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Protocol for Faecal Microbiota Transplantation in Ulcerative Colitis (FMTUC) – a Randomised Feasibility study

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Complete List of Authors:	Jitsumura, Maki; Singleton Hospital, Department of Colorectal Surgery Cunningham , Andrew; Singleton Hospital, Department of Colorectal Surgery Hitchings, Matthew; Swansea University Medical School, Medical Microbiology and Infectious Diseases Islam, Saiful; Swansea University, Swansea Trial Unit Davies, Angharad; Swansea University Medical School, Medical Microbiology and Infectious Diseases Row, Paula; Swansea University Medical School, Biochemistry Group Riddell, Andrew; Redcliffe Hospital, Department of Colorectal Surgery Kinross, James; Imperial College London, Department of Surgery and Cancer, St. Mary's Hospital, Wilkinson, Tom; Swansea University Medical School, Medical Microbiology and Infectious Diseases Jenkins, GJ; Swansea University Medical School, Molecular Carcinogenesis, Institute of Life Science Williams, John; Swansea University Medical School, Institute of Life Science 2 Harris, Dean; Abertawe Bro Morgannwg University Health Board, Department of Colorectal Surgery
Keywords:	Ulcerative colitis, Faecal microbiota transplantation, Microbiome, Feasibility study, Randomised trial, Quality of life

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Authors:

M. Jitsumura[1], A. L. Cunningham[1], M.D. Hitchings[2], S. Islam[3], A. P. Davies[4], P. E. Row[5], A.D. Riddell[6], J Kinross[7], T. S. Wilkinson[2], G. J. Jenkins[7], J.G. Williams[9], D.A. Harris [1]

Correspondence:

Professor D.A. Harris, MD FRCS MB ChB

Consultant Colorectal Surgeon, Singleton Hospital, Sketty Lane, Swansea SA2 8QA

Honorary Clinical Professor, Swansea University

Email: WPMDAH2@cardiff.ac.uk

Telephone: (01792) 285459

Author affiliations:

1 – Department of Colorectal Surgery, Singleton Hospital, Swansea, Wales

2 – Medical microbiology and Infectious disease, Swansea University Medical School, Swansea, Wales

3 – Swansea Trials Unit, Swansea University, Swansea, Wales

4 – Public Health Wales Microbiology, Singleton Hospital, Swansea University Medical School, Swansea, Wales

5 – Biochemistry Group, Swansea University Medical School, Swansea, Wales

6- Department of Colorectal Surgery, Redcliffe Hospital, Brisbane, Australia

7 – Department of Surgery and Cancer, St. Mary's Hospital, Imperial College London, London

8- Molecular Carcinogenesis, Institute of Life Science, Swansea University Medical School, Swansea , Wales

9 – Institute of Life Science 2, Swansea University Medical School, Swansea, Wales

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Abstract

Introduction: The interaction of the gut microbiota with the human host is implicated in the pathogenesis of inflammatory and immunological diseases including ulcerative colitis (UC). Faecal microbiota transplantation (FMT) as a method of restoring gut microbial diversity is of increasing interest as a therapeutic approach in the management of UC. The current literature lacks consensus about dose of FMT, route of administration and duration of response.

Methods and Analysis: This single-blinded randomised trial will explore the feasibility of FMT in 30 treatment-naïve patients with histologically confirmed distal UC limited to the recto-sigmoid region (up to 40 cm from the anal verge). This study aims to estimate the magnitude of treatment response to FMT under controlled conditions. The intervention (FMT) will be administered by rectal retention enema.

It will test the feasibility of randomising patients to: i) single FMT dose, ii) five daily FMT doses or iii) control (no FMT dose). All groups will receive standard antibiotic gut decontamination and bowel preparation before FMT. Recruitment will take place over a 24-month period with a 12-week patient follow up.

Trial objectives include evaluation of magnitude of treatment response to FMT, investigation of the clinical utility of metabolic phenotyping for predicting the clinical response to FMT and testing the recruitment rate of donors and patients for a study in FMT. This feasibility trial will enable an estimate of number of patients needed, help determine optimal study conditions and inform the choice of endpoints for a future definitive phase III study.

Ethics and dissemination

The trial is approved by the regional ethics committee and is sponsored by ABM University Health Board. Written informed consent from all patients will be obtained. Serious adverse events will be reported to the sponsor. Trial results will be disseminated via peer review publication and shared with trial participants.

Strengths and limitations of this study

- This is one of the first such trials to have a homogenous study group of newly diagnosed and treatment naïve UC patients

- Strictly defined UC disease extent at trial entry
- The trial will help to define the optimal number of doses of FMT for treatment of UC
- The efficacy of FMT treatment by rectal administration will be shown
- Metabolomic analysis will demonstrate mechanism of action of FMT in treatment responders

Sponsor

Abertawe Bro Morgannwg University Health Board will assume overall responsibility for the trial as sponsor.

Trial registration: ISRCTN 58082603, REC reference 15/WA/0262 (Wales REC6)

1. Introduction

Ulcerative Colitis (UC) is a chronic relapsing-remitting mucosal inflammatory bowel disease (IBD). Clinical features include rectal bleeding, diarrhoea, faecal urgency, fatigue and weight loss. The aetiology of UC is believed to be multifactorial involving immune dysregulation, mucosal disruption and genetic predisposition, though the precise cause is poorly understood (1).

There is no curative treatment at present; thus the aim of current management is induction and maintenance of remission with immunosuppressive agents. These are usually required lifelong incurring estimated costs of £2427 in year one and over £500 per patient per year thereafter (2). Failure of medical therapy or refractory disease may require major resectional surgery with temporary or permanent ostomy formation. Moreover, UC is a recognised risk factor for colorectal cancer requiring lifelong surveillance (3).

The human gut microbiota consists of a diverse biological environment comprising bacteria, viruses and fungi within the gut lumen and lining mucosa. The biodiversity of the gut microbiota is a dynamic process and is known to be affected by age, diet and lifestyle (4,5). It has been is referred to as a hidden metabolic organ through its major role as a driver of metabolic and immunological communications and the regulation of the immunological processes within the intestinal mucosa (6–9). Disruption of the gut microbiota, also called dysbiosis, has been suggested to be responsible for not only intestinal pathology such as *Clostridium difficile* infection, but also for systemic conditions such as obesity, diabetes mellitus and IBD including UC (4,10). The role of gut microbiota with host-microbiome interactions are likely to be a key driver in the pathogenesis of UC (11,12). Antibiotics, which alter the human gut microbiome, have been shown to contribute to UC activity (13), whereas probiotics have been implicated in UC remission (14). The gut microbiota of UC patients lacks diversity (15,16) and the Bacteroidetes are found significantly more in UC patients' microbiota (17,18). Furthermore, reduced amounts of bacterial producers of short-chain fatty acids (SCFA) (butyrate, propionate and acetate) are found in the microbiota of UC patients (19,20). These SCFAs are products of resistant starch fermentation from gut bacteria and are believed to have anti-inflammatory properties. Moreover, recent studies have shown that butyrate produced from *Faecalibacterium prausnitzii* not only has anti-inflammatory properties, but also provides the major nutrient for colonocytes (21), and prevents intestinal mucosa atrophy and colonocyte autophagy (22). A number of studies demonstrate that the butyrate producer *Faecalibacterium prausnitzii* was less abundant in UC patients (23–25). Moreover, recent studies have suggested that not only living

bacteria may be responsible inflammatory process of UC, but also bacterial specific components and structures, antimicrobial compounds and metabolites produced by bacteria may contribute to the gut microenvironment thus its inflammatory process (26). Understanding of a critical role of secondary metabolites has also been highlighted recently by Buffie *et al.* recently as they have indicated that certain species may inhibit *C. difficile* with their secondary metabolites, including secondary bile acids by *C. scindens* (27,28). Although the role of fungi in the human microbiome has not yet been fully understood, recent studies suggest microfragments of chitin, which is a substance produced by fungi and insects, display a significant immunomodulatory impact in the inflammatory process (29,30). This suggests that not only viable common gut anaerobic microorganisms, but also products and particles from other microorganisms may be responsible for dysregulation of the immune response. Despite extensive studies no single pathogen has been identified as responsible for the pathogenesis of UC. The current consensus is that the loss of certain bacterial strains with immunomodulatory as well as mucosal regulatory functions leads to gut dysbiosis, resulting in the pathogenesis of UC. Faecal Microbiota Transplantation (FMT) is an infusion of a faecal suspension from a healthy individual (donor) to restore the dysbiosis of affected individuals (recipient). Since the approval of FMT in the management of recurrent *C. difficile* infections in 2014 by the National Institute for Health and Care Excellence (NICE), FMT has been of increasing interest as a therapeutic approach in the management of UC. If we can successfully and durably alter the colonic microbiota (31), it may be possible to engender complete remission of this chronic debilitating disease without lifelong immunosuppression use or the need for major gastrointestinal (GI) surgery. The ability to induce remission and establish the microbiological basis for this would change the treatment paradigm for UC. Recent years have seen several randomised clinical studies emerging to investigate FMT in the management of UC with encouraging results (16,32–34). Despite these studies, many unknown aspects remain in the clinical application of FMT in UC, such as the optimum dose, route of administration and frequency of treatments. Equally it is not known whether FMT is effective as a first line treatment in drug-naïve patients. To study the optimum parameters for delivering FMT in UC and estimating the clinical response this randomised feasibility trial was designed.

The objectives of this feasibility study include: evaluation of the magnitude of treatment response to FMT, investigation of the functional metabolic changes associated with FMT using a metabolic phenotyping methodology and testing the recruitment rate of donors and patients. Furthermore,

we aim to measure the duration of clinical response with microbiome identification through 16S rRNA sequencing and metabolomic analysis. This will facilitate the design of a definitive multi-centred study to confirm the efficacy of FMT as a first-line treatment option in UC.

Primary Objectives:

The primary objective of this phase II study is to estimate the magnitude of the treatment response to FMT in treatment naïve patients with UC.

Secondary Objectives:

- Determine the recruitment rate of donors and participants for a study of FMT
- Determine the optimal study conditions and choice of endpoints for phase III study to include dosage and frequency of FMT treatments.
- Establish how many participants would be required for phase III to demonstrate the efficacy of FMT in the treatment of UC.

2. Methods

This is a single-blinded interventional randomised feasibility study to estimate the magnitude of the treatment response to FMT in newly diagnosed patients with distal UC who are treatment naïve. Recruitment is proposed over a two-year period with a twelve-week post-treatment follow up period. This feasibility trial will help determine the recruitment rate of donors and participants, define the optimal study conditions and choice of endpoints for a phase III definitive study. It will also allow us to establish how many participants would be required at phase III to demonstrate the efficacy of FMT in the treatment of UC.

1. Trial design

We aim to recruit thirty subjects with histologically confirmed UC, whose disease is confined to the recto-sigmoid area (defined here as within forty centimetres from the anal verge) and who are treatment naïve. Participants will be randomly assigned to study groups through a web based application hosted by University of Aberdeen.

Eligible patients will be randomised into one of three groups with an allocation ratio of 2:2:1 as shown in Table 1. Groups 1 and 2 are the intervention arms and twelve subjects will be assigned to each group respectively. Group 3 is the control arm and six subjects will be randomised into this group.

Intervention arms: Groups 1 and 2

Participants randomly allocated to group 1 will receive one single FMT treatment administered as a rectal retention enema. Participants in group 2 will receive a single FMT treatment on five consecutive days (total of 5 treatments) also administered by rectal retention enema.

Control arm: Group 3

Participants randomly allocated to group 3 will receive the pre-FMT preparation with antibiotics and bowel preparation but will not receive active FMT treatment.

Table 1: Intervention arms (Group 1 and 2) and Control arm (Group 3)

	Group 1	Group 2	Group 3
Bowel decontamination and preparation	Yes	Yes	Yes
FMT treatment dose	1	5 consecutive days (single treatment per day)	None
Number of participants	12	12	6

2. Endpoints

2.1 Paired primary endpoints

- Remission of UC (mucosal healing) at 12 weeks as assessed by blinded sigmoidoscopy. Assessment defined as Mayo score ≤ 2 with an endoscopic Mayo score of 0
- Proportion of successful engraftment of donor faecal microbiota at 12 weeks in each group as analysed by 16S sequencing

2.2 Secondary endpoints

- Rate of recruitment of patients
- Disease specific scores after treatment using IBDex severity scoring index (35), CUCQ-32 severity scoring index (36) and Mayo scoring system (37)
- Histological grading of colitis severity after treatment
- Mucosal immunological response to treatment (tissue IL-10 and IL-12 by ELISA)
- Rate of development of adverse effects to FMT

3. Participant selection

Potential participants will be identified by their usual clinicians in clinics and endoscopy units within the Health Board. Each potential participant will be screened for eligibility once referred to the research team. All subjects must have a definitive histological diagnosis of UC before enrolment. A written patient information sheet will be provided and participants will be offered a minimum of 24 hours to consider enrolment before providing written informed consent. During the screening visit, the study will be fully explained, and consent will be obtained if the subject satisfies all inclusion and exclusion criteria (Table 2).

