PROTOCOL FULL TITLE:
PROMISE Trial: A PROspective randomised double-blind parallel group placebo-controlled multicentre trial of faecal MIcrobiota tranSplantation to improve the primary outcomE (first hospitalisation due to infection) in patients with cirrhosis over 24 months.

Protocol Short Title/ Acronym:

A **PRO**spective faecal **MI**crobiota tran**S**plantation trial to improve outcom**E**s in patients with cirrhosis (**PROMISE)**

Protocol Version: V3.0 – 03/11/2023

Trial Identifiers

NIHR EME Project Reference Number:	NIHR EME130730
EudraCT Number:	2022-000300-35
IRAS Number:	1004822
ISRCTN Number:	ISRCTN17863382

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1 Revision History

Version Number	Description of changes from previous revision	Effective Date
1.0	Not Applicable, first version	05 Sep 2022
2.0	**Section 1 Study Synopsis:** • Research Ethics Committee updated to East of England Cambridge South. • To evaluate the safety of encapsulated FMT added as secondary objective. Safety assessments clarified in the secondary endpoints. • Incidence of AMR including skin and nose colonisation to include linezolid-resistant enterococci (LRE). **Section 4.2 Primary Endpoints:** • Skin definition of infection updated to: "Acute Bacterial Skin/ Soft Tissue Infection" in Table 1. • Blood (bacteraemia) definition of infection updated to exclude common skin contaminants e.g., coagulase-negative Staphylococci or Corynebacteria isolated on a single blood culture set only, in Table 1. **Section 6.6 Randomisation Procedure/ Code break:** • Update to procedure of PIN allocation • Blinding to treatment allocation updated. • Updates to blinding for IMP packs 'cannot be fully blinded due to a difference in the temperature storage conditions, expiry dates and batch number configurations for the FMT and placebo products. • Emergency unblinding: A 24-hour telephone-based emergency code break service added. **Section 7.2.2. Study Specific Tests:** • Specified blood and surplus samples will be stored at the King's Liver Research Tissue Bank for use in future research programmes. • Plasma updated to serum for bloods. **Section 9 Assessment of Safety:** • Removal of section 'Adverse events which don't require reporting'. **Section 10.1 Sample Size:** • P-value and minimum number of events updated for formal interim analysis.	10 Mar 2023
3.0	• Co-Sponsor contact details updated. • Trial Statistician updated under Key Trial Contacts. **Section 1 Revision History added into protocol.** **Section 2 Study Synopsis:** • Nomenclature updated from ALD and MAFLD to ALD, MASLD and MASLD-ALD Overlap Cirrhosis. Terminology updated throughout the protocol. • Clarification on gastrointestinal resection procedures under exclusion criteria 9 added.	03 Nov 2023

	Section 5 Trial Objectives and Design: • References to 'patient' corrected to 'participant'. Terminology updated throughout the protocol. • Time-windows for Screening, Baseline, Post-randomisation IMP dispensing updated. Screening and Baseline Repeat schedule added on Table 2. • Timepoints for Urinary b-HCG test, Liver Severity Score (MELD/Child Pugh/UKELD) and Adverse Event Monitoring updated. Liver Profile assessment updated to include MASLD-ALD Overlap Cirrhosis participants. A row for Urinary ethyl glucuronide/Ethyl sulphate test timepoints added on Table 2. **Section 6 Trial Medication:** • Fasting requirements prior to IMP administration were clarified. • Update to include how participant's IMP dosing will be managed in the event of relapsing back to alcohol drinking or the prescription of short term/long term antibiotics during the treatment period. • Additional categories of concomitant medication (anti-depressants/anti-anxiety and proton pump inhibitors) added. **Section 7 Selection and Withdrawal of participants:** • Recommended MELD score calculator and MELD score pre-screening window added. • Guidance on the Randomisation System made clearer. • Expected duration and timeline of the trial revised. • Extra guidance on reimbursement of travel added. **Section 8 Trial Procedures:** • Data collection on current or past history of psychiatric disorders added to the Medical History assessment at baseline visit. • Clearer explanation on how the Physical Examination will be performed. • Clearer explanation on how the urine and stool sample will be collected. • Clearer explanation on how the participant will be randomised for the trial. • Bilirubin and Urinary ethyl glucuronide/ Ethyl sulphate test added to the list of standard of care assessments. • Lithium heparinised green blood tube x 1 added to the list of study-specific laboratory analyses at the non-London sites. **Section 11 Statistics:** • Criteria for the Internal Pilot 'Go'/'No Go' progression was clarified.	

