live microbial therapeutics for the treatment of UC and other chronic diseases.

The objective of this preliminary investigation was to assess the safety and feasibility of using at-home frozen, encapsulated FMT (cFMT) following FMT induction by colonoscopy as a novel, long-term maintenance strategy in the treatment of UC. We also investigated the ability of this novel treatment regimen to induce significant and durable microbial changes utilizing 16S-based compositional analysis, and we assessed if there were longitudinal changes in T cell populations utilizing multicolor flow cytometry.

Methods

Study Design

This was a single center, double-blinded, placebo-controlled, randomized control trial intended to investigate the safety and feasibility of performing induction FMT by initial colonoscopic infusion followed by 12 weeks of ambulatory oral maintenance therapy with frozen FMT capsules. The study protocol was approved by the Institutional Review Board at the University of Vermont (UVM) and the UVM Medical Center (UVMCC) Committee on Human Research in the Medical Sciences (CHRMS) (Supplementary Material). All participants provided written informed consent. The study was registered on ClinicalTrials.gov (NCT02390726) under and FDA Investigational New Drug number (IND 16395). After acquisition of all samples, all authors had access to the study data and reviewed and approved the final manuscript.

Study Subjects

Adult subjects were recruited through the IBD Center at UVMCC, or by tertiary or quaternary referral. Study subjects were required to have an established diagnosis of UC, with inflammation extending proximally to at least the recto-sigmoid junction. Subjects with proctitis only were excluded. Subjects were required to be on stable doses of UC-specific medications for at least 6 weeks prior to screening, including anti-TNF α , oral immunomodulators, oral and topical 5-ASA, and methotrexate; cortico-steroid use was excluded. A baseline total Mayo score between 4-10, with an endoscopic Mayo subscore ≥ 1 , rectal bleeding subscore ≥ 1 , and stool frequency subscore ≥ 1 , was required for participation. Asymptomatic subjects or those with severe, refractory disease (defined as a Mayo score ≥ 10 , or an endoscopic subscore ≥ 3) were excluded, as were patients with a known infectious cause of colitis or exacerbation of baseline symptoms. Patients with a history of colectomy, documented gastrointestinal motility disorder, limited life expectancy (< 12 months), pregnancy, lactation, severe immunodeficiency or a history of anaphylaxis were also excluded. Subjects did not use antibiotics within 6 weeks or probiotics within 4 weeks prior to enrollment. After trial commencement, eligibility criteria were changed to not allow probiotics usage within 1 week prior to enrollment in order to increase recruitment; this change was not expected to undermine the scientific integrity of the study. All study visits and data collection were performed at UVMCC.

Randomization, Blinding and Sample Size Calculation

Eligible subjects were randomized 1:1 by a computer-generated randomization list maintained off-site at OpenBiome (Cambridge, MA) to ensure concealment of allocation and double blinding. The treatment allocation was blinded to the subject, and all on-site investigators and staff. As a pilot feasibility trial, the target sample size of this study was determined by clinical availability of willing subjects, and not based on a formal sample-size calculation.

Baseline Assessments

Baseline characterization of participants utilized validated measures of UC activity including the Inflammatory Bowel Disease Questionnaire (IBDQ) and the Mayo symptom score [19, 20]. Endoscopic and histologic disease activity was recorded at week 0 prior to FMT induction and fecal calprotectin levels obtained.

Donor Stool

In order to limit FMT microbial variability and account for any potential FMT donor effect [21], all donor material was derived from two healthy stool donors selected for high (top quintile, 11.11 mmol/g and 12.67 mmol/g stool) butyrate production, as measured by gas chromatography. Donors were rigorously screened by a universal stool bank (OpenBiome, Somerville, MA, USA) and stool allocated to each subject was shipped to the study site frozen on dry ice and maintained at -20°C prior to use.

Antibiotic Pretreatment

Subjects in both arms of the study were pretreated with antibiotics (ciprofloxacin 250mg PO q12 and metronidazole 500mg PO q8 x7 days) for 7 days prior to FMT (or placebo) procedure. This regimen was chosen for its ability to disrupt luminal microbial communities prior to FMT and to promote microbiota reprogramming [22].

Fecal Microbiota Transplantation Induction and Maintenance Therapy

Each subject in the active treatment arm received fecal material derived from a single donor as induction therapy, delivered by colonoscopic infusion (120 mL at a concentration of 1g of stool/2.5 mL) following standard bowel preparation. Twelve-week maintenance therapy consisted of an alternating schedule of the same two pre-defined donors at a dose of 1 daily 550µL FMT capsule (~ 0.5g of stool). Capsules were distributed in 4-week increments, transported on dry ice and maintained in subject's freezers. Subjects were instructed to follow strict guidelines regarding maintenance of capsule temperature and to not transfer the capsules between freezers, meaning that they could not to spend >24 hours away from home during the dosing period. A daily medication adherence log was maintained and monitored at follow-up visits. Subjects allocated to the placebo arm were given sham colonoscopic infusion and sham capsules designed to visually resemble fecal material.

Clinical Outcomes

Clinical follow-up was performed at 4, 8, 12, 18 and 36 weeks with endoscopic and histopathologic evaluations performed at 0 and 12 weeks. Endoscopic and histologic scorings were performed by a single gastroenterologist and surgical pathologist blinded to treatment allocation. Pinch biopsies obtained from the worst affected mucosal surface as determined endoscopically were immediately fixed in 10% formalin and underwent routine tissue processing. Histologic scoring was performed using the Geboes grading system for IBD-associated disease activity [23]. Additional measures of clinical and endoscopic (IBDQ, Mayo and UCEIS scores), as well as inflammatory response (fecal calprotectin) were recorded at 0 and 12 weeks. Adverse events (AEs) were assessed by phone call 24 hours following induction, at four clinic visits (weeks 4, 8, 12, and 18) and again by phone call at 36 weeks. AE severity and relatedness were assessed by clinical staff blinded to treatment assignment.

