Multidonor intensive faecal microbiota transplantation for active ulcerative colitis: a randomised placebo-controlled trial

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Summary

Background The intestinal microbiota is implicated in the pathogenesis of ulcerative colitis. Faecal microbiota transplantation is a novel form of therapeutic microbial manipulation, but its efficacy in ulcerative colitis is uncertain. We aimed to establish the efficacy of intensive-dosing, multidonor, faecal microbiota transplantation in active ulcerative colitis.

Methods We conducted a multicentre, double-blind, randomised, placebo-controlled trial at three hospitals in Australia. We randomly allocated patients with active ulcerative colitis (Mayo score 4–10) in a 1:1 ratio, using a pre-established randomisation list, to either faecal microbiota transplantation or placebo colonoscopic infusion, followed by enemas 5 days per week for 8 weeks. Patients, treating clinicians, and other study staff were unaware of the assigned treatment. Faecal microbiota transplantation enemas were each derived from between three and seven unrelated donors. The primary outcome was steroid-free clinical remission with endoscopic remission or response (Mayo score ≤2, all subscores ≤1, and ≥1 point reduction in endoscopy subscore) at week 8. Analysis was by modified intention-to-treat and included all patients receiving one study dose. We performed 16S rRNA stool analysis to assess associated microbial changes. This trial is registered with ClinicalTrials.gov, number NCT01896635. The trial has ended; this report presents the final analysis.

Findings From November, 2013, to May, 2015, 85 patients were enrolled to our trial, of whom 42 were randomly assigned faecal microbiota transplantation and 43 were allocated placebo. One patient assigned faecal microbiota transplantation and three allocated placebo did not receive study treatment and were excluded from the analysis. The primary outcome was achieved in 11 (27%) of 41 patients allocated faecal microbiota transplantation versus three (8%) of 40 who were assigned placebo (risk ratio 3·6, 95% CI 1·1–11·9; p=0·021). Adverse events were reported by 32 (78%) of 41 patients allocated faecal microbiota transplantation and 33 (83%) of 40 who were assigned placebo; most were self-limiting gastrointestinal complaints, with no significant difference in number or type of adverse events between treatment groups. Serious adverse events occurred in two patients assigned faecal microbiota transplantation and in one allocated placebo. Microbial diversity increased with and persisted after faecal microbiota transplantation. Several bacterial taxa were associated with clinical outcome; in particular, the presence of *Fusobacterium* spp was associated with lack of remission.

Interpretation Intensive-dosing, multidonor, faecal microbiota transplantation induces clinical remission and endoscopic improvement in active ulcerative colitis and is associated with distinct microbial changes that relate to outcome. Faecal microbiota transplantation is, thus, a promising new therapeutic option for ulcerative colitis. Future work should focus on precisely defining the optimum treatment intensity and the role of donor–recipient matching based on microbial profiles.

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Introduction

A substantial proportion of patients with ulcerative colitis are resistant or intolerant to standard drug treatment. In view of the pathophysiological role of the enteric microbiota in inflammatory bowel disease,[1] microbial manipulation could be an alternative therapeutic approach. Evidence for use of antibiotics, probiotics, and prebiotics is inconsistent in active ulcerative colitis.[2] By contrast, faecal microbiota transplantation entails transfer of the entire enteric microbiota and might have augmented capacity to correct the complex microbial disturbances associated with ulcerative colitis.[3]

Faecal microbiota transplantation is highly efficacious for treatment of *Clostridium difficile* infection,[4] with the ability to restore healthy microbial ecology. However, enteric microbiota alterations in ulcerative colitis might be more resistant to restoration than those in *C difficile* infection, possibly requiring more intense and prolonged therapy. To date, two controlled trials evaluating faecal microbiota transplantation in ulcerative colitis[5,6] have differed in their infusion protocols, entailed at most weekly treatment, and provided conflicting outcomes. In both studies, one donor was used for every patient, with findings of one

Research in context

Evidence before this study

We searched PubMed, MEDLINE, the Cochrane Library, BioMed Central, and Embase from database inception to the end of February, 2016, with terms including "fecal microbiota transplantation", "fecal transplant*", "faecal transplant*", "FMT", "inflammatory bowel disease", "IBD", "ulcerative colitis", and "UC". We also searched major conference proceedings to identify abstract publications. We included reports of clinical efficacy or safety of faecal microbiota transplantation in inflammatory bowel disease in human beings. We excluded studies if data for subtypes of inflammatory bowel disease (eg, ulcerative colitis, Crohn's disease, and pouchitis) were not reported individually or if they included patients who had co-infection with *Clostridium difficile* or other pathogens that were not reported separately. Our search yielded 5938 results, of which 27 were clinical studies in ulcerative colitis. These reports included 292 patients with ulcerative colitis, with pooled clinical remission of 36%. The quality of the evidence was low, comprising primarily small retrospective or uncontrolled prospective studies, with only two single-centre randomised controlled trials. The two randomised trials differed in route of administration of faecal microbiota transplantation (enema or nasoduodenal), number of infusions (one infusion every week for 6 weeks or two infusions 3 weeks apart), and had conflicting outcomes. As such, the role of faecal microbiota transplantation in ulcerative colitis was unclear.

Added value of this study

Our three-centre study is, to date, the largest randomised controlled trial of faecal microbiota transplantation in inflammatory bowel disease. It included the most intensive dosing schedule (40 infusions over 8 weeks) reported to date and is the first to utilise multidonor infusions for faecal microbiota transplantation. Therapeutic effect was assessed with a composite primary endpoint of steroid-free clinical remission together with endoscopic remission or response at week 8. 27% (11 of 41) of patients assigned faecal microbiota transplantation met the primary endpoint, compared with 8% (three of 40) of those allocated placebo. No significant difference in adverse events was noted between the two treatment groups. Microbial diversity increased with and persisted 8 weeks after faecal microbiota transplantation, and specific bacterial taxa were associated with clinical outcomes. Our findings suggest multidonor, intensive-dosing faecal microbiota transplantation is an effective treatment alternative in active ulcerative colitis.

Implications of all the available evidence

Therapeutic microbial manipulation using faecal microbiota transplantation might offer a complementary or alternative treatment option to currently available immune-based therapies for patients with active ulcerative colitis. Additional studies are necessary to further define the underlying microbiological mechanisms and predictors of response, and to optimise and personalise microbial-based treatments in ulcerative colitis.

study showing a possible difference in outcome related to individual donors.[5]

In the Fecal Microbiota Transplantation for Chronic Active Ulcerative Colitis (FOCUS) study, we aimed to assess the efficacy and safety of multidonor, intensive-dosing, faecal microbiota transplantation in patients with active ulcerative colitis.

Methods

Study design and participants

We conducted a randomised, double-blind, placebo-controlled trial of multidonor, intensive-dosing faecal microbiota transplantation in patients with active ulcerative colitis (appendix p 10). Patients aged 18–75 years with ulcerative colitis for greater than 3 months were enrolled at three Australian hospital centres: St Vincent's Hospital, Sydney; Bankstown-Lidcombe Hospital, Sydney; and Nambour General Hospital, Nambour.

We obtained written informed consent from all patients before screening. Inclusion criteria for patients are detailed in the appendix (p 2). Patients had to have clinically and endoscopically active ulcerative colitis, with a total Mayo score[7] of 4–10. The Mayo endoscopy subscore had to be 1 or greater and physician's global assessment subscore 2 or less. We included any disease extent except proctitis confined to the distal 5 cm. Exclusion criteria for patients are detailed in the appendix (pp 3, 4). We excluded individuals with indeterminate colitis, major comorbid chronic disease, major food allergy, irritable bowel syndrome, or a history of bowel cancer, those who were pregnant, and patients who had previous gastrointestinal surgery apart from appendicectomy more than 3 months before the study. We excluded gastrointestinal infection at study entry, including parasitic and *C difficile* infections.