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2 Study Synopsis

Title of clinical trial	A **PRO**spective randomised double-blind parallel group placebo-controlled multicentre trial of faecal **MI**crobiota tran**S**plantation to improve the primary outcom**E** (first hospitalisation due to infection) in patients with cirrhosis over 24 months
Protocol Short Title/Acronym	A **PRO**spective faecal **MI**crobiota tran**S**plantation trial to improve outcom**E**s in patients with cirrhosis (PROMISE)
Trial Phase if not mentioned in title	Phase III (CTIMP; multicentre randomised double-blind parallel group placebo controlled)
(Co)-Sponsor(s) name	King's College London and King's College Hospital NHS Foundation Trust
Chief Investigator	Professor Debbie Shawcross
EudraCT number	2022-000300-35
IRAS number	1004822
ISRCTN Number	ISRCTN17863382
Research Ethics Committee (REC)	East of England Cambridge South
Medical condition or disease under investigation	Cirrhosis
Aim	We have previously shown that FMT administered endoscopically into the jejunum in patients with cirrhosis is safe and feasible and have identified some potential mechanisms of action that warrant further interrogation. The aim of the PROMISE Trial is to evaluate the efficacy and mechanisms of action of encapsulated FMT (versus placebo) to reduce infection and mortality in patients with alcohol-related (ALD) and metabolic dysfunction-associated Steatotic liver disease (MASLD) and MASLD-ALD Overlap cirrhosis.
Primary Objective	1. To evaluate the efficacy of encapsulated FMT to reduce the susceptibility of infection in patients with cirrhosis measured by the time to first infection resulting in hospitalisation.
Secondary Objective(s)	1. To evaluate the efficacy of encapsulated FMT to reduce decompensating cirrhotic events including acute variceal bleeding, new-onset ascites spontaneous bacterial peritonitis, hepatic encephalopathy and progression to acute-on chronic liver failure (ACLF). 2. To evaluate the impact of encapsulated FMT on patient outcomes including all-cause hospitalisation, disease progression, quality of life, mental health, alcohol use and survival. 3. To assess the impact of FMT on antibiotic usage and loss/reduction of antimicrobial-resistance (AMR) genes. 4. To evaluate the safety of encapsulated FMT.
Mechanistic Aims & Objectives	Following the assessment of efficacy, our **mechanistic aims** are: 1. To determine the mechanisms of action based on the hypothesis that the characteristics of the gut microbiome

	in cirrhosis drive the development of cirrhosis-associated immune dysfunction (CAID). 2. To determine if FMT will favourably modify key contributors by improving gut microbiome diversity and reducing bacterial translocation into the systemic circulation. The **mechanistic objectives** are: 1. Determine whether FMT increases the diversity of the gut microbiome in cirrhosis. 2. Determine whether FMT reduces bacterial translocation and endotoxemia. 3. Evaluate whether FMT improves CAID and reduces systemic inflammation.
Trial design	UK multicentre randomised (1:1) double-blind parallel group placebo-controlled trial of FMT versus placebo with two-year follow up.
Setting	NHS Gastroenterology/hepatology outpatient clinics
Endpoints	**Primary endpoint:** Time to the first infection resulting in hospital admission **i.e., the date patient is admitted to the hospital due to infection.** **Secondary endpoints:** 1. Incidence of decompensating events including hepatic encephalopathy, new-onset ascites, variceal bleeding and spontaneous bacterial peritonitis over 24-month follow-up period. 2. Progression to ACLF (the development of one or more organ failures). 3. Infection rates and antibiotic usage following randomisation over 24-month follow-up period. 4. Incidence of AMR including skin and nose colonisation with methicillin-resistant *Staphylococcus aureus (MRSA)*, vancomycin-resistant *Enterococci (VRE)*, linezolid-resistant *enterococci* (LRE), extended-spectrum beta-lactamase producing bacteria (ESBL), fluoroquinolone-resistant Gram negative and carbapenem-resistant Enterobacterales (over 24-month follow up period). 5. Hospitalisation rates (liver-related and all-cause) including the length of stay and admission to high dependency/intensive care (over 24-month follow up period). 6. Change in liver disease severity scores over 24 months post-randomisation (recorded every 3 months) 7. Change in quality of life (EQ-5D-5L) scores at 3, 6,12 and 24 months post-randomisation. 8. Change in depression and anxiety scores (using HADS) at 3, 6, 12 and 24 months post-randomisation. 9. All-cause mortality and liver-related mortality. 10. Change in alcohol use disorder-related events in patients enrolled with alcohol-related cirrhosis as assessed by the alcohol-use disorders identification test (AUDIT score) at 3, 6, 12 and 24 months post-randomisation, and urinary

ethyl glucuronide/ethyl sulphate levels if tested as part of the standard of care.

11. Safety of FMT based on safety assessments including physical examination, clinical laboratory evaluation, vital signs and reported as adverse events (AEs) or serious adverse events (SAEs) over 24 month follow-up period.

Mechanistic endpoints:

1. **Matched faecal and plasma biomarkers** (D-lactate, fatty acid-binding protein 2 and ammonia) measured by enzyme-linked immunosorbent assay (ELISA).
2. **Faecal proteases** as markers of DNA replication, tissue re-modelling and immune response as a proxy of gut mucosal barrier integrity.
3. Matched **plasma and faecal cytokine profiles** using a combination of ELISA and by electrochemiluminescence using the Mesoscale discovery platform (MSD) [Tumour necrosis factor-alpha, interferon-gamma, interleukin (IL)-1-beta, IL-6, IL-8, IL-10, IL-17A, IL-17E, IL-17F, IL-21 and IL-22].
4. **Plasma, faecal and urinary metabonomics**. Short–chain fatty acids measured by proton magnetic resonance spectroscopy (^{1}NMR). Tryptophan metabolites and primary, secondary and tertiary bile acids measured with liquid chromatography tandem mass spectrometry (LC-MS) [Butyrate and Succinate].
5. **Faecal proteomics** will identify protein changes using quantitative high-resolution mass spectrometry coupled with liquid chromatography LC-MS/MS analysis.
6. Matched **faecal and blood microbiome composition** (including mycome, virome and phagome) and **microbial diversity** by metagenomic sequencing [20 million reads using NovaSeq as a marker of bacterial translocation].
7. **Faecal PCR for pathogenic species of interest** including copy number of DNA of cytolysin positive and negative *E. faecalis, enteropathogeneic E. coli* and *Candida albicans*.
8. Analysis of the presence and dynamics of the **microbial 'resistome'** (ensemble of genes encoding AMR in a given microbiome using the comprehensive antibiotic resistance database [CARD]).
9. The effect of FMT in cirrhosis on the human circulating immune profile will be explored in stored peripheral blood mononuclear cells (PBMCs) by assessing monocyte surface expression of CD16, HLA-DR, MerTK, Axl, CD163, PD-1, PD-L1, bile acid receptors TGR5 and FXR, transcriptomic profile and functional capacity (LPS-induced TNF-α, IL-6 and IL-10 production, phagocytosis and oxidative burst).
10. Mucosa-associated invariant T (MAIT) cell numbers, phenotype and function isolated from PBMCs.
11. Change in lipid profile (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol) in those with metabolic dysfunction-associated Steatotic liver disease (MASLD) and MASLD-ALD Overlap cirrhosis, and HbA1c for diabetic patients only.