Stool Microbiota Analysis by 16S Sequencing

Subject stool samples were obtained weekly throughout the study period, beginning prior to antibiotic pretreatment and ending at 18-weeks follow-up. Study subjects were provided with home stool collection kits and instructed to collect samples weekly at roughly the same time of day. DNA extraction was performed using the MoBio Powersoil 96 kit with minor modifications and 16S rRNA gene libraries targeting the V4 region of the 16S rRNA gene were prepared. Each sample was given a unique reverse barcode and replicates were then pooled, cleaned and normalized prior to sequencing on an Illumina MiSeq 300. Raw sequence reads were then processed and OTU calling performed using the Qiime2 – dada2 pipeline. Measures of microbial alpha diversity (Shannon index) and beta diversity (Jensen-Shannon divergence) between subjects and donor samples, and to their own baseline samples, were calculated.

Immunologic Profiling of Peripheral Blood T cells

Dynamic evaluation of lymphocyte subpopulations and cytokine production was performed in subjects at baseline and at weeks 4, 8, and 12 during the maintenance period. Control peripheral blood from healthy individuals without gastrointestinal disease and/or immunodeficiency (n = 10) were obtained at a single time point. Peripheral blood mononuclear cells (PBMC) were isolated via Ficoll gradient centrifugation, and cell staining protocols optimized to assess TCRαβ (CD4+ and CD8+) subsets. Special attention was paid to mucosal associated invariant T cells (MAIT), defined herein as TCR αβ +CD4+MR1+, and T regulatory cells (TCRαβ +CD4+CD25^{hi}). Intracellular cytokine production (IFN γ , IL-10 and IL-17A) was measured by flow cytometry following 5 hours of ex vivo stimulation with phorbol myristate acetate and ionomycin. Details of cell processing and staining are provided in the supplementary material. Immunophenotyping was performed with a flow cytometer FACS LSR II (BD Biosciences, San Jose, CA) using fluorochrome-labeled monoclonal antibodies (TCR αβ (AF 488), CD45 (AF 700), CD8 (BUV395), CD4 (BV510), CD13 (PE/Dazzle), CD25 (BV650), hMR1 (NIH tetramer facility APC, 5-OP-RU 2017-04-07, Atlanta, GA), IL-17 (BPERCP/Cy5.5), IL-10 (PE/Cy7), IFN- γ (PE)). Data were analyzed with FlowJo software (v 10.4.1, Tree Star, Inc., Ashland, OR).

Statistical Analysis

Adverse events were compared using a modified intention to treat analysis to include all subjects receiving at least one study treatment. Differences, including in AE frequency, were compared by Student's t-test or Fisher's exact test. For descriptive statistics, means and standard deviations were computed for continuous variables and proportions were computed for categorical variables. These analyses were conducted using SAS version 9.4 (Cary, NC: SAS Institute Inc.) and Prism software (version 7.0a; GraphPad Software, San Diego, CA). Differences with *p* values \leq 0.05 were considered significant.

Results And Discussion

Patient Characteristics

From February 2016 to September 2017, 154 UC patients were assessed for eligibility, and ultimately 15 subjects were recruited and randomized. Of these, 7 individuals were randomly assigned to the FMT and 8 to the placebo arm. Three subjects (1 in the FMT and 2 in the placebo group) did not meet endoscopic criteria for inclusion (Mayo score ≥ 1) and were excluded from the study (Fig. 1). The remaining 12 subjects (6 in each group) received at least one dose of study treatment. While all 6 subjects allocated to the FMT arm completed all treatments and follow-up assessments, 1 patient in the placebo group dropped out at 6 weeks due to worsening disease. The two study groups were exhibited comparable baseline demographic and clinical characteristics (Table 1).

Table 1. Baseline Subject Demographics

Variable		Group	
		Active	Placebo
N		6	6
Age	Mean(SD)	41 (15)	52 (15)
Sex	#(%) Male	4 (67%)	3 (50%)
Duration UC	Mean(SD) yrs	8.9 (9.1)	9.8 (10.6)
BMI*	Mean(SD)	24 (3)	29 (4)
Treatment with Biologic	#(%) yes	2 (33%)	1 (17%)
Fecal calprotectin	Mean(SD)	573 (659)	408 (277)
Total Mayo score	Mean(SD)	6.3 (2.0)	6.7 (1.2)
Mayo Symptom subscore	Mean(SD)	4.8 (1.5)	4.3 (1.0)
Mayo Endoscopic subscore	Mean(SD)	1.5 (0.8)	2.3 (0.5)
Endoscopic UCEIS [^] score	Mean(SD)	7.0 (1.8)	8.5 (1.8)
Histologic Severity Score ^{**}	Mean(SD)	3.4 (1.2)	4.3 (2.0)
IBDQ ^{^^} total score	Mean(SD)	142.8 (16.8)	120.2 (25.1)
IBDQ bowel system subscore	Mean(SD)	4.2 (0.7)	4.3 (0.9)

Baseline clinical characteristics of subjects randomized to both the active FMT and placebo study arms. *Body Mass Index [^]Ulcerative Colitis Endoscopic Index of Severity ^{**}Geboes Score ^{^^}Inflammatory Bowel Questionnaire

Safety Evaluation

Among study subjects that received at least one dose of active or placebo therapy, adverse events possibly or probably related to FMT were few (4 total) and were equally distributed between groups (2/6 vs 2/6; $p = 1.00$) (Table 2). The only serious adverse event was a worsening of disease activity, which occurred in one subject from each group. Both of these subjects required escalation of therapy (prednisone taper) during the treatment period (at 6 and 4 weeks following initial FMT, respectively). Mild adverse events included nausea (36 hours prior to colonoscopic delivery of placebo material) and fever (24 hours following FMT). Notably, this febrile patient also reported fever 24 hours prior to the initial FMT procedure, making causality uncertain. No infectious complications occurred.

Frozen FMT capsules were distributed to subjects in 4-week allotments (28 pills). cFMT capsules were maintained in home freezers for a total of 84 doses per subject. Medication adherence logs revealed strong adherence with <1% of missed doses across both arms (9/1,008); however, many subjects expressed frustration regarding the strict study guidelines imposed to ensure capsule temperature stability, particularly travel restrictions.

Table 2. Adverse Events by Treatment Assignment

Adverse events	FMT	(n = 6)	Placebo	(n = 6)	p value
AE possibly or probably related to FMT or sham FMT, n (%)	2/6 (33)		2/6 (33)		1.0
AE Type and severity, n (%)					
nausea, mild	0		1 (50)		1.0
fever, mild	1 (50)		0		1.0
worsening disease, severe	1 (50)		1 (50)		1.0

Adverse events by treatment group that were possibly or probably related to FMT.

