Gastroenterology 2015;149:102–109

Fecal Microbiota Transplantation Induces Remission in Patients With Active Ulcerative Colitis in a Randomized Controlled Trial

Paul Moayyedi,[1] Michael G. Surette,[1] Peter T. Kim,[2,3] Josie Libertucci,[1] Melanie Wolfe,[1] Catherine Onischi,[3] David Armstrong,[1] John K. Marshall,[1] Zain Kassam,[4] Walter Reinisch,[1] and Christine H. Lee[3]

[1]Farncombe Family Digestive Health Research Institute, Department of Medicine, McMaster University, Hamilton, Ontario, Canada; [2]Department of Mathematics and Statistics, University of Guelph, Guelph, Ontario, Canada; [3]Department of Pathology and Molecular Medicine, St Joseph's Healthcare, McMaster University, Hamilton, Ontario, Canada; and [4]Department of Biological Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts

CLINICAL AT

See Covering the Cover synopsis on page 1; see editorial on page 15; related article, Rossen et al, on page 110; and review, Kelly et al, on page 223.

BACKGROUND & AIMS: Ulcerative colitis (UC) is difficult to treat, and standard therapy does not always induce remission. Fecal microbiota transplantation (FMT) is an alternative approach that induced remission in small series of patients with active UC. We investigated its safety and efficacy in a placebo-controlled randomized trial. **METHODS:** We performed a parallel study of patients with active UC without infectious diarrhea. Participants were examined by flexible sigmoidoscopy when the study began and then were randomly assigned to groups that received FMT (50 mL, via enema, from healthy anonymous donors; n = 38) or placebo (50 mL water enema; n = 37) once weekly for 6 weeks. Patients, clinicians, and investigators were blinded to the groups. The primary outcome was remission of UC, defined as a Mayo score $\leq$2 with an endoscopic Mayo score of 0, at week 7. Patients provided stool samples when the study began and during each week of FMT for microbiome analysis. The trial was stopped early for futility by the Data Monitoring and Safety Committee, but all patients already enrolled in the trial were allowed to complete the study. **RESULTS:** Seventy patients completed the trial (3 dropped out from the placebo group and 2 from the FMT group). Nine patients who received FMT (24%) and 2 who received placebo (5%) were in remission at 7 weeks (a statistically significant difference in risk of 17%; 95% confidence interval, 2%−33%). There was no significant difference in adverse events between groups. Seven of the 9 patients in remission after FMT received fecal material from a single donor. Three of the 4 patients with UC $\leq$1 year entered remission, compared with 6 of 34 of those with UC >1 year (P = .04, Fisher's exact test). Stool from patients receiving FMT had greater microbial diversity, compared with baseline, than that of patients given the placebo (P = .02, Mann-Whitney U test). **CONCLUSIONS:** FMT induces remission in a significantly greater percentage of patients with active UC than placebo, with no difference in adverse events. Fecal donor and time of UC appear to affect outcomes. ClinicalTrials.gov Number: NCT01545908.

Keywords: IBD; Treatment; Microbe; Colon.

Ulcerative colitis (UC) is a chronic inflammatory disorder of the colorectum that has a peak incidence in early adulthood.[1] The cardinal symptom of UC is bloody diarrhea,[2] which is associated with a significant reduction in quality of life.[3] The etiology of the disease is unknown, but it is thought to arise from an aberrant immune response to a change in colonic environment in a genetically susceptible individual.[2,4] Current medical treatment remains imperfect[5] and a significant minority of patients need colectomy to manage their disease.[6] There have been advances in therapy,[7] but gains have been modest. The focus of drug development has been on altering the immune response[8] rather than reducing factors that stimulate the aberrant immune response.[9] A likely candidate that could drive the immune response in UC is the colonic microbiome, as this is altered in patients with the disease compared with healthy controls[10] and animal models of colitis require gut bacteria to induce inflammation.[11] Fecal microbiota transplantation (FMT) has emerged as a novel approach to altering the colonic microbiome and can successfully treat antibiotic-resistant *Clostridium difficile* colitis.[12,13] The concept of FMT has captured the imagination of the public, and this approach is being advocated for a number of diseases, including UC. The efficacy of FMT is unclear in other situations and there have only been a few case reports of FMT in UC, with conflicting results.[14,15] We report the first randomized trial of FMT to treat active UC.

Methods

Study Design

This is a double-blind randomized controlled trial of FMT vs placebo in active UC conducted in Hamilton Health Sciences, St Joseph's Healthcare Hamilton, and McMaster University, Hamilton, Canada. The local research ethics committee at McMaster University approved the trial and Health Canada had no objection to the use of FMT for this study. All participants

Abbreviations used in this paper: **FMT, fecal microbial transplantation; UC, ulcerative colitis.**

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2015.04.001

provided written informed consent and an independent Data Monitoring and Safety Committee evaluated the trial annually.

Study Population

Eligible patients were 18 years or older with active UC defined as a Mayo Clinic score[16] ≥4 with an endoscopic Mayo Clinic score ≥1. Concomitant treatments for UC, such as mesalamine, glucocorticoids, immunosuppressive therapy (eg, azathioprine), or tumor necrosis factor antagonists were permitted, provided these had been used at a stable dose for at least 12 weeks (4 weeks for glucocorticoids) and disease remained active. Five patients who were previously exposed to topical mesalamine or steroids had a 30-day washout period before being enrolled. Patients were excluded if they had taken antibiotics or probiotics in the last 30 days, had concomitant *C difficile* infection or another enteric pathogen, had a disease severity that required hospitalization, were pregnant, or were unable to give informed consent.

Baseline Assessments

Potentially eligible patients were scheduled for a flexible sigmoidoscopy and also completed baseline questionnaires to obtain demographic information, Mayo score,[16] Inflammatory Bowel Disease Questionnaire score (a validated disease specific quality of life measure; range of scores from 0 to 224 with higher score indicating better quality of life),[17] and EuroQol (EQ-5D) score (a validated general quality of life measure; range score 0 to 100 with higher score indicating better quality of life).[18] Blood samples were drawn for inflammatory markers (complete blood count, erythromycin sedimentation rate, and C-reactive protein) and serology for human immunodeficiency virus; hepatitis A, B, and C; and syphilis. Stool samples were provided by the participant and were screened for ova, cysts, and parasites, as well *C difficile* toxin gene tested by polymerase chain reaction. In addition, stool samples were collected in a sterile, airtight container for microbiota assessment.

Randomization

Eligible patients were randomized 1:1 according to a computer-generated randomization list that was stratified for patients with UC diagnosed within 1 year. The randomization was held centrally at the McMaster Gastroenterology Clinical Trials Unit to ensure concealment of allocation. The treatment location was masked to the patient, health care workers caring for the patient, and investigators. The technician administering FMT or placebo was aware of the treatment being administered, as the nature of the intervention meant that it was not possible to make it identical to the placebo.