We permitted the following drugs as long as the dose was stable preceding enrolment: oral 5-aminosalicylates (stable dose for 4 weeks); thiopurines and methotrexate (on medication for ≥90 days and dose stable for 4 weeks); and oral prednisone (dose ≤20 mg daily and stable for 2 weeks). During the study, patients remained on the same dose of 5-aminosalicylate, thiopurine, and methotrexate. For oral prednisone, we did a mandatory taper of up to 2·5 mg per week so that patients would be steroid-free by week 8. We did not allow rectal therapies, including: corticosteroids or 5-aminosalicylate (for 2 weeks before enrolment); antibiotics or probiotics (for 4 weeks before enrolment); and biological therapies or calcineurin inhibitors (for 12 weeks before enrolment).

Screening of study donors has been described previously.[8] We recruited healthy donors by advertisement

See Online for appendix

and used rigorous selection criteria and screening investigations (appendix pp 5–7). The Centre for Digestive Diseases in Sydney recruited and screened donors and produced study infusions. Donors provided written informed consent. The Centre for Digestive Diseases had no role in recruitment or assessment of study patients and did not have access to study patient data.

Randomisation and masking

Patients were randomised centrally by the Centre for Digestive Diseases after screening in a 1:1 ratio to either faecal microbiota transplantation or placebo, using a pre-established computer-generated randomisation list with permutated blocks of four and stratified for study site and concomitant corticosteroid use. Patients and investigators were unaware of treatment allocation. To ensure masking, we added food colourant and odourant to all study infusions (investigational and placebo) to replicate faecal colour and odour, respectively, and all study infusions were 150 mL. We assessed the placebo preparation on a test group of patients before the trial, who did not identify it as a placebo. Study investigators who played a part in patients' assessment did not see the investigational product at any time. A non-study investigator gave the initial colonoscopic infusion after the study investigator had done the endoscopic scoring assessment and then left the room; subsequent frozen infusions were dispensed to patients in opaque bags. Patients randomly allocated placebo were eligible for open-label faecal microbiota transplantation either after the double-blind study period had ended at week 8 or if they were withdrawn by the study investigator before week 8 because of treatment failure.

Procedures

We prepared investigational infusions from the blended stool of between three and seven donors, to increase microbial diversity. Each patient assigned faecal microbiota transplantation received all their infusions from the same donor batch to ensure consistency and reproducibility of the infused faecal microbiota. We randomly selected the number and specific donors for each batch based on availability.

Donors had to provide faeces within 4 h of a bowel movement, which was inspected visually for suitability (formed stool, no blood or mucous). We homogenised all donor stool for a given batch on each day in a biosafety cabinet. We added 37·5 g of blended stool to isotonic saline then filtered it to formulate every investigational infusion. Placebo infusions comprised isotonic saline. We added brown food colourant, odourant, and glycerol cryoprotectant (concentration 10%) to all study infusions (investigational and placebo). The volume of each infusion was 150 mL. We stored all infusions at –80°C until dispensation to patients at fortnightly study visits for home freezer storage at –20°C before daily administration.

Before the trial began, we tested the antimicrobial potential of the placebo against common gastrointestinal microorganisms (*Escherichia coli*, *Escherichia faecalis*, *Lactobacillus casei*, *Clostridium perfringens*, *Bifidobacterium longum*, and *Bacteroides fragilis*) and compared the placebo with incubation in 0·9% saline (control). We noted no significant difference in the viability of these micro-organisms.

We did identical stool and blood screening investigations in patients and donors (appendix p 7). At the start of the study, we performed colonoscopy after bowel preparation and administered the initial infusion directly into the terminal ileum or caecum. The next day, patients began self-administration of enemas five times per week (5 days on and 2 days off) for 8 weeks, for a total of 40 enemas. We scheduled study visits for weeks 1, 2, 4, 6, and 8. At each visit, study investigators assessed patients for bowel frequency and bleeding, gastrointestinal symptoms, adverse events, and medication changes. Adverse events were assessed with the Common Terminology Criteria for Adverse Events (CTCAE), version 4.03. We calculated a partial Mayo score for bowel frequency, rectal bleeding, and physician's global assessment.[7] Patients kept a log of enema administration, which we reviewed to monitor treatment accountability. At 8 weeks, we reassessed mucosal activity with sigmoidoscopy to at least the colonic segment of previous worst inflammation.

After the 8-week masked study period, we offered patients who were assigned placebo 8 weeks of open-label faecal microbiota transplantation (enemas five times per week), without the initial colonoscopic infusion. We repeated sigmoidoscopy examination after 8 weeks of open-label faecal microbiota transplantation. We did a final review of patients 8 weeks after completion of final enema treatment (masked or open-label).

We assessed the site of worst inflammation at every endoscopy procedure (which were done at the start of treatment, at week 8, and [if applicable] at the end of open-label treatment) using the Mayo endoscopy subscore[7] and the Ulcerative Colitis Endoscopic Index of Severity (UCEIS) score.[9] Five of us (SP, AJW, RWLL, SC, and MAK), who are inflammatory bowel disease subspecialist gastroenterologists, conducted a central blinded review and consensus scoring of all endoscopic images.

We collected blood and stool samples for biochemical and microbiological analyses at weeks 4 and 8 of masked and (if applicable) open-label study treatment, and at the final review 8 weeks after completion of masked or open-label treatment. At these same timepoints, we assessed quality of life with the Inflammatory Bowel Disease Questionnaire (IBDQ).[10] We did not do clinical and stool microbiological analyses planned for 1 year after trial completion, for logistical reasons.

We performed molecular microbiological analyses of faecal samples from patients, individual donors, and faecal microbiota transplantation batches

for gastrointestinal microbial community profiling. We obtained samples (masked and open-label) for these analyses from all study patients recruited at two of the three study centres (St Vincent's Hospital, Sydney; and Bankstown-Lidcombe Hospital, Sydney). We analysed faecal samples from all individual donors and faecal microbiota transplantation infusion batches. We stored samples at –80°C. We extracted faecal bacterial DNA using the Mobio PowerViral Environmental RNA/DNA Isolation kit (Mobio Laboratories, Carlsbad, CA, USA). We amplified the 16S rRNA gene fragment using F27 and 519R primers (Sigma-Aldrich, St Louis, MO, USA) then did high-throughput sequencing of this gene fragment on an Illumina MiSeq platform (2×300 base pairs chemistry; Illumina, San Diego, CA, USA) to ascertain microbiota diversity and abundance. We analysed raw sequences using mothur software, version 1.36.0,[11] which included alignment of sequences with a SILVA reference (release 123),[12] removal of singletons, chimera checking using UCHIME,[13] and classification with the RDP training set version 14 (trainset14_032015).[14] We did statistical tests on counts and relative abundances.

Outcomes

The primary outcome was a composite of steroid-free clinical remission and endoscopic remission or response at week 8, which we defined as a total Mayo score of 2 or less, with all Mayo subscores of 1 or less, and at least a 1 point reduction from baseline in the endoscopy subscore. Secondary outcomes were: steroid-free clinical remission (defined as combined Mayo subscores of 1 or less for rectal bleeding plus stool frequency); steroid-free clinical response (defined as either a decrease of 3 points or more on the Mayo score, a 50% or greater reduction from baseline in combined rectal bleeding plus stool frequency Mayo subscores, or both); steroid-free endoscopic response (defined as a Mayo endoscopy subscore of 1 or less, with a reduction of at least 1 point from baseline); steroid-free endoscopic remission (defined as a Mayo endoscopy subscore of 0); quality of life (assessed with the IBDQ);[10] and safety (assessed by adverse events).