Comparisons were made by Fisher's exact test.

Clinical and Histologic Outcomes

At baseline, no significant differences in histologic or endoscopic scoring were detected between the two groups (Table 1). At 12-week follow-up, the mean endoscopic UCEIS score decreased from 6.6 ± 2.0 to 6.2 ± 2.3 in the FMT group and increased from 7.4 ± 2.6 to 7.6 ± 1.8 in the placebo group. The mean histologic Geboes score decreased from 3.4 ± 1.2 to 2.3 ± 2.4 in FMT-treated subjects, and from 4.3 ± 2.0 to 4.1 ± 2.0 in the placebo group. While this study was not powered to predict a clinical response, values for clinical and physiologic markers of disease activity are presented for informative purposes (Table 3). Cumulatively, these observations enabled identification of 3 FMT subjects who responded favorably to treatment (Subjects E, W, F). All showed consistent evidence of mucosal and systemic healing. No such individuals were identified in the placebo group. These 3 potential 'responders' exhibited decreases in their average total Mayo score by 3.7 ± 3.1 points, endoscopic UCEIS scores by 1.3 ± 0.6 points, histologic Geboes scores by 2.1 ± 0.9 points, and average fecal calprotectin levels by 212.6 ± 64 mcg/g. Total IBDQ scores increased by an average of 51.6 ± 38 points. None of these subjects required escalation or changes in their IBD-related pharmaceutical regimens during the course of the trial. Alternatively, the remaining FMT subjects ($n = 3$) had inconsistent histologic and endoscopic changes, compounded by a less impressive increase in their total IBDQ score (average increase 33 ± 26 points) and all required prednisone tapers prior to the end of the trial. Changes in fecal calprotectin are unfortunately difficult to assess in this group of 'non-responders' due to a missing data point, and one subject (N) being above the limit of detection at both timepoints (Table 3). Photomicrographs of biopsy samples obtained before and after treatment are shown in Figure 2. Placebo subjects exhibited inconsistent changes over time, with no clear improvements in symptomatology or clinical evidence of disease. Four of six (66%) placebo subjects required escalation or adjustments in their pharmacologic treatment regimens: one (subject I) at week 2 (this subject dropped out of the trial at this point) and the other 3 study subjects at the end of the treatment period (weeks 12 and 13).

Table 3. Changes in clinical, endoscopic, and histologic evidence of disease by subject

	Study Code	Age	Sex	Extent of Disease	Duration of Disease (yrs)	Maintenance Therapy	BMI	Change in Total Mayo Score	Change in Endoscopic UCEIS Score	Change in Endoscopic Mayo Score	Change in Histologic Score	Change in Fecal Calprotectin (mcg/g)
FMT	E	46	F	pan-colitis	5.5	Mesalamine	20.9	-7	-2	0	-3	-285
	W	35	M	pan-colitis	7.5	Vedolizumab	27.8	-3	-1	0	-1.3	-189
	F	20	M	pan-colitis	3.8	Mesalamine	25	-1	-1	0	-2	-164
	A	65	F	L-Sided	26.2	Mesalamine	20.9	3	0	1	-1.2	? => 375
	N	44	M	pan-colitis	0.2	Sulfasalazine	25.6	1	-3	0	0.9	>1000 => >1000
	P	38	M	pan-colitis	10.2	Mercaptopurine	25.2	-3	2	-1	0	-42
Placebo	B	68	M	pan-colitis	4.4	Mesalamine	28.8	-2	-1	0	0.8	-132
	G	58	M	L-Sided	27.8	Mesalamine	26.9	1	0	0	-2	4
	Y	65	M	L-Sided	0.4	Mesalamine	36.15	0	0	0	0	74
	V	47	F	pan-colitis	8.8	Adalimumab	29.2	-1	0	0	0	27
	T	31	F	pan-colitis	0.8	Mesalamine	29.1	0	-1	0	0	-137
	I	40	F	pan-colitis	16.3	Mesalamine	25	DROPPED OUT DUE TO WORSENING DISEASE ACTIVITY				

[^]Ulcerative Colitis Endoscopic Index of Severity *Geboes Score; IBDQ, Inflammatory Bowel Disease Questionnaire; L-Sides, left-sided disease; BMI, Body Mass Index; yrs, years; wks, weeks

A retrospective analysis to identify baseline differences between FMT subjects who responded favorably relative to non-responders revealed greater mucosal damage at the time of FMT induction in those who responded poorly, including increased total Mayo score (7.0 ± 2.0 vs. 5.7 ± 2.1), endoscopic Mayo score (2.0 ± 1.0 vs. 1.0 ± 0.0), UCEIS score, (8.3 ± 1.5 vs. 5.7 ± 2.1), histologic Geboes score (4.3 ± 0.1 vs. 2.5 ± 1.0), and fecal calprotectin levels (929 ± 1144 vs. 335 ± 50 mcg/g) (Table 4).

Table 4. Baseline Clinical Characteristics by FMT Response

	Responders	n=3	(Mean ± SD)	Non-Responders	n=3	(Mean ± SD)
Clinical Variables						
Age (years)	34 (13)			49 (14)		
Sex (# (%) male)	2 (67%)			2 (67%)		
BMI	24.6 (3.5)			23.9 (2.6)		
Number current UC meds	1.0 (0.0)			1.7 (0.6)		
Number prior UC meds	3.0 (2.6)			2.7 (1.5)		
Total Mayo score	5.7 (2.1)			7.0 (2.0)		
Endo Mayo score	1.0 (0.0)			2.0 (1.0)		
UCEIS score	5.7 (0.6)			8.3 (1.5)		
Calprotectin	335 (50)			929 (1144)		
IBDQ total score	137 (12)			149 (21)		
Histologic score	2.5 (1.0)			4.3 (0.1)		
Duration of disease (years)	5.6 (1.9)			12.2 (13.1)		

Longitudinal Phenotyping of Peripheral Blood T-cells

Baseline T cell populations of interest were first compared between UC subjects and healthy controls. The frequency of total lymphocytes obtained following PBMC separation, as well as the CD4:CD8 ratio, were similar between groups. T regulatory cell frequencies were also similar (mean of $3.12\% \pm 0.41$ in UC patients vs. a mean of $3.42\% \pm 0.54$ in controls) with comparable proportions positive for IL-17A and IL-10. No T regulatory cells were IFN γ . The frequency of mucosal-associated invariant T (MAIT) cells was decreased in UC patients ($0.62\% \pm 0.15$ vs. $1.67\% \pm 0.46$) and IL-17A positivity occurred almost exclusively in UC-derived MAIT cell populations (3.42 ± 1.27 vs. 0.1759 ± 0.09). Alternatively, IFN γ secretion was increased in MAIT cells from healthy controls (46.97 ± 7.15 vs. 24.16 ± 6.01), (Fig. 3).