Interventions and Follow-Up

FMT was prepared from stool donated by volunteers who were between 18 and 60 years of age and were otherwise healthy, as assessed by a screening questionnaire (see Supplementary Material). Donor stool was screened for enteric pathogens, including *Salmonella*, *Shigella*, *Campylobacter*, *Escherichia coli* O157 H7, *Yersinia*, as well as ova, cysts, and parasites and *C difficile* toxin. The donor had to have negative serology for human immunodeficiency 1/2, hepatitis A IgM, hepatitis B surface antigen, hepatitis C antibody, syphilis, and human T- lymphotrophic virus 1/II and be screened negative for vancomycin-resistant *Enterococcus* or methicillin-resistant *Staphylococcus aureus*. All donors were prospectively screened and rescreened every 6 months. Stool was retested whenever a donor returned from travel outside of North America. The donor could not provide stool samples if they had taken antibiotics in the previous 3 months. Donor stool was delivered for processing within 5 h of collection. Fifty grams of donor stool was collected in the preweighed container and mixed with 300 mL commercial bottled drinking water. The mixture was emulsified for 3 to 5 minutes using a clean wooden/plastic spatula and then allowed to settle for 5 minutes. Filter paper was placed over the mixture until the supernatant filtered to the top and this was then decanted using a 60-mL syringe. The supernatant was either administered immediately to the patient or stored at −20°C.

Participants were randomized to receive 50 mL FMT or placebo consisting of 50 mL water given as a retention enema once per week for 6 weeks. The enema was administered with the patient in the left lateral position with instructions to retain this for at least 20 minutes. Patients provided stool samples each week before receiving their retention enema and samples were stored at −20°C for fecal microbiota analysis. The donor samples that were analyzed were those that were given to the patient. If the samples were frozen−thawed, it was the thawed sample that was sent for microbiome analysis. Adverse events were assessed at every weekly visit and the intervention was administered in a different hospital from where the patient was assessed for response to therapy.

Participants returned to complete a further Mayo Clinic score, Inflammatory Bowel Disease Questionnaire, EQ-5D, and have an exit flexible sigmoidoscopy at week 7 (±3 days). One investigator (PM) performed all flexible sigmoidoscopies at baseline and week 7, with the exception of 2 baseline sigmoidoscopies and 1 exit sigmoidoscopy (performed by JKM). Two rectal, sigmoid, and descending colon biopsies were taken for histology at baseline and at week 7.

Clinical Outcomes

The primary outcome was UC remission at week 7, defined as a full Mayo score <3 and complete healing of the mucosa at flexible sigmoidoscopy (endoscopic Mayo score = 0). Secondary outcomes included improvement in UC symptoms (defined as ≥3 improvement in full Mayo score), as well as change in Mayo, Inflammatory Bowel Disease Questionnaire, and EQ-5D scores. There was a 12-month follow-up phase of the trial planned with re-randomization to either open label no therapy or FMT once per month for 12 months. This part of the trial was discontinued, as there were insufficient patients entering remission at week 7. We did, however, record changes in UC medication, serious adverse events, hospitalization for UC and colectomies, as well as partial Mayo score at month 12.

Assessment and Analysis of the Microbiome

Microbial community profiling was conducted by extracting genomic DNA from patient and donor stool samples using a protocol described previously,[19] which enhances total DNA recovery. After genomic DNA extraction, the V3 region of the 16s ribosomal RNA gene was amplified (total polymerase chain reaction volume of 50 μL [25 pmol of each

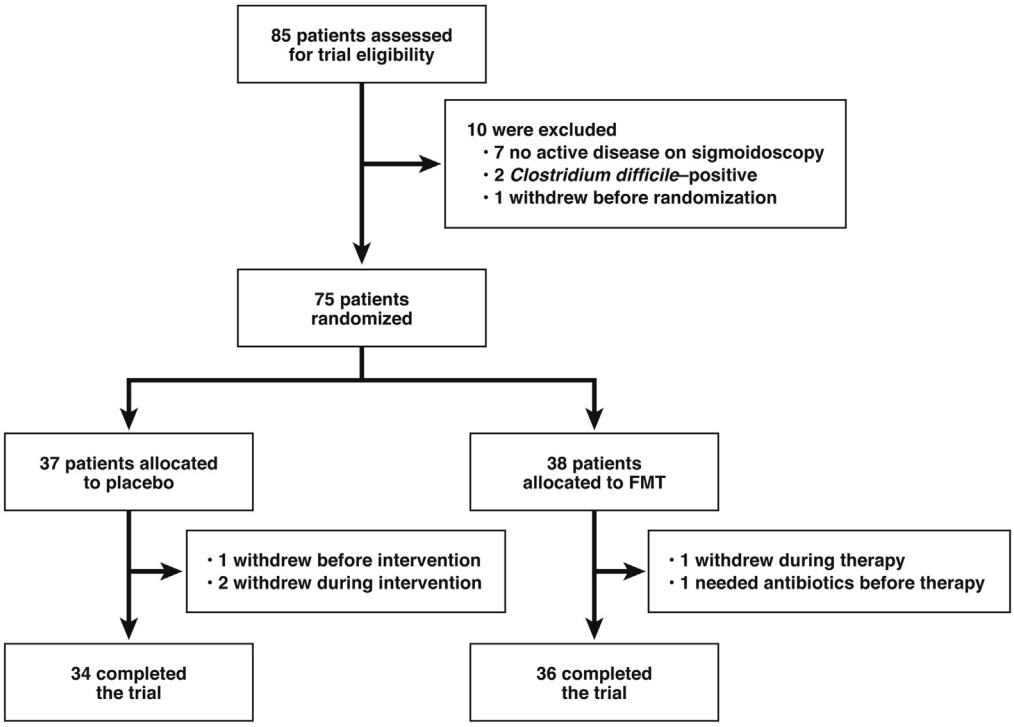


Figure 1. Flow of patients in the trial.

primer, 200 μL each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$, and Taq polymerase] divided into triplicate for greater efficiency). Samples were sequenced using the Illumina MiSeq platform, as per manufacturer's instructions and raw sequence reads were processed as described previously.[20] The stool microbiota was sampled to mean depth of 103,341 reads per sample (median = 87,509). Beta diversity (Bray-Curtis dissimilarity) was calculated using the Phyloseq R package[21] and statistical differences between pairwise comparisons were calculated using Mann-Whitney U test, with significance defined as $P < .05$. Relative abundance and taxonomic profiles were computed using Quantitative Insights Into Microbial Ecology.[22]

Statistical Analysis

We aimed to recruit 130 active UC patients (65 in each arm) using sample size calculations that assumed a 25% remission rate in the placebo arm[23] and a 50% remission rate in the FMT arm, using 80% power and 5% significance, and assuming a 10% attrition rate.[23]

Pearson's χ^2 or Fisher's exact test was used to analyze the primary outcome of the proportion of patients in remission at study completion. The primary analysis was intention to treat with an evaluable patient analysis also planned. All other categorical data were analyzed using Pearson's χ^2 or Fisher's exact test. Continuous data were analyzed using t tests and linear regression, with the baseline value being one of the covariates. Logistic regression and least absolute shrinkage and selection operator were used to evaluate independent predictors of response to therapy. All analyses were conducted using R version 3.1.0. PM, MW, and PTK had full access to the data and PM was responsible for submitting the manuscript.