Statistical analysis

Based on limited available data[15] and anecdotal experience at the time the study was designed, we predicted remission would be 60% with faecal microbiota transplantation and 15% with placebo; we estimated the proportion of dropouts at 30%. We planned 40 patients per group for recruitment, to ensure a greater than 80% probability of showing a difference between treatment groups, with a two-sided α of 0·05 on modified intention-to-treat analysis.

The modified intention-to-treat analysis included all patients who received at least one study dose. Patients who needed increased therapy, breached study protocol, failed to cease corticosteroids by week 8, or terminated the study for any reason were deemed treatment failures. We assigned missing and incomplete data the worst value in the cohort for statistical analyses. We also did per-protocol analyses, including all patients who completed the 8-week course of masked study therapy without protocol breach. We did no interim analyses.

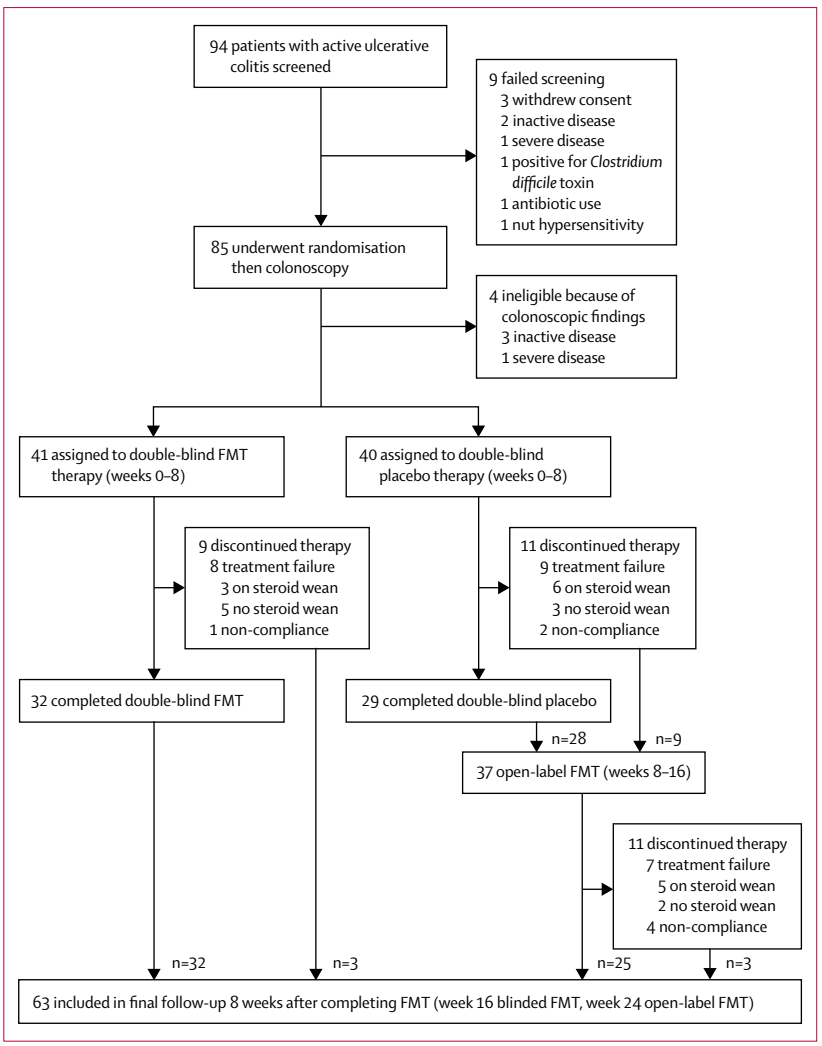

Figure 1: **Trial profile**
FMT=faecal microbiota transplantation.

We calculated descriptive statistics for all variables. We expressed normally distributed continuous data as mean (SD) and analysed them with the unpaired *t* test. We expressed data not normally distributed as median (IQR) and analysed them with the Wilcoxon rank sum test. We assessed categorical data with the χ^2 test and Fisher's exact test. We expressed results as risk ratios (RRs) with 95% CIs. We judged a two-sided p value less than 0·05 significant. We did statistical analyses with SPSS version 23.0.

To assess the gastrointestinal microbiota, we did diversity (α and phylogenetic) and statistical analyses—including principal component analysis, CLUSTER with SIMPROF testing, permutational MANOVA (PERMANOVA), and

	Faecal microbiota transplantation (n=41)	Placebo (n=40)
Age (years)	35·6 (27·8–48·9)	35·4 (27·7–45·6)
Male	22 (54%)	25 (63%)
Female	19 (46%)	15 (38%)
White ethnic origin	27 (66%)	27 (68%)
Non-smoker	23 (56%)	21 (53%)
Disease duration (years)	5·8 (3·4–9·0)	5·8 (2·7–9·4)
Duration <1 year	2 (5%)	2 (5%)
Disease extent		
Proctitis	4 (10%)	8 (20%)
Left-sided colitis	28 (68%)	20 (50%)
Pancolitis	9 (22%)	12 (30%)
Concomitant drugs		
None	9 (22%)	6 (15%)
Oral 5-aminosalicylate	26 (63%)	28 (70%)
Oral immunomodulator	20 (49%)	15 (38%)
Oral steroids	9 (22%)	11 (28%)
Previous anti-TNF therapy	9 (22%)	6 (15%)
Previous other biological therapy	2 (5%)	0 (0%)
Total Mayo score*	8 (6–9)	8 (6–9)
Mayo endoscopic subscore*		
1	1 (2%)	7 (18%)
2	27 (66%)	15 (38%)
3	13 (32%)	18 (45%)
UCEIS score†	4 (3·5–5·5)	4 (3–5)
IBDQ score‡	123 (99–157)	119 (109–149)
Faecal calprotectin (ug/g)	705 (226–1220)	505 (193–1475)
Erythrocyte sedimentation rate (mm/h)	14 (5·5–29·5)	10 (5–20)
C-reactive protein (mg/L)	2·6 (1·0–7·1)	2·9 (0·8–5·8)
White-cell count (×10⁹ cells per L)	7·8 (6·2–9·7)	8·0 (6·3–9·9)
Neutrophil count (×10⁹ cells per L)	4·8 (3·5–6·9)	5·7 (3·7–6·7)
Haemoglobin (g/L)	134 (129–143)	136 (127–148)
Platelet count (×10⁹ cells per L)	299 (248–352)	306 (251–362)
Albumin (g/L)	46 (43–48)	45 (43–48)

Data are number of patients (%) or median (IQR). IBDQ=Inflammatory Bowel Disease Questionnaire. TNF=tumour necrosis factor. UCEIS=Ulcerative Colitis Endoscopic Index of Severity. *The total Mayo score ranges from 0 to 12, and subscores from 0 to 3, with higher scores indicating more severe disease. †The UCEIS score ranges from 0 to 8, with a higher score indicating greater severity of endoscopic disease. ‡The IBDQ score ranges from 32 to 224, with a higher score indicating better quality of life.