T cell populations were examined before FMT, and then at weeks 4, 8, and 12 during the cFMT maintenance period. By week 4, the frequency of total PBMC lymphocytes and CD4:CD8 T cell ratios increased in all FMT subjects with poor clinical response, while variable changes were observed in the remaining FMT and placebo subjects. One notable exception was subject F who showed a prominent decrease in CD4:CD8 ratio through week 8, after which a reverse dynamic to baseline occurred. Longitudinal frequencies of T regulatory and MAIT cell populations remained relatively constant across groups. By week 4, IL-17A+ MAIT and IFN γ + MAIT cells decreased in FMT subjects with positive clinical responses, remained suppressed through week 8, and then returned to baseline by week 12. IL-17A + MAIT cells remained suppressed in subject E. The number of subjects is insufficient to evaluate the statistical significance of these observed changes.

Intestinal Microbiota Analysis by 16S Sequencing

Relative abundances

Across all time points, stool samples were dominated at the phylum level by *Firmicutes*, and *Bacteroidetes*, which accounted for 88.90% of all sequence reads. Bacteria present at lower proportions included *Proteobacteria*, and *Actinobacteria*, accounting for 6.9% and 4.0% of total reads, respectively. At the genus level, samples were dominated by *Clostridiales* and *Bacteroidales*, with a lower proportion of *Burkholderiales*, *Bifidobacteriales*, *Selenomonadales*, *Enterobacteriales*, *Lactobacillales* observed at various time points. A strong antibiotic effect was observed following a 7-day course of Metronidazole and Ciprofloxacin in all subjects. Changes included a decrease in gram negative and anaerobic bacteria of the *Firmicutes* and *Bacteroidetes* phyla and an increase in gram positive *Actinobacteria*, including from the genus *Bifidobacteriales*, and *Lactobacillales* Fig. 4. This effect was associated with a decrease in alpha (Shannon) diversity and increased divergence (Jensen-Shannon) from baseline. While these changes were mitigated by the cessation of antimicrobials, neither group returned to their own baseline by 18-week follow-up (Figs 5 and 6).

Alpha and Beta Diversity

No difference in alpha or beta diversity was observed between treatment groups at baseline (Fig. 5). FMT did not increase alpha (Shannon) diversity in recipients but did lead to community-level changes in the gut microbiota creating measurable similarity (beta diversity, Jensen-Shannon divergence index) between FMT subjects and their donor. This convergence, which we termed 'Donor Divergence Index', remained statistically significant through 8 weeks of dosing ($p < 0.01$) and although losing significance ($p = 0.16$), could still be detected at week 20, > 8 weeks following cessation of oral cFMT therapy (Fig. 6).

Discussion

FMT is a promising new therapy that may alleviate the microbial dysbiosis observed in IBD. Practical dosing strategies, however, are needed to test and optimize the clinical efficacy of long term FMT-based treatment strategies. The goal of the current study was to evaluate the safety and subject tolerance of a treatment plan that involved colonoscopic FMT followed by maintenance treatments of daily cFMT. In this small prospective, randomized controlled trial, we found daily oral FMT to be extremely well tolerated with strong adherence to the treatment plan. Only very minimal side effects that could be attributed to the

treatment were detected, and importantly, no infectious complications occurred. While this study provides promising evidence to support the further investigation of oral FMT formulations for control of UC and other chronic disease, it suggests that temperature stable formulations should be pursued. Lyophilized fecal preparations or derived bacterial communities may offer viable solutions [25].

Prior randomized control trials, involving multiple enemas and/or colonoscopy-based administrations have shown early clinical impact from FMT in UC populations [11–14]. Only one other study is known to us in which daily cFMT was trialed in the treatment of UC [24]. In it, 7 subjects took 25 frozen FMT capsules daily (~ 12 g of fecal matter) for 50 days in an open label trial design. As the authors did not report on adverse events, this is the first study to our knowledge to provide early evidence regarding the safety and tolerability of cFMT in UC patients.

While this study was not adequately powered to evaluate the effects of treatment on clinical outcome, we provide additional data to assess the potential impact of FMT on UC disease severity, including clinical, endoscopic, and histologic markers of disease. Our results reveal a striking differentiation in patient response to FMT with subjects separating into potential responder and non-responder phenotypes. This is consistent with previous reports suggesting that FMT efficacy is limited to a subset of UC patients [26]. In our experience, the subjects who responded more favorably were characterized by lower degrees of mucosal damage at the time of FMT induction, as evidenced by lower endoscopic (UCEIS, 5.7 ± 2.1 vs. 8.3 ± 1.5 vs.) and histologic scores (Geboes, 2.5 ± 1.0 vs. 4.3 ± 0.1), as well as lower fecal calprotectin levels (335 ± 50 vs. 929 ± 1144 mcg/g). Immunomodulating effects of the gut microbiota are well-established [27, 28] and, in the setting of gross and microscopic mucosal ulceration, FMT may induce uncontrolled and potentially damaging mucosal inflammation secondary to bacterial translocation and systemic immune activation [29, 30]. Targeting of UC patients with mild to moderate disease for FMT may therefore be advantageous.

