CLINICAL STUDY REPORT

PROFIT

A PROspective, randomised placebo controlled feasibility trial of Faecal mIcrobiota Transplantation in cirrhosis

Sponsor Protocol Code:	3576
EudraCT Number:	2017-003629-13
ClinicalTrials.gov Identifier:	NCT02862249
REC Number:	17/LO/2081
Investigational Drugs (IMPs):	Faecal microbiota transplantation
Indication:	Cirrhosis
Development Phase:	3
Study Begin (FPFV):	23/05/2018
Study End (LPLV):	17/10/2019
Report Version & Issue Date:	Version 1.0 17/09/2020
Co-sponsor Name and Address:	**Amy Holton** Quality Manager King's Health Partners Clinical Trials Office F16 Tower Wing | Guy's Hospital | Great Maze Pond | SE1 9RT
Co-sponsor contact details:	T: 020 7188 5732 M: 07540678025 E: amy.holton@kcl.ac.uk
Chief Investigator:	Professor Debbie Shawcross

SIGNATURE PAGE

By signing below I approve the contents of this Clinical Study Report, and confirm that to the best of my knowledge it accurately describes the conduct and results of the study. The clinical trial reported herein was conducted in accordance with the principles contained in the Declaration of Helsinki, Good Clinical Practice (GCP) and all applicable laws and regulations.

Chief Investigator:

Printed name	**Signature**	**Date: 16/09/2020**
Professor Debbie Shawcross		

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APPENDICES

1. Ethics

Independent Ethics Committee or Institutional Review Board

The study protocol and amendments were reviewed and approved by the London South East Research Ethics Committee (ref 17/LO/2081) on 31/01/2018.

Ethical conduct of the study

The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) as amended, the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial protocol and substantial amendments were reviewed by the United Kingdom (UK) Medicines and Healthcare products Regulatory Agency (MHRA).

Subject information and consent

Patients were identified by their treating clinicians and by review of clinic lists. Patients were approached by the trials team to see if they were interested in participating in the study and given a written Patient Information Sheet (PIS). Patients were given sufficient time to consider trial participation and to discuss this with family, friends and their treating clinicians. If in agreement, patients signed the informed consent form (ICF) and had bloods taken to confirm their MELD score (1) and HIV serology.

Data Monitoring

The study was overseen by an independent Data Monitoring and Ethics Committee (DMEC) and a Trial Steering Committee (TSC).

The DMEC was chaired by Dr Richard Aspinall, Consultant Gastroenterologist and Hepatologist at Portsmouth hospital. The DMEC panel consisted of an independent statistician, the chair and independent Consultant Hepatologist, Dr Phil Berry, from Guy's and St Thomas' hospital. The DMEC met twice to review the interim statistical analyses and trial data (prepared by the Trial Statistician, Dr Clare Flach). The DMEC's role was to ensure safety of trial participants and review the interim data to ensure safety of trial continuation.

The TSC was chaired by an independent hepatologist, Dr William Alazawi, Consultant Hepatologist at Bart's and the Royal London Hospital and consisted of Dr Nicholas Taylor, Dr Andrew Yeoman, Consultant Hepatologists, patient representative, Alister Pollock, Liver Research Nurse Ane Zamalloa, CI Professor Shawcross and Dr Charlotte Woodhouse, Clinical Research Fellow. The TSC met every six months to review trial progress and discuss any issues arising during the trial. For example, the TSC recommended reaching out to other hospitals in the area to increase recruitment, resulting in three patients being referred from Kingston

Hospital. Our patient representative also provided useful patient insight and recommended that patients be kept updated with the results of the trial once they had been published.

2. Sponsors, Investigators and Trial Sites

Co-Sponsors	
Co-Sponsors Contact: ***Amy Holton*** ***Quality Manager*** ***King's Health Partners Clinical Trials Office*** ***F16 Tower Wing | Guy's Hospital | Great Maze Pond | SE1 9RT*** ***T: 020 7188 5732 M: 07540678025*** ***F: 020 7188 8330*** ***E: amy.holton@kcl.ac.uk***	***King's College Hospital NHS Foundation Trust*** ***Denmark Hill*** ***London*** ***SE5 9RS*** ***King's College London*** ***Strand*** ***London*** ***WC2R 2LS***
Chief investigator: ***Professor Debbie Shawcross*** ***Professor of Hepatology & Chronic Liver Failure*** ***Institute of Liver Studies, King's College London and King's College Hospital NHS Foundation Trust*** ***T: 020 3299 3713 F: 020 3299 3167*** ***E: debbie.shawcross@kcl.ac.uk*** ***Denmark Hill | London | SE5 9RS***	

4. Co-Investigator(s), Statistician, Laboratories, Database Management

Co-investigator: ***Dr Charlotte Woodhouse*** ***Clinical Research Fellow*** ***Institute of Liver Studies, King's College Hospital NHS Foundation Trust*** ***Denmark Hill | London | SE5 9RS*** ***T: 020 3299 2504 F: 020 3299 3167*** ***E: charlottewoodhouse@nhs.net***	
Co-investigator ***Dr Vishal Patel*** ***Senior Clinical Lecturer and Honorary Consultant Hepatologist*** ***Institute of Liver Studies, King's College Hospital NHS Foundation Trust***	

Denmark Hill | London | SE5 9RS
T: 020 3299 2504 F: 020 3299 3167
E: vish.patel@kcl.ac.uk
Denmark Hill | London | SE5 9RS

Co-investigator:
Prof Alberto Sanchez-Fueyo
Academic Lead for Institute of Liver Studies and Professor in Hepatology
Institute of Liver Studies, King's College London and King's College Hospital NHS Foundation Trust
Denmark Hill | London | SE5 9RS
T: 020 3299 3695
E: sanchez_fueyo@kcl.ac.uk

Co-investigator
Dr Simon Goldenberg
Consultant Microbiologist
Guy's and St Thomas' Hospital
London SE1 7EH
Westminster Bridge Road | London | SE1 7EH
T: 020 7188 3152
E: simon.goldenberg@gstt.nhs.uk

Biostatistician and Co-investigator:
Dr Abdel Douiri
Senior Lecturer in Biostatistics
Department of Primary Care and Public Health Sciences
King's College London. Guy's Campus
4th Floor Addison House | London | SE1 1UL
T: 0207 848 8224
E: abdel.douiri@kcl.ac.uk

Biostatistican and Co-investigator:
Dr Clare Flach
Lecturer in Biostatistics
Department of Primary Care and Public Health Sciences
King's College London. Guy's Campus
4th Floor Addison House | London | SE1 1UL
E: clare.flach@kcl.ac.uk

Collaborators:

Dr Saeed Shoaie
Lecturer in Systems & Synthetic Biology
Centre for Host and Microbial Interactions
King's College London
Guy's Hospital
Great Maze Pond / London / SE1 1UL

Dr Alistair O'Brien
Clinical Senior Lecturer
University College London
Room G3, Ground Floor, Rayne Institute
5 University Street | London | WC1E 6JF
E: a.o'brien@ucl.ac.uk

Dr Louise China
Clinical Research Fellow
University College London
Department of Clinical Pharmacology, Rayne Institute
5 University Street | London | WC1E 6JF
E: louise.china@ucl.ac.uk

5. Study Synopsis

Title of clinical trial	A **PRO**spective, randomised placebo controlled feasibility trial of **F**aecal m**I**crobiota **T**ransplantation in cirrhosis
Protocol Short Title/Acronym	PROFIT Trial
Study Phase	3
Sponsor name	King's College Hospital and King's College London
Chief Investigator	Professor Debbie Shawcross
Eudract number	2017-003629-13
REC number	17/LO/2081
IRAS project ID:	197327
Medical condition or disease under investigation	Cirrhosis
Purpose of clinical trial	To determine the safety and feasibility of faecal microbiota transplantation in patients with advanced but stable cirrhosis
Primary objective	Safety and feasibility of FMT in cirrhosis • Assessment of the feasibility of FMT a. Assess recruitment rates b. Assess tolerability of FMT e.g reflux rates • Assessment of the safety of FMT
Secondary objective (s)	**Secondary Objectives:** The secondary objectives of the study are to provide preliminary evidence of efficacy for a larger randomised trial, with the purpose of: (i) Choosing the optimal primary outcome, and (ii) Estimating the parameters for sample size calculation. (a) As measured by an improvement in global liver synthetic function as assessed by

	the MELD score [a composite score of serum bilirubin, creatinine and INR] at 90 days. (b) Development of overt hepatic encephalopathy (grade 1 or more as measured by the Westhaven Criteria(2)). (c) The development of organ failure (hypotension requiring inotropic support, respiratory failure requiring ventilator support or the development of acute kidney injury requiring renal replacement therapy) and infection (d) The development of any infection during the 90 day follow up including chest, urinary, stool, ascites and blood infection. (e) Stability of the transplanted gut microbiome by comparing the % composition of the stool microbiota on day 7, 30 and 90 with the donor microbiome. (f) Comparison of the composition of the salivary microbiome with the stool microbiome as a surrogate marker of gut dysbiosis at baseline, day 7, day 30 and day 90 . **Mechanistic Outcome(s):** (i) Plasma endotoxin and bacterial DNA quantification at 7, 30 and 90 days. (ii) Changes in faecal biomarkers (calprotectin, lactoferrin and M2-Pyruvate Kinase) at 7, 30 and 90 days. (iii) Changes in leucocyte function including measurement by lipopolysaccharide-induced macrophage tumour necrosis alpha production and immunological markers using flow cytometry (HLA-DR and TLR-4 expression) at 7, 30 and 90 days.
Trial Design	Single-blinded randomized placebo