Results

Patients and Termination of the Trial

Patients were recruited during an 18-month period commencing in July 2012. The Data Monitoring and Safety Committee reviewed the data at the approximate 50% recruitment point of the trial and, at that time, there were 4 of 27 patients in remission in the FMT arm and 2 of 26 in the placebo arm. The Data Monitoring and Safety Committee advised that the trial should be discontinued for futility because the primary end point was unlikely to be achieved as specified in the protocol. They also recommended that patients already enrolled in the trial should complete their allocated therapy. At the point that the Data Monitoring and Safety Committee made this decision, there were 22 participants that had been screened and were eligible and awaiting entry to the trial or were already receiving intervention, but had not yet completed the trial. Overall, 85 patients were considered for the trial, 75 were randomized and 70 completed therapy (Figure 1). Baseline characteristics of the participants were similar, although those allocated to placebo were slightly older, with a female preponderance (Table 1).

Donors

We initially recruited two donors (donor A and B) to provide FMT for the study to minimize the variability of the intervention and aid interpretation of the microbiome analysis. However, after the first 17 participants were enrolled, donor B was prescribed antibiotics and did not donate for 4 months. Four other donors' (donor C to F) stools were used during this period, which ended shortly

before the Data Monitoring and Safety Committee's assessment. The remaining participants allocated to active therapy all received FMT from donor B exclusively, as we had not experienced any success with donor A (Figure 2). In all but one case, donors were anonymous, but 1 patient requested their spouse be used as a donor. This person was randomized to active therapy and was a treatment failure. Twenty-one patients received frozen–thawed stool, 15 patients received fresh stool, and 1 patient was given both fresh and frozen stool on different weeks.

Clinical Outcomes

Overall, there was a statistically significant effect of FMT on inducing remission in UC, with 9 of 38 (24%) patients in the FMT arm vs 2 of 37 (5%) in the placebo arm in remission at the end of treatment ($P = .03$, Pearson's χ^2; Table 2). There were no patients with an endoscopic Mayo score = 0 and a total Mayo score ≥3, so an end point of an endoscopic Mayo score = 0 gave the same result.

The proportion of patients with improvement in symptoms and the various quality of life scores were not statistically significant (Table 2). There was no difference in serious adverse events between the FMT and placebo groups (Table 2). We evaluated factors that might predict treatment success using logistic regression and least absolute shrinkage and selection operator. Of the 19 covariates, 10 were chosen by least absolute shrinkage and selection operator with randomization being one of them. When we ran logistic regression on these covariates, randomization was the only statistically significant positive covariate ($P = .05$, Supplementary Tables 1–9). There were also no statistically significant differences in change scores between groups (Supplementary Table 10).

We also assessed the histology of patients who were in remission at week 7 in a post-hoc analysis. Seven of the patients in remission in the FMT arm had no active inflammation in any biopsy, and the other 2 patients had mild patchy inflammation in the rectum with no active inflammation in sigmoid and descending colon biopsies (both had inflammation on histologic assessment at these sites at baseline) at week 7. One of the 2 patients that were in remission with placebo had no active inflammation in any biopsy, and the other had mild active inflammation in all biopsies.

There were 38 patients randomized to FMT. Treatment successes attributable to donor B were 7 of 18 (39%) vs 2 of 20 (10%) with other donors ($P = .06$, Fisher's exact test), suggesting statistical evidence for donor dependence. Other notable features included a trend toward those taking immunosuppressant therapy to have a greater benefit from FMT (5 of 11 [46%] in those taking immunosuppression vs 4 of 27 [15%] in those not on immunosuppressive therapy; $P = .09$, Fisher's exact test). Patients with a recent diagnosis of UC (defined as ≤1 year) were statistically significantly more likely to respond to FMT (3 of 4 [75%] compared with 6 of 34 [18%] in those with chronic disease; $P = .04$, Fisher's exact test). There was also a trend for frozen stool to have greater efficacy than fresh stool, although most of the treatment successes were also using donor B

Table 1. Baseline Characteristics of Patients in the Trial

Variable (denominator: placebo or FMT)	Placebo (n = 37)	FMT (n = 38)
Age, *y* (37, 38)	35.8 ± 12.1	42.2 ± 15.0[a]
Male sex, n (%) (37, 38)	26 (70)	18 (47)[b]
White race, n (%) (37, 38)	29 (78)	36 (95)
Nonsmoker, n (%) (37, 38)	21 (57)	19 (50)
UC <1 year, n (%) (37, 38)	4 (11)	4 (11)
Pancolitis, n (%) (30, 36)	12 (37.5)	20 (62.5)
Concomitant medications, n (%)		
Mesalamine therapy (37, 38)	20 (54)	21 (55)
Glucocorticoids (37, 38)	13 (35)	15 (39)
Immunosuppressants (37, 38)	6 (16)	11 (29)
Anti-TNF therapy (37, 38)	2 (5)	5 (13)
Years had UC (37, 38)	7.0 ± 6.8	7.9 ± 5.6
Hemoglobin concentration, *g/L* (37, 37)	128.6 ± 22.4	129.3 ± 17.3
White cell count, $\times 10^9$/*L* (37, 37)	8.8 ± 2.6	8.0 ± 2.5
ESR, *mm/h* (27, 26)	21.1 ± 16.3	18.9 ± 15.6
CRP, *mg/L* (27, 26)	7.2 ± 7.7	10.6 ± 16.6
High ESR, n (%) (27, 26)	14 (52)	8 (31)
High CRP, n (%) (27, 26)	13 (48)	11 (42)
Full Mayo Clinic score (37, 38)	7.86 ± 2.28	8.24 ± 2.61
IBDQ score (37, 37)	134.4 ± 32.3	130.3 ± 36.3
EQ-5D score (37, 36)	78.2 ± 15.4	75.7 ± 20.4

NOTE. All values are mean ± SD unless otherwise stated, with missing values not counted. Race, pancolitis, years, hemoglobin, white cell count, ESR, CRP, IBDQ, had missing values in the range of 1 to 23. All $P > .05$ except where indicated.
CRP, C-reactive protein (high CRP >5 mg/L); ESR, erythrocyte sedimentation rate (high ESR >20 mm/h); FMT, fecal microbial transplantation; IBDQ, Inflammatory Bowel Disease Questionnaire; TNF, tumor necrosis factor; UC, ulcerative colitis.
[a]$P = .045$.
[b]$P = .044$.