Table 1: **Baseline patients' characteristics**

PERMDISP—using mothur (version 1.36.0) and Primer-E (version 6) software. We did linear discriminant analysis effect size analyses[16] using the Galaxy web application.[17]

For more on the **Galaxy web application** see http://usegalaxy.org

Our investigator-initiated study was approved by the St Vincent's Hospital Sydney Human Research Ethics Committee (HREC/13/SVH/69) and registered with ClinicalTrials.gov (NCT01896635) and the Australian Therapeutic Goods Administration Clinical Trial Notification Scheme (2013/0523).

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 130 donors who were initially prescreened by telephone, 16 (12%) passed all screening criteria and investigations, of whom 14 ultimately served as study donors. Three to seven individual donors contributed to each of the 21 batches for faecal microbiota transplantation that were used in the study.

Between November, 2013, and May, 2015, 94 patients were recruited and screened for the trial, after meeting initial telephone prescreening criteria. Of these, 85 individuals were randomly allocated either faecal microbiota transplantation (n=42) or placebo (n=43). 81 patients received at least one dose of study treatment (41 assigned faecal microbiota transplantation, 40 allocated placebo) and were included in the modified intention-to-treat analysis (figure 1). The two study groups were well matched for baseline demographic and clinical characteristics (table 1). However, by chance, significantly more patients allocated placebo had the mildest endoscopic disease (Mayo score 1) severity; assessment for residual confounding by multivariate analysis was limited by the sample size.

Table 2 presents outcome data at week 8. 11 (27%) of 41 patients assigned faecal microbiota transplantation and three (8%) of 40 allocated placebo achieved the primary outcome on modified intention-to-treat analysis (RR 3·6, 95% CI 1·1–11·9; p=0·021; figure 2A, 2B; appendix p 11). By per-protocol analysis, 11 (34%) of 32 patients treated with faecal microbiota transplantation and three (10%) of 29 patients receiving placebo achieved the primary outcome (3·3, 1·0–10·7; p=0·026). On logistic regression analysis, the primary endpoint remained significant while considering disease extent, severity, and concomitant medication use (data not shown).

At week 8, steroid-free clinical remission was reported in 18 (44%) of 41 patients assigned faecal microbiota transplantation compared with eight (20%) of 40 allocated placebo (RR 2·2, 95% CI 1·1–4·5; p=0·021); steroid-free clinical response was achieved, respectively, by 22 (54%) of 41 and nine (23%) of 40 patients (2·4, 1·3–4·5; p=0·004; table 2; appendix p 11). Steroid-free endoscopic response was noted in 13 (32%) of 41 patients assigned faecal microbiota transplantation compared with four (10%) of 40 allocated placebo (RR 3·2, 95% CI 1·1–8·9; p=0·016); however, endoscopic remission did not differ between study groups (five [12%] of 41 *vs* three [8%] of 40, respectively; RR 1·6, 95% CI 0·4–6·4; p=0·48; table 2; appendix p 12). Endoscopic outcomes were similar with UCEIS scoring (appendix p 13).

Week 4 clinical assessments showed that clinical response was significantly greater in patients allocated faecal microbiota transplantation than in those assigned placebo (17 [41%] of 41 *vs* five [13%] of 40; RR 3·3, 95% CI 1·4–8·1; p=0·003) but clinical remission did not differ significantly between study groups at this timepoint (12 [29%] of 41 *vs* five [13%] of 40, respectively; RR 2·3, 95% CI 0·9–6·0; p=0·064; appendix p 14). At week 8, the total Mayo score and decrease in total Mayo score differed significantly between study groups; however, IBDQ score and inflammatory markers did not differ significantly (appendix p 8).

37 patients assigned placebo proceeded to open-label faecal microbiota transplantation, of whom ten (27%) met criteria for the primary endpoint on completion of open-label treatment. 17 (46%) of 37 were in clinical remission and eight (22%) had endoscopic remission after 8 weeks of open-label treatment (figure 2C, 2D).

Post-hoc exploratory analyses were done to assess factors associated with the primary outcome in patients assigned faecal microbiota transplantation (appendix p 9). No relation was noted between the primary outcome and anatomical disease extent (p=0·16). Endoscopic severity was associated inversely with the primary outcome (p=0·020). No patient who entered the study while on corticosteroids achieved the primary outcome at the end of masked treatment, but one patient on corticosteroids at the start of open-label faecal microbiota transplantation met the primary outcome on completion of treatment.

63 patients attended the final study follow-up visit 8 weeks after completion of double-blind or open-label faecal microbiota transplantation. Of 35 patients in clinical remission at completion of faecal microbiota transplantation (masked or open-label), 23 (66%) remained in clinical remission at final follow-up. Five patients who were not in clinical remission at completion of either masked or open-label faecal microbiota transplantation had continued improvement after cessation of treatment and were in clinical remission at final follow-up without any additional treatment. 20 patients needed an escalation in treatment for ulcerative colitis before follow-up at 8 weeks after either masked or open-label faecal microbiota transplantation.

Nine (22%) of 41 patients assigned faecal microbiota transplantation and 11 (28%) of 40 allocated placebo (p=0·56) discontinued treatment or had protocol failure before week 8 (figure 1). Reasons for study discontinuation included symptom worsening on steroid reduction (three faecal microbiota transplantation *vs* six placebo), symptom persistence or progression in the absence of steroid reduction (five *vs* three), and non-compliance (one *vs* two). 11 patients who began open-label faecal microbiota transplantation discontinued study treatment: five because of symptom worsening on steroid reduction, two for symptom persistence or progression in the absence of steroid reduction, and four due to non-compliance.

	Faecal microbiota transplantation (n=41)	Placebo (n=40)	Risk ratio (95% CI)	p value
Primary outcome				
Steroid-free clinical remission and endoscopic remission or response*	11 (27%)	3 (8%)	3·6 (1·1–11·9)	0·021
Secondary outcomes				
Steroid-free clinical remission†	18 (44%)	8 (20%)	2·2 (1·1–4·5)	0·021
Steroid-free clinical response‡	22 (54%)	9 (23%)	2·4 (1·3–4·5)	0·004
Steroid-free endoscopic remission§	5 (12%)	3 (8%)	1·6 (0·4–6·4)	0·48
Steroid-free endoscopic response¶	13 (32%)	4 (10%)	3·2 (1·1–8·9)	0·016

*Total Mayo score ≤2, with all subscores ≤1, and ≥1 point reduction from baseline in endoscopy subscore. †Combined Mayo subscores of ≤1 for rectal bleeding plus stool frequency. ‡Decrease of ≥3 points or ≥50% reduction from baseline (or both) in combined Mayo subscores for rectal bleeding plus stool frequency. §Mayo endoscopy subscore 0. ¶Mayo endoscopy subscore ≤1, with ≥1 point reduction from baseline.

Table 2: **Primary and secondary outcomes at week 8**

32 (78%) of 41 patients assigned faecal microbiota transplantation and 33 (83%) of 40 allocated placebo had at least one adverse event during the 8-week period of masked treatment, with no significant difference between study groups in number or type of adverse events (table 3). The most common adverse events were self-limiting gastrointestinal complaints.

Six serious adverse events occurred during study treatment: two in patients assigned faecal microbiota transplantation, one in a patient allocated placebo, and three in patients who progressed to open-label faecal microbiota transplantation (table 3). One patient with refractory ulcerative colitis who was assigned faecal microbiota transplantation withdrew at week 2 because of clinical and endoscopic deterioration (change in Mayo endoscopic subscore from 2 to 3, and in UCEIS score from 5 to 7) and underwent colectomy. One patient with moderately severe colitis who was assigned faecal microbiota transplantation remained unwell at week 3, withdrew from the study, and was admitted for intravenous corticosteroid therapy. One patient with moderately severe colitis who was allocated placebo withdrew at week 3 and needed hospitalisation. Three patients who were assigned placebo progressed to open-label faecal microbiota transplantation but failed to improve and needed hospitalisation for intravenous corticosteroids and anti-tumour necrosis factor therapy.