	controlled trial
Endpoints	Assessment of the feasibility and tolerability of FMT: • >25% consent rate (of all patients screened ~250) • >50% fulfil inclusion/exclusion criteria (of all patients consented ~64) • >80% randomised patients treated successfully and completing study up to day 90 (out of those randomised ~22) • Availability of obtaining sufficient stool donors for the study • Reflux rates of transplanted material <20% (e.g. foul taste, smell, nausea and vomiting, indigestion) • Significant gastrointestinal side effects (including diarrhoea, constipation, abdominal pain, flatulence and bloating) of <20% (ii) Assessment of the safety of FMT: • Incidence of any transmissable bacterial or viral infection that is deemed to have been acquired from the donor including *Clostrium difficile* infection. • The development of any Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR) that is not pre-specified or is a known consequence of disease progression or complication of cirrhosis as outlined in section 7.2.5.1 that: o Results in death o Is life-threatening o Required hospitalisation or prolongation of existing hospitalisation o Results in persistent or significant disability or incapacity

	o Consists of a congenital anomaly or birth defect
Planned number of subjects	32
Summary of eligibility criteria	Inclusion • 18-75 years old • Confirmed advanced cirrhosis of any aetiology with a MELD score of 10-16. The diagnosis of cirrhosis will be based on clinical, radiological or histological criteria • Patients with alcohol-related liver disease must have been abstinent from alcohol for a minimum of 6 weeks • Patients must be deemed to have capacity to consent to study (if patients lose capacity during the trial a legal representative will be appointed to act on their behalf) Exclusion • Severe or life-threatening food allergy • Pregnancy or breastfeeding • Patients treated for active variceal bleeding, infection, overt hepatic encephalopathy, bacterial peritonitis or acute-on-chronic liver failure within the past 14 days. • Patients who have received antibiotics in the past 14 days. • Active alcohol consumption of >20 grams/day. • Has had a previous liver transplant. • Hepatocellular carcinoma outside of the Milan Criteria (3). • Inflammatory bowel disease. • Coeliac disease. • A history of prior gastrointestinal resection such as gastric bypass. • Patient is not expected to survive the duration of the study (90 days). • Severe renal impairment (creatinine

	>150 µmol/L) • HIV positive • Immunosuppression e.g. more than two weeks treatment with corticosteroids within 8 weeks of intervention, active treatment with tacrolimus, mycophenylate mofetil, azathioprine
IMP, dosage and route of administration	200mL Faecal microbiota transplant (50g stool homogenized in 200ml 0.9% saline with 12.5% glycerol as a cryo-preservant), delivered via nasojejunal tube inserted at gastroscopy.
Active comparator product(s)	Placebo (200ml 0.9% saline with 12.5% glycerol without faecal material)
Maximum duration of treatment of a subject	90 days
Version and date of protocol amendments	Version 2.0 11/01/2018

6. Glossary of terms

AE	Adverse Event
AMR	Anti-microbial resistance
AR	Adverse Reaction
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation

CTIMP	Clinical Trial of Investigational Medicinal Product
CTU	Clinical Trials Unit
DMC	Data Monitoring Committee
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
EC	European Commission
EMEA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
FMT	Faecal Microbiota Transplant
GCP	Good Clinical Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
HE	Hepatic Encephalopathy
HIV	Human immunodeficiency virus
IB	Investigator Brochure
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File (This forms part of the TMF)
ISRCTN	International Standard Randomised Controlled Trials Number
KCH	King's College Hospital
MA	Marketing Authorisation
MC+S	Microscopy, culture and sensitivity
MELD	Model for end stage liver disease
MHRA	Medicines and Healthcare products Regulatory Agency

NAFLD	Non-Alcoholic Fatty Liver Disease
NBM	Nil by mouth
NIMP	Non-Investigational Medicinal Product
NJ	Naso-jejunal
PI	Principal Investigator
PIC	Participant Identification Centre
PIS	Participant Information Sheet
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person
RCT	Randomised Control Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SDV	Source Data Verification
SIBO	Small intestinal bacterial overgrowth
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee

7. Publication (reference)

Woodhouse CA, Patel VC, Goldenberg S, et al. PROFIT, a PROspective, randomised placebo controlled feasibility trial of Faecal mIcrobiota Transplantation in cirrhosis: study protocol for a single-blinded trial. BMJ Open 2019;9:e023518. doi:10.1136/bmjopen-2018-023518

8. Study period (years)

The first patient visit was on 23/05/2018.

The last patient visit was 17/10/2019.

There were no interruptions to the trial but the trial was extended by 6-months (no cost extension) to allow for completion of recruitment and analyses due to a delay in starting the trial necessitated by completion of several regulatory hurdles including MHRA approval which took longer than anticipated. The trial ended on 1st December 2019.

9. Phase of development

PROFIT was a phase 3 randomised placebo controlled trial.

10. Objectives

This study assessed whether stabilising gut dysbiosis with FMT in patients with advanced cirrhosis was both feasible and safe.

Primary Endpoints:

The primary endpoints of the study are twofold:
(i) Assessment of the feasibility of FMT
- Assess recruitment rates
- Assess tolerability of FMT e.g reflux rates

(ii) Assessment of the safety of FMT

Success Criteria of Primary Endpoints:

(i) Assessment of the feasibility and tolerability of FMT:
- >25% consent rate (of all patients screened ~250)
- >50% fulfil inclusion/exclusion criteria (of all patients consented ~64)
- >80% randomised patients treated successfully and completing study up to day 90 (out of those randomised ~22)
- Availability of obtaining sufficient stool donors for the study
- Reflux rates of transplanted material <20% (e.g. foul taste, smell, nausea and vomiting, indigestion)
- Significant gastrointestinal side effects (including diarrhoea, constipation, abdominal pain, flatulence and bloating) of <20%

(ii) Assessment of the safety of FMT:
- Incidence of any transmissable bacterial or viral infection that is deemed to have been acquired from the donor including *Clostridiodes difficile* infection.
- The development of any Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR) that is not pre-specified or is a known consequence of disease progression or complication of cirrhosis as

outlined in section 7.2.5.1 that:

- Results in death
- Is life-threatening
- Required hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability or incapacity
- Consists of a congenital anomaly or birth defect

Secondary Objectives:

The secondary objectives of the study are to provide preliminary evidence of efficacy for a larger randomised trial, with the purpose of:
(i) Choosing the optimal primary outcome, and
(ii) Estimating the parameters for sample size calculation.
(a) As measured by an improvement in global liver synthetic function as assessed by the MELD score [a composite score of serum bilirubin, creatinine and INR] at 90 days.
(b) Development of overt hepatic encephalopathy (grade 1 or more as measured by the Westhaven Criteria(2)).
(c) The development of organ failure (hypotension requiring inotropic support, respiratory failure requiring ventilator support or the development of acute kidney injury requiring renal replacement therapy) and infection
(d) The development of any infection during the 90 day follow up including chest, urinary, stool, ascites and blood infection.
(e) Stability of the transplanted gut microbiome by comparing the % composition of the stool microbiota on day 7, 30 and 90 with the donor microbiome.
(f) Comparison of the composition of the salivary microbiome with the stool microbiome as a surrogate marker of gut dysbiosis at baseline, day 7, day 30 and day 90 .

Mechanistic Outcome(s):

(i) Plasma endotoxin and bacterial DNA quantification at 7, 30 and 90 days.
(ii) Changes in faecal biomarkers (calprotectin, lactoferrin and M2-Pyruvate Kinase) at 7, 30 and 90 days.
(iii) Changes in leucocyte function including measurement by lipopolysaccharide-induced macrophage tumour necrosis alpha production and immunological markers using flow cytometry (HLA-DR and TLR-4 expression) at 7, 30 and 90 days.

11. Background and Context

Cirrhosis is the third biggest cause of mortality and loss of working life (behind ischaemic heart disease and self-harm) in the UK. Mortality rates for cirrhosis have risen 400% since 1970, whereas the mortality rates for most other chronic diseases have fallen considerably over the same period (4). Patients with cirrhosis are at increased risk of developing hepatocellular carcinoma and can develop serious non-malignant complications of their cirrhosis including variceal bleeding, hepatic encephalopathy and ascites, which often require hospital admission. In 2016/17, there were 68,364 admissions to hospital due to liver disease. Currently £2.1 billion is spent on treating liver disease in the UK (5).

Cirrhotic patients have altered intestinal motility, which predisposes to small intestinal bacterial overgrowth (SIBO) (6). Patients with cirrhosis have an over-abundance of potential pathogens in the gut e.g. *Enterobacteriaceae* and a reduction in potentially 'healthy' gut bacteria such as *Lachnospiraceae* and *Ruminococcaceae,* which worsens with increasing severity of liver disease (7). This disruption to the intestinal gut microbiota is termed 'dysbiosis'(8). Patients with cirrhosis also develop increased intestinal permeability, with disruption of tight junctions (TJs) that usually maintain the integrity of the intestinal epithelial barrier (9), allowing movement of potential pathogens and chemicals such as endotoxin (lipopolysaccharide or LPS, a component of bacterial cell walls), pre-disposing to infection and driving systemic inflammation and subsequent deterioration of liver disease.