	12. To interpret the faecal microbiome sequencing and metabolome analyses and how it impacts the patients gut microbiome composition in relation to dietary habits.
Sample size	300 (1:1)
Summary of eligibility criteria	**Inclusion criteria:** 1. Aged ≥ 18 years 2. Confirmed alcohol-related (ALD) cirrhosis or metabolic dysfunction-associated steatotic liver (MASLD) and MASLD-ALD Overlap cirrhosis based on clinical, radiological and/or histological criteria. 3. MELD score 8-16 4. Patients with alcohol-related cirrhosis must have been abstinent for a minimum of 4-weeks prior to randomisation. 5. Patients must be deemed to have the capacity to provide written informed consent to participate. **Exclusion criteria:** 1. Severe or life-threatening food allergy (*e.g.*, peanut allergy) 2. Pregnancy or planned pregnancy. Urine testing will be performed at randomisation to rule out pregnancy in females. 3. Breast-feeding. 4. Patients treated for acute variceal bleeding, infection, overt hepatic encephalopathy, bacterial peritonitis or ACLF within 14 days prior to randomisation. 5. Active alcohol consumption of >20 grams/day [1 unit of alcohol contains 10mLs or 8g of alcohol]. 6. Previous liver transplantation. 7. Patients with inflammatory bowel disease. 8. Patients with coeliac disease. 9. Patients with a history of prior gastrointestinal resection such as gastric bypass or surgery that could change the gut microbiome or result in bacterial overgrowth e.g. gastric bypass. 10. Active malignancy including hepatocellular carcinoma. 11. Patients with an expected life expectancy <6 months or listed for liver transplantation. 12. Infected with HIV, hepatitis B or C [patients who have undetectable hepatitis B or C DNA/RNA can be recruited]. 13. Patients who have received antibiotics or probiotics within 7 days prior to randomisation. 14. Swallowing disorder, oral-motor dyscoordination or likely inability/unwillingness to ingest study medication. 15. Patients who have received another investigational drug or device within 4 months prior to randomisation. 16. Patients, who in the opinion of the PI, have a medical condition, or other relevant psychological, familial, or social factor that may jeopardise their health, compliance, or influence the trial integrity in any way.
Intervention (description, dose, frequency of dosing and route of administration)	Faecal Microbiota Transplantation (FMT) capsules taken orally; 5 capsules once every 3 months

Comparator Intervention	Matched placebo taken orally; 5 capsules once every 3 months
Maximum duration of treatment of a participant	24 months
Target duration of the trial	60 months
Electronic CRF	InferMED MACRO (programmed by King's Clinical Trials Unit [KCTU])

3 Glossary of Terms

AASLD	American Association for the Study of Liver Disease
ACLF	Acute-on-Chronic Liver Failure
AE	Adverse Event
ALD Cirrhosis	Alcohol-related Liver Disease Cirrhosis
AMR	Anti-microbial Resistance
AR	Adverse Reaction
AUDIT	Alcohol-use disorders identification test
BASL	British Association for the Study of the Liver
BLT	The British Liver Trust
BMI	Body Mass Index
BSG	British Society of Gastroenterology
CA	Competent Authority
CAID	Cirrhosis-Associated Immune Dysfunction
CARD	Comprehensive Antibiotic Resistance Database
CI	Chief Investigator
CLD	Chronic Liver Disease
CRA	Clinical Research Associate
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
CTU	Clinical Trials Unit
CTO	Clinical Trials Office
DMC	Data Monitoring Committee
DSUR	Development Safety Update Report
EC	European Commission
EDC	Electronic Data Capture
EEA	European Economic Area
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency
EPR	Electronic Patient Record
EQ-5D	EuroQol-5 Dimension
ESBL	Extended-spectrum beta-lactamase producing bacteria
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
FBC	Full Blood Count
FDA	The Food and Drug Administration
FMT	Faecal Microbiota Transplant
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GSTT	Guys and St Thomas' NHS Foundation Trust
HADS	Hospital Anxiety and Depression Scale
HC	Healthy Controls
HE	Hepatic Encephalopathy
HRA	Health Research Authority
HTA	Human Tissue Authority
IB	Investigator Brochure
ICF	Informed Consent Form
IME	Important Medical Events
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
INR	International Normalised Ratio
ISF	Investigator Site File (This forms part of the TMF)
ISRCTN	International Standard Randomised Controlled Trials Number
KCTU	King's Clinical Trials Unit
LCMS	Liquid Chromatography tandem Mass Spectrometry

LRE	Linezolid-resistant *enterococci*
MASLD Cirrhosis	Metabolic dysfunction-Associated Steatotic Liver Disease Cirrhosis
MASLD-ALD Overlap Cirrhosis	Metabolic dysfunction-Associate Steatotic Liver Disease – Alcohol related Liver Disease Overlap Cirrhosis
NHS	National Health Services
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NIMP	Non-Investigational Medicinal Product
PBMC	Peripheral Blood Mononuclear Cells
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
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SDV	Source Data Verification
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
UAR	Unexpected Adverse Reaction
VRE	Vancomycin-resistant *Enterococci*