Table 2: Participant selection criteria

Inclusion Criteria
• Newly diagnosed histologically confirmed UC with inflammation limited to the rectum or recto-sigmoid (within 40cm of anal verge as measured by flexible sigmoidoscopy) • Age 18 years and above • Able to give full informed written consent • Willing to return for sequential FMT dosing and endoscopic assessment

• Not in receipt of conventional medical treatment for colitis such as steroids or 5-aminosalicylic acid (5-ASA) i.e. treatment naïve
Exclusion Criteria
• Patients without a definitive diagnosis of UC (for example diagnosis of Crohn's disease or infectious colitis) • Colitis extending beyond 40cm from the anal verge • Diagnosis of severe acute colitis (defined as greater than 6 blood-stained stools per 24 hrs with one of the following: pulse rate >90/ temperature >37.8 degree/ haemoglobin <105g/L / ESR>30) • Abdominal tenderness on examination • Already commenced standard medical therapy for UC • Contraindication to oral bowel preparation • Allergy to study antibiotics • Age less than 18 • Patient is within a vulnerable group • Pregnant • Immuno-suppressed e.g. transplant patient • Known communicable disease or at least 2 weeks full recovery from infectious disease e.g. chickenpox, • Systemic autoimmunity, or atopic diseases • Previous prosthetic implant (for example metallic heart valve, joint replacement, ventricular-peritoneal shunt, cardiac stent) • Chronic pain syndromes (for example: fibromyalgia, chronic fatigue) • Neurologic, neuro-developmental or neurodegenerative disorders • Depression (requiring therapy) • Obesity (BMI>35) • Malignancy • Use of antibiotics for any indication within the past 3 months • Foreign travel to areas of enteric disease prevalence within 3 months • High risk sexual behaviour (examples: sexual contact with anyone with HIV/HTLV/AIDS or hepatitis B/C carrier, men who have sex with men (MSM))

- Known exposure to HIV or hepatitis B/C
- Current/previous use of injected drugs or intranasal cocaine
- Tattooing, piercing, cosmetic botulinum toxin or permanent makeup within 120 days (as per Welsh Blood Transfusion guidelines)
- Recent blood transfusion, tissue / organ transplant or skin graft
- Risk factors for variant Creutzfeldt-Jakob disease e.g. blood transfusion or transplant after 1st January 1980

4. Interventions and Investigational products

All three study groups will complete a ten-day course of oral antibiotics (Metronidazole 400mg, vancomycin 500mg, rifampicin 150mg twice daily), which should complete at least forty-eight hours before the first FMT treatment. This will allow the poorly absorbed vancomycin to wash out of the gastrointestinal tract. Patients should therefore start the ten-day course of antibiotics twelve days before the first FMT is given. Additionally, all participants will receive bowel preparation (polyethylene glycol, 2 litres) on the day before transplantation to prepare the lumen for engraftment of the FMT treatment and to minimise interference from the existing gut microbiota.

4.1 Investigational Product

The investigational product is donated faecal material from healthy volunteers who are unrelated and non-cohabiting to the study participants. The FMT products are obtained either from Wessex stool bank or material that has been locally processed using the identical FMT preparation technique as Wessex stool bank by a physician for the purposes of the research trial. Despite many discussions around FMT preparation techniques, including whether to utilise pellet or supernatant from donor stools, the FMT products employed in this feasibility study are processed to make the most of pellet in order to maintain as much of the donor microbiota and its diversity as possible. The products are frozen in 20% glycerol and stored for up to 8 weeks at -80°C until the day of treatment. Donors are screened for infections in accordance with current best practice (38) (Table 3). In the case of multiple treatments (group 2) all doses are obtained from the same donor to minimise variation. Faecal microbiota of the donated faecal samples is studied using 16S rRNA analysis. This will be used as a reference for the effect of FMT treatments, evaluation of magnitude of treatment response to FMT and durability of engulfment after FMT.

Table 3: Screening infections

Blood tests	• Cytomegalovirus • Epstein-Barr virus • Hepatitis A • HBV • HCV • Hepatitis E virus • Syphilis • HIV-1 and HIV-2 • *Entamoeba histolytica* • Human T-lymphotropic virus types I and II antibodies • *Strongyloides stercoralis*
Faecal tests	• Detection of *C. difficile* • Detection of enteric pathogens, including *Salmonella, Shigella* • *Campylobacter, Escherichia coli O157 H7, Yersinia*, Vanocomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, Gram-negative mutidrug-resistant bacteria • Norovirus • Antigens and/or acid fast staining for *Giardia sp* and *Cryptosporidium sp* • Protozoa (including *Blastocystis hominis*) and helminths

4.2 Administration of Investigational Product

All three study groups will complete a ten-day course of oral antibiotics (vancomycin 500mg; metronidazole 400mg, rifampicin 150mg – all taken twice daily) and bowel preparation (polyethylene glycol 2 litres on the day before transplantation). The first FMT treatment dose will be commenced 48 hours after the final dose of antibiotics to preserve the activity of the FMT. Frozen FMT will be thawed over four hours at room temperature prior to infusion, which will subsequently be diluted to 250mL with non-bacteriostatic normal saline prior to infusion. The subjects of Group 1 and 2, who receive FMT treatment, will also be given loperamide 4mg orally thirty minutes prior to administration to maximise the chance of enema retention. Each participant receives 50mL of enema every 15 minutes over 60 minutes. The subjects will be encouraged to retain the treatment samples as long as possible (ideally more than one hour).

5. Study setting

Recruitment will take place from clinics and endoscopy units within the Abertawe Bro Morgannwg University Health Board, Swansea. FMT will be administered at the Joint Clinical Research Facility within Swansea University.

6. Randomisation

Study participants will be randomised 2:2:1 by a web-based method hosted by the University of Aberdeen's Health Services Research Unit. The simple randomisation process employed in this allocation was not stratified by any factors (e.g. age, gender). We aim to update the randomisation process based on the results of this feasibility study for potential stratifying factors in phase III.

7. Blinding

The trial statistician, the assessing independent endoscopist and the pathologist undertaking macroscopic and microscopic disease assessments will be blinded to the treatment allocation.

8. Participant timeline and Schedule of Assessment

Figure 1 and Table 4 show the follow up schedule and assessment for the trial. At baseline the study participants will undergo assessment for disease activity with validated tools (CUCQ-32, IBDex and Mayo Score) alongside a full history and physical examination. Baseline biopsies of the rectum for 16S rRNA analysis and immunological studies (IL-10 and IL-21), faecal samples for 16S rRNA analysis and metabolomic profile, blood tests (renal function, liver function, full blood count, C-reactive protein, metabolomic profile) will be obtained. Furthermore, 16S rRNA analysis for the donors' faecal samples is performed. Subsequently, this will be studied together with 16S rRNA analysis of the participant's faecal samples for the study of durability of engulfment after FMT treatments during a 12-week follow up period.

Follow-up visits will take place at week 1, 4, 8 and 12 for all three study groups. Participants will undergo clinical examination, blood and faecal testing to include faecal microbiota profiling using the 16S rRNA analysis and metabolomic profile, and complete disease activity scoring questionnaires (CUCQ-32 and IBDex). At the final assessment (week 12), all subjects will also undertake a repeat flexible sigmoidoscopy for macroscopic assessment and biopsies for degree of inflammation or confirmation of remission. Participants who relapse or fail to improve after FMT will be offered conventional medical therapy.

Study participants will be instructed to inform the treating physician of any infectious symptom or new medical condition which develops after receiving FMT and a patient registry will be maintained.

Table 4: Follow up schedule and assessments

	Baseline	Week 1	Week 4	Week 8	Week 12
Questionnaires					
CUCQ-32	•	•	•	•	•
IBDex	•	•	•	•	•
Mayo score	•				•
Endoscopy assessment					
Sigmoidoscopy	•				•
Rectal biopsy	•				•
Histology assessment					
Histological grading	•				•
Mucosal 16S sequencing	•				•
Mucosal IL-10	•				•
Mucosal IL-21	•				•
Blood tests					
Renal profile	•	•	•	•	•
Liver profile	•	•	•	•	•
Full blood count	•	•	•	•	•
C-reactive protein	•	•	•	•	•
Metabolomic profile	•	•	•	•	•
Faecal sample assessment					
16S sequencing	•	•	•	•	•
Metabolomic profile	•	•	•	•	•

Withdrawal

Participants may be withdrawn from the study if;

- they wish to terminate treatment and/or follow-up assessments
- clinical features worsen during FMT or the 12-week follow-up period

- the participant is non-compliant with the study in a manner that is either harmful to their health or interferes with the validity of the study results
- participants who withdraw their consent may not wish for their data to be used – if this is the case then it will be deleted

Data collection and management

Data collection will be performed at baseline, week 1, 4, 8 and 12 as described in Table 4. All data is to be recorded on the case report form (CRF) in an anonymised format against a unique participant number.

Data will be transferred to a computer database without patient identifiable data and analysed once all results have been collected. The trial database will have built in measures to assess data quality at time of input and stored securely.

Participant rights and confidentiality

The Chief Investigator will be the custodian of the data. Information with regards to study participants will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval.

There will be no patient identifiable data on the CRF and a unique participant number will be allocated. The Principal investigator will hold the key to the coded number of the participants only. Only the Principal investigator will have access to the patient identifiable information.

Statistical Analysis

The analysis strategy of this feasibility study is mainly descriptive and exploratory data analysis. For each group, we will calculate the number of participants approached, and/or assessed for eligibility, randomised and received the treatment. Thus, we will calculate the recruitment and retention rate along with the rate of adverse events. Descriptive statistics (mean, standard deviation, 95% confidence interval) for continuous outcomes (e.g. CUCQ-32 Score, Mayo Scoring) and raw count

(n, %) for categorical outcomes (e.g. renal profile, liver profile, histological grading) will be reported as per the clinical endpoints.

All these summary statistics will be provided as per baseline and other follow ups (as appropriate to the outcome measure) and with respect to the three treatment arms. All the analysis and data preparation will be performed using SPSS v.22.0 as a validated statistical software for clinical trials.

Safety measures

An adverse event (AE) is defined as any untoward medical occurrence in a patient after administration of the study intervention (FMT) that does not necessarily have to have a causal relationship with this treatment. Serious adverse events (SAEs) will be notified to the study sponsor within twenty-four hours and to the Research Ethics Committee (REC) within fifteen days. AEs that are expected for patients undergoing FMT, and symptoms expected from UC, will be specified and will not require to be reported as adverse events.

Quality Assurance

The Research and Development Quality Assurance Officer has performed a Monitoring Prioritisation Assessment to assess the impact of trial participation on the rights and safety of participants and the reliability of trial results. This has guided the development of procedures in the trial with respect to informed consent, confidentiality and trial monitoring. Monitoring visits to the site will be made every three months during the study to ensure that all aspects of the protocol are followed. The Quality Assurance Officer will also monitor the study after the first participant has been recruited. The monitoring visit timeframe can be changed depending on the monitoring findings. A Quality Assurance programme is also in place to ensure adherence to the study protocol. Major and minor deviations will be collected.

Endosocopy: one of several JAG accredited gastroenterologists or colorectal surgeons from hospitals of the Abertawe Bro Morgannwg University Health Board will perform the sigmoidoscopy assessment at baseline and week twelve. The study team will ensure that the endoscopist performing the 12-week assessment is blinded to the intervention that the patient has received. Endoscopic photographs taken at baseline and at final assessment will be independently assessed by a blinded expert to provide quality assurance for this outcome measure.

Pathology: A standardised protocol based on RCPath guidelines will be used for histological assessment of the disease as per standard of care by consultant pathologists.

Ethics and Dissemination

The Chief Investigator will ensure that the trial is conducted in compliance with the principles of the Declaration of Helsinki (1996), and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework, Trust and Research Office policies and procedures and any subsequent amendments. The trial is approved by the regional ethics committee (REC) and Health Research Authority (HRA) or equivalent. Written informed consent will be obtained from all participants. Serious adverse events will be reported to the study sponsor and the regional ethics committee. Trial results will be disseminated through oral presentations at national conferences and through peer reviewed publication, which will include named members of the Trial Management Group (TMG) who meet the three criteria of scholarship (design, execution, analysis and/or interpretation of the data), authorship (drafting, reviewing and revision of the manuscript) and approval (approving the manuscript to be published). Participants in the study will be given a copy of the results and a final report will be written by the TMG for the funding body and the REC. Results will be used to aid in the development of a definitive phase III trial.