No individual donor or donor batch was associated significantly with the primary outcome or serious adverse events, although the study was not powered to evaluate this possibility. One donor (D054) seemed to be associated with benefit; in a post-hoc analysis, 14 (37%) of 38 patients treated with faecal microbiota transplantation that contained a sample from this donor (blinded and open-label) met criteria for the primary outcome compared with seven (18%) of 40 patients whose faecal microbiota transplantation did not include a sample from this donor (p=0·054).

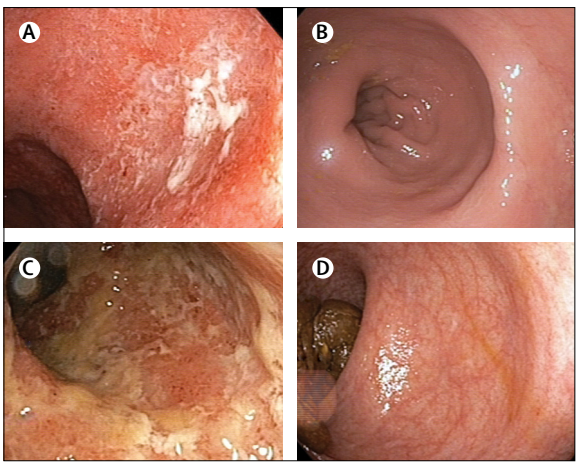

Figure 2: **Case examples of primary outcome after faecal microbiota transplantation**

(A, B) 37-year-old woman with a 4-year history of left-sided ulcerative colitis and acute colitis (diarrhoea six times per day with bleeding) despite maximum oral and topical 5-aminosalicylate treatment. (A) Baseline endoscopic appearance of 25 cm rectosigmoid active colitis; total Mayo score 8, endoscopic subscore 2. (B) Endoscopic appearance at end of week 8 after masked faecal microbiota transplantation; total Mayo score 0, endoscopic subscore 0. The female patient remained in clinical remission at final study follow-up, 8 weeks after completion of masked faecal microbiota transplantation. (C, D) 28-year-old woman with a 7-year history of extensive ulcerative colitis. The patient had failed treatment with mesalazine, probiotics, and adalimumab. She was maintained on azathioprine and allopurinol and was dependent on oral steroids (budesonide 9 mg/day). At study entry, the patient had diarrhoea eight times per day with bleeding and abdominal pain. (C) Baseline endoscopic appearance of extensive colitis to the hepatic flexure; total Mayo score 10, endoscopic subscore 3. The female patient received masked placebo treatment during the primary study, but was unable to taper corticosteroids and was, therefore, a treatment failure for the primary outcome. (D) Endoscopic appearance at completion of 8 weeks of open-label faecal microbiota transplantation; total Mayo score 0, endoscopic subscore 0. After 8 weeks of open-label faecal microbiota transplantation, the female patient had weaned corticosteroids completely and was in clinical and endoscopic remission.

	Faecal microbiota transplantation (n=41)	Placebo (n=40)	Open-label faecal microbiota transplantation (n=37)	Follow-up (n=63)
Total adverse events	78	80	35	14
Total patients with adverse events	32 (78%)	33 (83%)	18 (49%)	9 (14%)
Total patients with a serious adverse event	2 (5%)	1 (3%)	3 (8%)	1 (2%)
Total infection-related adverse events	11	17	9	3
Total patients with infection-related adverse events	10 (24%)	14 (35%)	8 (22%)	3 (5%)
Abdominal pain	12 (29%)	11 (28%)	5 (14%)	1 (2%)
Colitis	10 (24%)	9 (23%)	3 (8%)	4 (6%)
Flatulence	10 (24%)	8 (20%)	2 (5%)	0
Bloating	8 (20%)	11 (28%)	3 (8%)	0
Upper respiratory tract infection	7 (17%)	6 (15%)	4 (11%)	2 (3%)
Headache	4 (10%)	2 (5%)	2 (5%)	0
Dizziness	3 (7%)	3 (8%)	0	0
Fever	3 (7%)	2 (5%)	0	0
Rash	3 (7%)	0	0	0

(Table 3 continues on next page)

Microbiota analyses were done on 314 faecal samples from 70 patients and 113 donor faecal samples (55 individual donor and 58 batch). The mean number of clean sequences obtained per sample was 26 976 (SD 540). Rarefaction curves suggested sampling had reached saturation (appendix p 15).

Operational taxonomic units (OTUs: taxonomically related groups of bacteria) and phylogenetic diversity were significantly higher in donor batches than in individual donor samples, and in donor samples (batch and individual) than in baseline samples from patients (all $p<0·0001$; figure 3A, 3B). These bacterial taxa were confirmed as viable at the time of transplant by RNA extraction, cDNA conversion, and cDNA 16S amplicon sequencing, which showed consistent results with DNA sequencing (data not shown). OTUs and phylogenetic diversity increased significantly relative to baseline in all patients treated with faecal microbiota transplantation, at 4 weeks and 8 weeks, and persisted at 8 weeks after treatment (all $p<0·0001$; figure 3A, 3B; appendix p 16). Similar patterns were observed for species richness and Shannon's diversity (data not shown).

In diversity analyses, significant differences in microbial profiles and reduced dispersion levels were noted after faecal microbiota transplantation, from OTU to class taxonomic levels (appendix pp 22–29). Principal component analysis confirmed the changes in microbial profiles of patients undergoing faecal microbiota transplantation (figure 3C; appendix pp 17–21). Patients' profiles shifted from a dominance of *Bacteroides* spp to *Prevotella* spp (figure 3C; appendix p 30). The shift in microbial profiles of patients undergoing faecal microbiota transplantation towards the donor was most notable at the OTU level (appendix p 31).

Using linear discriminant analysis effect size analysis, patients' baseline samples were compared with samples taken at week 4, week 8, and 8 weeks after faecal microbiota transplantation to identify taxa altered by the treatment (appendix pp 32–42) and with donor samples to identify OTUs associated with donor batches and those associated with the patient (appendix pp 43–51). Across all taxonomic levels, 295 microbial taxa were differentially abundant after faecal microbiota transplantation, of which 78 showed strong associations (linear discriminant analysis score >3). A decrease in baseline patient-derived *Bacteroides* spp (eg, OTU 8, 15, 69) and an increase in donor-derived *Prevotella* spp (eg, OTU 2) and donor-derived *Bacteroides* spp (eg, OTU 12, 26, 56) was noted with both masked and open-label faecal microbiota transplantation, independent of clinical outcome. This pattern was more apparent when OTUs were picked at higher resolution (appendix pp 52–77).

Significant changes in the microbiota were seen at week 4. Microbial diversity and composition at week 4 were very similar to that noted at week 8 of faecal microbiota transplantation (figure 3A, 3B, 3C; appendix p 16). This finding raises the possibility that 4 weeks of

intensive infusions could be sufficient to induce microbial changes associated with remission. However clinical remission was not significantly different between faecal microbiota transplantation and placebo at week 4.