4 Background & Rationale

1. Addressing the unmet need: Tackling infection and anti-microbial resistance in chronic liver disease

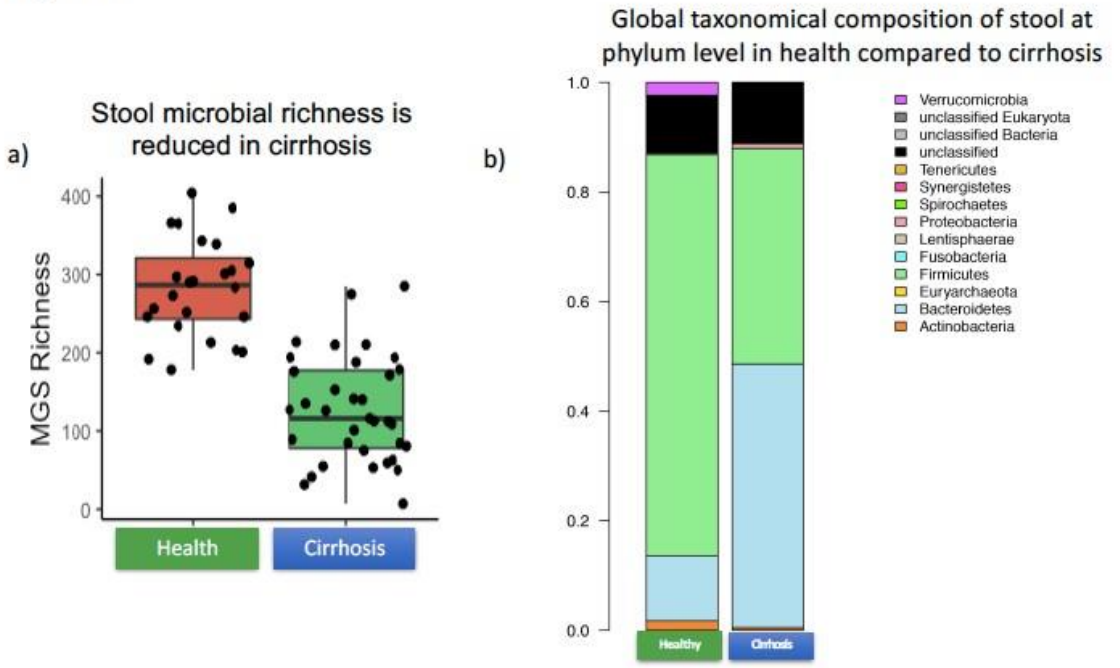
There is an evolving crisis of chronic liver disease (CLD) in the UK with the prevalence and mortality increasing exponentially. End-stage CLD, known as cirrhosis, is the third biggest cause of mortality and loss of working life behind ischaemic heart disease and self-harm. It is the only major cause of mortality and morbidity, which is on the increase in England. Over the last decade, the number of people dying with an underlying cause of liver disease in England rose by 40%.[1 2] Most liver disease deaths are from cirrhosis and its complications. People die from liver disease at a young age with 90% under 70 years old and more than one in ten in their 40s. It is estimated that 10-20% of the UK population are at risk of developing some degree of CLD within their lifetime, with 600,000 people living with significant CLD and 60,000 with cirrhosis. [3] 70% of patients with cirrhosis die in hospital. Whilst one in five of those who die have had five or more admissions to hospital in the last year of life, one in five die in their first admission. [1] This reflects the severe decompensating events, which precede death from CLD that often arise following the development of an infection.

2. Assessment of the current knowledge gaps

Cirrhosis is associated with an increased incidence of infection resulting in hospitalisation and complicates hospital admissions in up to 40% of cases. [4] In patients with cirrhosis, disruption of the immune system has been shown to be a common pathway and susceptibility to infection is one of the most common complications. Infection can lead to worsening liver function and precipitate complications including bleeding, hepatic encephalopathy, acute kidney injury and multi-organ failure, contributing to high mortality. [5] 37% of patients with advanced cirrhosis will be re-admitted to hospital within 30-days [6] and the prevalence of multidrug anti-microbial resistance (AMR) increased from 29 to 38% in culture-positive infections from 2011 to 2017/18. [7] With poor outcomes following infection, the propagation of AMR bacterial species and increasing demand for liver transplantation, there is an urgent need for novel approaches to reduce the rate of infection in this cohort and preventing the spread of AMR in patients with CLD. Patients with cirrhosis represent a particularly high risk for AMR because they are frequently prescribed antibiotics (25% are on prophylactic antibiotics), undergo many invasive procedures (*e.g.* endoscopy and large volume paracentesis) and have recurrent hospital admissions. [7 8]

A diverse and balanced gut microbiome is key to remaining healthy and becomes perturbed in patients particularly with metabolic-associated fatty liver disease (MAFLD) and alcohol-related liver disease, which contributes to the gut becoming more permeable with bacterial translocation into the systemic circulation. [9] The liver is closely linked to the gut and is the first organ to challenge bacteria and their by-products escaping from the gut. This causes activation of the immune system, which becomes exhausted and is then unable to fight infection. We and others have shown striking differences in the gut microbial composition between patients with cirrhosis and healthy controls (HCs) **[Figure 1].**[10-12] Patients with cirrhosis have reduced stool microbial diversity on metagenomic sequencing with reduced metagenomic sequences (MGS) compared to HCs **[Figure 1a].** Patients with cirrhosis have a higher abundance of Bacteroidetes and a reduced abundance of Actinobacteria and Firmicutes **[Figure 1b].**

Figure 1



Patel VC et al. Results of a placebo-controlled double blind randomised trial to investigate the efficacy of rifaximin-α versus placebo in improving systemic inflammation in patients with cirrhosis and chronic hepatic encephalopathy (RIFSYS Trial). Journal of Hepatology 2018; 68: S105-364. LBA 005.