Discussion

A recently published systematic review on the usage of FMT in IBD concluded that overall 36% of patients with UC achieved clinical remission (a total of 41 studies and 4 randomised controlled trials (RCTs))(39). Meta-analysis which included 4 RCTs (a total of 140 individuals) demonstrated that FMT was significantly linked to clinical remission with a pooled odd ratio (P-OR) of 2.89, 95% confidence interval (CI) of 1.36-6.13 and p-value of 0.016.

The number of FMT studies with high methodological quality has increased of late, yet the optimal conditions for durable FMT engraftment and maximal remission are presently unclear for UC. Table 4 summarises the current knowledge gaps in the application of FMT in UC.

Table 4: Uncertainties for the optimal application of FMT in UC

Human gut microbiota	• Responsible pathogens and their roles • Microbiome profiling techniques
FMT preparations	• Frozen vs Fresh • Donor screening protocol • Preparation methodology
Donors	• Related vs unrelated • Single donor vs multiple donors
Pre-medications/preparation	• Bowel preparation • Antibiotics vs non-antibiotics
FMT in clinical application	• Dose • Administration routes • FMT alone vs with other traditional medications • Durability of engraftment • Who to treat – active, remission, refractory • Adverse effects • Long-term effects and safety • Long term effects after transplant
Clinical remission	• How to assess clinical response • How to define clinical remission • When to stop FMT treatment • Maintenance dose required for remission • Post-remission dietary modification

Current studies are difficult to interpret as there is no universally agreed definition of remission as an endpoint in UC clinical trials to date (40). Furthermore, a lack of homogeneity of clinical trial protocols makes comparison of such studies more difficult to comprehend and these clinical trials are no exception. Moreover, different clinical trials use different patient groups, donors, treatment

dose, routes, frequency and pre-treatment medications. These multiple variables make the comparison of studies very challenging, although all studies appear to demonstrate promising results for the usage of FMT in active UC. Finally, and most importantly, patients recruited in published RCTs had been on previous conventional medical therapy until given the FMT treatment if not being assigned to further medical treatment. This makes the interpretation of the magnitude of treatment response to FMT very difficult.

Although the efficacy of FMT in UC appears to be promising, more clarity is required around optimal treatment conditions through rigorous study. This study will estimate the efficacy of rectally administered FMT in treatment naïve patients towards the design of a definitive trial. This phase II study allows us not only to estimate the magnitude of treatment response to FMT in UC, but also to determine the changes and durability of engraftment of the gut microbiota after FMT treatment. Furthermore, we will study the dose response by comparing one dose only and five daily doses towards establishing the optimum dosage of rectally administered FMT treatment for UC. There is a fundamental lack of mechanistic data to support the use of FMT in clinical practice. Bacteria represent a diverse and highly active chemical engine, that creates a suite of biologically active small molecules through secondary metabolism. The critical function of these target metabolites in the initiation and maintenance of systemic inflammation remains poorly defined and this trial will provide a detailed insight into the role of the gut microbiome in UC therapy that have the potential to stratify care in the future and improve the precision of this intervention.

Competing interests

None

Collaborators

Department of Colorectal Surgery (MD, ME, GT, SA, CS, JK, JB)

Author Contributions

DAH and AR are responsible for the idea for the trial

MJ, ALC, MDH, SI, AD, PER, ADR, JK, TW, GJ, JGW, DAH have drafted and the manuscript and/or provided critical revision.

MDH, SI, AD, PER, ADR, JK, TW, GJ, JGW, DAH have made substantial contributions to the conception and design of the work and subsequent protocol revisions.

MJ, ALC, MDH, SI, AD, PER, ADR, JK, TW, GJ, JGW, DAH all agree to be accountable for all aspects of work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figure legends

Figure 1: Study scheme flowchart

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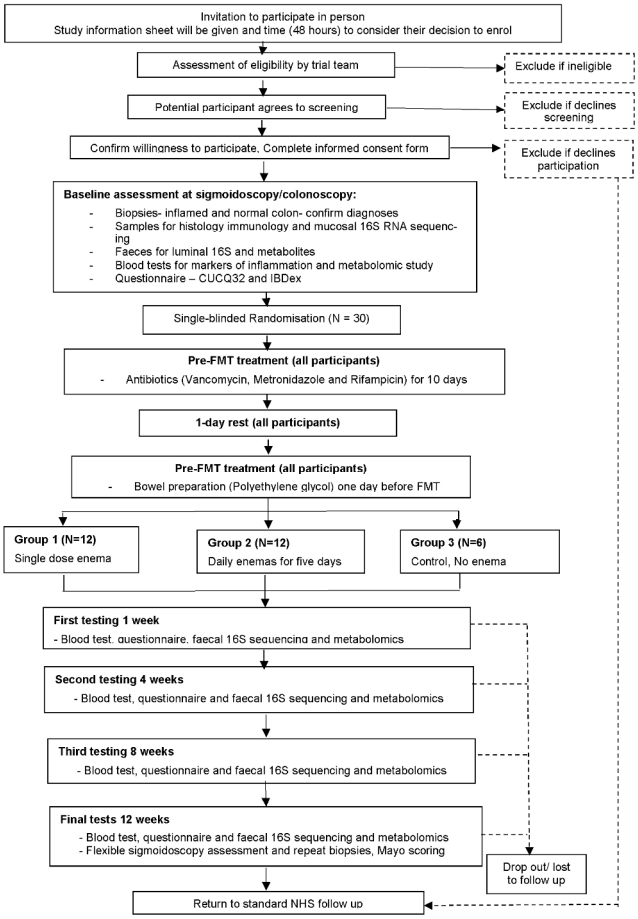

Figure 1: Study scheme flowchart

209x297mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	6
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	6
Funding	4	Sources and types of financial, material, and other support	6
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	4
	5b	Name and contact information for the trial sponsor	3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	____7_____
	6b	Explanation for choice of comparators	____7-9_____
Objectives	7	Specific objectives or hypotheses	____9_____
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	____10_____
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	____10_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	____13-15_____

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	___11, Table 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	____12_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	____18_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	____14_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	____13_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	____17. Table 4, Figure 1_____
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	____13_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___13_____
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___17_____

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	____17__
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____17__
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	_____17____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____17_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_19
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	__19___
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	__19_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_19_____

	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	N/A_____
	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomised analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	___N/A _____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_19_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	___n/a__
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___20_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_20_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21_____
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	n/a_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5,13, 21,_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	n/a_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	21_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	21
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A __

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__N/A_____
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	__N/A____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Protocol for Faecal Microbiota Transplantation in Ulcerative Colitis (FMTUC) – a Randomised Feasibility study

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Complete List of Authors:	Jitsumura, Maki; Singleton Hospital, Department of Colorectal Surgery Cunningham , Andrew; Singleton Hospital, Department of Colorectal Surgery Hitchings, Matthew; Swansea University Medical School, Medical Microbiology and Infectious Diseases Islam, Saiful; Swansea University, Swansea Trial Unit Davies, Angharad; Swansea University Medical School, Medical Microbiology and Infectious Diseases Row, Paula; Swansea University Medical School, Biochemistry Group Riddell, Andrew; Redcliffe Hospital, Department of Colorectal Surgery Kinross, James; Imperial College London, Department of Surgery and Cancer, St. Mary's Hospital, Wilkinson, Tom; Swansea University Medical School, Medical Microbiology and Infectious Diseases Jenkins, GJ; Swansea University Medical School, Molecular Carcinogenesis, Institute of Life Science Williams, John; Swansea University Medical School, Institute of Life Science 2 Harris, Dean; Abertawe Bro Morgannwg University Health Board, Department of Colorectal Surgery
Primary Subject Heading:	Gastroenterology and hepatology
Secondary Subject Heading:	Evidence based practice, Research methods
Keywords:	Ulcerative colitis, Faecal microbiota transplantation, Microbiome, Feasibility study, Randomised trial, Quality of life

BMJ Open

Protocol for a Randomised Feasibility Trial evaluating Faecal Microbiota Transplantation in Ulcerative Colitis (FMTUC)

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Authors	Jitsumura, Maki; Department of Colorectal Surgery, Singleton Hospital, Swansea, Wales Cunningham, Andrew Laurence; Department of Colorectal Surgery, Singleton Hospital, Swansea, Wales Hitchings, Matthew David; Medical Microbiology and Infectious Diseases, Swansea University Medical School Islam, Saiful; Swansea Trial Unit, Swansea University Davies, Angharad; Medical Microbiology and Infectious Diseases, Swansea University Medical School Row, Paula; Biochemistry Group, Swansea University Medical School Riddell, Andrew; Department of Colorectal Surgery, Redcliffe Hospital, Brisbane, Australia Kinross, James; Department of Surgery and Cancer, St. Mary's Hospital, Imperial College London, London Wilkinson, Tom; Medical Microbiology and Infectious Diseases, Swansea University Medical School Jenkins, Gareth; Molecular Carcinogenesis, Institute of Life Science, Swansea University Medical School Williams, John; Institute of Life Science 2, Swansea University Medical School Harris, Dean Anthony; Department of Colorectal

	Surgery Abertawe Bro Morgannwg University Health Board, Swansea, Wales
Primary Subject Heading	Colitis, Ulcerative
Secondary Subject Heading	Microbiota
Keywords	Faecal microbiota transplantation, microbiome, Ulcerative colitis, Randomised trial, Quality of life

Title: Protocol for Faecal Microbiota Transplantation in Ulcerative Colitis (FMTUC) – a Randomised Feasibility study

Authors:

M. Jitsumura[1], A. L. Cunningham[1], M.D. Hitchings[2], S. Islam[3], A. Davies[4], P. Row[5], A.D. Riddell[6], J Kinross[7], T Wilkinson[2], G. Jenkins[7], J.G. Williams[9], D.A. Harris [1]

Correspondence:

Professor D.A. Harris, MD FRCS MB ChB

Consultant Colorectal Surgeon, Singleton Hospital, Sketty Lane, Swansea SA2 8QA

Honorary Clinical Professor, Swansea University

Email: WPMDAH2@cardiff.ac.uk

Telephone: (01792) 285459

Author affiliations:

1 – Department of Colorectal Surgery, Singleton Hospital, Swansea, Wales

2 – Medical microbiology and Infectious disease, Swansea University Medical School, Swansea, Wales

3 – Swansea Trials Unit, Swansea University, Swansea, Wales

4 – Public Health Wales Microbiology, Singleton Hospital, Swansea University Medical School, Swansea, Wales

5 – Biochemistry Group, Swansea University Medical School, Swansea, Wales

6- Department of Colorectal Surgery, Redcliffe Hospital, Brisbane, Australia

7 – Department of Surgery and Cancer, St. Mary's Hospital, Imperial College London, London

8- Molecular Carcinogenesis, Institute of Life Science, Swansea University Medical School, Swansea , Wales

9 – Institute of Life Science 2, Swansea University Medical School, Swansea, Wales

Keywords

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Abstract

Background: The interaction of the gut microbiota with the human host is implicated in the pathogenesis of inflammatory and immunological diseases including ulcerative colitis (UC). Faecal microbiota transplantation (FMT) as a method of restoring gut microbial diversity is of increasing interest as a therapeutic approach in the management of UC. The current literature lacks consensus about dose of FMT, route of administration and duration of response.

Methods: This single-blinded randomised trial will explore the feasibility of FMT in 30 treatment-naïve patients with histologically confirmed distal UC limited to the recto-sigmoid region (up to 40 cm from the anal verge). This study aims to estimate the magnitude of treatment response to FMT under controlled conditions. The intervention (FMT) will be administered by rectal retention enema. It will test the feasibility of randomising patients to: i) single FMT dose, ii) five daily FMT doses or iii) control (no FMT dose). All groups will receive standard antibiotic gut decontamination and bowel preparation before FMT. Recruitment will take place over a 24-month period with a 12-week patient follow up.

Trial objectives include evaluation of magnitude of treatment response to FMT, investigation of the clinical value of metabolic phenotyping for predicting the clinical response to FMT and testing the recruitment rate of donors and patients for a study in FMT. This feasibility trial will enable an estimate of number of patients needed, help determine optimal study conditions and inform the choice of endpoints for a future definitive phase III study.

Ethics and dissemination

The trial is approved by the regional ethics committee and is sponsored by ABM University Health Board. Written informed consent from all patients will be obtained. Serious adverse events will be reported to the sponsor. Trial results will be disseminated via peer review publication and shared with trial participants.

Strengths and limitations of this study

- This is one of the first such trials to have a homogenous study group of newly diagnosed and treatment naïve UC patients

- The trial will not only show the efficacy of FMT treatment by rectal administration, but also help to define the optimal number of doses of FMT for treatment of UC
- Metabolomic analysis will demonstrate mechanism of action of FMT in treatment responders
- Patient reported quality of life measures will be reported
- This study is limited by a short (12 week) follow up period

Sponsor

Abertawe Bro Morgannwg University Health Board will assume overall responsibility for the trial as a sponsor.