(Supplementary Table 9). We evaluated factors that might predict treatment success in the FMT arm, and there was no impact of sex, age, Mayo score at baseline, disease extent, smoking history, current steroid therapy, or tumor necrosis factor antagonist therapy on treatment success (Supplementary Table 2–8).

Five patients had significant adverse events. One person in the placebo group developed worsening colitis and was admitted to hospital 3 weeks into the trial and had an

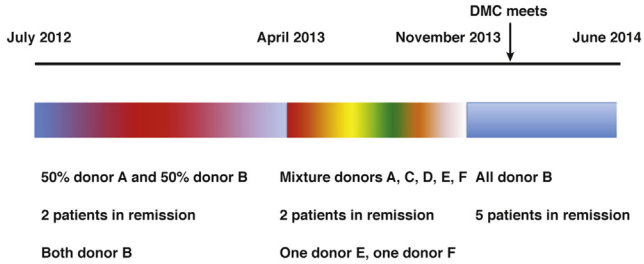


Figure 2. Description of the timeline of the use of donors through the trial. DMC, Data Monitoring Committee.

Table 2. Outcome Measures Comparing Fecal Microbial Transplantation With Placebo

Outcome	Placebo (n = 37)	FMT (n = 38)	*P* value
Clinical remission,[a] n (%)	2 (5)	9 (24)	.03
Clinical response,[b] n (%)	9 (24)	15 (39)	.16
Full Mayo score	6.34	6.09	.42
IBDQ score	149.38	152.13	.44
EQ-5D score	70.07	68.52	.99
CRP, *mg/L* (n = 17 placebo, n = 15 FMT)	3.3 ± 3.4	4.9 ± 5.9	.38
ESR, *mm/h* (n = 17 placebo, n = 15 FMT)	13.1 ± 11.2	15.9 ± 17.0	.59
Proportion with high ESR, n (%)	4 (24)	3 (20)	1.0
Proportion with high CRP, n (%)	5 (29)	2 (13)	.40
Patients with serious adverse events n (%)	2[c] (5)	3[d] (8)	1.0

NOTE. All continuous data evaluated using analysis of covariance adjusting for baseline value are mean ± SE. Full Mayo and IBDQ had missing values, which were replaced by their means. The Full Mayo, IBDQ, and EQ-5D are adjusted means for baseline, and *P* value is the significance of the randomization to placebo or FMT. All analyses are intention to treat.
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IBDQ, Inflammatory Bowel Disease Questionnaire.
[a]Defined as full Mayo Clinic score <3 and an endoscopic Mayo Clinic score = 0.
[b]Defined as a reduction in full Mayo clinic score of at least 3 points.
[c]One patient had the diagnosis changed to Crohn's colitis and one was admitted to hospital with active ulcerative colitis.
[d]Two patients had their diagnoses changed to Crohn's colitis and one was *Clostridium difficile* toxin positive at the end of therapy.

urgent colectomy. Three patients in the trial (2 in the FMT group and 1 in the placebo group) developed patchy inflammation of the colon and also rectal abscess formation, which resolved with antibiotic therapy. One patient in the FMT group with worsening abdominal discomfort tested positive for *C difficile* toxin after study exit.

We have followed up the 37 patients that received FMT in this randomized trial for 12 months, although in 5 patients, follow-up has only been for 9−11 months and 6 patients failed to respond. It is important to note that patients were informed of their randomization status once all trial information on that patient was obtained, so these data are open label. Eight of the 9 patients that were in remission at week 7 on FMT have remained in remission at week 52 without any relapse in their symptoms. Two of these patients have only been followed up for 9−11 months. Four patients have elected to stop all their UC medications (1 patient on mesalamine, 1 on long-term corticosteroids, 1 on both mesalamine and azathioprine, and 1 on infliximab) and have remained remission free to date. Three of these patients have been receiving FMT once per month (2 electively and 1 as part of the trial that was discontinued). One patient experienced a relapse of their symptoms after taking a course of antibiotic therapy and elected not to have any further FMT therapy. This patients started infliximab and still has some UC symptoms, with a partial Mayo score of 5. Eleven patients believed their symptoms were improving in the trial and when informed that they had been randomized to FMT elected to have further open-label FMT therapy for 6−12 weeks. Four of these patients went into remission with no inflammation at additional 6- to 12-week flexible sigmoidoscopy, and all have remained in remission at week 52. The remaining 20 patients had no further FMT therapy and 14 of these have completed follow-up questionnaires. One patient had a colectomy for failure of UC medical therapy, but there was no significant change in the disease status of the other participants.

Microbiome Outcomes

Patients were separated into their respective cohort (FMT treatment [active] and placebo) and community similarity (or stability) over time was computed using pairwise comparisons of week 0 to week 6. There was a statistically significant change in microbiota composition with more diversity in the treatment group compared with the placebo group at week 6 vs baseline ($P = .02$, Mann-Whitney U test) (Figure 3). When similarity was compared between the active cohort after FMT (week 6) and their respective donors, there was a statistically significant effect of the active therapy group being more similar to their donor than a control fecal sample ($P = .04$, Mann Whitney U test; Figure 4*A*). Taxonomic profiles of the donors highlighted distinct microbial differences between the 2 most common donors (A and B) used in this study (Figure 5). Notable differences include a significant enrichment for the family Lachnospiraceae and the genera *Ruminococcus* in donor B, and donor A displayed enrichment for the genera *Escherichia* and *Streptococcus*. Notably donors B and F had similar profiles and both were associated with successful FMT. As most of the responders received donor B, we evaluated whether responders in this group had more

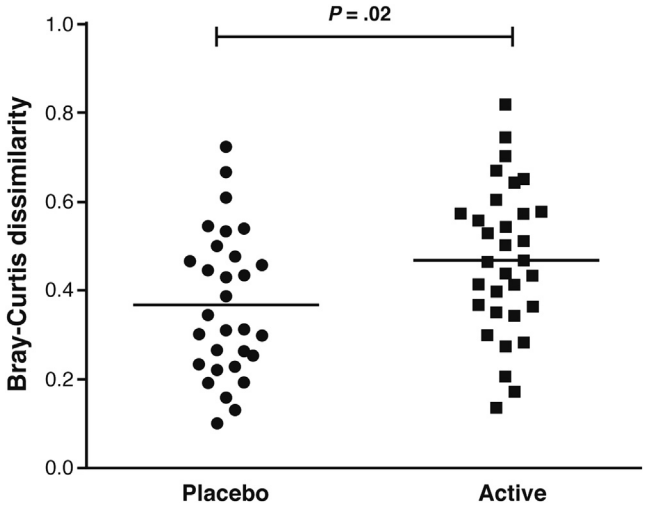


Figure 3. Similarity of microbial composition in those receiving FMT vs placebo at the start and end of the trial.

similarity at the end of therapy with donor B than non-responders. There was a trend for responders having microbiota that was more similar to donor B than non-responders, but this did not achieve statistical significance ($P = .07$, Mann Whitney U test) (Figure 4*B*).