Figure 1: Results of a placebo-controlled double blind randomised trial to investigate the efficacy of rifaximin-α versus placebo in improving systemic inflammation in patients with cirrhosis and chronic hepatic encephalopathy (RIFSYS Trial).

Current understanding on the composition and function of the gut microbiome and how this relates to the progression and outcomes in patients with cirrhosis remains in its infancy and is based on descriptive snapshots afflicted with confounders and lacks robust clinical validation. As perturbations in the gut microbiome are a hallmark of advanced CLD and influence the rate of progression to liver failure, driving the susceptibility to infection, unlocking the potential of the microbiome and developing antibiotic-free therapies to tackle these unmet needs becomes a research priority.

3. Evidence-based rationale and review of existing evidence

The gut microbiome has prime importance in the pathogenesis of cirrhosis with the evolution from a healthy gut microbiome to one characterised by dysregulation of gut microbial composition and functionality or 'dysbiosis' which is associated with the progression to end-stage cirrhosis. [10] 75,245 microbial genes have been identified as different between patients with cirrhosis and healthy individuals [11] and dysbiosis has been shown to be greater in patients with cirrhosis who develop complications correlating with plasma endotoxin and 30-day mortality. [10] Pre-cirrhotic liver diseases particularly alcohol and MAFLD, are associated with increased intestinal permeability and dysbiosis in cirrhosis and there is an imbalance between healthy and pathogenic gut bacteria with skewed microbiota populations in favour of increased numbers of pro-inflammatory and ammonia producing bacteria including Enterobacterales, Firmicutes, Archaea and *Prevotella.* [13] Bacterial translocation is a significant driver of cirrhosis-associated immune dysfunction (CAID), although the mechanisms by which intestinal dysbiosis drives immune cell dysfunction remain unknown **[Figure 2].** [14]

Figure 2

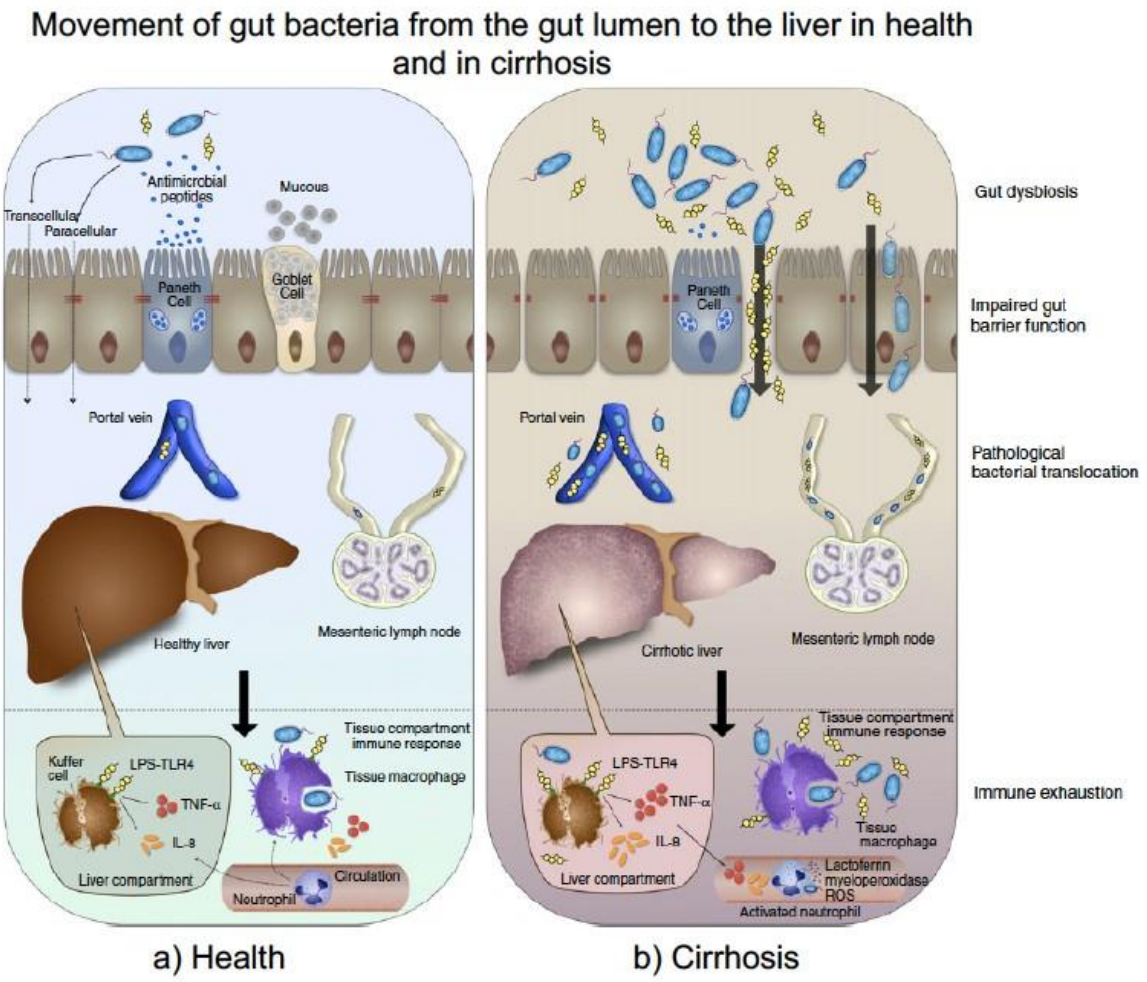


Figure 2: Bacterial Translocation: This figure demonstrates the movement of gut bacteria from the gut lumen to the liver in health and in cirrhosis.