Longitudinal phenotyping of peripheral T-cell populations provides additional insights into the differential clinical responses observed following FMT. In particular, T regulatory and MAIT cells have previously been shown to be altered in UC patients, [31–33] While small sample sizes preclude attribution or causality, this report offers a rare window into the host T cell response following FMT. MAIT cells are of particular interest when examining the immunologic impact of FMT as they represent an evolutionarily conserved subset of effector T cells whose natural ligands are bacterially derived vitamin B2 (riboflavin) metabolites [34]. MAIT cell development is dependent upon the presence of commensal bacteria and they play an important role in bacterial defense at mucosal surfaces [35–38]. Previous reports have revealed lower levels of MAIT cells in the peripheral blood of UC patients with their concurrent accumulation in inflamed mucosal tissues where they display an enhanced Th17 phenotype [33]. Our data from peripheral blood samples are consistent with this, supporting the notion that MAIT cell populations traffic to the mucosal surface of UC patients where they modulate microbial stimuli. The decrease in IL-17 and IFN γ MAIT cell positivity observed in subjects with improved clinical scores associated with cFMT treatment further supports a potential role for MAIT cells in the response of UC patients to FMT, and warrants further study. Ultimately, an enhanced understanding of the defining clinical and immunological characteristics of UC patients poised to respond favorably to FMT is critical in moving FMT and other microbially-based therapies forward.

FMT can significantly alter the gut microbiota of recipients [9, 17–18]. We show that, by 2 weeks after FMT induction, the gut microbiota of UC patients is highly correlated with that of donors, and that these changes persist up to 20 weeks. cFMT appears to reinforce the donor convergence period, extending the currently reported interval by 4 weeks [11–14]. It does not, however, appear that the degree of donor correlation is an indicator of positive FMT clinical effects as no difference in donor convergence was observed between potential FMT “responders” and “non-responders” in our small sample.

A disconnect between donor convergence and clinical response has also been reported individuals treated for recurrent *C. difficile*. These patients demonstrate variable patterns of donor microbial divergence following FMT with little correlation to clinical outcome. This observation suggests that a more granular and complex understanding of FMT-induced microbial changes is desirable [17, 18, 39]. While the ultimate durability of FMT-induced changes in the microbiota is unknown, studies in recurrent *C. difficile* patient populations have shown high donor correlations to persist for greater than 1 year [9, 18]. The durability of FMT-induced microbial changes in IBD populations is less well established. In prior studies using serial endoscopic or enema-based regimens, persistent microbiota changes have been detected for up to 16 weeks in UC patients [11–14]. The only report to our knowledge of microbial changes following oral FMT monotherapy in UC patients showed no differences in alpha diversity nor evidence of donor convergence [24], raising the possibility that large volume FMT induction may be necessary to achieve initial community-level donor convergence.

As a small pilot trial, this study was not powered to statistically demonstrate a difference in clinical outcome and thus preliminary observations must be replicated in larger trials. Despite its small size however, the observed changes were consistent with previously published clinical studies and immunologic observations recapitulated those of others [33]. Another limitation of the present study is its lack of mucosal immunologic data. Further inquiry should include immune profiling data from mucosal tissues at the site of disease activity to better understand FMT-induced immunologic responses to alterations in the colonic microbiome.

Conclusions

Oral cFMT formulations are likely to gain acceptance by some individuals as a therapeutic alternative in UC, and may enhance the potential for longterm microbially-based treatment strategies. To date, scientific data regarding the feasibility, safety and efficacy of these regimens are lacking. This study provides initial evidence that cFMT is a safe, and well-tolerated adjunctive mechanism by which to support and extend FMT-induced shifts in the gut microbiota of UC patients.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Medical Ethics Committee of the University of Vermont (document number CHRMS15-373). Written informed consents from voluntary subjects were obtained.

Consent for publication

No identifiable data is presented.

Competing interests

EJA, ZK and MS are cofounders of OpenBiome and Finch Therapeutics

ZK, MS, RJE, EV are employees of Finch Therapeutics

JWC consults for Finch Therapeutics

WFW, RJE, EV are employees of OpenBiome

GMM has research funding from Takeda Pharmaceuticals and is on the Scientific Advisory Board of Dignify Therapeutics

Availability of data and materials

The 16S datasets generated and analysed during the current study are available in the NCBI's SRA database repository, (BioProject PRJNA475599). The clinical datasets generated and analysed during the current study are not publicly available due privacy issues, but are available from the corresponding author on reasonable request.

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Authors' contributions

JWC, MS, ZK, GM, EJA, and PLM conceived of this study and were major contributors in the writing of the manuscript. JWC, MP, RE, CC, WFW, EV, MV, AC, RB and PLM carried out the clinical trial component of this study. JWC, RB, RDG, KR, BL, and CC developed, generated, and analyzed the flow cytometric data. NDC, and LTN generated and analyzed the 16S data presented. PC performed statistical analysis. All authors read and approved the final manuscript."

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None.

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Figures

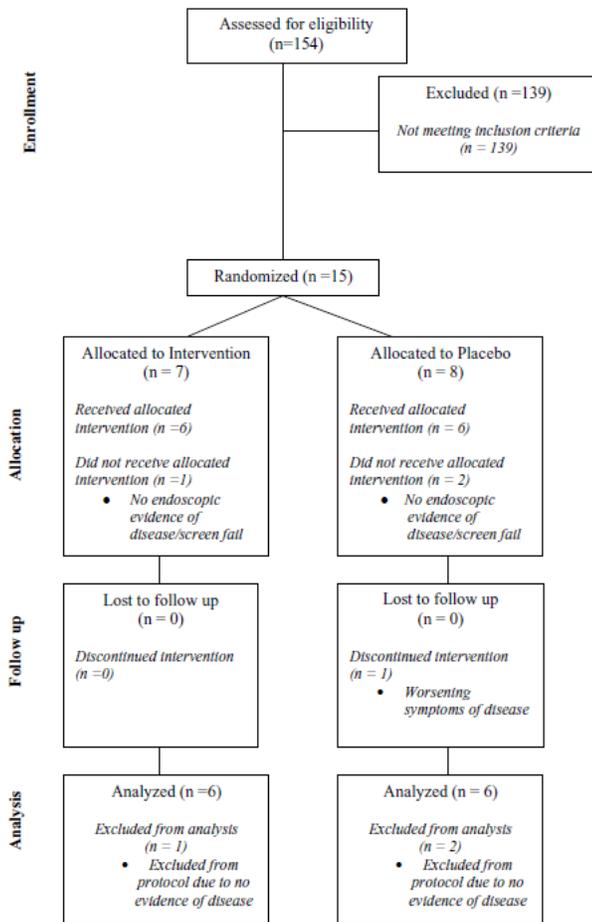


Figure 1

CONSORT diagram showing the flow of subjects through the study. Following randomization, but prior to administration of designated intervention, 1 subject in the treatment group and 2 subjects in the placebo group had no evidence of disease upon endoscopic evaluation and were excluded from the remainder of the study.