Discussion

This is the first randomized, placebo-controlled trial, to evaluate the efficacy of FMT in active UC and suggests that this approach induces remission in a statistically significant proportion of cases. FMT may be more efficacious in patients with a recent diagnosis of UC, and this is biologically plausible, as a perturbation in the microbiome might be more easily restored early in the course of the disease. The efficacy of this approach may also be donor dependent and this may explain why some case series have shown promise,[14] and others have had disappointing results.[15] The benefit was relatively modest, but our end point for treatment success was more stringent than most trials in UC[23] and remission rates seen with FMT were consistent with a similar end point for a novel biologic therapy.[7] The secondary end points were not met in this trial. Softer end points often have higher placebo response rates,[5] and this can limit the power to show a statistically significant difference between active therapy and placebo.[24] There was also no statistically significant effect of FMT over placebo on disease-specific or general quality of life. Quality of life is multidimensional and influenced by other factors, as well as UC activity, and this might explain why there was no significant impact of FMT on these outcome measures.[24]

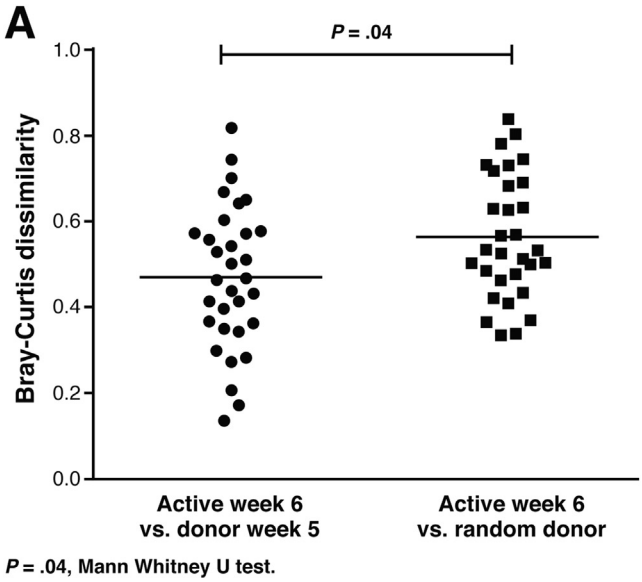


Figure 4. Similarity of FMT patients stool to the donor they received vs a control donor that they did not receive. (*A*) All patients receiving FMT compared with the donor they received vs different donor. (*B*) Similarity of patients to donor B in patients receiving that donor in those that did and did not experience remission at week 7.

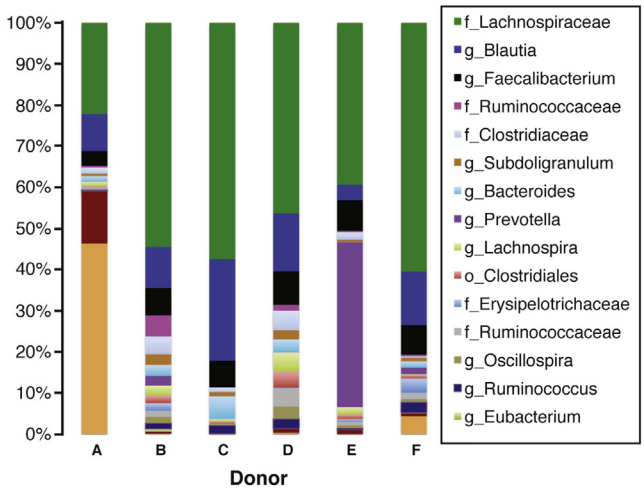


Figure 5. Taxonomic profiles of donors used in the trial.

There are a number of questions on the administration of FMT that remain unanswered. Many protocols recommend giving bowel lavage before donor feces administration and also giving antibiotics before the transplantation[25] to facilitate the colonization of microbiota from the donor. We did not adopt this in the trial FMT protocol, as currently there is a paucity of data to support this practice,[26] and if colonic lavage and/or antibiotic therapy had any impact on UC activity,[27] this would complicate the interpretation of the study. There is also uncertainty as to whether fresh or frozen–thawed fecal samples should be used for FMT.[28] Data from patients with antibiotic-resistant *C difficile* suggest that although the frozen stool may have a different microbiota distribution, both are equally efficacious,[29,30] and there was no difference in outcome seen in this study. There was a trend for frozen–thawed stool to perform better, although evaluation of this is confounded by the fact that most of the treatment successes were also related to donor B. Studies that have evaluated FMT in *C difficile* and in inflammatory bowel disease have used a variety of routes of administration, including retention enemas, colonoscopy, and the nasoduodenal route.[14,25] We chose the retention enema approach, as

one systematic review[25] of the *C difficile* literature suggested this was more effective than the nasoduodenal route, and also UC is characterized by a disorder that commences in the rectum but can extend the length of the large bowel.[4] We speculate that the dysbiosis of UC[31] is therefore likely to start in the rectum and it is plausible that this is best targeted by retention enema. It is interesting to note that patients with pancolitis due to *C difficile* colitis have been effectively treated by FMT using a retention enema.[32] We also chose the rectal route for safety reasons. A systematic review of the literature suggests that the nasojejunal approach might have more adverse events,[25] and, in a small case series,[5] all patients were given FMT by the nasojejunal route and developed a fever and rise in C-reactive protein.