In light of the disruption to intestinal bacteria present in cirrhosis, patients with cirrhosis have been shown to benefit from treatment with antibiotics, such as the non-absorbable antibiotic, rifaximin. Rifaxmin-α is licensed by NICE in the UK for the treatment of recurrent hepatic encephalopathy. Rifaximi-α has been shown to reduce all-cause admissions, including those for spontaneous bacterial peritonitis (SBP) and variceal bleeding in patients on the liver transplant waiting list (10) and also improves outcomes post-transplantation, reducing the risk of early liver injury after transplantation (11). However, long-term use of antibiotics may increase the risk of anti-microbial resistance (AMR). For example in a study of cirrhotic patients treated with rifaximin-α, 44% were found to have rifampin resistant-resistant *Staphylococcal* isolates within 7 weeks of starting treatment with rifaximin for hepatic encephalopathy (12).

Faecal microbiota transplantation (FMT) is a way of replacing abnormal gut bacteria with healthy gut microbiota from donor stool. Interest in the use of FMT for a variety of indications has grown exponentially since FMT was shown to be highly successful in the treatment of recurrent *Clostridiodes diffficile* infection, with cure rates of over 90% (13). FMT aims to replace the abnormal bacteria present in the intestine of a patient with disease, with a 'normal' or 'healthy' gut microbiota from a healthy donor, who is free from disease. Bajaj and colleagues showed FMT given after broad spectrum antibiotics to be safe in men with cirrhosis when given via enema, however the control group were not treated with antibiotics so it remains to be ascertained whether the treatment effect observed was due to FMT itself (14).

PROFIT was devised to assess the safety and feasibility of FMT in advanced stable cirrhosis and to assess whether FMT might be a viable treatment in this vulnerable patient group. We elected to deliver FMT directly into the small bowel via an NJ tube (inserted at gastroscopy) as this is where the 'dysbiosis' in liver disease is maximal and FMT can be better retained than via the lower gastrointestinal (GI) route of administration.

12. Methodology

Thirty-two patients were recruited from outpatient clinics and the hepatology wards at King's college hospital, including two patients who were referred from Kingston Hospital. Twenty-three patients went on to be randomized, with 21 receiving IMP (15 FMT, 6 placebo.) Patients were allocated to FMT or placebo in a 3:1 ratio.

The first patient visit was on 23/05/2018 and the last patient visit was on 17/10/2019. Patients were seen at the screening visit to sign the consent form and check bloods for the MELD score and HIV serology. If the patient met the inclusion criteria, the patient attended the baseline visit where they were reviewed in the Clinical Research Facility by the research team. Concomitant medications, medical and surgical histories were confirmed, and adverse events were recorded. The patient underwent a full physical examination and provided samples of stool, saliva, urine and blood for study sampling. Additional samples were supplied at baseline for serum save for serology (for retrospective testing in the event of transmissible infection), urine MC+S and stool for MC+S and *C. Difficile* to ensure no infection was present at baseline. Patients were given instructions regarding bowel preparation for the endoscopy and Moviprep® was dispensed at the baseline visit. The informed consent form for the gastroscopy was signed at this point. Patients were consented for the risks of endoscopy including bleeding, perforation, damage to teeth and dental work and reaction to sedation. The risks of FMT/placebo treatment were included on the ICF e.g. risk of infection and aspiration of IMP/gastric secretions.

If the baseline sample results were satisfactory, the inclusion and exclusion criteria were confirmed and the patient was randomised to a treatment group using the electronic randomization system.

On the day prior to the endoscopy the patient drank one sachet of Moviprep® dissolved in 1L of water at 6pm. The second litre was consumed at 6am on the day of the procedure. Patients were asked to remain nil-by-mouth (NBM) for 6h prior to the endoscopy. On the day of IMP administration, patients were reviewed in the Clinical Research Facility to ensure they remained well enough to undergo the endoscopy. FMT was delivered to the Clinical Research Facility at King's College Hospital from the FMT laboratory at St Thomas' hospital via courier, with constant temperature monitoring. FMT was instilled within 6h of removal from the freezer at the point of origin. The placebo was dispensed from the King's College Hospital (KCH) pharmacy, following manufacture at Guy's Hospital (after manufacturing the placebo was delivered in a batch to KCH pharmacy to allow local dispensation.)

IMP was instilled at gastroscopy if there were no contra-indications. FMT or placebo (200mL) was delivered via an NJ tube inserted into the proximal jejunum through the working channel of the endoscope. Patients received conscious sedation with fentanyl and midazolam, titrated to clinical response and blood pressure. IMP was delivered out of the patient's sight, so as not to unblind them to the treatment allocation. All efforts were made to maintain blinding of the treatment allocation to the patient, but the study investigators were not blinded as the placebo and FMT solutions were not matched. Carbon dioxide, instead of air, was insufflated via the endoscope to reduce belching/retching during the procedure as instillation of the total 200mL volume took several minutes. Patients were sat upright post-endoscopy and observed for 2h post-procedure in endoscopy recovery. Observations were recorded prior to discharge and any adverse events were recorded. Specifically vomiting or diarrhea within two hours of the procedure were recorded. Patients were required to return home with a responsible adult, due to the use of sedation during the procedure, as per KCH endoscopy unit protocol.

Patients returned to the Clinical Research Facility at Day 7 (+/-5 days), day 30 (+/- 7 days) and

90 days (+/- 7 days) for clinical review. At each visit adverse events were recorded, along with concomitant medications, physical examination and study sampling (stool, urine, saliva, blood). Quality of life (EQ-5D-3L) and dietary questionnaires were completed at each visit.

At the end of the trial period patients returned to their usual clinical pathways.

Figure 1 Schedule of events

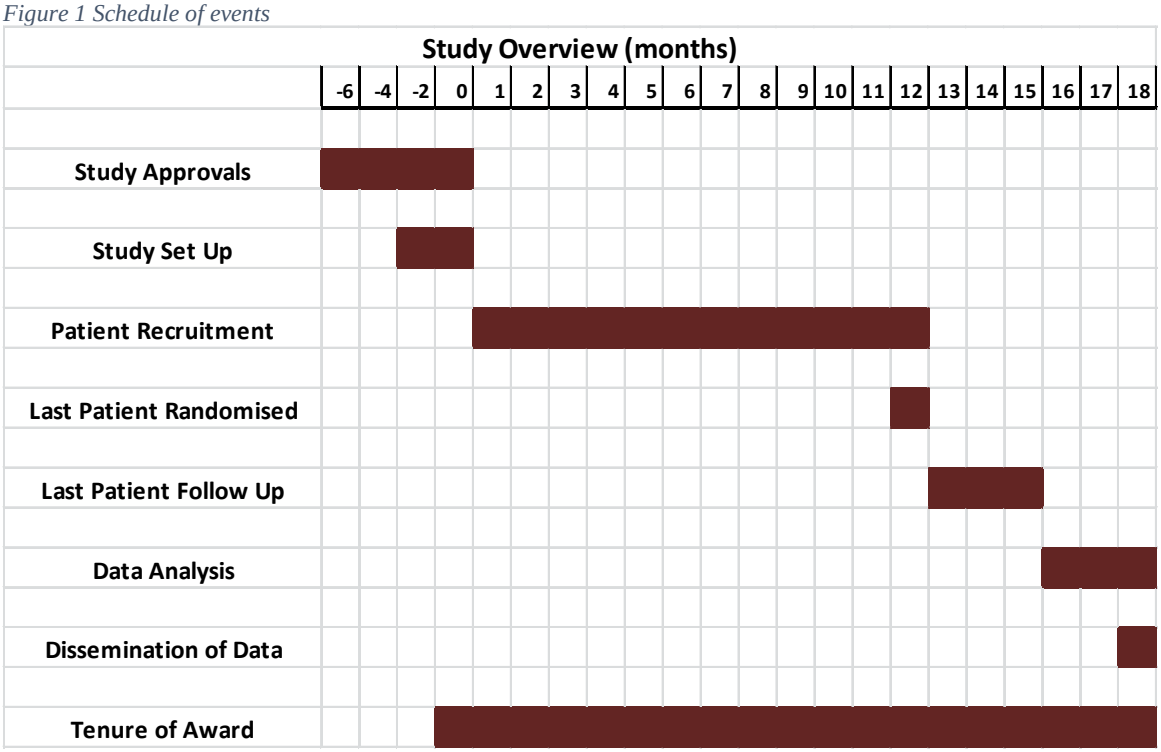

Table 1 study procedure schedule of events

Procedure	Screening	Baseline (<=7d prior to IMP)	Randomisation	D1 endoscopy	D7 +/- 5 days	D30 +/- 5 days	D90 +/- 5 days
Signed informed consent	X						
Eligibility criteria	X						
Participant demographics	X						
Medical/surgical Histories	X				X	X	X
Dietary questionniare		X			X	X	X
Concomittant medication use		X			X	X	X
Clinical examination		X		X	X	X	X
Local bloods (FBC, clotting, U+E, LFT etc)		X			X	X	X

Urine for MC+S		X					
Stool for MC+S and C. difficile		X					
MELD score		X			X	X	X
Randomisation			X				
FMT/Placebo administration				X			
Adverse events monitoring				X	X	X	X
Serum sample for archiving							
QOL questionnaire (EQ-5D-3L)					X	X	X

Trial Medication

The trial medication was FMT, 50g of stool homogenized in 200mL 0.9% saline, with 12.5% glycerol as a cryo-preservant. 10mL of the FMT preparation was removed from the total volume for storage at St Thomas' hospital for retrospective testing in the event of transmissible infection, leaving a total volume for instillation of 190mL. The placebo was 200mL 0.9% saline with 12.5% glycerol, without faecal material.

Dosing Regimen

The IMP was administered at gastroscopy following bowel preparation with 2L Moviprep® and was delivered via an NJ tube, directly into the proximal jejunum. The amount of IMP delivered was recorded in the CRF.