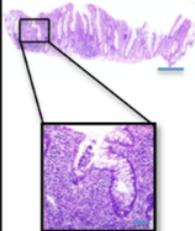
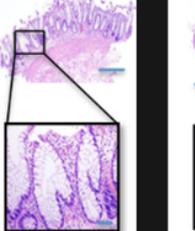
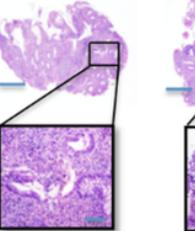
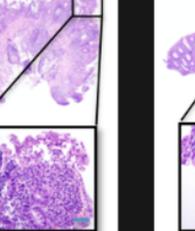
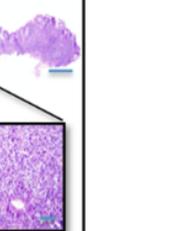
	FMT Responder (E)		FMT Non-Responder (N)		Placebo (Y)	
	Pre-FMT (day 0)	Post-FMT (day 84)	Pre-FMT (day 0)	Post-FMT (day 84)	Pre-FMT (day 0)	Post-FMT (day 84)
						
Geboes	3.1	0.1	4.3	5.2	5.1	5.1
UCEIS	6	4	10	7	8	8
Fecal Calprotectin	285	0	>1000	>1000	286	360
IBDQ	123	215	141	200	149	153

Figure 2

Histologic, endoscopic and clinical parameters of a represent FMT 'responder' (E), 'non-responder'(N), and placebo subject (Y) before and after treatment. Hematoxylin-eosin staining of intestinal mucosa highlight acute and chronic changes and are accompanied by Geboes score (0, structural change only; 1, chronic inflammation; 2, lamina propria neutrophils; 3, neutrophils in epithelium; 4, crypt destruction; and 5, erosions or ulcers), 2x, insets at 20x, scale bar, 50 micrometers; UCEIS, Ulcerative Colitis Endoscopic Index of Severity; fecal calprotectin (mcg/g), and IBDQ, inflammatory bowel disease questionnaire (scale ranging from 32 (worst) to 224 (best))

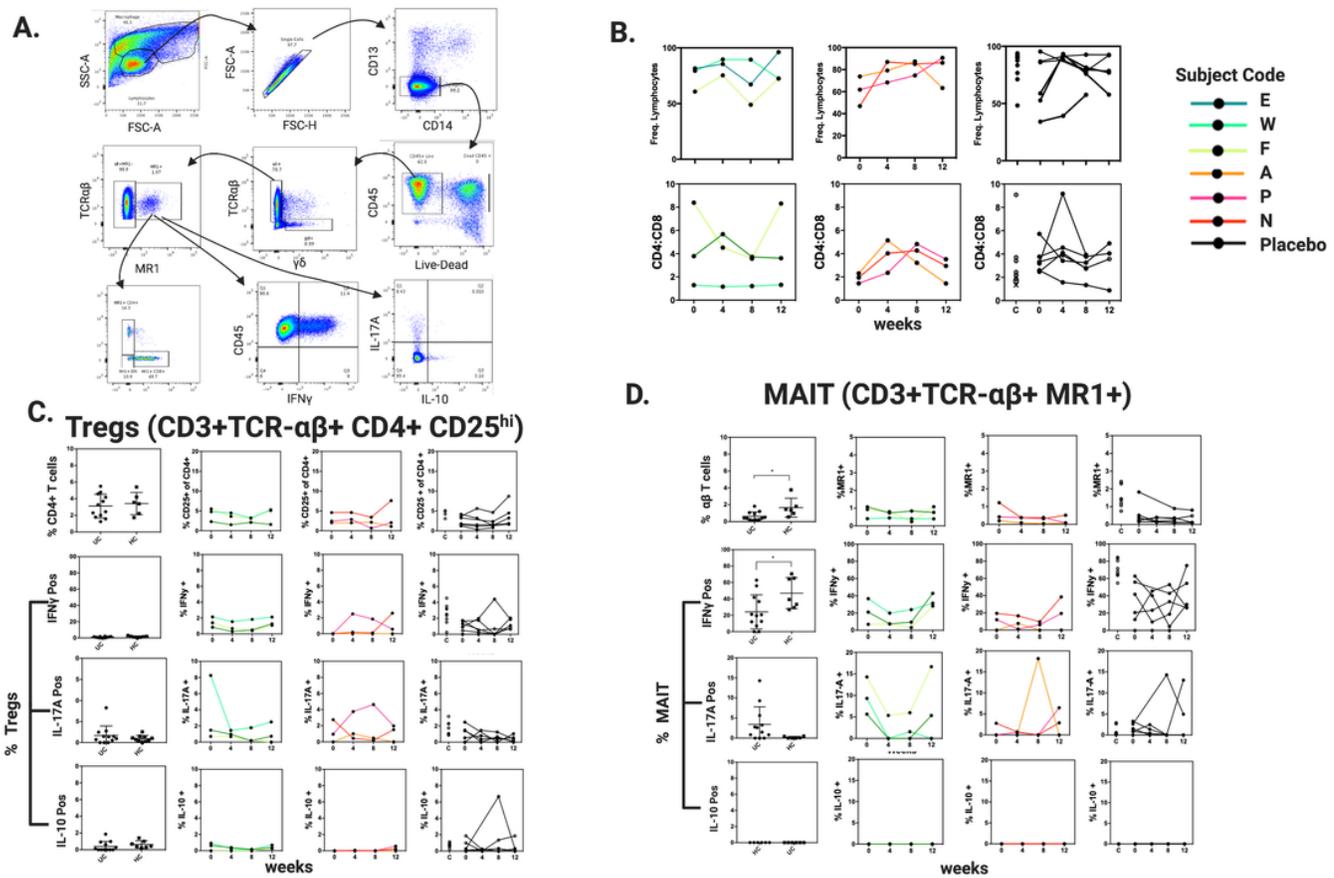


Figure 3

Longitudinal T cell profiling of subjects by flow cytometry. a Representative gating scheme; b Frequency of total lymphocytes within peripheral blood mononuclear cell isolations and their CD4:CD8 T cell ratios. c Comparison of T regulatory cell frequencies between UC patients and healthy controls (HC) with longitudinal frequencies and INF γ +, IL-17A+, and IL-10+ proportions displayed by treatment group and clinical response; c Comparison of MAIT cell frequencies between UC patients and healthy controls (HC) with longitudinal frequencies and INF γ +, IL-17A+, and IL-10+ proportions displayed by treatment group and clinical response (black, placebo; red, poor response; green, good response). Each line is an individual subject. Controls are displayed with placebo results (C).