Double-blind faecal microbiota transplantation was associated with significantly increased diversity in all patients. Those who achieved the primary outcome had greater diversity at baseline, during faecal microbiota transplantation, and at 8 weeks after treatment, achieving levels higher than individual donors though lower than donor batches (figure 3D). However, these differences were not significant. Increased α diversity was specific to faecal microbiota transplantation; three patients allocated placebo who met criteria for the primary outcome showed no change in diversity (appendix p 78).

To identify taxa associated with the primary outcome in patients assigned faecal microbiota transplantation, linear discriminant analysis effect size analyses were done in masked and open-label patients (appendix pp 79–85). 87 taxa were associated significantly with the primary outcome in masked patients (appendix pp 79–82) versus 46 taxa in open-label patients (appendix pp 83–85). Several microbial taxa were associated with remission after double-blind faecal microbiota transplantation (eg, *Barnesiella* spp, *Parabacteroides* spp, *Clostridium* cluster IV, and *Ruminococcus* spp) and after open-label faecal microbiota transplantation (eg, *Blautia* spp, *Dorea* spp, *Ruminococcus 2*, and *Clostridium* cluster XVIII). Both *Fusobacterium* spp and *Sutterella* spp were associated consistently with no remission in patients who had double-blind and open-label faecal microbiota transplantation (appendix pp 79–85); for *Fusobacterium* spp, this association entailed either lack of eradication in patients who did not achieve remission, increased abundance in patients without remission, or eradication in patients who achieved remission (appendix p 86).

Discussion

Our study findings suggest that intensive-dosing faecal microbiota transplantation is an effective treatment for patients with active ulcerative colitis, as defined by a rigorous composite primary endpoint of corticosteroid-free clinical remission and endoscopic remission or response. Faecal microbiota transplantation was associated with clinical remission in 44% (18 of 41) of patients. Endoscopic remission did not differ between patients assigned faecal microbiota transplantation and placebo, which was defined stringently as a steroid-free Mayo endoscopic subscore of 0, but endoscopic response was achieved in three times as many individuals allocated faecal microbiota transplantation compared with those assigned placebo (32% [13 of 41] *vs* 10% [four of 40]). Data from patients assigned placebo who subsequently received open-label faecal microbiota transplantation provided further intra-patient support for the validity of the masked treatment findings (27% [ten of 37] met criteria for the primary endpoint).

	Faecal microbiota transplantation (n=41)	Placebo (n=40)	Open-label faecal microbiota transplantation (n=37)	Follow-up (n=63)
(Continued from previous page)				
Nausea	2 (5%)	5 (13%)	1 (3%)	0
Alanine aminotransferase elevated	2 (5%)	2 (5%)	0	1 (2%)
Chills	2 (5%)	2 (5%)	0	1 (2%)
Vomiting	2 (5%)	1 (3%)	0	0
Back pain	2 (5%)	0	0	0
Influenza-like symptoms	1 (2%)	4 (10%)	3 (8%)	0
Enterocolitis	1 (2%)	3 (8%)	0	0
Diarrhoea	1 (2%)	0	1 (3%)	0
Fracture (foot)	1 (2%)	0	0	0
Reflux symptoms	1 (2%)	0	0	0
Sinusitis	1 (2%)	0	0	0
Haemorrhoids	1 (2%)	0	0	0
Elective surgical procedure	1 (2%)	0	0	0
Anxiety	0	1 (3%)	1 (3%)	1 (2%)
Lung infection	0	1 (3%)	1 (3%)	0
Anal fissure	0	1 (3%)	0	0
Faecal incontinence	0	1 (3%)	0	0
Fatigue	0	1 (3%)	0	0
Genital herpes	0	1 (3%)	0	0
Irritability	0	1 (3%)	0	0
Lip infection	0	1 (3%)	0	0
Otitis media	0	1 (3%)	0	0
Sore throat	0	1 (3%)	0	0
Urticaria	0	1 (3%)	0	0
Arthralgia	0	0	1 (3%)	0
Aspartate aminotransferase elevated	0	0	1 (3%)	0
Blurred vision	0	0	1 (3%)	0
Depression	0	0	1 (3%)	0
Dry skin	0	0	1 (3%)	0
Insomnia	0	0	1 (3%)	0
Myalgia	0	0	1 (3%)	0
Palpitations	0	0	1 (3%)	0
Productive cough	0	0	1 (3%)	0
Anaemia	0	0	0	1 (2%)
Non-elective surgical procedure (intraoperative urinary injury)	0	0	0	1 (2%)
Soft-tissue infection (axillary abscess)	0	0	0	1 (2%)
Tremor	0	0	0	1 (2%)

Data are number of events or number of patients (%).

Table 3: **Adverse events**

Because many patients were steroid-dependent, the mandatory steroid wean was demanding and resulted in several individuals withdrawing from the study. This criterion has not been included in the primary endpoint of previous studies of faecal microbiota transplantation, and rarely during drug studies, for acute induction therapy in ulcerative colitis. In a non-study setting, the rate of steroid withdrawal could be tailored individually.