A limitation of this trial is that we did not perform colonoscopy at study exit and it is possible that patients with extensive colitis have active disease beyond the limit of the flexible sigmoidoscope. It is, however, unlikely that they would be symptom free and 1 patient with extensive colitis that had successful FMT did require colonoscopy for screening purposes after exiting the trial and had no active inflammation throughout the colon to the cecum either endoscopically or histologically (Supplementary Figure 1). This is the largest randomized trial of FMT in any disease and, in addition, the number of patients enrolled in the trial is double that seen in a systematic review of all case series of FMT in UC.[14] Nevertheless, the sample size of the trial is modest and the magnitude of the effect of FMT in UC remains uncertain. The trial was also stopped early, as we did not anticipate the extent of positive effect seen at completion of the study. This may relate to the effect being donor dependent and it is notable that donor B was not available in the time leading up to the Data Monitoring and Safety Committee meeting and all patients subsequent to this were treated with donor B.

In conclusion, this is the first randomized trial to demonstrate efficacy of FMT in UC. Many questions remain, but this provides interesting data suggesting that altering the gut microbial flora may be promising for treating UC.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.04.001.

References

1. Kappelman MD, Rifas-Shiman SL, Kleinman K, et al. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. Clin Gastroenterol Hepatol 2007;5:1424–1429.
2. Danese S, Fiocchi C. Ulcerative colitis. N Engl J Med 2013;365:1713–1725.
3. Graff LA, Walker JR, Clara I, et al. Stress coping, distress, and health perceptions in inflammatory bowel disease and community controls. Am J Gastroenterol 2009; 104:2959–2969.
4. Ford AC, Moayyedi P, Hanauer SB. Ulcerative colitis. BMJ 2013;346:f432.
5. Talley NJ, Abreu MT, Achkar JP, et al. An evidence-based systematic review on medical therapies in inflammatory bowel disease. Am J Gastroenterol 2011; 106(Suppl 1):S2–S25.
6. Sandborn WJ, Rutgeerts P, Feagan BG, et al. colectomy rate comparison after treatment of ulcerative colitis with placebo or infliximab. Gastroenterology 2009;137: 1250–1260.
7. Feagan BG, Rutgeerts P, Sands BE, et al. Vedolizumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med 2013;369:699–710.
8. Danese S. New therapies for inflammatory bowel disease: from the bench to the bedside. Gut 2012; 61:918–932.
9. Torres J, Danese S, Colombel J-F. New therapeutic avenues in ulcerative colitis: thinking out of the box. Gut 2013;62:1642–1652.
10. Michail S, Durbin M, Turner D, et al. Alterations in the gut microbiome of children with severe ulcerative colitis. Inflamm Bowel Dis 2012;18:1799–1808.
11. Nell S, Suerbaum S, Josenhans C. The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. Nat Rev Microbiol 2010; 8:564–577.
12. van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent clostridium difficile. N Engl J Med 2013;368:407–415.
13. Moayyedi P, Marshall JK, Yuan Y, et al. Canadian Association of Gastroenterology position statement: fecal microbiota transplant therapy. Can J Gastroenterol 2014;28:66–68.
14. Anderson JL, Edney RJ, Whelan K. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. Aliment Pharmacol Ther 2012;36:503–516.
15. Angelberger S, Reinisch W, Makristathis A, et al. Temporal bacterial community dynamics vary among ulcerative colitis patients after fecal microbiota transplantation. Am J Gastroenterol 2013;108: 1620–1630.
16. D'Haens G, Sandborn WJ, Feagan BG, et al. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. Gastroenterology 2007;132:763–786.
17. Irvine EJ, Feagan B, Rochon J, et al. Quality of life: a valid and reliable measure of therapeutic efficacy in the treatment of inflammatory bowel disease. Gastroenterology 1994;106:287–296.
18. Konig HH, Ulshofer A, Gregor M, et al. Validation of the EuroQol questionnaire in patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol 2002;14: 1205–1215.
19. Whelan FJ, Verschoor CP, Stearns JC, et al. The loss of topography in the microbial communities of the upper respiratory tract in the elderly. Ann Am Thorac Soc 2014; 11:513–521.
20. Bartram AK, Lynch MDJ, Stearns JC, et al. Generation of multimillion-sequence 16S rRNA gene libraries from

complex microbial communities by assembling paired-end illumina reads. Appl Environ Microbiol 2011; 77:3846–3852.
21. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 2013;8:e61217.
22. Caporaso JG, Kuczynski J, Stombaugh J, et al. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 2010;7:335–336.
23. Ford AC, Achkar JP, Khan KJ, et al. Efficacy of 5-aminosalicylates in ulcerative colitis: systematic review and meta-analysis. Am J Gastroenterol 2011;106:601–616.
24. Reinisch W, Sandborn WJ, Hommes DW, et al. Adalimumab for induction of clinical remission in moderately to severely active ulcerative colitis: results of a randomised controlled trial. Gut 2011;60:780–787.
25. Kassam Z, Lee CH, Yuan Y, et al. Fecal microbiota transplantation for *Clostridium difficile* infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108:500–508.
26. Brandt LJ. American Journal of Gastroenterology Lecture: intestinal microbiota and the role of fecal microbiota transplant (FMT) in treatment of *C. difficile* infection. Am J Gastroenterol 2013;108:177–185.
27. Khan KJ, Ullman TA, Ford AC, et al. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol 2011;106:661–673.
28. Borody TJ, Brandt LJ, Paramsothy S. Therapeutic faecal microbiota transplantation: current status and future developments. Curr Opin Gastroenterol 2014;30:97–105.
29. Hamilton MJ, Weingarden AR, Sadowsky MJ, et al. Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium difficile* infection. Am J Gastroenterol 2012;107:761–767.
30. Youngster I, Russell GH, Pindar C, et al. Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium difficile* infection. JAMA 2014;312:1772–1778.
31. Rajilic-Stojanovic M, Shanahan F, Guarner F, et al. Phylogenetic analysis of dysbiosis in ulcerative colitis during remission. Inflamm Bowel Dis 2013;19:481–488.
32. Kassam Z, Hundal R, Marshall JK, et al. Fecal transplant via retention enema for refractory or recurrent *Clostridium difficile* infection. Arch Inter Med 2012; 172:191–193.