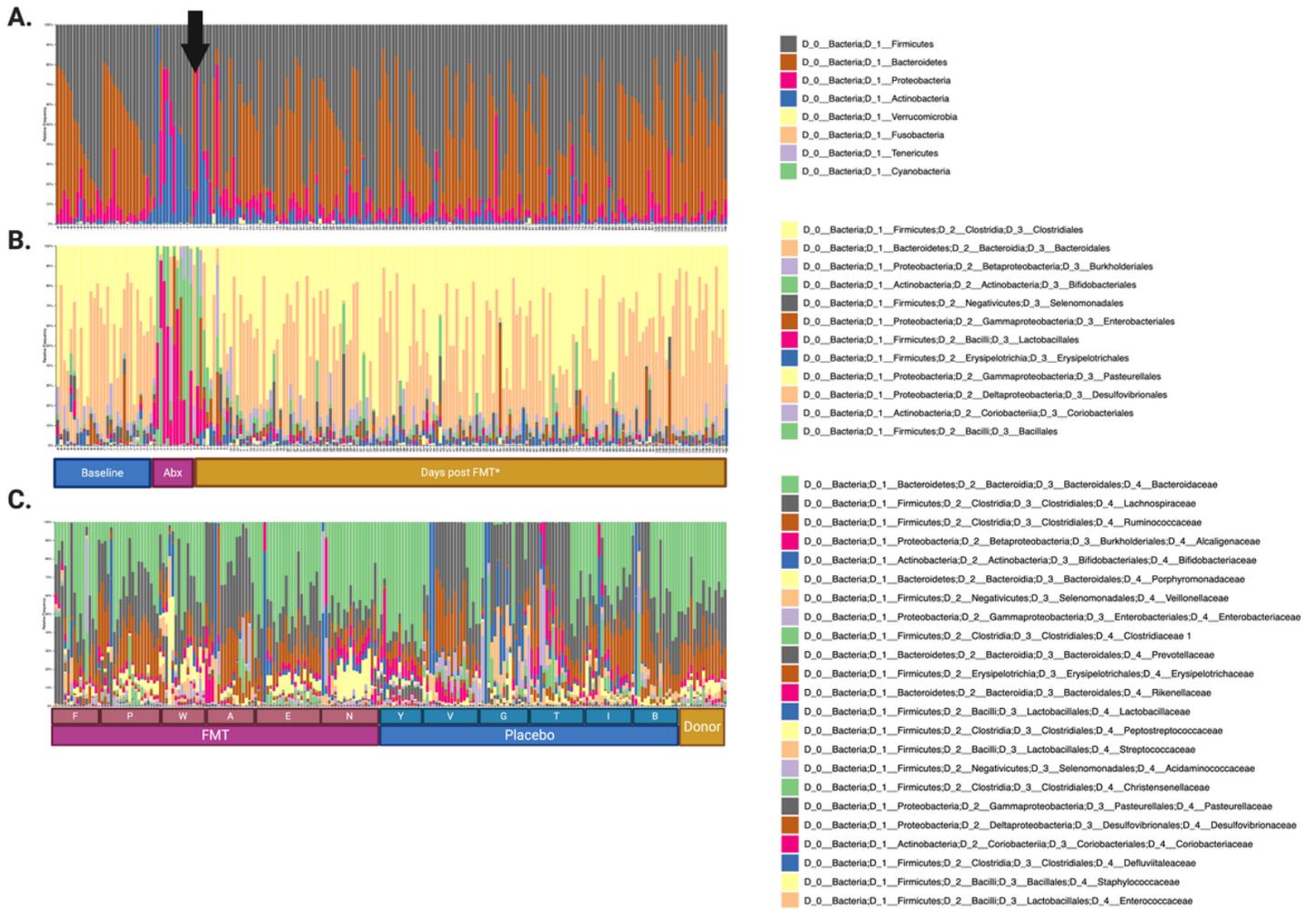


Figure 4
 Relative abundance of fecal microbiota in subjects with ulcerative colitis (UC) before and after treatment at the phylum and genus levels. Different colors represent different bacterial species, each bar represents one patient sample. a, b. most abundant taxa by phylum and genus level, respectively. Arrow denotes day of FMT (*or placebo); c 23 most abundant taxa of donors and subject at species level, arranged by subject, treatment group, and day.