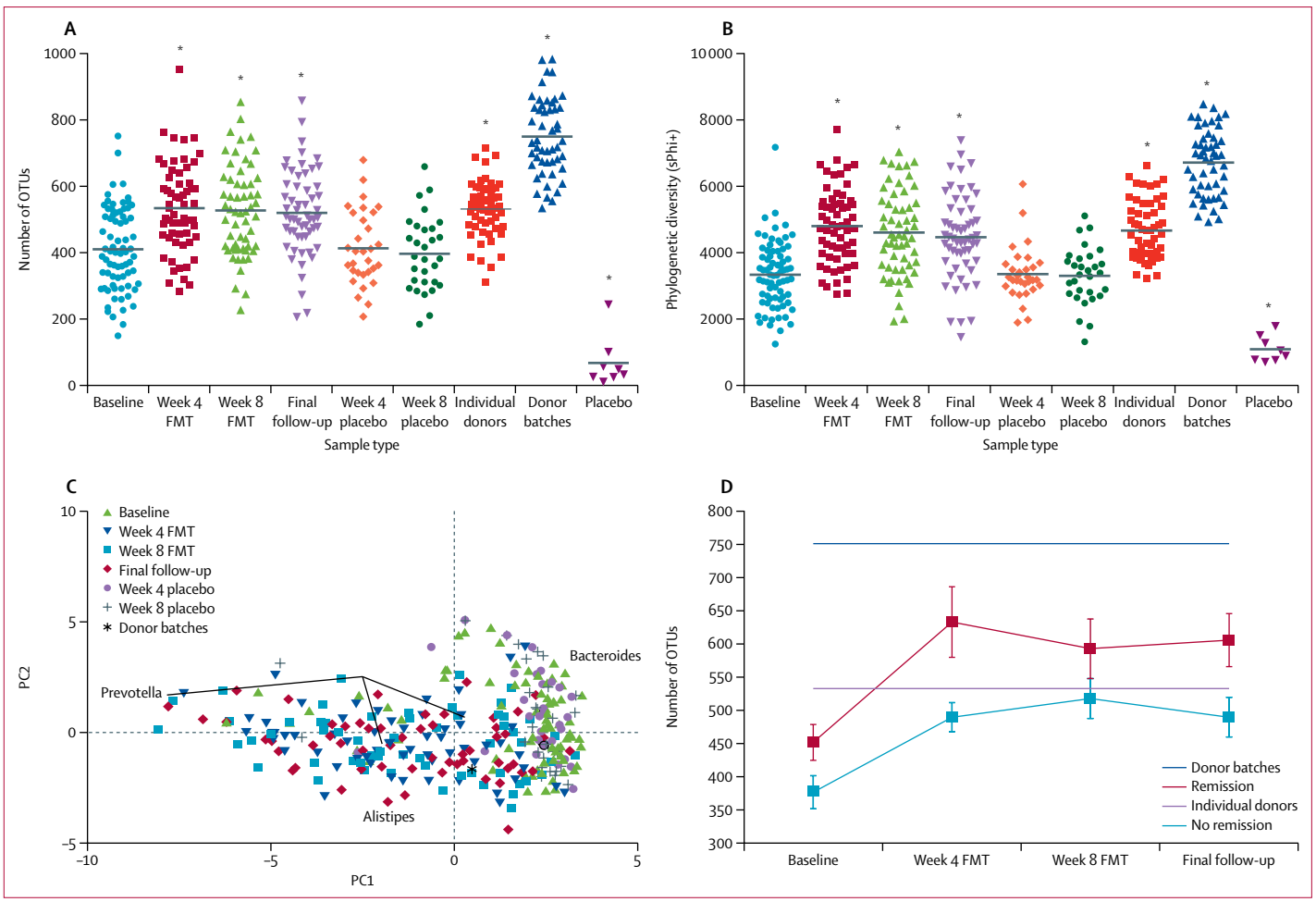

Figure 3: **Microbiota analyses of faecal samples from patients and donors**

FMT=faecal microbiota transplantation. OTU=operational taxonomic unit. PC=principal component. sPhi+=total phylogenetic diversity. (A) Number of OTUs per sample. Horizontal lines show the mean value. *p<0·0001. Significance was calculated by comparing values against baseline samples using ANOVA. (B) Phylogenetic diversity within each sample. Horizontal lines show the mean value. *p<0·0001. Significance was calculated by comparing values against baseline samples using ANOVA. (C) Principal component analysis of square-root transformation of percentage relative abundances of samples at the genus taxonomic level (averaged). Further β-diversity statistical tests (showing the degree of differentiation between regional and local microbial diversity) can be found in the appendix (pp 17–25). (D) Number of OTUs in masked study patients assigned faecal microbiota transplantation (according to primary outcome [achieved or not]), in individual donors, and in donor batches.

Possible predictors of the efficacy of faecal microbiota transplantation identified by post-hoc analyses were the severity of endoscopic inflammation and concomitant corticosteroid use. Some patients with the most severe endoscopic disease, or dependence on corticosteroids, responded to faecal microbiota transplantation but were less likely to achieve the primary outcome. This finding could reflect disease that is resistant to faecal microbiota transplantation treatment, or it could be that prolonged therapy and slower steroid reduction is needed in these patients.

The key features distinguishing our trial from previous studies of faecal microbiota transplantation in ulcerative colitis were the intensity of treatment and the use of multiple donors for each faecal microbiota transplantation infusion. 40 multidonor, preprocessed, frozen faecal microbiota transplantation infusions were administered over 8 weeks. Previous studies of *C difficile* infection suggest that microbial engraftment and efficacy are not altered significantly by freezing faecal microbiota transplantation.[18,19] The two published controlled trials of faecal microbiota transplantation in ulcerative colitis to date both utilised single-donor infusions and a more limited treatment schedule—ie, one enema per week for 6 weeks[5] or two nasoduodenal infusions 3 weeks apart.[6] Both trials were stopped early for presumed futility, but the Canadian study[5] ultimately achieved the primary endpoint of remission on final analysis. These findings and those of previous studies,[5,6,20–22] along with anecdotal reports, suggest that unlike for *C difficile* infection, which generally responds to a single infusion, faecal microbiota transplantation treatment for ulcerative colitis needs to be more intense. In ulcerative colitis, the complex interaction between

genetic, immunological, and environmental factors[23] might result in a disease state more resistant to therapeutic microbial manipulation.

Other aspects of our study in relation to the Canadian study[5] deserve consideration. Patients in our study had to be weaned off corticosteroids to achieve the primary and secondary outcomes, and steroid-free clinical response was achieved in 54% (22 of 41) of patients assigned faecal microbiota transplantation versus 23% (nine of 40) of those allocated placebo, by contrast to clinical response in the Canadian study of 39% (15 of 38) versus 24% (nine of 37), respectively (p=0·16). Whether these differences relate to single-donor versus multidonor treatment, particular donors used, or intensity of treatment, are unknown.

The optimum intensity and duration of faecal microbiota transplantation in ulcerative colitis remains to be defined. In our study, patients varied in their time to achieve clinical remission. Overall, although significant clinical improvement was noted by week 4 (appendix p 14), only at 8 weeks was a significant increase in clinical remission recorded with faecal microbiota transplantation. The duration and intensity of faecal microbiota transplantation therapy might need to be individualised: treatment once a week could be effective in some patients,[5] whereas more intensive therapy might be needed in others.

Follow-up at 8 weeks after faecal microbiota transplantation treatment revealed sustained clinical remission in a proportion of patients. Long-term benefit from faecal microbiota transplantation, and the role of maintenance treatment in responsive patients, is unclear and requires further study; as with all treatments for ulcerative colitis, it is likely that some form of maintenance faecal microbiota transplantation will be needed.

Multidonor faecal microbiota transplantation infusions were utilised in our study, both to ensure an adequate supply of infusions for faecal microbiota transplantation and to minimise the possibility of patients receiving only therapeutically ineffective donor stool. The multidonor batches had greater microbial diversity than the individual donors (figure 3A, 3B); previous study findings have suggested donor species richness is a predictor of therapeutic benefit of faecal microbiota transplantation in inflammatory bowel disease.[24] However multidonor treatment might limit the ability to define beneficial or detrimental donor-specific and microbial content-specific effects.[5] The interaction between donor and patient factors probably has an important role in determining both therapeutic benefit and safety of faecal microbiota transplantation in ulcerative colitis, and identification of someone as a good or bad donor might be an oversimplification.[25]

Multidonor, intensive-dosing faecal microbiota transplantation in patients with ulcerative colitis seems to be safe in the short term. Most serious adverse events related to either corticosteroid-dependent or corticosteroid-refractory patients unable to tolerate steroid wean, or patients with moderate-to-severe colitis. The patient who underwent a colectomy while on faecal microbiota transplantation suggests that a small subset with ulcerative colitis might be susceptible to disease worsening. Long-term safety of faecal microbiota transplantation, including the potential to transplant donor microbial disposition to other chronic diseases linked to the enteric microbiota,[26] remains unknown. Despite rigorous donor screening, an infection transmission risk remains with any biological product, which could be theoretically increased with pooling from multiple donors and increased number of infusions.

Microbial analyses have begun to characterise changes associated with faecal microbiota transplantation and identify who might benefit and by what mechanisms. Faecal microbiota transplantation therapy was associated with a significant increase in α diversity, which was durable 8 weeks after therapy completion. In our study, patients achieving the primary outcome seemed to have higher baseline microbial diversity before faecal microbiota transplantation and a greater increase in α diversity with faecal microbiota transplantation, compared with those not achieving the primary outcome (figure 3D). Specific taxa were associated with faecal microbiota transplantation outcomes, with many genera associated with therapeutic benefit; a negative outcome was associated consistently with other taxa, including the presence of *Fusobacterium* spp and *Sutterella* spp. The findings in relation to *Fusobacterium* spp are of interest because this bacterial genus has been implicated in ulcerative colitis pathogenesis.[27] Furthermore, findings show that isolates of *Fusobacterium nucleatum* from patients with inflammatory bowel disease are more invasive than are those from controls.[28]

Our study has limitations. First, intensive faecal microbiota transplantation therapy is challenging, both in terms of enema preparation and patients' convenience; future faecal microbiota transplantation capsule preparations could be helpful in this respect.[29] Second, the suitability of faecal microbiota transplantation for patients with severe active ulcerative colitis remains unclear. Third, from a microbial perspective, the study design did not allow determination of the number of infusions needed for donor microbial engraftment, and the relation between microbial engraftment and clinical outcome remains unclear. Finally, the multidonor approach restricted our ability to identify individual donor-specific microbial effects.