13. Number of patients (planned and analysed)

Three hundred and eighteen patients were pre-screened via review of clinic lists, inpatient ward lists and patients identified by their treating physicians. Two hundred and eight six were deemed ineligible (for reasons for in-eligibility please see table 4). Thirty-two patients consented to enroll in the study. Twenty-three patients were randomized, with twenty-one patients receiving treatment. Two patients withdrew after randomization, but prior to treatment. All patients that received IMP attended all scheduled visits as planned, excluding one patient who missed his day 90 review (having attended all visits apart prior to D90). He was contacted by the trial nurse, but was unable to attend due to personal reasons.

Figure 2 Trial flow chart

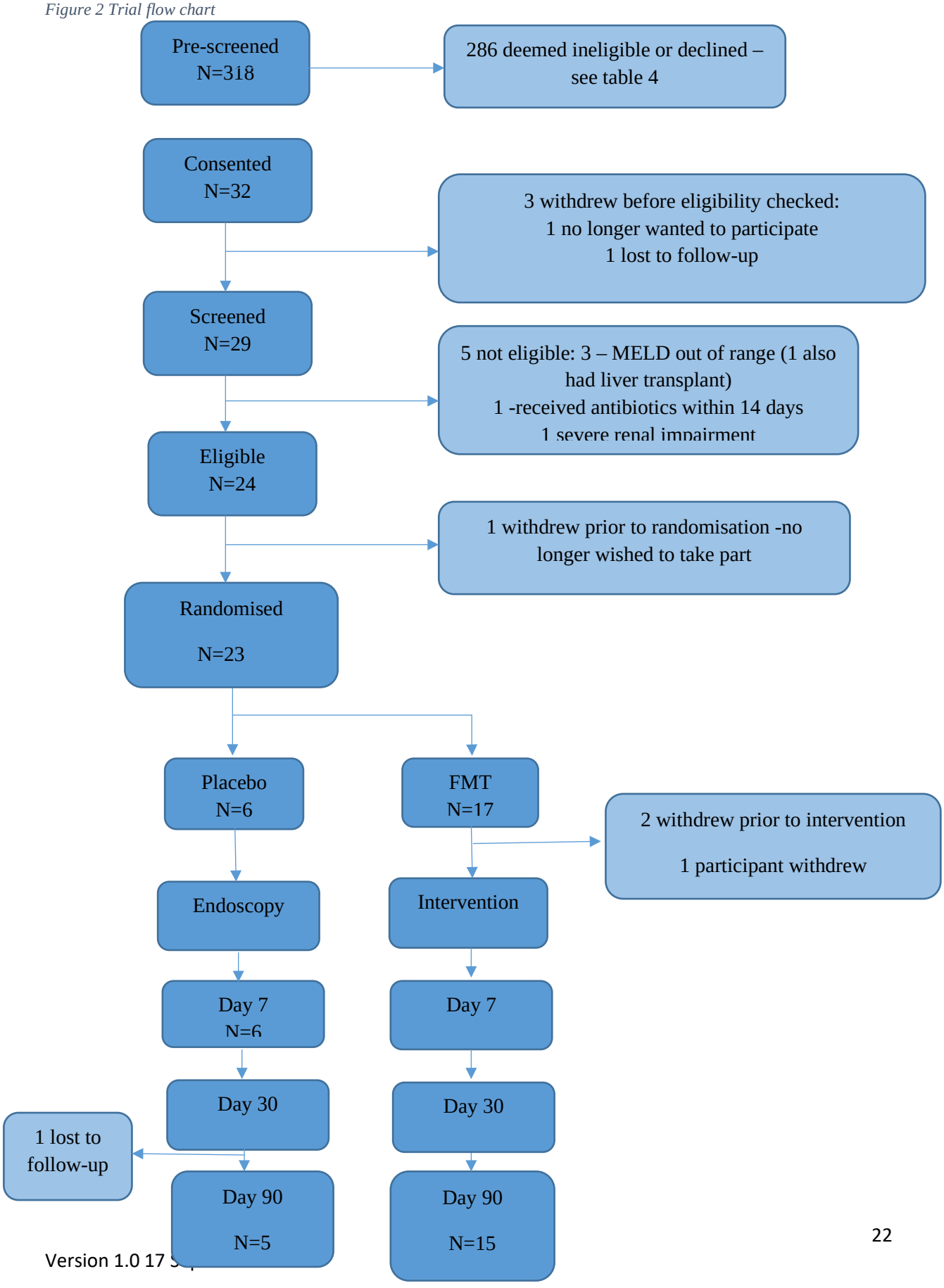

Table 2 Pre-screen reasons for non-eligibility

Reason	N excluded pre-screen	N excluded after consent
Total screened	**319**	**32**
Total excluded	**286**	**5***
Not between 18-75 years	5	
Does not have confirmed advanced cirrhosis of any aetiology with MELD score between 10-16	82	3
Has not been abstinent from alcohol for >6 weeks	35	
Does not have capacity to consent	0	
Severe or life-threatening food allergy	0	
Pregnant or breastfeeding	0	
Treated for active variceal bleeding, infection, bacterial peritonitis, overt hepatic encephalopathy or acute-on-chronic liver failure within the past 14 days.	0	
Received antibiotics in the past 14 days.	51	1
Active alcohol consumption of >20 grams/day.	0	
Has had a previous liver transplant	1	1
Hepatocellular carcinoma outside of the Milan Criteria(3)	1	
A history of prior gastrointestinal resection such as gastric bypass	7	
Patient is not expected to survive the duration of the study (90 days).	10	
Severe renal impairment (creatinine >150 µmol/L)	6	1
Inflammatory bowel disease (IBD)	4	
Coeliac disease	1	

HIV positive	4	
Immunosuppression e.g. more than two weeks treatment with corticosteroids within 8 weeks of intervention, active treatment with tacrolimus, mycophenylate mofetil, azathioprine	23	
Other reasons		
Physician declined	7	
In another trial	1	
Does not speak English	1	
Unable to contact	5	
Too far to travel	6	
Declined	36	

**One individual had both MELD out of range and a previous liver transplant*

Table 3 patient demographics screened vs randomised

	Consented		Randomised	
	N	Mean (SD)	N	Mean (SD)
Age	32	58.2 (10.6)	23	57.1 (11.0)
	N	Percent	N	Percent
Gender: female	10	31%	6	26%
Ethnicity: White	26	81%	18	78%
Black	1	3%	1	4%
Asian	3	10%	2	9%
Mixed	0	0	0	0
Other	2	6%	2	9%
Height (cm)	32	170.2 (10.4)	23	169.5 (10.8)
Smoking status: Current	8	25%	6	26%
Ex-smoker	6	19%	6	26%

Never smoker	18 56%	11 48%
Use recreational drugs: Yes	2 6%	1 4%
Inject drugs: Yes	0 0%	0 0%

Table 4 Baseline characteristics by trial arm

	Placebo		FMT	
	N	Mean (SD)	N	Mean (SD)
Age	6	56.8 (11.8)	17	57.3 (11.1)
	N	Percent	N	Percent
Gender: female	2	33%	4	23%
Ethnicity: White	3	50%	15	88%
Black	1	17%	0	0%
Asian	2	33%	0	0%
Mixed	0	0%	0	0%
Other	0	0%	2	12%
Height (cm)	6	165.5 (11.3)	17	170.9 (10.6)
Weight (kg)	6	73.7 (20.9)	17	86.9 (19.7)
Smoking status: Current	1	17%	5	29%
Ex-smoker	1	17%	5	29%
Never smoker	4	67%	7	41%
Use recreational drugs: Yes	0	0%	1	6%
Inject drugs: Yes	0	0%	0	0%
Gastro Intestinal History - had any of the following previously				
TIPSS: Yes	0	0%	2	12%

Surgical shunt in situ: Yes	0	0%	0	0%
Hepatitis B: Yes	3	50%	1	6%
Hepatitis C: Yes	0	0%	3	17%
Variceal bleeding: Yes	3	50%	6	35%
Pancreatitis: Yes	0	0	0	0%
Encephalopathy: Yes	4	67%	10	59%
Spontaneous Bacterial Peritonitis: Yes	0	0%	1	6%
Ascites: Yes	3	50%	9	53%
Diabetes Mellitus: Yes	1	17%	3	18%
Clinical examination				
Grade of hepatic encaphalopathy: 0 none	5	83%	12	71%
1. Lack of awareness	1	17%	5	29%
2. Lethargy	0	0%	0	0%
3. Somnolence	0	0%	0	0%
4. Comatose	0	0%	0	0%
Current Ascites	0	0%	2	12%
Blood panels				
White Blood Cell Count (x10^9/L)	6	4.64 (2.40)	17	4.45 (1.47)
Neutrophils (x10^9/L)	6	2.79 (1.58)	17	2.79 (0.97)
Lymphocytes (x10^9/L)	6	1.16 (0.59)	17	1.07 (0.45)
Haemoglobin (g/L)	6	123 (21.9)	17	124.8 (19.1)
Mean Cell Volume (MCV) (fL)	6	97.9 (10.6)	17	98.2 (8.74)
Platelet count (x10^9/L)	5	119.6 (92.5)	17	115.7 (55.6)
International Normalised Ratio (INR)	6	1.40 (0.22)	16	1.45 (0.37)
Sodium (mmol/L)	6	136.5 (3.08)	17	137.5 (4.87)
Potassium (mmol/L)	6	4.17 (0.18)	17	4.19 (0.34)
Creatinine (µmol/L)	6	69.2 (18.9)	17	74.1 (32.8)