Trial registration: ISRCTN 58082603, REC reference 15/WA/0262 (Wales REC6)

1. Introduction

Ulcerative Colitis (UC) is a chronic relapsing-remitting mucosal inflammatory bowel disease (IBD). Clinical features include rectal bleeding, diarrhoea, faecal urgency, fatigue and weight loss. The aetiology of UC is believed to be multifactorial involving immune dysregulation, mucosal disruption and genetic predisposition, though the precise cause is poorly understood (1).

There is no curative treatment at present; thus the aim of current management is induction and maintenance of remission with immunosuppressive agents. Failure of medical therapy or refractory disease may require major resectional surgery with temporary or permanent ostomy formation. UC is also a recognised risk factor for colorectal cancer requiring lifelong surveillance (2). However, it is uncertain how to predict which group of patients will respond to medical therapy.

The human gut microbiota consists of a diverse biological environment comprising bacteria, viruses and fungi within the gut lumen and lining mucosa. The biodiversity of the gut microbiota is a dynamic process and is known to be affected by age, diet and lifestyle (3,4). It has been referred as a hidden metabolic organ through its major role as a driver of metabolic and immunological communications and the regulation of the immunological processes within the intestinal mucosa (5–8). Disruption of the gut microbiota, also called dysbiosis, has been suggested to be responsible for not only intestinal pathology such as *Clostridium difficile* infection, but also for systemic conditions such as obesity, diabetes mellitus and IBD including UC (3,9). The role of gut microbiota with host-microbiome interactions are likely to be a key driver in the pathogenesis of UC (10,11). Antibiotics, which alter the human gut microbiome, have been shown to contribute to UC activity (12), whereas probiotics have been implicated in UC remission (13). The gut microbiota of UC patients lacks diversity (14,15) and *Bacteroidetes* and *Firmicutes* are found significantly less in UC patients' microbiota (14,16). Furthermore, reduced amounts of bacterial producers of short-chain fatty acids (SCFA) (butyrate, propionate and acetate) are found in the microbiota of UC patients (17,18). These SCFAs are products of starch fermentation from gut bacteria and are believed to have anti-inflammatory properties. Moreover, recent studies have shown that butyrate produced from *Faecalibacterium prausnitzii* not only has anti-inflammatory properties, but also provides the major nutrient for colonocytes (19), and prevents intestinal mucosa atrophy and colonocyte autophagy (20). A number of studies demonstrate that the butyrate producer *Faecalibacterium prausnitzii* was less abundant in UC patients (21–23). Moreover, recent studies have suggested that not only living bacteria may be responsible inflammatory process of UC, but also bacterial specific

components and structures, antimicrobial compounds and metabolites produced by bacteria may contribute to the gut microenvironment thus its inflammatory process (24). Understanding of a critical role of secondary metabolites has also been highlighted recently by Buffie *et al.* recently as they have indicated that certain species may inhibit *C. difficile* with their secondary metabolites, including secondary bile acids by *C. scindens* (25,26). Although the role of fungi in the human microbiome has not yet been fully understood, recent studies suggest microfragments of chitin, which is a substance produced by fungi and insects, display a significant immunomodulatory impact in the inflammatory process (27,28). This suggests that not only viable common gut anaerobic microorganisms, but also products and particles from other microorganisms may be responsible for dysregulation of the immune response. Despite extensive studies no single pathogen has been identified as responsible for the pathogenesis of UC. The current consensus is that the loss of certain bacterial strains with immunomodulatory as well as mucosal regulatory functions leads to gut dysbiosis, resulting in the pathogenesis of UC. Faecal Microbiota Transplantation (FMT) is an infusion of a faecal suspension from a healthy individual (donor) to restore the dysbiosis of affected individuals (recipient). Since the approval of FMT in the management of recurrent *C. difficile* infections in 2014 by the National Institute for Health and Care Excellence (NICE), FMT has been of increasing interest as a therapeutic approach in the management of UC. If we can successfully and durably alter the colonic microbiota (29), it may be possible to achieve complete remission of this chronic debilitating disease without lifelong immunosuppression use or the need for major gastrointestinal (GI) surgery. The ability to induce remission and establish the microbiological basis for this would change the treatment paradigm for UC. Recent years have seen several randomised clinical studies emerging to investigate FMT in the management of UC with encouraging results (15,30–32). Despite these studies, many unknown aspects remain in the clinical application of FMT in UC, such as the optimum dose, route of administration and frequency of treatments. Equally it is not known whether FMT is effective as a first line treatment in drug-naïve patients. To study the optimum parameters for delivering FMT in UC and estimating the clinical response this randomised feasibility trial was designed.

The objectives of this feasibility study include: evaluation of the magnitude of treatment response to FMT, investigation of the functional metabolic changes associated with FMT using a metabolic phenotyping methodology and testing the recruitment rate of donors and patients. Furthermore, we aim to measure the duration of clinical response with microbiome identification through 16S

rRNA sequencing and metabolomic analysis. This will facilitate the design of a definitive multi-centred study to confirm the efficacy of FMT as a first-line treatment option in UC.

Primary Objectives:

The primary objective of this phase II study is to estimate the magnitude of the treatment response to FMT in treatment naïve patients with UC.

Secondary Objectives:

- Determine the recruitment rate of donors and participants for a study of FMT
- Determine the optimal study conditions and choice of endpoints for phase III study to include dosage and frequency of FMT treatments.
- Establish how many participants would be required for phase III to demonstrate the efficacy of FMT in the treatment of UC.

2. Methods

This is a single-blinded interventional randomised feasibility study to estimate the magnitude of the treatment response to FMT in newly diagnosed patients with distal UC who are treatment naïve. Recruitment is proposed over a two-year period with a twelve-week post-treatment follow up period. This feasibility trial will help determine the recruitment rate of donors and participants, define the optimal study conditions and choice of endpoints for a phase III definitive study. It will also allow us to establish how many participants would be required at phase III to demonstrate the efficacy of FMT in the treatment of UC.

1. Trial design

We aim to recruit thirty subjects with histologically confirmed UC, whose disease is confined to the recto-sigmoid area (defined here as within forty centimetres from the anal verge) and who are treatment naïve. Participants will be randomly assigned to study groups through a web-based application hosted by University of Aberdeen.

Eligible patients will be randomised into one of three groups with an allocation ratio of 2:2:1 as shown in Table 1. Groups 1 and 2 are the intervention arms and twelve subjects will be assigned to each group respectively. Group 3 is the control arm and six subjects will be randomised into this group.

Intervention arms: Groups 1 and 2

Participants randomly allocated to group 1 will receive one single FMT treatment administered as a rectal retention enema. Participants in group 2 will receive a single FMT treatment on five consecutive days (total of 5 treatments) also administered by rectal retention enema.

Control arm: Group 3

Participants randomly allocated to group 3 will receive the pre-FMT preparation with antibiotics and bowel preparation but will not receive active FMT treatment.

Table 1: Intervention arms (Group 1 and 2) and Control arm (Group 3)

	Group 1	Group 2	Group 3
Bowel decontamination and preparation	Yes	Yes	Yes
FMT treatment dose	1	5 consecutive days (single treatment per day)	None
Number of participants	12	12	6

2. Endpoints

2.1 Paired primary endpoints

- Remission of UC (mucosal healing) at 12 weeks as assessed by blinded sigmoidoscopy. Assessment defined as Mayo score ≤ 2 with an endoscopic Mayo score of 0
- Proportion of successful engraftment of donor faecal microbiota at 12 weeks in each group as analysed by 16S sequencing and longitudinal diversity index.

2.2 Secondary endpoints

- Rate of recruitment of patients
- Disease specific scores after treatment using IBDex severity scoring index (33), CUCQ-32 severity scoring index (34) and Mayo scoring system (35)
- Histological grading of colitis severity after treatment
- Mucosal immunological response to treatment (tissue IL-10 and IL-12 by ELISA)
- Rate of development of adverse effects to FMT

3. Participant selection

Potential participants will be identified by their usual clinicians in clinics and endoscopy units within the Health Board. Each potential participant will be screened for eligibility once referred to the research team. All subjects must have a definitive histological diagnosis of UC before enrolment as made by a GI pathologist with a special interest in colitis. Minimum required microscopic features include cryptitis, crypt abcesses, crypt distortion and mucin depletion in the absence of granulomata. Participants with any features not consistent with UC will be excluded. A minimum

time period of one month from identification to screening will exclude participants with acute self-limiting colitis.

A written patient information sheet will be provided and participants will be offered a minimum of 24 hours to consider enrolment before providing written informed consent.

During the screening visit, the study will be fully explained, and consent will be obtained if the subject satisfies all inclusion and exclusion criteria (Table 2).

Table 2: Participant selection criteria

Inclusion Criteria
• Newly diagnosed histologically confirmed UC with inflammation limited to the rectum or recto-sigmoid (within 40cm of anal verge as measured by flexible sigmoidoscopy) • Age 18 years and above • Able to give full informed written consent • Willing to return for sequential FMT dosing and endoscopic assessment • Not in receipt of conventional medical treatment for colitis such as steroids or 5-aminosalicylic acid (5-ASA) i.e. treatment naïve
Exclusion Criteria
• Patients without a definitive diagnosis of UC (for example diagnosis of Crohn's disease or infectious colitis) • Colitis extending beyond 40cm from the anal verge • Diagnosis of acute severe colitis (defined as greater than 6 blood-stained stools per 24 hrs with one of the following: pulse rate >90/ temperature >37.8 degree/ haemoglobin <105g/L / ESR>30) • Abdominal tenderness on examination • Already commenced standard medical therapy for UC • Contraindication to oral bowel preparation • Allergy to study antibiotics • Age less than 18 • Patient is within a vulnerable group, defined as people who are unable to take care of him or herself, or unable to protect him or herself against significant

harm or exploitation

- Pregnant
- Immuno-suppressed e.g. transplant patient
- Known communicable disease or at least 2 weeks full recovery from infectious disease e.g. chickenpox,
- Systemic autoimmunity, or atopic diseases
- Previous prosthetic implant (for example metallic heart valve, joint replacement, ventricular-peritoneal shunt, cardiac stent)
- Chronic pain syndromes (for example: fibromyalgia, chronic fatigue)
- Neurologic, neuro-developmental or neurodegenerative disorders
- Depression (requiring therapy)
- Obesity (BMI>35)
- Malignancy
- Use of antibiotics for any indication within the past 3 months
- Foreign travel to areas of enteric disease prevalence within 3 months
- High risk sexual behaviour (examples: sexual contact with anyone with HIV/HTLV/AIDS or hepatitis B/C carrier, men who have sex with men (MSM))
- Known exposure to HIV or hepatitis B/C
- Current/previous use of injected drugs or intranasal cocaine
- Tattooing, piercing, cosmetic botulinum toxin or permanent makeup within 120 days (as per Welsh Blood Transfusion guidelines)
- Recent blood transfusion, tissue / organ transplant or skin graft
- Risk factors for variant Creutzfeldt-Jakob disease e.g. blood transfusion or transplant after 1st January 1980

Comment [DAH1]: have changed this

4. Interventions and Investigational products

All three study groups will complete a ten-day course of oral antibiotics (Metronidazole 400mg, vancomycin 500mg, rifampicin 150mg twice daily), which should complete at least forty-eight hours before the first FMT treatment. This will allow the poorly absorbed vancomycin to wash out of the gastrointestinal tract. Patients should therefore start the ten-day course of antibiotics twelve days before the first FMT is given. These antibiotics were chosen following the recently published

guidelines on FMT in clinical practice (36) towards whole gut decontamination. Additionally, all participants will receive bowel preparation (polyethylene glycol, 2 litres) on the day before transplantation to prepare the lumen for engraftment of the FMT treatment and to minimise interference from the existing gut microbiota.

4.1 Investigational Product

The investigational product is donated faecal material from healthy volunteers who are unrelated and non-cohabiting to the study participants. The FMT products are obtained either from Wessex stool bank or material that has been locally processed using the identical FMT preparation technique by a physician for the purposes of the research trial. The pellet is resuspended and frozen in 20% glycerol and stored for up to 8 weeks at -80°C until the day of treatment. Donors are screened for infections in accordance with current best practice (36) (Table 3). In the case of multiple treatments (group 2) all doses are obtained from the same donor to minimise variation. Faecal microbiota of the donated faecal samples is studied using 16S rRNA analysis. This will be used as a reference for the effect of FMT treatments, evaluation of magnitude of treatment response to FMT and durability of engraftment after FMT.