In health, gut barrier integrity is maintained by several factors including mucus from goblet cells, secretory IgA, anti-microbial peptides, bile acids and tight junctions. This prevents unregulated movement of bacteria from the gut to the liver via the portal vein. In cirrhosis, gut dysbiosis and bacterial overgrowth disrupt the usual mechanisms, resulting in pathological bacterial translocation and endotoxin uptake. These products reach the liver and mesenteric lymph nodes activating immune cells and causing proinflammatory cytokine release. Circulating neutrophils degranulate in response to TNF-a and IL-8 released from hepatic macrophages (Kupffer cells) in response to endotoxin exposure. Granules include lactoferrin, myeloperoxidase and reactive oxygen species initiating systemic inflammation and innate immune dysfunction.[13]

Manipulation of the cirrhotic gut microbiome to improve liver-related outcomes has been explored by treating with non-absorbable antibiotics such as rifaximin. [15] This has shown a number of benefits with significant reductions in hospitalisation, bed days, emergency department attendances and 30-day re-admissions. [16] Patients on the liver transplant waiting list treated with rifaximin have reduced all-cause admissions, episodes of spontaneous bacterial peritonitis (resulting from bacterial translocation), variceal bleeding, and reduced length of stay. [17] Furthermore, the use of rifaximin by patients on the liver transplant waiting list has been linked to reduced early allograft dysfunction following transplantation. [18] In our recent double-blind randomised placebo-controlled longitudinal trial (RIFSYS trial) [**Figure 1**], rifaximin led to a reduction in markers of gut-derived systemic inflammation and gut mucin degradation. Those on rifaximin were less likely to develop infection (odds ratio 0.21; 95% CI 0.05-0.96). [12]

These data constitute "proof of principle" that modifying the gut microbiota in patients with cirrhosis improves clinical outcomes. However, considerable concern remains regarding whether long term antibiotic prescription may drive AMR. Indeed, in a recent study, 50% of patients prescribed rifaximin for hepatic encephalopathy developed rifaximin-resistant *Staphylococcal* isolates after as little as 1-7 weeks of rifaximin treatment [19] raising concern that this drug may contribute to AMR in cirrhosis. This begs the question therefore as to whether changing the gut microbiota utilising faeces from healthy donors may be a safer and more durable therapy and might offer an antibiotic alternative for patients with cirrhosis.

Faecal microbiota transplantation (FMT) is an established treatment to stably modify the gut microbiome and has been shown to be safe, [20] cost-effective [21] and efficacious in several disease states resulting from gut dysbiosis [22] including recurrent *Clostridioides difficile* infection where it has become a standard of care in many UK hospitals [NICE IPG 485] [23] Bajaj *et al.* have shown in the first-ever performed open-label phase 1 pilot study of 10 patients treated with FMT from one stool donor, that FMT is safe and potentially efficacious in treating hepatic encephalopathy. This study had significant limitations as patients were treated with broad-spectrum antibiotics prior to administration of the FMT (administered *via* enema) and the favourable impact may have been related to the antibiotic administration (not given to the standard of care arm). [24] This would still support FMT as having utility in restoring antibiotic induced disruption in microbial diversity and function in patients with cirrhosis. [25] A further phase 1 FMT trial enrolling 20 men with alcohol-related cirrhosis with alcohol misuse showed that FMT *versus* placebo was associated with a short-term reduction in alcohol craving and consumption. There was also a reduction in alcohol-use disorder-related events over 6 months in patients assigned to FMT. [26]

We have demonstrated in a recently completed NIHR-RfPB funded feasibility study [**PROFIT trial**: **PRO**spective, randomised placebo-controlled feasibility trial of **F**aecal m**I**crobiota **T**ransplantation in cirrhosis NCT02862249][27] that FMT was well tolerated and safe. The primary outcome of the study was to assess the safety and feasibility of a single 50-gram FMT [in 200mLs 0.9% saline and 12.5% glycerol] or placebo [200mLs diluent only] (3:1 allocation) administered by upper gastrointestinal endoscopy into the jejunum in patients with cirrhosis and MELD score 10-16 who were not being treated with antibiotics. [28] There were no serious adverse events related to FMT in the 15 patients treated or an excess in gastrointestinal side effects compared to placebo. Interestingly, mean venous ammonia reduced at day-30 in the FMT cohort (p=0.0007) whereas it increased in the placebo group. This was associated with a significant reduction by day-90 in stool *Enterococcus faecalis* (p=0.000006) [**Figure 3a**] and enteropathogenic *Escherichia coli* (p=0.0033) [**Figure 3b**] in the FMT-treated group but not placebo. *E. faecalis* hydrolyses arginine to produce ammonia and this may explain the reduction in blood ammonia known to drive the complication of hepatic encephalopathy in cirrhosis.