Our study shows that intensive-dosing multidonor faecal microbiota transplantation is a promising treatment in ulcerative colitis and that microbial manipulation with faecal microbiota transplantation could offer a new treatment paradigm, beyond immune-based therapies. Further studies are needed to define long-term outcomes and better characterise the

underlying microbiological mechanisms. In particular, identification of specific bacteria associated with a positive or negative response, and matching donors and recipients based on microbial profiles, could lead to improved personalised faecal microbiota transplantation or defined microbial consortia manipulation.

Contributors

SP, MAK, AJW, JvdB, DS, EL, HMM, and TJB designed the study. EL and TJB were responsible for donor recruitment and study product manufacture. SP, AJW, JvdB, DS, RWLL, SC, and WN acquired clinical data. SP, MAK, RP, and WX analysed clinical data and interpreted the results. SP, NOK, and HMM did microbial analyses and interpreted the results. SP, MAK, NOK, RP, and HMM drafted the report. AJW, JvdB, DS, RWLL, SC, WN, WX, EL, and TJB critically reviewed the report. All authors read and approved the final version of the report.

Declaration of interests

SP, MAK, JvdB, DS, WN, RP, NOK, HMM, WX, and EL declare no competing interests. AJW declares advisory board fees from Abbvie, Ferring, Hospira, and Janssen; and speaking fees from Abbvie, Janssen, and Shire. RWLL declares grants from Hospira, Janssen, Shire, and the National Health and Medical Research Council, Australia; and personal fees from Abbvie, Aspen, Celgene, Ferring, Hospira, Janssen, and Takeda. SC declares grants to her institution from Abbvie, Aspen, Ferring, Janssen, Shire, and Takeda; advisory board fees from Abbvie, Celgene, Ferring, Janssen, MSD, Pfizer, and Takeda; speaking fees from Abbvie, Ferring, Janssen, Shire, and Takeda; and travel grants from Pfizer, Shire, and Takeda. TJB declares a grant to his institution (Centre for Digestive Diseases, Sydney) from Australia Research LLC, for research into faecal microbiota transplantation, and he has filed patents in this field (numbers US 5,443,826 and US 6,645,530).

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References

1 Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology* 2014; **146:** 1489–99.

2 Shanahan F, Quigley EM. Manipulation of the microbiota for treatment of IBS and IBD: challenges and controversies. *Gastroenterology* 2014; **146:** 1554–63.

3 Borody TJ, Brandt LJ, Paramsothy S. Therapeutic faecal microbiota transplantation: current status and future developments. *Curr Opin Gastroenterol* 2014; **30:** 97–105.

4 van Nood E, Vrieze A, Nieuwdorp M, et al. Duodenal infusion of donor feces for recurrent *Clostridium difficile*. *N Engl J Med* 2013; **368:** 407–15.

5 Moayyedi P, Surette MG, Kim PT, et al. Fecal microbiota transplantation induces remission in patients with active ulcerative colitis in a randomized controlled trial. *Gastroenterology* 2015; **149:** 102–09.e6.

6 Rossen NG, Fuentes S, van der Spek MJ, et al. Findings from a randomized controlled trial of fecal transplantation for patients with ulcerative colitis. *Gastroenterology* 2015; **149:** 110–18 e4.

7 Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis: a randomized study. *N Engl J Med* 1987; **317:** 1625–29.

8 Paramsothy S, Borody TJ, Lin E, et al. Donor recruitment for fecal microbiota transplantation. *Inflamm Bowel Dis* 2015; **21:** 1600–06.

9 Travis SP, Schnell D, Krzeski P, et al. Developing an instrument to assess the endoscopic severity of ulcerative colitis: the Ulcerative Colitis Endoscopic Index of Severity (UCEIS). *Gut* 2012; **61:** 535–42.

10 Guyatt G, Mitchell A, Irvine EJ, et al. A new measure of health status for clinical trials in inflammatory bowel disease. *Gastroenterology* 1989; **96:** 804–10.

11 Schloss PD, Westcott SL, Ryabin T, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 2009; **75:** 7537–41.

12 Yilmaz P, Parfrey LW, Yarza P, et al. The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Res* 2014; **42:** D643–48.

13 Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011; **27:** 2194–200.

14 Cole JR, Wang Q, Fish JA, et al. Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Res* 2014; **42:** D633–42.

15 Anderson JL, Edney RJ, Whelan K. Systematic review: faecal microbiota transplantation in the management of inflammatory bowel disease. *Aliment Pharmacol Ther* 2012; **36:** 503–16.

16 Segata N, Izard J, Waldron L, et al. Metagenomic biomarker discovery and explanation. *Genome Biol* 2011; **12:** R60.

17 Goecks J, Nekrutenko A, Taylor J, et al. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol* 2010; **11:** R86.

18 Lee CH, Steiner T, Petrof EO, et al. Frozen vs fresh fecal microbiota transplantation and clinical resolution of diarrhea in patients with recurrent *Clostridium difficile* infection: a randomized clinical trial. *JAMA* 2016; **315:** 142–49.

19 Hamilton MJ, Weingarden AR, Unno T, et al. High-throughput DNA sequence analysis reveals stable engraftment of gut microbiota following transplantation of previously frozen fecal bacteria. *Gut Microbes* 2013; **4:** 125–35.

20 Kump PK, Grochenig HP, Spindelbock W, et al. Preliminary clinical results of repeatedly fecal microbiota transplantation (FMT) in chronic active ulcerative colitis. *United European Gastroenterol J* 2013; **1** (suppl 1)**:** A57.

21 Kump PK, Grochenig HP, Lackner S, et al. Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. *Inflamm Bowel Dis* 2013; **19:** 2155–65.

22 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:** 1620–30.

23 Sartor RB. Genetics and environmental interactions shape the intestinal microbiome to promote inflammatory bowel disease versus mucosal homeostasis. *Gastroenterology* 2010; **139:** 1816–19.

24 Vermeire S, Joossens M, Verbeke K, et al. Donor species richness determines faecal microbiota transplantation success in inflammatory bowel disease. *J Crohns Colitis* 2016; **10:** 387–94.

25 Li SS, Zhu A, Benes V, et al. Durable coexistence of donor and recipient strains after fecal microbiota transplantation. *Science* 2016; **352:** 586–89.

26 Alang N, Kelly CR. Weight gain after fecal microbiota transplantation. *Open Forum Infect Dis* 2015; **2:** ofv004.

27 Ohkusa T, Okayasu I, Ogihara T, et al. Induction of experimental ulcerative colitis by *Fusobacterium varium* isolated from colonic mucosa of patients with ulcerative colitis. *Gut* 2003; **52:** 79–83.

28 Strauss J, Kaplan GG, Beck PL, et al. Invasive potential of gut mucosa-derived *Fusobacterium nucleatum* positively correlates with IBD status of the host. *Inflamm Bowel Dis* 2011; **17:** 1971–78.

29 Youngster I, Russell GH, Pindar C, et al. Oral, capsulized, frozen fecal microbiota transplantation for relapsing *Clostridium difficile* infection. *JAMA* 2014; **312:** 1772–78.