Table 3: Screening infections

Blood tests	• Cytomegalovirus • Epstein-Barr virus • Hepatitis A • HBV • HCV • Hepatitis E virus • Syphilis • HIV-1 and HIV-2 • *Entamoeba histolytica* • Human T-lymphotropic virus types I and II antibodies • *Strongyloides stercoralis*
Faecal tests	• Detection of *C. difficile* • Detection of enteric pathogens, including *Salmonella, Shigella* • *Campylobacter, Escherichia coli O157 H7, Yersinia*, Vanocomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, Gram-negative mutidrug-resistant bacteria • Norovirus • Antigens and/or acid fast staining for *Giardia sp* and *Cryptosporidium sp*

	• Protozoa (including *Blastocystis hominis*) and helminths

4.2 Administration of Investigational Product

All three study groups will complete a ten-day course of oral antibiotics (vancomycin 500mg; metronidazole 400mg, rifampicin 150mg – all taken twice daily) and bowel preparation (polyethylene glycol 2 litres on the day before transplantation). The first FMT treatment dose will be commenced 48 hours after the final dose of antibiotics to preserve the activity of the FMT. Frozen FMT will be thawed over four hours at room temperature prior to infusion, which will subsequently be diluted to 250mL with non-bacteriostatic normal saline prior to infusion. The subjects of Group 1 and 2, who receive FMT treatment, will also be given loperamide 4mg orally thirty minutes prior to administration to maximise the chance of enema retention. Each participant receives 50mL of enema every 15 minutes over 60 minutes. The subjects will be encouraged to retain the treatment samples as long as possible (ideally more than one hour).

5. Study setting

Recruitment will take place from clinics and endoscopy units within the Abertawe Bro Morgannwg University Health Board, Swansea. FMT will be administered at the Joint Clinical Research Facility within Swansea University.

6. Randomisation

Study participants will be randomised 2:2:1 by a web-based method hosted by the University of Aberdeen's Health Services Research Unit. The simple randomisation process employed in this allocation was not stratified by any factors (e.g. age, gender). We aim to update the randomisation process based on the results of this feasibility study for potential stratifying factors in phase III.

7. Blinding

The trial statistician, the assessing independent endoscopist and the pathologist undertaking macroscopic and microscopic disease assessments will be blinded to the treatment allocation.

8. Participant timeline and Schedule of Assessment

Figure 1 and Table 4 show the follow up schedule and assessment for the trial. At baseline the study participants will undergo assessment for disease activity with validated tools (CUCQ-32, IBDex and

Mayo Score) alongside a full history and physical examination. Baseline biopsies of the rectum for 16S rRNA analysis and immunological studies (IL-10 and IL-21), faecal samples for 16S rRNA analysis and metabolomic profile, blood tests (renal function, liver function, full blood count, C-reactive protein, metabolomic profile) will be obtained. Furthermore, 16S rRNA analysis for the donors' faecal samples is performed. Subsequently, this will be studied together with 16S rRNA analysis of the participant's faecal samples for the study of durability of engraftment after FMT treatments during a 12-week follow up period.

Follow-up visits will take place at week 1, 4, 8 and 12 for all three study groups. Participants will undergo clinical examination, blood and faecal testing to include faecal microbiota profiling using the 16S rRNA analysis and metabolomic profile, and complete disease activity scoring questionnaires (CUCQ-32 and IBDex) at baseline and thereafter at 1 week. At the final assessment (week 12), all subjects will also undertake a repeat flexible sigmoidoscopy for macroscopic assessment and biopsies for degree of inflammation or confirmation of remission. Participants who relapse or fail to improve after FMT will be offered conventional medical therapy.

Study participants will be instructed to inform the treating physician of any infectious symptom or new medical condition which develops after receiving FMT and a patient registry will be maintained.

Table 4: Follow up schedule and assessments

	Baseline	Week 1	Week 4	Week 8	Week 12
Questionnaires					
CUCQ-32	•	•	•	•	•
IBDex	•	•	•	•	•
Mayo score	•				•
Endoscopy assessment					
Sigmoidoscopy	•				•
Rectal biopsy	•				•
Histology assessment					
Histological grading	•				•
Mucosal 16S sequencing	•				•
	•				•

Mucosal IL-10 Mucosal IL-21	•				•
Blood tests					
Renal profile	•	•	•	•	•
Liver profile	•	•	•	•	•
Full blood count	•	•	•	•	•
C-reactive protein	•	•	•	•	•
Metabolomic profile	•	•	•	•	•
Faecal sample assessment					
16S sequencing	•	•	•	•	•
Metabolomic profile	•	•	•	•	•

9. Withdrawal

Participants may be withdrawn from the study if;

- they wish to terminate treatment and/or follow-up assessments
- clinical features worsen during FMT or the 12-week follow-up period
- the participant is non-compliant with the study in a manner that is either harmful to their health or interferes with the validity of the study results
- participants who withdraw their consent may not wish for their data to be used – if this is the case then it will be deleted

10. Data collection and management

Data collection will be performed at baseline, week 1, 4, 8 and 12 as described in Table 4. All data is to be recorded on the case report form (CRF) in an anonymised format against a unique participant number.

Data will be transferred to a computer database without patient identifiable data and analysed once all results have been collected. The trial database will have built in measures to assess data quality at time of input and stored securely.

11. Metabolic profiling

We will use both untargeted (1H NMR) and targeted quantitative approaches such as HPLC-MS to analyse a panel of gut microbial co-metabolites involved in cell signalling, namely short chain fatty acids, bile acids, indoles and cresols and branch chain amino acids. This will include a novel eicosanoid assay (37) for precision measurement of pro and anti-inflammatory regulators and the use of a bile acid assay (38). Metabolome data will be analysed by several multivariate ordinations including principal component analyses, nonmetric multidimensional scaling Kruskal-Wallis independent tests, and multivariate ANOVA with Bonferroni correction. We will create receiver operating curves for both multivariate models and individual metabolites for key clinical outcomes. Metabolic reaction networks of metabolites found differentially expressed between different transplants, will be created using the MetaboNetworks software (39).

12. Participant rights and confidentiality

The Chief Investigator will be the custodian of the data. Information with regards to study participants will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval.

There will be no patient identifiable data on the CRF and a unique participant number will be allocated. The Principal investigator will hold the key to the coded number of the participants only. Only the Principal investigator will have access to the patient identifiable information.

13. Statistical Analysis

Both descriptive and exploratory data analysis will be performed. For each group, we will calculate the number of participants approached, and/or assessed for eligibility, randomised and received the treatment. Thus, we will calculate the recruitment and retention rate along with the rate of adverse events. Descriptive statistics (mean, standard deviation, 95% confidence interval) for continuous outcomes (e.g. CUCQ-32 Score, Mayo Scoring) and raw count (n, %) for categorical outcomes (e.g. renal profile, liver profile, histological grading) will be reported as per the clinical endpoints.

Comment [KJM2]: Not sure if you want this...

Comment [DAH3]: I'd keep this in I think.

All these summary statistics will be provided as per baseline and other follow ups (as appropriate to the outcome measure) and with respect to the three treatment arms. All the analysis and data preparation will be performed using SPSS v.22.0 as a validated statistical software for clinical trials.

14. Safety measures

An adverse event (AE) is defined as any untoward medical occurrence in a patient after administration of the study intervention (FMT) that does not necessarily have to have a causal relationship with this treatment. Serious adverse events (SAEs) is any adverse experience occurring during or after FMT that results in either death, life-threatening experience or requiring inpatient hospitalisation, persistent or significant disability or incapacity. SAEs will be notified to the study sponsor within twenty-four hours and to the Research Ethics Committee (REC) within fifteen days. AEs that are expected for patients undergoing FMT, and symptoms expected from UC, are specified in the protocol and will not require to be reported as adverse events. FMT related AEs are procedure related symptoms such as bloating, transient fever or abdominal discomfort as reported by previously reported studies.

15. Quality Assurance

The Research and Development Quality Assurance Officer has performed a Monitoring Prioritisation Assessment to assess the impact of trial participation on the rights and safety of participants and the reliability of trial results. This has guided the development of procedures in the trial with respect to informed consent, confidentiality and trial monitoring. Monitoring visits to the site will be made every three months during the study to ensure that all aspects of the protocol are followed. The Quality Assurance Officer will also monitor the study after the first participant has been recruited. The monitoring visit timeframe can be changed depending on the monitoring findings. A Quality Assurance programme is also in place to ensure adherence to the study protocol. Major and minor deviations will be collected.

Endosocopy: one of several JAG accredited gastroenterologists or colorectal surgeons from hospitals of the Abertawe Bro Morgannwg University Health Board will perform the sigmoidoscopy assessment at baseline and week twelve. The study team will ensure that the endoscopist performing the 12-week assessment is blinded to the intervention that the patient has received.

Endoscopic photographs taken at baseline and at final assessment will be independently assessed by a blinded expert to provide quality assurance for this outcome measure.

Pathology: A standardised protocol based on RCPath guidelines will be used for histological assessment of the disease as per standard of care by consultant pathologists.

16. Patient and Public Involvement

Patients are recruited self-referral as well as clinician-referral. Those patients who meet strict inclusion criteria will be included in this study. Participants in the FMTUC study will be consented voluntarily after consultations when they have enough time to ask any questions about the study.

After FMT treatment, their symptoms will be scored using CUCQ-32 and IBDex at the time of follow ups. Any adverse symptoms reported by participants will be recorded.

17. Ethics and Dissemination

The Chief Investigator will ensure that the trial is conducted in compliance with the principles of the Declaration of Helsinki (1996), and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework, Trust and Research Office policies and procedures and any subsequent amendments. The trial is approved by the regional ethics committee (REC) and Health Research Authority (HRA) or equivalent. Written informed consent will be obtained from all participants. Serious adverse events will be reported to the study sponsor and the regional ethics committee. Trial results will be disseminated through oral presentations at national conferences and through peer reviewed publication, which will include named members of the Trial Management Group (TMG) who meet the three criteria of scholarship (design, execution, analysis and/or interpretation of the data), authorship (drafting, reviewing and revision of the manuscript) and approval (approving the manuscript to be published). Participants in the study will be given a copy of the results and a final report will be written by the TMG for the funding body and the REC. Results will be used to aid in the development of a definitive phase III trial.

Discussion

A recently published systematic review on the usage of FMT in IBD concluded that overall 36% of patients with UC achieved clinical remission (a total of 41 studies and 4 randomised controlled trials (RCTs))(40). Meta-analysis which included 4 RCTs (a total of 140 individuals) demonstrated that FMT was significantly linked to clinical remission with a pooled odd ratio (P-OR) of 2.89, 95% confidence interval (CI) of 1.36-6.13 and p-value of 0.016.

The number of FMT studies with high methodological quality has increased of late, yet the optimal conditions for durable FMT engraftment and maximal remission are presently unclear for UC. Table 4 summarises the current knowledge gaps in the application of FMT in UC.

Table 4: Uncertainties for the optimal application of FMT in UC

Human gut microbiota	• Responsible pathogens and their roles • Microbiome profiling techniques
FMT preparations	• Frozen vs Fresh • Donor screening protocol • Preparation methodology
Donors	• Related vs unrelated • Single donor vs multiple donors
Pre-medications/preparation	• Bowel preparation • Antibiotics vs non-antibiotics
FMT in clinical application	• Dose • Administration routes • FMT alone vs with other traditional medications • Durability of engraftment • Who to treat – active, remission, refractory • Adverse effects • Long-term effects and safety • Long term effects after transplant
Clinical remission	• How to assess clinical response • How to define clinical remission • When to stop FMT treatment • Maintenance dose required for remission • Post-remission dietary modification

Current studies are difficult to interpret as there is no universally agreed definition of remission as an endpoint in UC clinical trials to date (41). Furthermore, a lack of homogeneity of clinical trial protocols makes comparison of such studies more difficult to comprehend and these clinical trials are no exception. Moreover, different clinical trials use different patient groups, donors, treatment

dose, routes, frequency and pre-treatment medications. These multiple variables make the comparison of studies very challenging, although all studies appear to demonstrate promising results for the usage of FMT in active UC. Finally, and most importantly, patients recruited in published RCTs had been on previous conventional medical therapy until given the FMT treatment if not being assigned to further medical treatment. This makes the interpretation of the magnitude of treatment response to FMT very difficult.

Although the efficacy of FMT in UC appears to be promising, more clarity is required around optimal treatment conditions through rigorous study. This study will estimate the efficacy of rectally administered FMT in treatment naïve patients towards the design of a definitive trial. This phase II study allows us not only to estimate the magnitude of treatment response to FMT in UC, but also to determine the changes and durability of engraftment of the gut microbiota after FMT treatment. Furthermore, we will study the dose response by comparing one dose only and five daily doses towards establishing the optimum dosage of rectally administered FMT treatment for UC. There is a fundamental lack of mechanistic data to support the use of FMT in clinical practice. Bacteria represent a diverse and highly active chemical engine, that creates a suite of biologically active small molecules through secondary metabolism. The critical function of these target metabolites in the initiation and maintenance of systemic inflammation remains poorly defined and this trial will provide a detailed insight into the role of the gut microbiome in UC therapy that have the potential to stratify care in the future and improve the precision of this intervention.