Figure 3

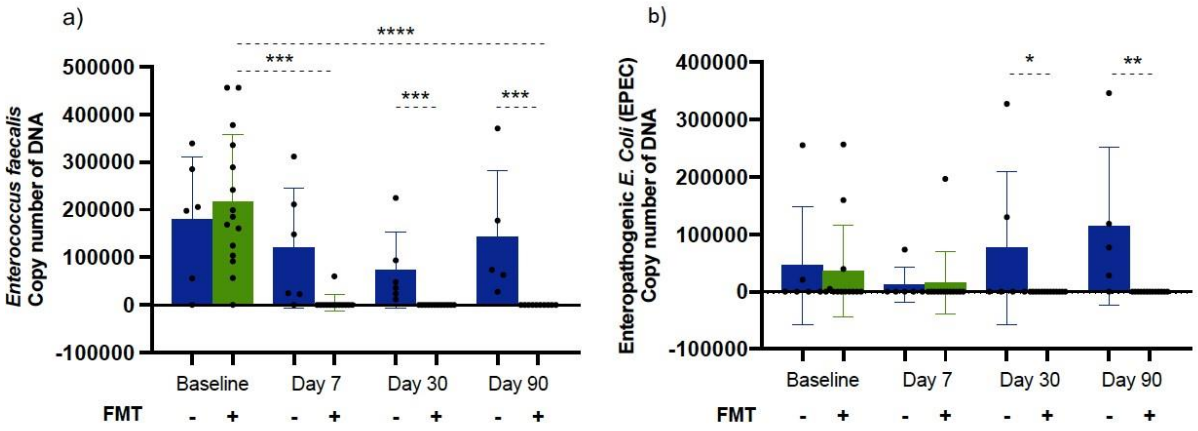


Figure 3: Stool bacterial DNA of Enterococcus faecalis and Enteropathogenic E. coli following FMT [green bars] versus placebo [blue bars] at baseline and days 7, 30 and 90.
FMT eradicates the presence of these pathogenic species in the stool in patients with cirrhosis with a significant reduction by day-90 in stool Enterococcus faecalis (p=0.000006) [Fig 3a] and enteropathogenic Escherichia coli (p=0.0033) [Fig 3b] in the FMT-treated group but not placebo.

This trial was not powered to detect differences in clinical outcomes. Mechanistic data from the 21 cirrhotic patients treated in this study demonstrate that at baseline they have **excessive non-specific inflammation and blunted anti-bacterial responses**. Using a whole blood culture system (TruCulture®) which preserves physiological cellular interactions to more accurately reflect the complexities of the immune system, capturing global immune cell activity at the time and place of sample collection, we confirmed that patients with cirrhosis have increased circulating levels of the proinflammatory cytokines interleukin (IL)-1b and IL-8 consistent with CAID and inflammasome activation. [14] Using this technique we have previously shown that cirrhotic patients exposed to bacterial products exhibit an exaggerated inflammatory response orchestrated by IFNs, IL-6 and IL-8. [29] Mechanistic data from the PROFIT trial has confirmed that patients with cirrhosis mount an augmented pro-inflammatory response to heat-killed *E.coli* 0111:B4 (HKEB) and lipopolysaccharide (LPS) exposure, with the production of TNF-α, as part of the antibacterial response, which was enhanced in the FMT group at day 7 ($p=0.0025$). Following FMT, we observed significantly increased production of IL-1b ($p<0.0001$) following HKEB exposure suggesting that FMT may improve immune responsiveness to bacterial exposure **[Figure 4]**. Furthermore, 16S rRNA sequencing of whole blood revealed that the O-antigen of *E. coli* is significantly downregulated at day 7 post-FMT ($p=9.39e^{-4}$) and persists to day 90. The O-antigen is the immune stimulatory part of LPS and this may account for the reduction in systemic inflammation post-FMT.

Figure 4

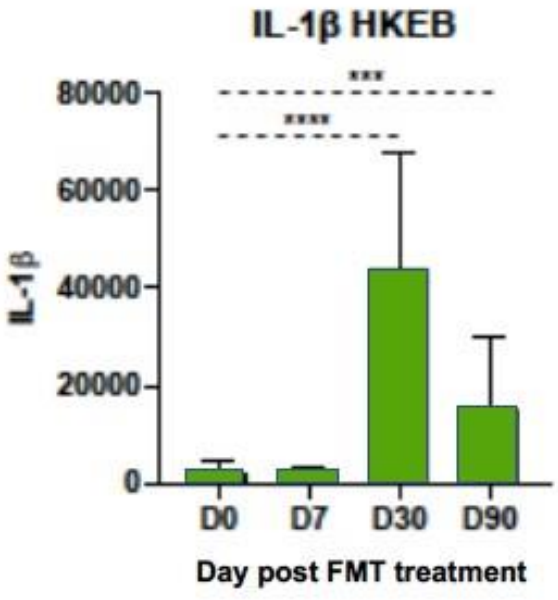


***Figure 4**: Supernatant IL-1ß concentration (mean fluorescence intensity) at baseline and then on days 7, 30 and 90 post FMT in 15 patients with advanced cirrhosis following incubation with heat-killed E.coli 0111:B4 (HKEB) coated TruCulture® tubes. Supernatant IL-1 ß was significantly elevated at days 30 ($p<0.001$) and 90 ($p<0.0001$) compared to baseline suggesting that FMT may improve immune responsiveness to bacterial exposure.*

The beneficial effect of the FMT was shown to wain at day-90 suggesting its efficacy was short to medium term. During this trial, it also became clear that not all patients enrolled were willing to undergo an invasive endoscopy to have the FMT instilled, and patients fed back that a strategy where the FMT is administered in a pill would be preferable and more practicable in the longer term. Indeed, Bajaj *et al*. have recently published a phase 1 study, demonstrating that oral FMT capsules are safe and well-tolerated in 10 patients with cirrhosis and recurrent hepatic encephalopathy. FMT was associated with improved duodenal mucosal diversity, dysbiosis, and anti-microbial peptide expression, reduced lipopolysaccharide-binding protein, and improved cognitive performance. [31] **Further studies are now needed to prove the efficacy of FMT in larger populations of patients with cirrhosis and to evaluate its mechanisms of action which remain unclear.**

5 Trial Objectives and Design

5.1 Trial Aims and Objectives

The aim of the PROMISE Trial will be to evaluate the efficacy and mechanisms of action of encapsulated FMT (*versus* placebo) to reduce infection and mortality in patients with alcohol-related (ALD) and Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD) and MASLD-ALD Overlap cirrhosis. This approach has the added benefit that removing the possibility of endoscopy-related adverse events will reduce risk further.