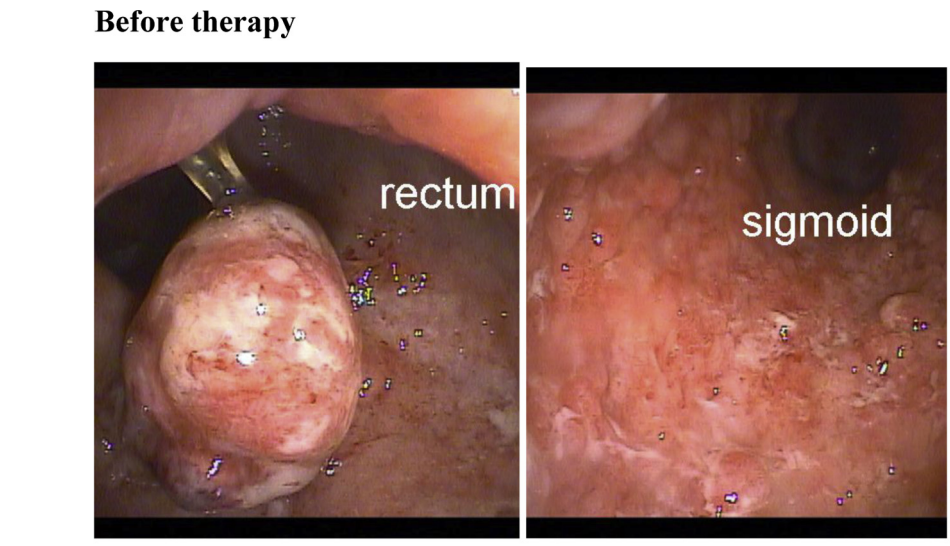
Reprint requests
Address requests for reprints to: Paul Moayyedi, MB, ChB, PhD and Christine H. Lee, MD, Division of Gastroenterology, McMaster University, Room 4W8E, 1200 Main Street West, Hamilton, Ontario, Canada L8N 3Z5. e-mail: moayyep@mcmaster.ca; fax: +1 905 521 4958.

Acknowledgments
This trial was supported by grants from Hamilton Academic Health Sciences Organization (HAHSO) and Crohn's and Colitis Canada (CCC). The authors are grateful to Lisa Dunlop and Rita Taraschi for clerical support of the trial and to Dr Ken Croitoru (chair), Josh Neufeld, and Noori Akhtar-Danesh for the services as the Data Monitoring and Safety Committee.

Author contributions: All authors contributed to the design of the study. PM and MW were responsible for the recruitment and assessment of most of the participants with support from DA, JKM, WR, and ZK. FMT was administered by CHL and CO and the fecal microbiome extraction and analyses were conducted by MGS and JL. PTK conducted all other analyses. PM wrote the initial draft of the manuscript with all authors providing interpretation of the analyses and contributions to the final draft.

Conflicts of interest
These authors disclose the following: Dr Moayyedi: Chair partly funded by an unrestricted donation given to McMaster University by AstraZeneca; received honoraria for speaking and/or serving on the advisory board for AstraZeneca, Actavis, and Shire Pharmaceuticals. Dr Marshall served as a speaker and/or served on the advisory board for Abbott/Abbvie, Actavis, Aptalis, Ferring, Janssen, Proctor & Gamble, Shire, and Takeda. Dr Reinisch served as a speaker and/or served on the advisory board for Abbott Laboratories, Abbvie, Aesca, Amgen, AM Pharma, Aptalis, Astellas, Astra Zeneca, Avaxia, Bioclinica, Biogen IDEC, Bristol-Myers Squibb, Cellerix, Chemocentryx, Celgene, Centocor, Celltrion, Danone Austria, Elan, Falk Pharma GmbH, Ferring, Galapagos, Genentech, Grünenthal, Inova, Janssen, Johnson & Johnson, Kyowa Hakko Kirin Pharma, Lipid Therapeutics, MedImmune, Millenium, Mitsubishi Tanabe Pharma Corporation, MSD, Novartis, Ocera, Otsuka, PDL, Pharmacosmos, Pfizer, Procter & Gamble, Prometheus, Robarts Clinical Trial, Schering-Plough, Setpointmedical, Shire, Takeda, Therakos, Tigenix, UCB, Vifor, Yakult, Zyngenia, and 4SC. Dr Armstrong has received speakers' fees, consulting fees, research funding, or unrestricted support for educational events from Abbott, Abbvie, Actavis, Aptalis, AstraZeneca, Cook, Cubist, Ferring, Forest, Janssen, Merck, Olympus, Pendopharm, Pentax, Shire, Takeda. and Warner-Chilcott. Dr Kassam was Chief Medical Officer for OpenBiome (after trial was completed). Dr Lee served as a speaker and/or served on the advisory board for Cubist, Merck, and Rebiotix. The remaining authors disclose no conflicts.

Funding
This work was funded by Hamilton Academic Health Sciences Organization (HAHSO) and Crohn's and Colitis Canada (CCC).



Supplementary Figure 1. Before and after endoscopic images of a patient responded to FMT that had a colonoscopy at week 20.

Supplementary Table 1. Summary of the Logistic Regression Model to Assess Independent Factors Predicting Remission

Logistic regression model:

Explanatory variables: The following are the variables used in this study and described in Table 1. The units, missing values, and imputation of missing values are described below.

Age: years; complete

Sex: 1 = male, 2 = female; complete

Race: 1 = Caucasian, 2 = Southeast Asian, 3 = other Asians, 4 = Afro-Carribean, 5 = Middle Eastern, 6 = Other; complete

Smoke: 1 = nonsmoker, 2 = former smoker; 3 = current smoker, 4 NAs changed to 1

UC < 1 year: 0 = No, 1 = Yes; complete

Pancolitis: 0 = No, 1 = Yes; 11 NAs changed to 0

Mesalamine: 1 = Yes, 2 = No; complete

Glucocorticoids: 1 = Yes, 2 = No; complete

Immunosuppressants: 1 = Yes, 2 = No; complete

Anti-TNF: 1 = Yes, 2 = No; complete

UC: years, complete

Hemoglobin: *g/L*, 1 NA replaced with mean

White cell count: 10^9 */L*, 1 NA replaced with mean

ESR: *mm/h*, 22 NAs replaced with mean

CRP: *mg/L*, 22 NAs replaced with mean

Full Mayo: score, complete

IBDQ: score, 1 NA replaced with mean

EQ5D: score, complete

Randomization: 1 = placebo, 2 = FMT, complete

LASSO: The R package "glmnet" was used on the above explanatory variables for logisitic regression. Of the 19 explanatory variables, LASSO using cross-validation selected: age, race, UC <1, pancolitis, immunosuppresants, hemoglobin, C-reactive protein, Full Mayo, and randomization. The intercept was also chosen and the dependent variable was remission.

Logistic regression: From these selected explanatory variables, we ran logisitic regression via "glm" in R and Randomization was the only statistically significant variable.

Final model: The model is summarized below.

Remission	Coefficient	SE	*P* value
Intercept	−4.554	1.503	.002
Randomization	1.692	0.821	.039

Final logistic regression model for UC remission. Null deviance 62.5, degrees of freedom 74; residual deviance 57.2, degrees of freedom 73; Akaike information criterion 61.2.
CRP, C-reactive protein; ESR, erythromycin sedimentation rate; IBDQ, Inflammatory Bowel Disease Questionnaire; LASSO, least absolute shrinkage and selection operator; NA, not answered; TNF, tumor necrosis factor.