Competing interests

None

Collaborators

Departments of Colorectal Surgery and Gastroenterology, Swansea (Mark Davies, Martyn Evans, Greg Taylor, Shahzad Ather, Chandra Sekaran, Umesh Khot, John Beynon, Umakant Dave, Mesbah Rahman, Linzi Thomas, Lisa Williams, Sophie Henson, Chin-Lye Ch'ng, Mithun Nagari, Jagadish Nagaraj, Praveen Eadala)

Departments of Gastroenterology and Colorectal Surgery at Cardiff and Vale University Health Board (lead investigator Barney Hawthorne) and Aneurin Bevan Health Board (lead investigators Vivek Goel and Gethin Williams)

Author Contributions

DAH and AR are responsible for the idea for the trial

MJ, ALC, ADR, MDH, SI, JK, AD, PR, TW, GJ, JGW, DAH have drafted and the manuscript and/or provided critical revision.

ADR, MH, SI, JK, AD, PR, TW, GJ, JGW, DAH have made substantial contributions to the conception and design of the work and subsequent protocol revisions.

MJ, ALC, ADR, MDH, SI, JK, AD, PR, TW, GJ, JGW, DAH all agree to be accountable for all aspects of work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding statement

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Figure legends

Figure 1: Study scheme flowchart

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	6
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	6
Funding	4	Sources and types of financial, material, and other support	6
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	4
	5b	Name and contact information for the trial sponsor	3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	____7_____
	6b	Explanation for choice of comparators	____7-9_____
Objectives	7	Specific objectives or hypotheses	____9_____
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	____10_____
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	____10_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	____13-15_____

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	___11, Table 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	____12_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	____18_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	____14_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	____13_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	____17. Table 4, Figure 1_____
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	____13_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___13_____

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___17_____

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	____17__
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____17__
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	_____17____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____17_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_19
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	__19___
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	__19_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_19_____

	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	N/A_____
	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomised analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	___N/A _____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_19_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	___n/a__
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___20_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_20_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21_____
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	n/a_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5,13, 21,_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	n/a_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	21_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	21
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A __

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__N/A_____
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	__N/A____

BMJ Open

Protocol for Faecal Microbiota Transplantation in Ulcerative Colitis (FMTUC) – a Randomised Feasibility study

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Complete List of Authors:	Jitsumura, Maki; Singleton Hospital, Department of Colorectal Surgery Cunningham , Andrew; Singleton Hospital, Department of Colorectal Surgery Hitchings, Matthew; Swansea University Medical School, Medical Microbiology and Infectious Diseases Islam, Saiful; Swansea University, Swansea Trial Unit Davies, Angharad; Swansea University Medical School, Medical Microbiology and Infectious Diseases Row, Paula; Swansea University Medical School, Biochemistry Group Riddell, Andrew; Redcliffe Hospital, Department of Colorectal Surgery Kinross, James; Imperial College London, Department of Surgery and Cancer, St. Mary's Hospital, Wilkinson, Tom; Swansea University Medical School, Medical Microbiology and Infectious Diseases Jenkins, GJ; Swansea University Medical School, Molecular Carcinogenesis, Institute of Life Science Williams, John; Swansea University Medical School, Institute of Life Science 2 Harris, Dean; Abertawe Bro Morgannwg University Health Board, Department of Colorectal Surgery
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Title: Protocol for Faecal Microbiota Transplantation in Ulcerative Colitis (FMTUC) – a Randomised Feasibility study

Authors:

M. Jitsumura[1], A. L. Cunningham[1], M.D. Hitchings[2], S. Islam[3], A. Davies[4], P. Row[5], A.D. Riddell[6], J Kinross[7], T Wilkinson[2], G. Jenkins[7], J.G. Williams[9], D.A. Harris [1]

Correspondence:

Professor D.A. Harris, MD FRCS MB ChB

Consultant Colorectal Surgeon, Singleton Hospital, Sketty Lane, Swansea SA2 8QA

Honorary Clinical Professor, Swansea University

Email: WPMDAH2@cardiff.ac.uk

Telephone: (01792) 285459

Author affiliations:

1 – Department of Colorectal Surgery, Singleton Hospital, Swansea, Wales

2 – Medical microbiology and Infectious disease, Swansea University Medical School, Swansea, Wales

3 – Swansea Trials Unit, Swansea University, Swansea, Wales

4 – Public Health Wales Microbiology, Singleton Hospital, Swansea University Medical School, Swansea, Wales

5 – Biochemistry Group, Swansea University Medical School, Swansea, Wales

6- Department of Colorectal Surgery, Redcliffe Hospital, Brisbane, Australia

7 – Department of Surgery and Cancer, St. Mary's Hospital, Imperial College London, London

8- Molecular Carcinogenesis, Institute of Life Science, Swansea University Medical School, Swansea , Wales

9 – Institute of Life Science 2, Swansea University Medical School, Swansea, Wales

Keywords

Ulcerative colitis; Microbiome; Feasibility study; Randomised trial, Quality of life

Word count: 4696

Abstract

Background: The interaction of the gut microbiota with the human host is implicated in the pathogenesis of inflammatory and immunological diseases including ulcerative colitis (UC). Faecal microbiota transplantation (FMT) as a method of restoring gut microbial diversity is of increasing interest as a therapeutic approach in the management of UC. The current literature lacks consensus about dose of FMT, route of administration and duration of response.

Methods and Analysis: This single-blinded randomised trial will explore the feasibility of FMT in 30 treatment-naïve patients with histologically confirmed distal UC limited to the recto-sigmoid region (up to 40 cm from the anal verge). This study aims to estimate the magnitude of treatment response to FMT under controlled conditions. The intervention (FMT) will be administered by rectal retention enema.

It will test the feasibility of randomising patients to: i) single FMT dose, ii) five daily FMT doses or iii) control (no FMT dose). All groups will receive standard antibiotic gut decontamination and bowel preparation before FMT. Recruitment will take place over a 24-month period with a 12-week patient follow up.

Trial objectives include evaluation of magnitude of treatment response to FMT, investigation of the clinical value of metabolic phenotyping for predicting the clinical response to FMT and testing the recruitment rate of donors and patients for a study in FMT. This feasibility trial will enable an estimate of number of patients needed, help determine optimal study conditions and inform the choice of endpoints for a future definitive phase III study.

Ethics and dissemination

The trial is approved by the regional ethics committee and is sponsored by ABM University Health Board. Written informed consent from all patients will be obtained. Serious adverse events will be reported to the sponsor. Trial results will be disseminated via peer review publication and shared with trial participants.

Strengths and limitations of this study

- This is one of the first such trials to have a homogenous study group of newly diagnosed and treatment naïve UC patients

- The trial will not only show the efficacy of FMT treatment by rectal administration, but also help to define the optimal number of doses of FMT for treatment of UC
- Metabolomic analysis will demonstrate mechanism of action of FMT in treatment responders
- Patient reported quality of life measures will be reported
- This study is limited by a short (12 week) follow up period

Sponsor

Abertawe Bro Morgannwg University Health Board will assume overall responsibility for the trial as a sponsor.

Trial registration: ISRCTN 58082603, REC reference 15/WA/0262 (Wales REC6)

1. Introduction

Ulcerative Colitis (UC) is a chronic relapsing-remitting mucosal inflammatory bowel disease (IBD). Clinical features include rectal bleeding, diarrhoea, faecal urgency, fatigue and weight loss. The aetiology of UC is believed to be multifactorial involving immune dysregulation, mucosal disruption and genetic predisposition, though the precise cause is poorly understood (1).

There is no curative treatment at present; thus the aim of current management is induction and maintenance of remission with immunosuppressive agents. Failure of medical therapy or refractory disease may require major resectional surgery with temporary or permanent ostomy formation. UC is also a recognised risk factor for colorectal cancer requiring lifelong surveillance (2). However, it is uncertain how to predict which group of patients will respond to medical therapy.

The human gut microbiota consists of a diverse biological environment comprising bacteria, viruses and fungi within the gut lumen and lining mucosa. The biodiversity of the gut microbiota is a dynamic process and is known to be affected by age, diet and lifestyle (3,4). It has been referred as a hidden metabolic organ through its major role as a driver of metabolic and immunological communications and the regulation of the immunological processes within the intestinal mucosa (5–8). Disruption of the gut microbiota, also called dysbiosis, has been suggested to be responsible for not only intestinal pathology such as *Clostridium difficile* infection, but also for systemic conditions such as obesity, diabetes mellitus and IBD including UC (3,9). The role of gut microbiota with host-microbiome interactions are likely to be a key driver in the pathogenesis of UC (10,11). Antibiotics, which alter the human gut microbiome, have been shown to contribute to UC activity (12), whereas probiotics have been implicated in UC remission (13). The gut microbiota of UC patients lacks diversity (14,15) and *Bacteroidetes* and *Firmicutes* are found significantly less in UC patients' microbiota (14,16). Furthermore, reduced amounts of bacterial producers of short-chain fatty acids (SCFA) (butyrate, propionate and acetate) are found in the microbiota of UC patients (17,18). These SCFAs are products of starch fermentation from gut bacteria and are believed to have anti-inflammatory properties. Moreover, recent studies have shown that butyrate produced from *Faecalibacterium prausnitzii* not only has anti-inflammatory properties, but also provides the major nutrient for colonocytes (19), and prevents intestinal mucosa atrophy and colonocyte autophagy (20). A number of studies demonstrate that the butyrate producer *Faecalibacterium prausnitzii* was less abundant in UC patients (21–23). Moreover, recent studies have suggested that not only living bacteria may be responsible inflammatory process of UC, but also bacterial specific

components and structures, antimicrobial compounds and metabolites produced by bacteria may contribute to the gut microenvironment thus its inflammatory process (24). Understanding of a critical role of secondary metabolites has also been highlighted recently by Buffie *et al.* recently as they have indicated that certain species may inhibit *C. difficile* with their secondary metabolites, including secondary bile acids by *C. scindens* (25,26). Although the role of fungi in the human microbiome has not yet been fully understood, recent studies suggest microfragments of chitin, which is a substance produced by fungi and insects, display a significant immunomodulatory impact in the inflammatory process (27,28). This suggests that not only viable common gut anaerobic microorganisms, but also products and particles from other microorganisms may be responsible for dysregulation of the immune response. Despite extensive studies no single pathogen has been identified as responsible for the pathogenesis of UC. The current consensus is that the loss of certain bacterial strains with immunomodulatory as well as mucosal regulatory functions leads to gut dysbiosis, resulting in the pathogenesis of UC. Faecal Microbiota Transplantation (FMT) is an infusion of a faecal suspension from a healthy individual (donor) to restore the dysbiosis of affected individuals (recipient). Since the approval of FMT in the management of recurrent *C. difficile* infections in 2014 by the National Institute for Health and Care Excellence (NICE), FMT has been of increasing interest as a therapeutic approach in the management of UC. If we can successfully and durably alter the colonic microbiota (29), it may be possible to achieve complete remission of this chronic debilitating disease without lifelong immunosuppression use or the need for major gastrointestinal (GI) surgery. The ability to induce remission and establish the microbiological basis for this would change the treatment paradigm for UC. Recent years have seen several randomised clinical studies emerging to investigate FMT in the management of UC with encouraging results (15,30–32). Despite these studies, many unknown aspects remain in the clinical application of FMT in UC, such as the optimum dose, route of administration and frequency of treatments. Equally it is not known whether FMT is effective as a first line treatment in drug-naïve patients. To study the optimum parameters for delivering FMT in UC and estimating the clinical response this randomised feasibility trial was designed.

The objectives of this feasibility study include: evaluation of the magnitude of treatment response to FMT, investigation of the functional metabolic changes associated with FMT using a metabolic phenotyping methodology and testing the recruitment rate of donors and patients. Furthermore, we aim to measure the duration of clinical response with microbiome identification through 16S

rRNA sequencing and metabolomic analysis. This will facilitate the design of a definitive multi-centred study to confirm the efficacy of FMT as a first-line treatment option in UC.

Primary Objectives:

The primary objective of this phase II study is to estimate the magnitude of the treatment response to FMT in treatment naïve patients with UC.

Secondary Objectives:

- Determine the recruitment rate of donors and participants for a study of FMT
- Determine the optimal study conditions and choice of endpoints for phase III study to include dosage and frequency of FMT treatments.
- Establish how many participants would be required for phase III to demonstrate the efficacy of FMT in the treatment of UC.