Urea (mmol/L)	5	5.04 (1.03)	17	5.29 (2.69)
C Reactive Protein (CRP) (mg/L)	6	6.42 (10.43)	17	4.53 (3.53)
Alanine transferase (ALT) (IU/L)	6	42.3 (26.9)	17	29.4 (14.5)
Aspartate transaminase (AST) (IU/L)	6	61.8 (45.0)	16	46.5 (14.4)
Total Bilirubin (µmol/L)	6	40.8 (35.2)	16	27.7 (13.7)
Alkaline Phosphatase (ALP) (IU/L)	6	141.5 (71.2)	17	112.9 (53.2)
Albumin (g/L)	6	34.3 (3.9)	16	38.1 (4.5)
Gamma glutamyl transferase (IU/L)	6	109.5 (85.4)	17	129.8 (130.8)
Total Protein (g/L)	6	67.3 (7.5)	16	65.3 (13.1)
Glucose (mmol/L)	6	9.7 (6.2)	16	6.24 (3.41)
Venous Ammonia (µmol/L)	6	64.7 (27.7)	17	67.2 (29.2)
Venous blood gas: pH	6	7.37 (0.04)	17	7.36 (0.08)
Venous blood gas: Bicarbonate (mmol/L)	6	21.7 (3.5)	17	20.6 (4.1)
Lactate (mmol /L)	6	1.68 (0.58)	17	1.57 (0.50)

Table 5 reason for patient withdrawal from study

Patient	Reason for withdrawal
P010002	Required antibiotics for UTI (positive urine MC+S)
P010003	Withdrew after randomization for personal reasons (new job so could not come for endoscopy)
P010008	Patient did not attend baseline visit (attempted to contact and rebooked appointment, but patient failed to attend rescheduled appointment and did not reply to messages subsequently)
P010010	Patient changed mind as was referred for transplantation and did not feel able to commit to study in addition to transplant assessment
P010012	Patient withdrew after feeling unwell after bowel preparation for the procedure and did not feel able to travel to the hospital (lived about 2h from King's Hospital)
P010014	Patient withdrew following discussion with family
P010018	Withdrawn from the study due to progressive frailty (not expected to survive duration of study)
P010018	Withdrawn as screening MELD <10 (9)
P010028	Withdrawn as screening MELD <10 (8)
P010030	Withdrawn as transplanted prior to baseline visit
P010032	Withdrawn as MELD>16 (18) and creatinine >150

14. Diagnosis and main criteria for inclusion

All patients enrolled in the study had a diagnosis of cirrhosis.

Inclusion criteria:

- 18-75 years old
- Confirmed advanced cirrhosis of any aetiology with a MELD score of 10-16. The diagnosis of cirrhosis will be based on clinical, radiological or histological criteria
- Patients with alcohol related liver disease must have been abstinent from alcohol for a minimum of 6 weeks
- Patients must be deemed to have capacity to consent to study (if patients lose capacity during the trial a legal representative will be appointed to act on their behalf)

15. Test product, dose and mode of administration

Baseline therapy

Patients received 2L of Moviprep®, one litre at 6pm the night before the endoscopy and the second litre at 6am on the morning of the endoscopy. Patients were required to be nil by mouth for 6 hours prior to the endoscopy.

16. Duration of treatment

IMP was given once at the endoscopy visit. There were no further doses.

17. Reference therapy, dose and mode of administration

IMP was delivered via an NJ tube inserted in to the proximal jejunum under direct vision at gastroscopy.

18. Criteria for evaluation: Endpoints

18.1 Efficacy

Primary end-point

This study will assess whether stabilising gut dysbiosis with FMT in patients with advanced cirrhosis is both feasible and safe.

Primary Endpoints:

The primary endpoints of the study will be two-fold:

(i) Assessment of the feasibility of FMT

c. Assess recruitment rates
d. Assess tolerability of FMT e.g reflux rates

(ii) Assessment of the safety of FMT

Success Criteria of Primary Endpoints:

(i) Assessment of the feasibility and tolerability of FMT:

- >25% consent rate (of all patients screened ~250)
- >50% fulfil inclusion/exclusion criteria (of all patients consented ~64)
- >80% randomised patients treated successfully and completing study up to day

90 (out of those randomised ~22)

- Availability of obtaining sufficient stool donors for the study
- Reflux rates of transplanted material <20% (e.g. foul taste, smell, nausea and vomiting, indigestion)
- Significant gastrointestinal side effects (including diarrhoea, constipation, abdominal pain, flatulence and bloating) of <20%

(ii) Assessment of the safety of FMT:

- Incidence of any transmissable bacterial or viral infection that is deemed to have been acquired from the donor including *Clostridium difficile* infection.
- The development of any Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR) that is not pre-specified or is a known consequence of disease progression or complication of cirrhosis as outlined in section 7.2.5.1 that:
  - Results in death
  - Is life-threatening
  - Required hospitalisation or prolongation of existing hospitalisation
  - Results in persistent or significant disability or incapacity
  - Consists of a congenital anomaly or birth defect

Secondary Efficacy Parameters

Secondary Objectives:

The secondary objectives of the study are to provide preliminary evidence of efficacy for a larger randomised trial, with the purpose of:

(i) Choosing the optimal primary outcome, and

(ii) Estimating the parameters for sample size calculation.

(a) As measured by an improvement in global liver synthetic function as assessed by the MELD score [a composite score of serum bilirubin, creatinine and INR] at 90 days.

(b) Development of overt hepatic encephalopathy (grade 1 or more as measured by the Westhaven Criteria(2)).

(c) The development of organ failure (hypotension requiring inotropic support, respiratory failure requiring ventilator support or the development of acute kidney injury requiring renal replacement therapy) and infection

(d) The development of any infection during the 90 day follow up including chest, urinary, stool, ascites and blood infection.

(e) Stability of the transplanted gut microbiome by comparing the % composition of the stool microbiota on day 7, 30 and 90 with the donor microbiome.

(f) Comparison of the composition of the salivary microbiome with the stool microbiome as a surrogate marker of gut dysbiosis at baseline, day 7, day 30 and day 90 .

Mechanistic Outcome(s):

(i) Plasma endotoxin and bacterial DNA quantification at 7, 30 and 90 days.

(ii) Changes in faecal biomarkers (calprotectin, lactoferrin and M2-Pyruvate Kinase) at 7, 30 and 90 days.

(iii) Changes in leucocyte function including measurement by lipopolysaccharide-induced macrophage tumour necrosis alpha production and immunological markers using flow cytometry (HLA-DR and TLR-4 expression) at 7, 30 and 90 days.

18.2 Safety

FMT was found to be safe in the patients treated within the study. There were no treatment related SAEs. There were no transmissible infections in either group that were deemed to have come from the donor material.

19. Statistical Methods

This feasibility study was designed to evaluate feasibility parameters using 95% Confidence Intervals. The sample size was proposed mainly to enable the trial to be conducted within the allocated budget and with acceptable precision for continuous outcomes. According to the simulation work by Teare MD et al.(15), even with the relatively small pilot sample size of 20, the planned studies would have at least 80% power to detect the target effect size (for continuous outcomes) more than 75% of the time. Teare et al. recommend that 60 to 100 subjects are sufficient to estimate an event rate (such as recruitment rates) with acceptable precision in a feasibility study, while sample sizes between 24 and 50 have been recommended for the accurate estimation of standard deviations. Therefore, we have chosen a sample size of 32 patients in this trial to have reliable data on all critical parameters (including event rates) which can also be utilised when planning a larger intervention trial. For event rates (e.g. recruitment rates) and particularly in the extreme case with lower rates e.g. 10%, we estimate a drop of precision by only 5% using our updated sample size and the minimum recommended by Teare et al. (0.16 for 60 patients vs 0.21 for 32 patients). Figure 3 below illustrates the reduction in precision of different rates when the sample size increases for binary outcomes. This sample size will also be feasible within the budget and will provide acceptable information for planning a future large clinical trial.

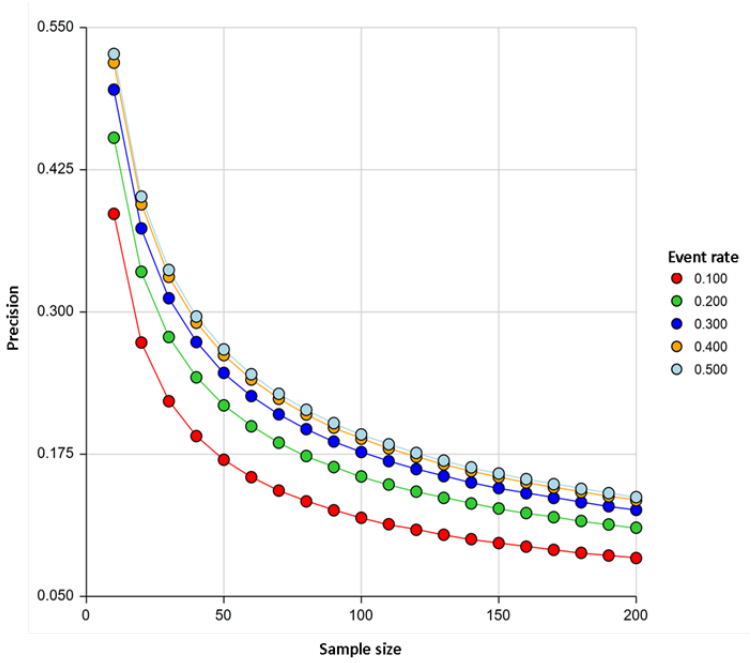

Figure 3

The sample size of 32 patients will undergo randomisation in a 3:1 ratio. This will allow the study to demonstrate feasibility of randomising, yet providing robust evidence with respect to the feasibility of the treatment, and preliminary evidence of efficacy parameters.