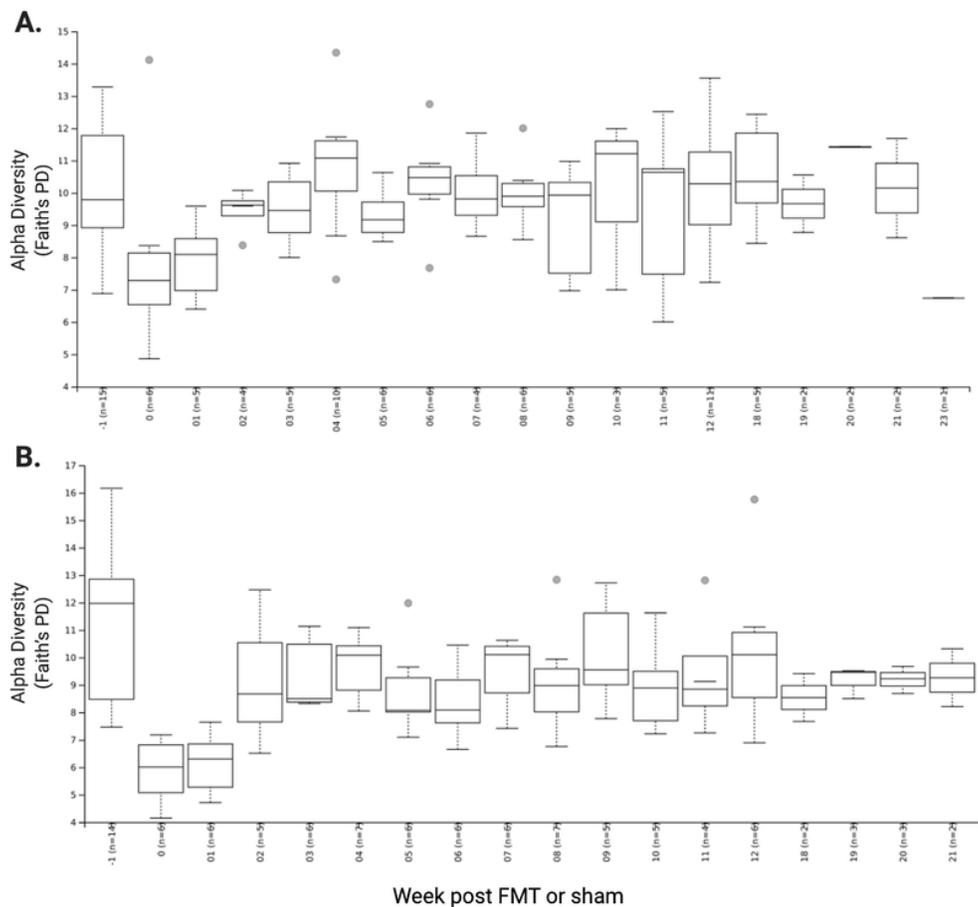


Figure 5

Alpha diversity measured by Shannon index. a alpha diversity in placebo subjects grouped by week; b alpha diversity in FMT subjects grouped by week. Comparisons between groups made by Student's t-test and p values of <0.05 were considered significant.

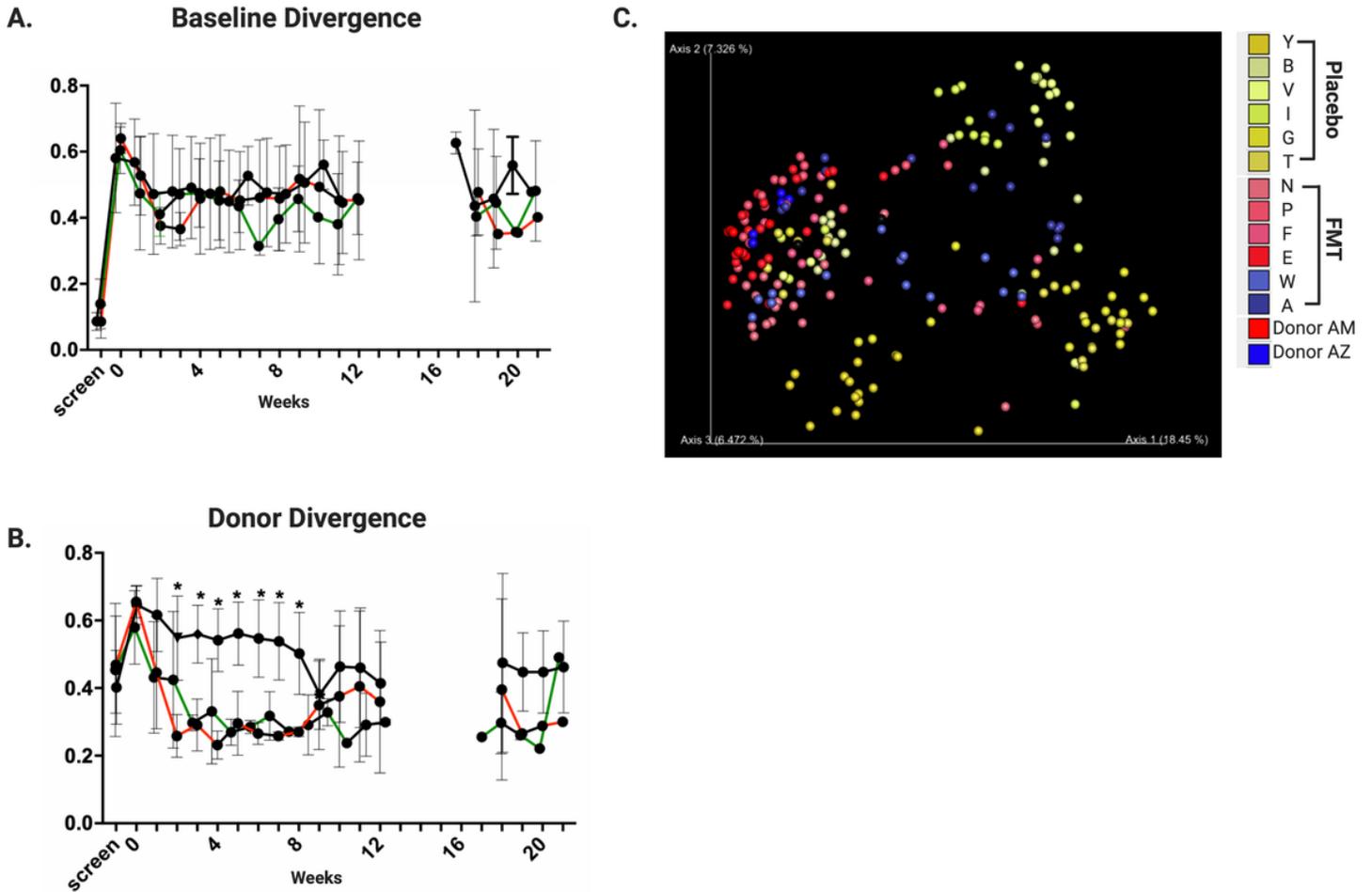


Figure 6

Beta diversity measured by Jenson-Shannon diversity. a Beta diversity comparing subjects to their own baseline overtime; b Beta diversity comparing subjects to donors. Aggregate data is presented by treatment and clinical response (black, placebo; red, poor response; green, good response). Comparisons between groups made by Student's t-test and p values of <0.05 were considered significant; c Principle component analysis of donors and study subjects. Each dot represents one sample, and subjects are colored by the treatment group (yellow shades represent placebo, blue and red represent primary donor).

Supplementary Files

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