2. Methods

This is a single-blinded interventional randomised feasibility study to estimate the magnitude of the treatment response to FMT in newly diagnosed patients with distal UC who are treatment naïve. Recruitment is proposed over a two-year period with a twelve-week post-treatment follow up period. This feasibility trial will help determine the recruitment rate of donors and participants, define the optimal study conditions and choice of endpoints for a phase III definitive study. It will also allow us to establish how many participants would be required at phase III to demonstrate the efficacy of FMT in the treatment of UC.

1. Trial design

We aim to recruit thirty subjects with histologically confirmed UC, whose disease is confined to the recto-sigmoid area (defined here as within forty centimetres from the anal verge) and who are treatment naïve. Participants will be randomly assigned to study groups through a web-based application hosted by University of Aberdeen.

Eligible patients will be randomised into one of three groups with an allocation ratio of 2:2:1 as shown in Table 1. Groups 1 and 2 are the intervention arms and twelve subjects will be assigned to each group respectively. Group 3 is the control arm and six subjects will be randomised into this group.

Intervention arms: Groups 1 and 2

Participants randomly allocated to group 1 will receive one single FMT treatment administered as a rectal retention enema. Participants in group 2 will receive a single FMT treatment on five consecutive days (total of 5 treatments) also administered by rectal retention enema.

Control arm: Group 3

Participants randomly allocated to group 3 will receive the pre-FMT preparation with antibiotics and bowel preparation but will not receive active FMT treatment.

Table 1: Intervention arms (Group 1 and 2) and Control arm (Group 3)

	Group 1	Group 2	Group 3
Bowel decontamination and preparation	Yes	Yes	Yes
FMT treatment dose	1	5 consecutive days (single treatment per day)	None
Number of participants	12	12	6

2. Endpoints

2.1 Paired primary endpoints

- Remission of UC (mucosal healing) at 12 weeks as assessed by blinded sigmoidoscopy. Assessment defined as Mayo score ≤ 2 with an endoscopic Mayo score of 0
- Proportion of successful engraftment of donor faecal microbiota at 12 weeks in each group as analysed by 16S sequencing and longitudinal diversity index.

2.2 Secondary endpoints

- Rate of recruitment of patients
- Disease specific scores after treatment using IBDex severity scoring index (33), CUCQ-32 severity scoring index (34) and Mayo scoring system (35)
- Histological grading of colitis severity after treatment
- Mucosal immunological response to treatment (tissue IL-10 and IL-12 by ELISA)
- Rate of development of adverse effects to FMT

3. Participant selection

Potential participants will be identified by their usual clinicians in clinics and endoscopy units within the Health Board. Each potential participant will be screened for eligibility once referred to the research team. All subjects must have a definitive histological diagnosis of UC before enrolment as made by a GI pathologist with a special interest in colitis. Minimum required microscopic features include cryptitis, crypt abcesses, crypt distortion and mucin depletion in the absence of granulomata. Participants with any features not consistent with UC will be excluded. A minimum

time period of one month from identification to screening will exclude participants with acute self-limiting colitis.

A written patient information sheet will be provided and participants will be offered a minimum of 24 hours to consider enrolment before providing written informed consent.

During the screening visit, the study will be fully explained, and consent will be obtained if the subject satisfies all inclusion and exclusion criteria (Table 2).

Table 2: Participant selection criteria

Inclusion Criteria
• Newly diagnosed histologically confirmed UC with inflammation limited to the rectum or recto-sigmoid (within 40cm of anal verge as measured by flexible sigmoidoscopy) • Age 18 years and above • Able to give full informed written consent • Willing to return for sequential FMT dosing and endoscopic assessment • Not in receipt of conventional medical treatment for colitis such as steroids or 5-aminosalicylic acid (5-ASA) i.e. treatment naïve
Exclusion Criteria
• Patients without a definitive diagnosis of UC (for example diagnosis of Crohn's disease or infectious colitis) • Colitis extending beyond 40cm from the anal verge • Diagnosis of acute severe colitis (defined as greater than 6 blood-stained stools per 24 hrs with one of the following: pulse rate >90/ temperature >37.8 degree/ haemoglobin <105g/L / ESR>30) • Abdominal tenderness on examination • Already commenced standard medical therapy for UC • Contraindication to oral bowel preparation • Allergy to study antibiotics • Age less than 18 • Patient is within a vulnerable group, defined as people who are unable to take care of him or herself, or unable to protect him or herself against significant

harm or exploitation

- Pregnant
- Immuno-suppressed e.g. transplant patient
- Known communicable disease or at least 2 weeks full recovery from infectious disease e.g. chickenpox,
- Systemic autoimmunity, or atopic diseases
- Previous prosthetic implant (for example metallic heart valve, joint replacement, ventricular-peritoneal shunt, cardiac stent)
- Chronic pain syndromes (for example: fibromyalgia, chronic fatigue)
- Neurologic, neuro-developmental or neurodegenerative disorders
- Depression (requiring therapy)
- Obesity (BMI>35)
- Malignancy
- Use of antibiotics for any indication within the past 3 months
- Foreign travel to areas of enteric disease prevalence within 3 months
- High risk sexual behaviour (examples: sexual contact with anyone with HIV/HTLV/AIDS or hepatitis B/C carrier, men who have sex with men (MSM))
- Known exposure to HIV or hepatitis B/C
- Current/previous use of injected drugs or intranasal cocaine
- Tattooing, piercing, cosmetic botulinum toxin or permanent makeup within 120 days (as per Welsh Blood Transfusion guidelines)
- Recent blood transfusion, tissue / organ transplant or skin graft
- Risk factors for variant Creutzfeldt-Jakob disease e.g. blood transfusion or transplant after 1st January 1980

4. Interventions and Investigational products

All three study groups will complete a ten-day course of oral antibiotics (Metronidazole 400mg, vancomycin 500mg, rifampicin 150mg twice daily), which should complete at least forty-eight hours before the first FMT treatment. This will allow the poorly absorbed vancomycin to wash out of the gastrointestinal tract. Patients should therefore start the ten-day course of antibiotics twelve days before the first FMT is given. These antibiotics were chosen following the recently published

guidelines on FMT in clinical practice (36) towards whole gut decontamination. Additionally, all participants will receive bowel preparation (polyethylene glycol, 2 litres) on the day before transplantation to prepare the lumen for engraftment of the FMT treatment and to minimise interference from the existing gut microbiota.

4.1 Investigational Product

The investigational product is donated faecal material from healthy volunteers who are unrelated and non-cohabiting to the study participants. The FMT products are obtained either from Wessex stool bank or material that has been locally processed using the identical FMT preparation technique by a physician for the purposes of the research trial. The pellet is resuspended and frozen in 20% glycerol and stored for up to 8 weeks at -80°C until the day of treatment. Donors are screened for infections in accordance with current best practice (36) (Table 3). In the case of multiple treatments (group 2) all doses are obtained from the same donor to minimise variation. Faecal microbiota of the donated faecal samples is studied using 16S rRNA analysis. This will be used as a reference for the effect of FMT treatments, evaluation of magnitude of treatment response to FMT and durability of engraftment after FMT.

Table 3: Screening infections

Blood tests	• Cytomegalovirus • Epstein-Barr virus • Hepatitis A • HBV • HCV • Hepatitis E virus • Syphilis • HIV-1 and HIV-2 • *Entamoeba histolytica* • Human T-lymphotropic virus types I and II antibodies • *Strongyloides stercoralis*
Faecal tests	• Detection of *C. difficile* • Detection of enteric pathogens, including *Salmonella, Shigella* • *Campylobacter, Escherichia coli O157 H7, Yersinia*, Vanocomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, Gram-negative mutidrug-resistant bacteria • Norovirus • Antigens and/or acid fast staining for *Giardia sp* and *Cryptosporidium sp*

	• Protozoa (including *Blastocystis hominis*) and helminths

4.2 Administration of Investigational Product

All three study groups will complete a ten-day course of oral antibiotics (vancomycin 500mg; metronidazole 400mg, rifampicin 150mg – all taken twice daily) and bowel preparation (polyethylene glycol 2 litres on the day before transplantation). The first FMT treatment dose will be commenced 48 hours after the final dose of antibiotics to preserve the activity of the FMT. Frozen FMT will be thawed over four hours at room temperature prior to infusion, which will subsequently be diluted to 250mL with non-bacteriostatic normal saline prior to infusion. The subjects of Group 1 and 2, who receive FMT treatment, will also be given loperamide 4mg orally thirty minutes prior to administration to maximise the chance of enema retention. Each participant receives 50mL of enema every 15 minutes over 60 minutes. The subjects will be encouraged to retain the treatment samples as long as possible (ideally more than one hour).

5. Study setting

Recruitment will take place from clinics and endoscopy units within the Abertawe Bro Morgannwg University Health Board, Swansea. FMT will be administered at the Joint Clinical Research Facility within Swansea University.

6. Randomisation

Study participants will be randomised 2:2:1 by a web-based method hosted by the University of Aberdeen's Health Services Research Unit. The simple randomisation process employed in this allocation was not stratified by any factors (e.g. age, gender). We aim to update the randomisation process based on the results of this feasibility study for potential stratifying factors in phase III.

7. Blinding

The trial statistician, the assessing independent endoscopist and the pathologist undertaking macroscopic and microscopic disease assessments will be blinded to the treatment allocation.

8. Participant timeline and Schedule of Assessment

Figure 1 and Table 4 show the follow up schedule and assessment for the trial. At baseline the study participants will undergo assessment for disease activity with validated tools (CUCQ-32, IBDex and

Mayo Score) alongside a full history and physical examination. Baseline biopsies of the rectum for 16S rRNA analysis and immunological studies (IL-10 and IL-21), faecal samples for 16S rRNA analysis and metabolomic profile, blood tests (renal function, liver function, full blood count, C-reactive protein, metabolomic profile) will be obtained. Furthermore, 16S rRNA analysis for the donors' faecal samples is performed. Subsequently, this will be studied together with 16S rRNA analysis of the participant's faecal samples for the study of durability of engraftment after FMT treatments during a 12-week follow up period.

Follow-up visits will take place at week 1, 4, 8 and 12 for all three study groups. Participants will undergo clinical examination, blood and faecal testing to include faecal microbiota profiling using the 16S rRNA analysis and metabolomic profile, and complete disease activity scoring questionnaires (CUCQ-32 and IBDex) at baseline and thereafter at 1 week. At the final assessment (week 12), all subjects will also undertake a repeat flexible sigmoidoscopy for macroscopic assessment and biopsies for degree of inflammation or confirmation of remission. Participants who relapse or fail to improve after FMT will be offered conventional medical therapy.

Study participants will be instructed to inform the treating physician of any infectious symptom or new medical condition which develops after receiving FMT and a patient registry will be maintained.

Table 4: Follow up schedule and assessments

	Baseline	Week 1	Week 4	Week 8	Week 12
Questionnaires					
CUCQ-32	•	•	•	•	•
IBDex	•	•	•	•	•
Mayo score	•				•
Endoscopy assessment					
Sigmoidoscopy	•				•
Rectal biopsy	•				•
Histology assessment					
Histological grading	•				•
Mucosal 16S sequencing	•				•
	•				•

Mucosal IL-10 Mucosal IL-21	•				•
Blood tests					
Renal profile	•	•	•	•	•
Liver profile	•	•	•	•	•
Full blood count	•	•	•	•	•
C-reactive protein	•	•	•	•	•
Metabolomic profile	•	•	•	•	•
Faecal sample assessment					
16S sequencing	•	•	•	•	•
Metabolomic profile	•	•	•	•	•

9. Withdrawal

Participants may be withdrawn from the study if;

- they wish to terminate treatment and/or follow-up assessments
- clinical features worsen during FMT or the 12-week follow-up period
- the participant is non-compliant with the study in a manner that is either harmful to their health or interferes with the validity of the study results
- participants who withdraw their consent may not wish for their data to be used – if this is the case then it will be deleted

10. Data collection and management

Data collection will be performed at baseline, week 1, 4, 8 and 12 as described in Table 4. All data is to be recorded on the case report form (CRF) in an anonymised format against a unique participant number.

Data will be transferred to a computer database without patient identifiable data and analysed once all results have been collected. The trial database will have built in measures to assess data quality at time of input and stored securely.

11. Metabolic profiling

We will use both untargeted (1H NMR) and targeted quantitative approaches such as HPLC-MS to analyse a panel of gut microbial co-metabolites involved in cell signalling, namely short chain fatty acids, bile acids, indoles and cresols and branch chain amino acids. This will include a novel eicosanoid assay (37) for precision measurement of pro and anti-inflammatory regulators and the use of a bile acid assay (38). Metabolome data will be analysed by several multivariate ordinations including principal component analyses, nonmetric multidimensional scaling Kruskal-Wallis independent tests, and multivariate ANOVA with Bonferroni correction. We will create receiver operating curves for both multivariate models and individual metabolites for key clinical outcomes. Metabolic reaction networks of metabolites found differentially expressed between different transplants, will be created using the MetaboNetworks software (39).