The following feasibility criteria have been established:

- 25% of screened patients will consent: 256 patients will allow the estimate of the 2-Sided 95% confidence interval of the proportion of consented patients, where the distance from the observed proportion to the limit is 0.058 units.
- 50% of patients screened will fulfil inclusion/exclusion criteria for the trial: screening 64 patients will allow the estimate of the 2-Sided 95% confidence interval of the proportion of patients eligible, where the distance from the observed proportion to the limit is 0.128 units.
- 80% of randomised patients will complete treatment and follow up: 26 of 32 patients completing will allow the estimate of the 2-Sided 95% confidence interval for a single proportion, where the distance from the observed proportion to the limit is 0.177 units.

When estimating the difference between the placebo and treatment group in efficacy binary outcomes, the two-sided 95% CI will be calculated with the half-width indicated below. Differences of 0.4 or higher will be distinguishable from 0.

Difference	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Width	0.308	0.333	0.359	0.372	0.372	0.372	0.359	0.333

The half-width of the 95% CI of the difference in means for continuous outcome will be 1.012 SD.

Clinical Endpoints

Clinical and safety events will be listed and summarised by intervention group. MELD scores will be calculated by visit and treatment group.

Data synthesis, analysis and presentation

Feasibility and efficacy outcomes will be summarised using the appropriate descriptive statistics, and 95% Confidence Intervals will be calculated to allow for success and go/no go decisions.

Biomarker data will be pre-processed according to the established standards for each platform, and statistical analyses will be performed using non-parametric and permutation based methods which are more appropriate for small sample sizes. When possible, internal cross-validation will be used as part of the analysis pipeline to avoid overfitting and reduce false-positive results.

Statistical Software

Analyses were performed using R and or Stata statistical software packages

20. Summary – Conclusions

20.1 Demographic data

20.2 Primary outcome

Table 6 Feasibility summary

Type	Total N (%, 95% CI)	Placebo	FMT
Total pre-screened	318		
Total declined	36/318 (11%, 8%-15%)		
Consented (eligible from pre-screen)	32 (10%, 7%-14%)		
Screened	29		
Eligible of those screened	24/29 (83%, 64%-94%)		
Eligible of screened and pre-screened	24/318 (8%, 5%-11%)		
Randomised	23	6	17
Received intervention (% of randomised)	21 (91%)	6 (100%)	15 (88%)
Completed 7 day follow-up (% of randomised)	21 (91%)	6 (100%)	15 (88%)
Completed 30 day follow-up (% of randomised)	21 (91%)	6 (100%)	15 (88%)
Completed 90 day follow-up (% of randomised)	20 (87%)	5 (80%)	15 (88%)

Table 7 Tolerability summary

	Total	Placebo	FMT
	N (%)	N (%)	N (%)
Intervention administered: Yes	22 (100%)	6 (100%)	15 (100%)
Missing	2 (withdrew before intervention)	-	2
Volume delivered if administration completed:			
100 mL	1 (5%)	0	1 (7%)
189/190 mL*	15 (71%)	1 (17%)	14 (93%)
200 mL	5 (24%)	5 (83%)	0
N (%) vomit within 2hrs	1 (5%)	0	1 (7%)
N (%) type 6/7 bowel motion within 2hrs	3 (14%)	0	3 (20%)

*10 mL of the intervention was removed for testing. 2 individuals withdrew before the intervention, both were in the FMT arm. in the FMT arm. 1 individual received less than the 190/200mL of intervention planned. 1 (7%) of individuals in the FMT arm vomited and 3 (20%) had a 6/7 bowel motion within 2 hrs

Table 8 gastrointestinal adverse events within 7 days of endoscopy

Event	**Placebo**	**FMT**
Total individuals received endoscopy	6	15
Reflux	-	-
Abdominal distention/bloating	-	2 (13%)
Flatulence	-	-
Diarrhoea	-	3 (20%)
Constipation	1 (17%)	
Vomiting	1 (17%)	2 (13%)
Nausea	-	1 (7%)

Foul taste/smell	-	-
Indigestion	-	-
Infection	-	-
Other gastro-intestinal	1 (17%)	1 (7%)
Any gastrointestinal related AE: # events, # individuals (% individuals)	3, 3 (50%)	9, 7 (47%)

20.3 Safety results

Table 9 Serious adverse events (SAEs)

Patient	SAE	Related	Description
P010004	Cellulitis	No	Background chronic lymphoedema and swelling
P010004	Lesion on leg	No	Area of skin loss on background of lymphoedema
P010007	Rectal bleeding	No	Fresh PR bleeding, haemorrhoids on sigmoidoscopy
P010007	Acute kidney injury	No	Worsening kidney function, admitted to local hospital for IV fluids
P010026	Hyperglycaemia	No	Started on prednisolone shortly before starting trial for unrelated Bell's palsy, high blood sugar on D7
P010021	Fever	No	Temperature and low white cell count, admitted for iv

			antibiotics (occurred about 3 weeks before the D90 visit, TSC did not feel related to treatment)
P010015	Urinary Tract Infection	No	admitted with abdominal discomfort and positive urine dipstick (August 2019)
P010015	Headache	No	admitted with headache, CT showed no acute findings (between D30 and D90)
P010015	Staph aureus bacteraemia	No	admitted with increased confusion, positive blood cultures (staph aureus)- no obvious source identified, occurred on scheduled D90 visit

Table 10 Secondary outcomes

		Placebo	FMT
MELD score	Baseline	9.76 (3.63)	9.70 (2.84)
	90 days	8.92 (3.03	10.20 (3.80)
	Change (90 days-Baseline)	-1.83 (1.77)	-0.58 (2.18)
Overt Hepatic Encephalopathy (Westhaven Criteria >=grade1)(2)	Baseline	1 (17%)	5 (29%)
	90 days	1(17%)	7 (47%)
Organ Failure	by 90 days	0	0
Infection	7 days	0	1 (7%)
	30 days	0	1 (7%)
	90 days	0	0

Infection reported in AEs	# events, # individuals (% individuals)	0	4, 2 (13%)

***** MELD Score = 10 * ((0.957 * ln(Creatinine)) + (0.378 * ln(Bilirubin)) + (1.12 * ln(INR))) + 6.43 with creatinine and bilirubin measured in mg/dL. Conversion from µmol/l is 1/88.42 for creatinine and 1/17.1 for bilirubin.

Table 11 Summary of adverse events since endoscopy to day 90 post endoscopy

Event	Adverse events				Serious adverse events	
	Placebo (N=6)		FMT (N=15)		Placebo (N=6)	FMT (N=15)
	#events (related* to intervention)	# individuals	#events (related* to intervention)	# individuals	#events (# individuals)	#events (# individuals)
Reflux						
Abdominal distention/bloating			3 (1)	2		1 (1)
Flatulence	0					
Diarrhoea	1	1	3 (1)	3		
Constipation	1	1				
Vomiting	1	1	3 (1)	3		
Nausea			2	2		
Foul taste/smell						
Infection			4	2		2 (2)

Other Gastro-intestinal	2	2	2	2		1 (1)
Musculo-skeletal	3	1	4	2		
Other Neurological	1	1	6	4		1 (1)
Other	5	5	29 (2)	13	1 (1)	4 (3)

*deemed possibly, probably or definitely related to the intervention by the study team. The 9 severe adverse events reported in the FMT arm were from 4 individuals.

Table: Listing of Adverse Events for all patients *(state which version of the MedDRA dictionary or other medical dictionary was used to code the events)*

Table 12 Adverse events individual listings

Patient ID	Date Consented	AE Event	Date Endoscopy	Date AE start	Time since endoscopy	Date AE end	Severity	Related	SAE	TrialArmName
Prior to endoscopy										
P010004	06/07/2018	Diarrhoea	02/08/2018	01/08/2018	-1	08/08/2018			No	FMT
P010004	06/07/2018	Dermatological - Rash	02/08/2018	01/08/2018	-1	03/08/2018			No	FMT
P010007	13/08/2018	Diarrhoea	04/10/2018	25/09/2018	-9	27/09/2018	Mild	Not related	No	FMT
P010007	13/08/2018	Vomiting	04/10/2018	25/09/2018	-9	27/09/2018	Mild	Not related	No	FMT
P010013	31/08/2018	Constipation	31/01/2019	03/01/2019	-28	01/04/2019	Mild	Possibly	No	FMT
P010013	31/08/2018	Other -	31/01/2019	03/01/2019	-28	02/02/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Other Neurological - syncope	25/04/2019	30/03/2019	-26	30/03/2019	Moderate	Not related	No	FMT
P010029	11/06/2019	Other - tiredness	27/06/2019	23/06/2019	-4		Mild	Not related	No	FMT
P010026	25/04/2019	Other Neurological - Bell's palsy	16/05/2019	09/05/2019	-7	ongoing	Moderate	Not related	No	Placebo