Supplementary Table 2. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of Sex on Remission Rates With Fecal Microbiota Transplantation

	Sex		
	Male	Female	Total
Week 6 remission			
No	14	13	27
Yes	4	5	9
% Within sex	22.2	27.8	
Total	18	18	36

χ^2 Tests	Value	df	Asymptomatic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson χ^2	.148[a]	1	.700		
Continuity correction[b]	.000	1	1.000		
Likelihood ratio	.148	1	.700		
Fisher's exact test				1.000	.500
Linear-by-linear association	.144	1	.704		
No. of valid cases	36				

df, degrees of freedom.
[a]Two cells (50.0%) have expected count <5. The minimum expected count is 4.50.
[b]Computed only for a 2 × 2 table.

Supplementary Table 3. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of Age on Remission Rates With Fecal Microbiota Transplantation

	Age as a category			
	<35 years old	35–50 years old	>50 years old	Total
Week 6 remission				
No	7	9	11	27
Yes	4	4	1	9
% Within age as a category	36.4	30.8	8.3	
Total	11	13	12	36

χ^2 Tests	Value	df	Asymptomatic significance (2-sided)
Pearson χ^2	2.766[a]	2	.251
Likelihood ratio	3.135	2	.209
Linear-by-linear association	2.384	1	.123
No. of valid cases	36		

df, degrees of freedom.
[a]Three cells (50.0%) have expected count <5. The minimum expected count is 2.75.

Supplementary Table 4. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of Severity of Disease at Baseline on Remission Rates With Fecal Microbiota Transplantation

	Baseline Mayo score as a category			
	Score = 4 to 5	Score = 6 to 9	Score >9	Total
Week 6 remission				
No	5	12	10	27
Yes	4	3	2	9
% Within baseline Mayo score as a category	44.4	20.0	16.7	
Total	9	15	12	36

χ^2 tests	Value	df	Asymptomatic significance (2-sided)
Pearson χ^2	2.459[a]	2	.292
Likelihood ratio	2.297	2	.317
Linear-by-linear association	1.890	1	.169
No. of valid cases	36		

[a]Three cells (50.0%) have expected count <5. The minimum expected count is 2.25.

Supplementary Table 5. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of Disease Extent on Remission Rates With Fecal Microbiota Transplantation

	Extent of UC		
	Left-sided colitis	Extensive colitis	Total
Week 6 remission			
No	12	13	25
Yes	3	6	9
% Within distribution of UC	20.0	31.6	
Total	15	19	34

χ^2 Tests	Value	df	Asymptomatic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson χ^2	.577[a]	1	.447		
Continuity correction[b]	.136	1	.713		
Likelihood ratio	.588	1	.443		
Fisher's exact test				.697	.360
Linear-by-linear association	.560	1	.454		
No. of valid cases	34				

[a]One cells (25.0%) have expected count <5. The minimum expected count is 3.97.
[b]Computed only for a 2 × 2 table.

Supplementary Table 6. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of Smoking on Remission Rates With Fecal Microbiota Transplantation

	Smoke				
	Nonsmoker	Former smoker	Smoker	NR	Total
Week 6 remission					
No	14	10	2	1	27
Yes	5	3	1	0	9
% Within smoke	26.3	23.1	33.3		
Total	19	13	3	1	36

χ^2 Tests	Value	df	Asymptomatic significance (2-sided)
Pearson χ^2	.488[a]	3	.922
Likelihood ratio	.723	3	.868
No. of valid cases	36		

[a]Six cells (75.0%) have expected count <5. The minimum expected count is .25.

Supplementary Table 7. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of Current Steroid Therapy on Remission Rates With Fecal Microbiota Transplantation

	Current therapy steroid		
	Yes	No	Total
Week 6 remission			
No	11	16	27
Yes	3	6	9
% Within current therapy steroid	21.4	27.3	
Total	14	22	36

χ^2 Tests	Value	df	Asymptomatic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson χ^2	.156[a]	1	.693		
Continuity correction[b]	.000	1	1.000		
Likelihood ratio	.158	1	.691		
Fisher's exact test				1.000	.506
Linear-by-linear association	.152	1	.697		
No. of valid cases	36				

[a]One cells (25.0%) have expected count <5. The minimum expected count is 3.50.
[b]Computed only for a 2 × 2 table.

Supplementary Table 8. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of current anti-TNF therapy on remission rates with Fecal Microbiota Transplantation

	Current therapy biologic		
	Yes	No	Total
Week 6 remission			
No	3	24	27
Yes	1	8	9
% Within current therapy biologic	25.0	25.0	
Total	4	32	36

χ^2 Tests	Value	df	Asymptomatic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson χ^2	.000[a]	1	1.000		
Continuity correction[b]	.000	1	1.000		
Likelihood ratio	.000	1	1.000		
Fisher's exact test				1.000	.702
Linear-by-linear association	.000	1	1.000		
No. of valid cases	36				

[a]Two cells (50.0%) have expected count <5. The minimum expected count is 1.00.
[b]Computed only for a 2 × 2 table.

Supplementary Table 9. Factors That Predict Remission in Evaluable Patients Allocated to Fecal Microbiota Transplantation: Impact of Fresh vs Frozen Stool on Remission Rates of Fecal Microbiota Transplantation

Week 6 remission * stool type cross tabulation

	Stool type			
	Fresh	Frozen	Mixed	Total
Week 6 remission				
No	14	12	1	27
Yes	1	8	0	9
% Within stool type	6.7	40.0	0.0	
Total count	15	20	1	36

χ^2 Tests	Value	df	Asymptomatic significance (2-sided)
Pearson χ^2	5.422[a]	2	.066
Likelihood ratio	6.220	2	.045
Linear-by-linear association	3.070	1	.080
No. of valid cases	36		

[a]Three cells (50.0%) have expected count <5. The minimum expected count is .25.

Supplementary Table 10. Summary of the Mean Change From Baseline Between Placebo and Fetal Microbiota Transplantation Groups at Week 7 for Continuous Variables (Equal Variances Not Assumed)

Variable	Placebo	FMT	*P* value
Mayo score	−1.29 ± 2.73	−2.06 ± 3.33	.30
IBDQ score	10.93 ± 25.86	18.69 ± 37.54	.33
EQ-5D score	2.00 ± 15.07	−3.71 ± 18.20	.18
CRP, *mg/L*	−0.275 ± 6.73	−0.59 ± 25.86	.86
ESR, *mm/h*	−3.06 ± 11.95	−3.07 ± 7.53	1.00

CRP, C-reactive protein; ESR, erythromycin sedimentation rate; IBDQ, Inflammatory Bowel Disease Questionnaire.