12. Participant rights and confidentiality

The Chief Investigator will be the custodian of the data. Information with regards to study participants will be kept confidential and managed in accordance with the Data Protection Act, NHS Caldicott Guardian, The Research Governance Framework for Health and Social Care and Research Ethics Committee Approval.

There will be no patient identifiable data on the CRF and a unique participant number will be allocated. The Principal investigator will hold the key to the coded number of the participants only. Only the Principal investigator will have access to the patient identifiable information.

13. Statistical Analysis

Both descriptive and exploratory data analysis will be performed. For each group, we will calculate the number of participants approached, and/or assessed for eligibility, randomised and received the treatment. Thus, we will calculate the recruitment and retention rate along with the rate of adverse events. Descriptive statistics (mean, standard deviation, 95% confidence interval) for continuous outcomes (e.g. CUCQ-32 Score, Mayo Scoring) and raw count (n, %) for categorical outcomes (e.g. renal profile, liver profile, histological grading) will be reported as per the clinical endpoints.

All these summary statistics will be provided as per baseline and other follow ups (as appropriate to the outcome measure) and with respect to the three treatment arms. All the analysis and data preparation will be performed using SPSS v.22.0 as a validated statistical software for clinical trials.

14. Safety measures

An adverse event (AE) is defined as any untoward medical occurrence in a patient after administration of the study intervention (FMT) that does not necessarily have to have a causal relationship with this treatment. Serious adverse events (SAEs) is any adverse experience occurring during or after FMT that results in either death, life-threatening experience or requiring inpatient hospitalisation, persistent or significant disability or incapacity. SAEs will be notified to the study sponsor within twenty-four hours and to the Research Ethics Committee (REC) within fifteen days. AEs that are expected for patients undergoing FMT, and symptoms expected from UC, are specified in the protocol and will not require to be reported as adverse events. FMT related AEs are procedure related symptoms such as bloating, transient fever or abdominal discomfort as reported by previously reported studies.

15. Quality Assurance

The Research and Development Quality Assurance Officer has performed a Monitoring Prioritisation Assessment to assess the impact of trial participation on the rights and safety of participants and the reliability of trial results. This has guided the development of procedures in the trial with respect to informed consent, confidentiality and trial monitoring. Monitoring visits to the site will be made every three months during the study to ensure that all aspects of the protocol are followed. The Quality Assurance Officer will also monitor the study after the first participant has been recruited. The monitoring visit timeframe can be changed depending on the monitoring findings. A Quality Assurance programme is also in place to ensure adherence to the study protocol. Major and minor deviations will be collected.

Endosocopy: one of several JAG accredited gastroenterologists or colorectal surgeons from hospitals of the Abertawe Bro Morgannwg University Health Board will perform the sigmoidoscopy assessment at baseline and week twelve. The study team will ensure that the endoscopist performing the 12-week assessment is blinded to the intervention that the patient has received.

Endoscopic photographs taken at baseline and at final assessment will be independently assessed by a blinded expert to provide quality assurance for this outcome measure.

Pathology: A standardised protocol based on RCPath guidelines will be used for histological assessment of the disease as per standard of care by consultant pathologists.

16. Patient and Public Involvement

Patients with ulcerative colitis were surveyed during the trial design stage to ascertain willingness to participate in the trial as described. All seven patients approached indicated by return of questionnaire their willingness to be recruited into the trial.

The investigators will invite IBD specific charitable organisations and their patient representatives to help disseminate the findings of the feasibility trial and to design phase 3.

17. Ethics and Dissemination

The Chief Investigator will ensure that the trial is conducted in compliance with the principles of the Declaration of Helsinki (1996), and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework, Trust and Research Office policies and procedures and any subsequent amendments. The trial is approved by the regional ethics committee (REC) and Health Research Authority (HRA) or equivalent. Written informed consent will be obtained from all participants. Serious adverse events will be reported to the study sponsor and the regional ethics committee. Trial results will be disseminated through oral presentations at national conferences and through peer reviewed publication, which will include named members of the Trial Management Group (TMG) who meet the three criteria of scholarship (design, execution, analysis and/or interpretation of the data), authorship (drafting, reviewing and revision of the manuscript) and approval (approving the manuscript to be published). Participants in the study will be given a copy of the results and a final report will be written by the TMG for the funding body and the REC. Results will be used to aid in the development of a definitive phase III trial.

Discussion

A recently published systematic review on the usage of FMT in IBD concluded that overall 36% of patients with UC achieved clinical remission (a total of 41 studies and 4 randomised controlled trials (RCTs))(40). Meta-analysis which included 4 RCTs (a total of 140 individuals) demonstrated that FMT was significantly linked to clinical remission with a pooled odd ratio (P-OR) of 2.89, 95% confidence interval (CI) of 1.36-6.13 and p-value of 0.016.

The number of FMT studies with high methodological quality has increased of late, yet the optimal conditions for durable FMT engraftment and maximal remission are presently unclear for UC. Table 4 summarises the current knowledge gaps in the application of FMT in UC.

Table 4: Uncertainties for the optimal application of FMT in UC

Human gut microbiota	• Responsible pathogens and their roles • Microbiome profiling techniques
FMT preparations	• Frozen vs Fresh • Donor screening protocol • Preparation methodology
Donors	• Related vs unrelated • Single donor vs multiple donors
Pre-medications/preparation	• Bowel preparation • Antibiotics vs non-antibiotics
FMT in clinical application	• Dose • Administration routes • FMT alone vs with other traditional medications • Durability of engraftment • Who to treat – active, remission, refractory • Adverse effects • Long-term effects and safety • Long term effects after transplant
Clinical remission	• How to assess clinical response • How to define clinical remission • When to stop FMT treatment • Maintenance dose required for remission • Post-remission dietary modification

Current studies are difficult to interpret as there is no universally agreed definition of remission as an endpoint in UC clinical trials to date (41). Furthermore, a lack of homogeneity of clinical trial protocols makes comparison of such studies more difficult to comprehend and these clinical trials are no exception. Moreover, different clinical trials use different patient groups, donors, treatment

dose, routes, frequency and pre-treatment medications. These multiple variables make the comparison of studies very challenging, although all studies appear to demonstrate promising results for the usage of FMT in active UC. Finally, and most importantly, patients recruited in published RCTs had been on previous conventional medical therapy until given the FMT treatment if not being assigned to further medical treatment. This makes the interpretation of the magnitude of treatment response to FMT very difficult.

Although the efficacy of FMT in UC appears to be promising, more clarity is required around optimal treatment conditions through rigorous study. This study will estimate the efficacy of rectally administered FMT in treatment naïve patients towards the design of a definitive trial. This phase II study allows us not only to estimate the magnitude of treatment response to FMT in UC, but also to determine the changes and durability of engraftment of the gut microbiota after FMT treatment. Furthermore, we will study the dose response by comparing one dose only and five daily doses towards establishing the optimum dosage of rectally administered FMT treatment for UC. There is a fundamental lack of mechanistic data to support the use of FMT in clinical practice. Bacteria represent a diverse and highly active chemical engine, that creates a suite of biologically active small molecules through secondary metabolism. The critical function of these target metabolites in the initiation and maintenance of systemic inflammation remains poorly defined and this trial will provide a detailed insight into the role of the gut microbiome in UC therapy that have the potential to stratify care in the future and improve the precision of this intervention.

Competing interests

None

Collaborators

Departments of Colorectal Surgery and Gastroenterology, Swansea (Mark Davies, Martyn Evans, Greg Taylor, Shahzad Ather, Chandra Sekaran, Umesh Khot, John Beynon, Umakant Dave, Mesbah Rahman, Linzi Thomas, Lisa Williams, Sophie Henson, Chin-Lye Ch'ng, Mithun Nagari, Jagadish Nagaraj, Praveen Eadala)

Departments of Gastroenterology and Colorectal Surgery at Cardiff and Vale University Health Board (lead investigator Barney Hawthorne) and Aneurin Bevan Health Board (lead investigators Vivek Goel and Gethin Williams)

Author Contributions

DAH and AR are responsible for the idea for the trial

MJ, ALC, ADR, MDH, SI, JK, AD, PR, TW, GJ, JGW, DAH have drafted and the manuscript and/or provided critical revision.

ADR, MDH, SI, JK, AD, PR, TW, GJ, JGW, DAH have made substantial contributions to the conception and design of the work and subsequent protocol revisions.

MJ, ALC, ADR, MDH, SI, JK, AD, PR, TW, GJ, JGW, DAH all agree to be accountable for all aspects of work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding statement

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Figure legends

Figure 1: Study scheme flowchart

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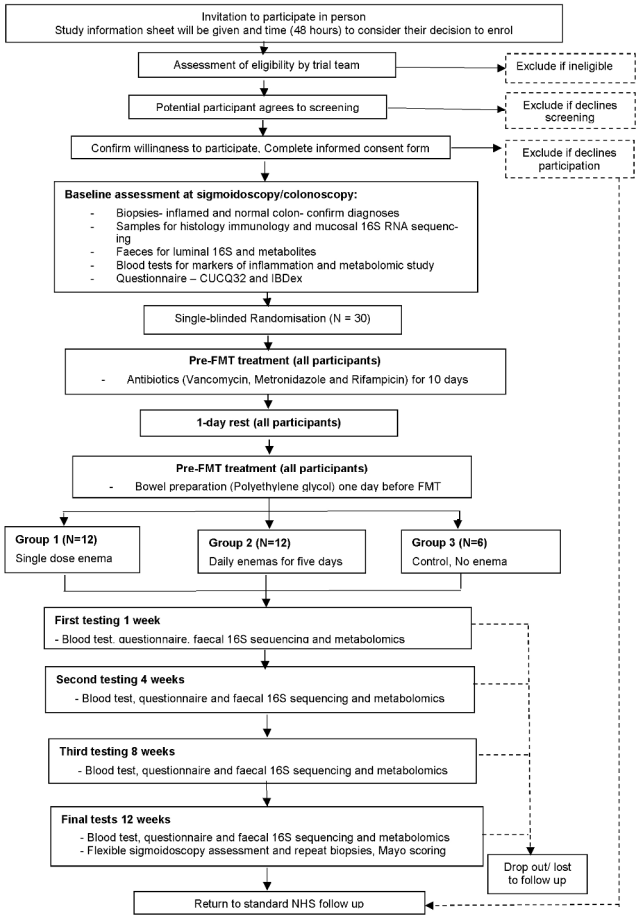

Figure 1: Study scheme flowchart

209x297mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	6
	2b	All items from the World Health Organization Trial Registration Data Set	
Protocol version	3	Date and version identifier	6
Funding	4	Sources and types of financial, material, and other support	6
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	4
	5b	Name and contact information for the trial sponsor	3
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	6

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	_____
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	____7_____
	6b	Explanation for choice of comparators	____7-9_____
Objectives	7	Specific objectives or hypotheses	____9_____
Trial design	8	Description of trial design including type of trial (e.g., parallel group, crossover, factorial, single group), allocation ratio, and framework (e.g., superiority, equivalence, noninferiority, exploratory)	____10_____
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	____10_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (e.g., surgeons, psychotherapists)	____13-15_____

Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	___11, Table 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (e.g., drug dose change in response to harms, participant request, or improving/worsening disease)	____12_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (e.g., drug tablet return, laboratory tests)	____18_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	____14_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (e.g., systolic blood pressure), analysis metric (e.g., change from baseline, final value, time to event), method of aggregation (e.g., median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	____13_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	____17. Table 4, Figure 1_____
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	____13_____
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	___13_____

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (e.g., computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (e.g., blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	___17_____

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (e.g., central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	____17__
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	____17__
Blinding (masking)	17a	Who will be blinded after assignment to interventions (e.g., trial participants, care providers, outcome assessors, data analysts), and how	_____17____
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	____17_____
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (e.g., duplicate measurements, training of assessors) and a description of study instruments (e.g., questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	_19
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	__19___
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (e.g., double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	__19_____
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	_19_____

	20b	Methods for any additional analyses (e.g., subgroup and adjusted analyses)	N/A_____
	20c	Definition of analysis population relating to protocol non-adherence (e.g., as randomised analysis), and any statistical methods to handle missing data (e.g., multiple imputation)	___N/A _____
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	_19_____
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	___n/a__
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	___20_____
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	_20_____
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	21_____
Protocol amendments	25	Plans for communicating important protocol modifications (e.g., changes to eligibility criteria, outcomes, analyses) to relevant parties (e.g., investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	n/a_____

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5,13, 21,_____
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a_____
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	19_____
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	n/a_____
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	n/a
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A_____
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (e.g., via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	21_____
	31b	Authorship eligibility guidelines and any intended use of professional writers	21
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A __

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	__N/A_____
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	__N/A____

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.