P010026	25/04/2019	Other Gastro-intestinal - abdominal discomfort	16/05/2019	15/05/2019	-1		Mild	Not related	No	Placebo
Post endoscopy										
P010004	06/07/2018	Other - Left leg cellulitis	02/08/2018	17/08/2018	15	28/08/2018	Moderate	Not related	Hospitalisation	FMT
P010004	06/07/2018	Dermatological - Rash	02/08/2018	24/08/2018	22	ongoing	Mild	Not related	No	FMT
P010004	06/07/2018	Other - Left leg pain	02/08/2018	06/09/2018	35	12/09/2018	Moderate	Not related	Hospitalisation	FMT
P010005	20/07/2018	Abdominal distention/bloating	16/08/2018	16/08/2018	0	16/08/2018	Mild	Probably	No	FMT
P010005	20/07/2018	Other - palmar erythema	16/08/2018	22/08/2018	6	ongoing	Mild	Not related	No	FMT
P010005	20/07/2018	ENT - stye right eye	16/08/2018	11/11/2018	87	ongoing	Mild	Not related	No	FMT
P010006	27/07/2018	ENT - Bilateral styes	23/08/2018	01/09/2018	9		Mild	Not related	No	FMT
P010007	13/08/2018	Diarrhoea	04/10/2018	05/10/2018	1	ongoing	Mild	Unlikely	No	FMT
P010007	13/08/2018	Vomiting	04/10/2018	07/10/2018	3	07/10/2018	Mild	Unlikely	No	FMT
P010007	13/08/2018	Other Gastro-intestinal - rectal bleeding	04/10/2018	01/11/2018	28	01/01/2019	Moderate	Not related	Hospitalisation	FMT
P010007	13/08/2018	Other Genito-urinary/renal - Acute Kidney Injury	04/10/2018	20/12/2018	77	24/12/2018	Moderate	Not related	Hospitalisation	FMT

P010013	31/08/2018	Other Respiratory - productive cough	31/01/2019	04/02/2019	4	01/04/2019	Mild	Not related	No	FMT
P010015	02/10/2018	Other Gastro-intestinal - abdominal discomfort	20/06/2019	25/06/2019	5	ongoing	Mild	Not related	No	FMT
P010015	02/10/2018	Musculo-skeletal - pain to right thumb	20/06/2019	25/06/2019	5	ongoing	Moderate	Not related	No	FMT
P010015	02/10/2018	Other - insomnia	20/06/2019	25/06/2019	5	ongoing	Moderate	Not related	No	FMT
P010015	02/10/2018	Other Hepatic - pheripheral oedema	20/06/2019	27/06/2019	7	ongoing	Moderate	Not related	No	FMT
P010015	02/10/2018	Other - confusion	20/06/2019	01/07/2019	11	18/07/2019	Moderate	Not related	Hospitalisation	FMT
P010015	02/10/2018	Musculo-skeletal - hematoma to lower back	20/06/2019	12/07/2019	22	ongoing	Mild	Not related	No	FMT
P010015	02/10/2018	Musculo-skeletal - weakness to legs	20/06/2019	17/07/2019	27	ongoing	Mild	Not related	No	FMT
P010015	02/10/2018	Other - dizziness	20/06/2019	19/07/2019	29	ongoing	Mild	Not related	No	FMT
P010015	02/10/2018	Infection	20/06/2019	03/08/2019	44	04/08/2019	Mild	Not related	No	FMT
P010015	02/10/2018	Infection	20/06/2019	19/08/2019	60	26/08/2019	Moderate	Not related	Hospitalisation	FMT
P010015	02/10/2018	Decompensation of liver disease	20/06/2019	10/09/2019	82	13/09/2019	Moderate	Not related	No	FMT

P010015	02/10/2018	Other Neurological - headache	20/06/2019	10/09/2019	82	13/09/2019	Moderate	Not related	Hospitalisation	FMT
P010016	17/10/2018	Decompensation of liver disease	07/02/2019	14/02/2019	7	ongoing	Mild	Not related	No	FMT
P010016	17/10/2018	Other Genito-urinary/renal - urinary tract infection	07/02/2019	29/04/2019	81	07/05/2019	Moderate	Not related	No	FMT
P010017	01/11/2018	Other Genito-urinary/renal - Urinary frequency	22/11/2018	09/12/2018	17	12/12/2018	Mild	Not related	No	FMT
P010017	01/11/2018	ENT- Epistaxis	22/11/2018	11/12/2018	19	11/12/2018	Mild	Not related	No	FMT
P010017	01/11/2018		22/11/2018							FMT
P010019	01/02/2019	Vomiting	14/02/2019	14/02/2019	0	14/02/2019	Mild	Definitely	No	FMT
P010019	01/02/2019	Other Neurological - Headache	14/02/2019	18/02/2019	4	19/04/2019	Moderate	Not related	No	FMT
P010019	01/02/2019	Dermatological - Itch	14/02/2019	20/02/2019	6	20/02/2019	Mild	Not related	No	FMT
P010019	01/02/2019	Other - fever	14/02/2019	29/04/2019	74	30/04/2019	Moderate	Not related	No	FMT
P010019	01/02/2019	Musculo-skeletal - Joint pain	14/02/2019			19/04/2019	Moderate	Not related	No	FMT
P010019	01/02/2019	Other - Reduced libido	14/02/2019			ongoing	Mild	Not related	No	FMT
P010020	13/02/2019	Diarrhoea	14/03/2019	15/03/2019	1	15/03/2019	Mild	Not related	No	FMT

P010020	13/02/2019	ENT - Epistaxis	14/03/2019	15/03/2019	1	15/03/2019	Mild	Probably	No	FMT
P010020	13/02/2019	Musculo-skeletal	14/03/2019			ongoing				FMT
P010021	06/03/2019	Other Neurological - dizzyness	25/04/2019	26/04/2019	1	01/05/2019	Moderate	Not related	No	FMT
P010021	06/03/2019	ENT - sore throat	25/04/2019	26/04/2019	1	27/04/2019	Mild	Definitely	No	FMT
P010021	06/03/2019	Abdominal distention/bloating	25/04/2019	29/04/2019	4	01/05/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Other Neurological - pre-syncope	25/04/2019	29/05/2019	34	29/05/2019	Moderate	Not related	No	FMT
P010021	06/03/2019	Other Hepatic - peripheral oedema	25/04/2019	03/07/2019	69	31/07/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Other - petechial rash	25/04/2019	03/07/2019	69	ongoing	Mild	Not related	No	FMT
P010021	06/03/2019	Abdominal distention/bloating	25/04/2019	08/07/2019	74	16/07/2019	Mild	Not related	Hospitalisation	FMT
P010021	06/03/2019	Vomiting	25/04/2019	10/07/2019	76	15/07/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Nausea	25/04/2019	10/07/2019	76	15/07/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Infection	25/04/2019	10/07/2019	76	31/07/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Infection	25/04/2019	10/07/2019	76	16/07/2019	Moderate	Not related	Hospitalisation	FMT

P010021	06/03/2019	Other - dysuria	25/04/2019	10/07/2019	76	10/07/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Other Cardiovascular - hypotension	25/04/2019	11/07/2019	77	15/07/2019	Mild	Not related	No	FMT
P010021	06/03/2019	Other Immunological - neutropenia	25/04/2019	11/07/2019	77	06/09/2019	Moderate	Not related	No	FMT
P010021	06/03/2019	Other Respiratory - shortness of breath	25/04/2019	14/07/2019	80	14/07/2019	Mild	Not related	No	FMT
P010023	11/03/2019	0	28/03/2019							FMT
P010024	05/04/2019	Other - tiredness	09/05/2019	04/08/2019	87		Mild	Not related	No	FMT
P010027	17/05/2019	Nausea	23/05/2019	29/05/2019	6		Moderate	Not related	No	FMT
P010027	17/05/2019	Other Neurological - headache	23/05/2019	29/05/2019	6	30/05/2019	Moderate	Not related	No	FMT
P010027	17/05/2019	Other Neurological - backache	23/05/2019	29/05/2019	6	05/06/2019	Mild	Not related	No	FMT
P010027	17/05/2019	Diarrhoea	23/05/2019	30/05/2019	7	06/06/2019	Moderate	Possibly	No	FMT
P010027	17/05/2019	Endocrine - hypoglycaemia	23/05/2019	30/05/2019	7	30/05/2019	Mild	Not related	No	FMT
P010027	17/05/2019	Musculo-skeletal - back pain	23/05/2019	01/07/2019	39		Moderate	Not related	No	FMT
P010027	17/05/2019	Diarrhoea	23/05/2019				Mild	Possibly	No	FMT

P010027	17/05/2019	Musculo-skeletal - knee pain	23/05/2019				Moderate	Not related	No	FMT
P010027	17/05/2019	Other	23/05/2019							FMT
P010001	23/05/2018	Endocrine - Hypoglycaemia	05/07/2018	12/07/2018	7	17/07/2018	Mild	Not related	No	Placebo
P010001	23/05/2018	Diarrhoea	05/07/2018	30/09/2018	87	01/10/2018	Mild	Not related	No	Placebo
P010001	23/05/2018	Other Gastro-intestinal - abdominal pain	05/07/2018			ongoing	Mild	Not related	No	Placebo
P010001	23/05/2018		05/07/2018							Placebo
P010009	15/08/2018	Vomiting	27/09/2018	02/10/2018	5	02/10/2018	Mild	Not related	No	Placebo
P010009	15/08/2018	ENT - Epistaxis	27/09/2018	02/10/2018	5	02/10/2018	Mild	Not related	No	Placebo
P010011	28/08/2018	Constipation	17/01/2019	18/01/2019	1	ongoing	Mild	Not related	No	Placebo
P010011	28/08/2018	Other - Tiredness	17/01/2019	18/01/2019	1	ongoing	Mild	Not related	No	Placebo
P010011	28/08/2018	Musculo-skeletal - back pain	17/01/2019			ongoing	Moderate	Not related	No	Placebo
P010022	11/03/2019	Other Gastro-intestinal - esophageal candidiasis	04/04/2019	04/04/2019	0		Moderate	Not related	No	Placebo
P010022	11/03/2019	Other Neurological - light headedness	04/04/2019	02/05/2019	28		Mild	Not related	No	Placebo

P010022	11/03/2019		04/04/2019							Placebo
P010022	11/03/2019		04/04/2019							Placebo
P010026	25/04/2019	Musculo-skeletal - bilateral ankle pain	16/05/2019	19/05/2019	3		Mild	Not related	No	Placebo
P010026	25/04/2019	Endocrine - -hyperglycaemia	16/05/2019	21/05/2019	5	24/05/2019	Moderate	Not related	Hospitalisation	Placebo
P010026	25/04/2019	Other Gastro-intestinal - abdominal discomfort	16/05/2019	28/05/2019	12		Mild	Not related	No	Placebo
P010026	25/04/2019	Musculo-skeletal - gaut-feet	16/05/2019	01/08/2019	77	03/08/2019	Mild	Not related	No	Placebo
P010026	25/04/2019	Musculo-skeletal - gaut-finger	16/05/2019	14/08/2019	90		Moderate	Not related	No	Placebo
P010026	25/04/2019	Other Neurological - tingling in feet	16/05/2019				Moderate	Not related	No	Placebo
P010031	15/07/2019	Other - bleeding gums	25/07/2019	17/09/2019	54		Mild	Not related	No	Placebo
P010031	15/07/2019	Dermatological - rash left chest	25/07/2019			04/08/2019	Mild	Not related	No	Placebo
>90 days since endoscopy										
P010015	02/10/2018	Decompensation of liver disease	20/06/2019	23/09/2019	95	09/10/2019	Moderate	Not related	No	FMT
P010015	02/10/2018	Musculo-skeletal - leg pain	20/06/2019	01/10/2019	103	ongoing	Moderate	Not related	No	FMT

P010015	02/10/2018	Infection	20/06/2019	23/09/2019	95	09/10/2019	Moderate	Not related	No	FMT
P010020	13/02/2019	Other - Tiredness	14/03/2019	15/06/2019	93	ongoing	Mild	Not related	No	FMT

Within the per protocol population (n= 23), a total of 99 AEs, including 9 SAE, were identified as treatment-emergent and included in the safety analysis. Summary tables for AEs and SAEs are presented above.

Overall, 21 patients (21/23= 91%) patients experienced at least one AE. The proportion that experienced at least one AE was 17% (4/23).

Incidence of adverse drug reactions (ADRs): 7 AEs were deemed definitely, probably or possibly related to the IMP out of a total of 90 AEs that had relatedness recorded (9 missing). The 7 AEs deemed related were in 6 patients, 5 of whom had one AE and one had two AEs. All of these were in the FMT arm, none in the placebo arm. 7/90 AEs (7.8 %) were assessed as related to at least one study drug and 6/23 patients (26%) experienced 7 ADR.

There were no Serious Adverse Reactions (SARs), no unexpected SARs and no SUSARs.

20.4 Conclusion

The aim of the PROFIT study was to assess the safety and feasibility (including tolerability and recruitment rates) of FMT in advanced but stable cirrhosis.

It was difficult to assess balance in baseline characteristics between trial arms, especially for categorical measures, due to small sample sizes, but the groups seem to be well-balanced. The majority of patients were male and of white ethnicity.

In respect of safety, nine SAEs occurred in total, eight of which were in the FMT group (patients were allocated to the FMT group in a 3:1 ratio). None of the SAEs were deemed treatment related. Four patients (n=15) accounted for the 8 SAEs in the FMT treated group.

With respect to tolerability, total GI adverse events within 7 days of endoscopy occurred at similar rates in the placebo and FMT treatment groups (FMT 47%, placebo 50%). Diarrhoea occurred more frequently in the FMT treated group, than the placebo treated group, occurring in 20% of FMT treated patients, but none of the placebo group. 13% of the FMT group experienced abdominal bloating or distension and 13% vomited within 7 days of endoscopy. 7% of the FMT group (n=1) reported nausea. In the placebo group 17% experienced constipation and 17% vomiting, with a further 17% experiencing ‘other’ GI AEs.

The gastrointestinal adverse events reported are consistent with the results of previously published studies of FMT (16). 21 patients out of 23 experienced one adverse event, 17 of whom experienced more than one AE. Six patients experienced 7 AEs that were deemed definitely, probably and possibly related to IMP (all in the FMT group). Five patients experienced one related AE, one experienced two. There were no SARs or SUSARs.

In respect of recruitment, the target number of patients (n=32) were recruited on schedule. Of the 318 pre-screened individuals, the main reasons for non-eligibility for the trial were MELD score out of range (n=82), recent antibiotics (n=51), declined to participate (n=36) and not abstinent from alcohol for six weeks (n=35). 10% (95% CI 7-14%) of all patients who were pre-screened were deemed likely to be eligible and consented to be screened. 83% (95% CI

64%-94%) of those that consented and were screened were found eligible to take part in the study. 87% (95% CI 66-97%) of all patients randomised were treated successfully and followed to completion at 90 days.

With respect to secondary outcomes, infection occurred in 13% of patients who received FMT, but none of the placebo treated group (however the sample size was small, with just 6 patients in the placebo group and 15 in the FMT group). No patients in either treatment group developed organ failure during the 90 day follow up period. Hepatic encephalopathy >/= grade 1 was present in 17% of placebo treated patients at baseline and this did not change by day 90. HE was present in 29% of the FMT treated group at baseline, increasing to 47% at day 90 (but the maximal grade of HE in both treatment groups was 1, without the development of higher grades of HE.) The higher rates of HE in the FMT group may be in part related to the presence of two patients (12%) who had a TIPSS (trans-jugular intrahepatic systemic shunt) in situ the FMT group (TIPSS can predispose to hepatic encephalopathy as it shunts blood past the liver, reducing the detoxification capacity of the liver). None of the patients in the placebo group had a TIPSS.

MELD score did not change much between baseline and day 90. The baseline MELD score in both groups was around 9, with a reduction of 1.83 in the placebo group and 0.58 in the FMT group.

We conclude that FMT is both safe and feasible in patients with stable, but advanced cirrhosis, with a MELD score of 10-16. A larger study population is required to determine the impact upon rates of infection and changes in MELD score and HE grade. Mechanistic analyses are in progress and the manuscript is currently being drafted.

21. Date of Report

This is version 1.0 of the Clinical Study Report synopsis, dated 16/09/2020.

APPENDICES

i) Summary of treatment-emergent AEs in the per protocol population

System Organ Class *(Current list of MedDRA SOC)*	**Preferred Term** *(below are examples of preferred terms)*	**Number of Subjects Experiencing the AE in Active Arm** *(ideally list number and percentage e.g. 10 /12 subjects would be listed as 10 (83.33%)*	**Total Number of Occurrences of the AE** *(10 subjects may have experienced the same AE multiple times throughout the trial e.g. there were 20 occurrences of the same event)*	**Number of Subjects Experiencing the AE in Placebo Arm** *(ideally list number and percentage e.g. 10 /12 subjects would be listed as 10 (83.33%)*	**Total Number of Occurrences of the AE** *(10 subjects may have experienced the same AE multiple times throughout the trial e.g. there were 20 occurrences of the same event)*
Blood and lymphatic system disorders	Leukopenia				
Cardiac disorders	Palpitations Cardiac death Sudden death Chest pain Chest discomfort				
Congenital, familial and genetic disorders	Hydrocele				
Ear and labyrinth disorders	Ear pain				
Eye Disorders	Tunnel vision				

	Visual impairment				
Gastrointestinal disorders	Diarrhoea Dyspepsia Constipation Nausea Paraesthesia oral				
General disorders and administration site conditions	Fatigue Impaired healing Oedema peripheral				
Hepatobiliary disorders					
Immune system disorders					
Infections and infestations	Viral upper respiratory tract infection Nasopharyngitis Ear infection Folliculitis Gastroenteritis viral Lower respiratory tract infection Polyomavirus-associated nephropathy Sinusitis Urinary tract infection				
Injury, poisoning and procedural complications	Incision site pain Procedural pain Wound secretion Contusion Post procedural contusion Post procedural haematuria Post procedural oedema Procedural nausea Seroma				

	Suture related complication				
Investigations	Polyomavirus test positive Blood creatinine increased Escherichia test positive White blood cell count decreased				
Metabolism and nutritional disorders	Glucose tolerance impaired Gout Hypercalcaemia				
Musculoskeletal and connective tissue disorders	Myalgia Arthralgia Joint swelling Musculoskeletal discomfort Osteoarthritis Pain in extremity				
Neoplasms benign, malignant and unspecified (incl cysts and polyps)					
Nervous system disorders	Dizziness Headache Dysgeusia Paraesthesia Tremor Burning sensation Dizziness postural				
Pregnancy, puerperium and perinatal conditions					
Product issues					
Psychiatric disorders	Anxiety Depression				

Renal and urinary disorders	Haematuria Pollakiuria Renal cyst haemorrhage Renal cyst ruptured				
Reproductive system and breast disorders	Epididymal cyst Erectile dysfunction				
Respiratory, thoracic and mediastinal disorders	Cough Dyspnoea exertional Productive cough				
Skin and subcutaneous tissue disorders	Acne Actinic keratosis Alopecia Dermatitis acneiform Night sweats Pruritus Rash generalised				
Social circumstances					
Surgical and medical procedures					
Vascular disorders	Hot flush				

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