The primary objective is:

1. **To evaluate the efficacy of encapsulated FMT to reduce the susceptibility of infection in patients with cirrhosis measured by the time to first infection resulting in hospitalisation.**

Secondary (clinical efficacy) objectives are as follows:

1. Evaluate the efficacy of encapsulated FMT to reduce decompensating cirrhotic events including acute variceal bleeding, new-onset ascites, spontaneous bacterial peritonitis, hepatic encephalopathy and progression to acute-on-chronic liver failure (ACLF).
2. To evaluate the impact of encapsulated FMT on patient outcomes including all-cause hospitalisation, disease progression, quality of life, mental health, alcohol use and survival.
3. To assess the impact of FMT on antibiotic usage and loss/reduction of antimicrobial resistance (AMR) genes.
4. To evaluate the safety of encapsulated FMT.

Following the assessment of efficacy, our **mechanistic aims are**:

1. To determine the mechanisms of action based on the hypothesis that the characteristics of the gut microbiome in cirrhosis drive the development of CAID.
2. To determine if FMT will favourably modify key contributors by improving gut microbiome diversity and reducing bacterial translocation into the systemic circulation.

The **mechanistic objectives** are:

1. Determine whether FMT increases the diversity of the gut microbiome in cirrhosis.
2. Determine whether FMT reduces bacterial translocation and endotoxemia.
3. Evaluate whether FMT improves CAID and reduces systemic inflammation.

The mechanistic objectives can be considered “exploratory” objectives and will not necessarily be covered in the primary paper.

5.2 Primary endpoints

Primary endpoint: Time to first infection resulting in hospital admission i.e., the date patient is admitted to the hospital due to infection.

Definition of infection

For the purposes of this study, infection is defined [32] as the development of a **lower respiratory tract infection** with new pulmonary infiltrate on chest x-ray in the presence of at least one respiratory symptom (cough, sputum production, dyspnoea, pleuritic pain) with at least one finding on auscultation (rales or crepitation) or one sign of infection (core body temperature >38°C or less than 36°C, shivering, or leukocyte count >10,000/mm^3 or <4,000/mm^3) in the absence of antibiotics; **urine infection** as a urine white blood cell >15/high-power field (or equivalent) with either positive urine Gram stain (or cytometry) or culture; **bacterial peritonitis** defined by an ascitic white cell count >250 cells/mm^3 and/or positive ascitic culture; **positive stool culture/PCR** including the presence of *Clostridioides difficile* infection/toxin if suspected; **confirmed acute bacterial skin/soft tissue infection** with a lesion size of >75 cm^2; confirmed **bacteraemia** with peripheral blood cultures, or **meningitis** confirmed by positive cerebrospinal fluid microscopy and culture. This includes both bacterial and fungal infection.

Table 1: Summary of definition of infections for the purposes of the trial

Lower respiratory tract	New pulmonary infiltrate on chest x-ray in the presence of at least one respiratory symptom (cough, sputum production, dyspnoea, pleuritic pain) with at least one finding on auscultation (rales or crepitation) or one sign of infection (core body temperature >38°C or less than 36°C, shivering, or leukocyte count >10,000/mm^3 or <4,000/mm^3) in the absence of antibiotics.
Urine	Urine white blood cell >15/high-power field (or equivalent) with either positive urine Gram stain (or cytometry) or culture.
Ascites (spontaneous bacterial peritonitis)	Ascitic Neutrophils count >250 cells/mm^3 and/or positive ascitic culture.
Stool	Positive stool culture/PCR including the presence of *Clostridioides difficile* infection/toxin if suspected.
Acute Bacterial Skin/ Soft Tissue Infection	Bacterial infection of the skin with a lesion size of >75 cm^2 (lesion size measured by area of redness, oedema or induration) as defined by FDA US Food and Drug Administration. Guidance for industry. Acute bacterial skin and skin structure infections: developing drugs for treatment. www.fda.gov/media/71052/download
Blood (bacteraemia)	A positive peripheral blood cultures. (Exclude common skin contaminants *e.g.*, coagulase-negative *Staphylococci* or *Corynebacteria* isolated on a single blood culture set only)
Central nervous system (meningitis)	Meningitis confirmed by positive cerebrospinal fluid microscopy and culture.

The diagnosis of incidental viral infection, including COVID-19, contracted in the community or whilst in hospital, will not qualify as meeting primary endpoint.

5.3 Secondary endpoints

1. Incidence of decompensating events including hepatic encephalopathy, new-onset ascites, variceal bleeding and spontaneous bacterial peritonitis over 24 month follow up period.
2. Progression to ACLF (the development of one or more organ failures). [33]
3. Infection rates and antibiotic usage following randomisation over 24 month follow up period.
4. Incidence of AMR including skin and nose colonisation with methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), linezolid-resistant *Enterococci* (LRE), extended-spectrum beta-lactamase producing bacteria (ESBL), fluoroquinolone-resistant Gram negative and carbapenem-resistant Enterobacterales (over 24 month follow up period). [8]
5. Hospitalisation rates (liver-related and all-cause) including the length of stay and admission to high dependency/intensive care (over 24 month follow up period).
6. Change in liver disease severity scores over 24 months post-randomisation (recorded every 3 months).
7. Change in quality of life (EQ-5D-5L score) at 3, 6, 12 and 24 months post-randomisation.
8. All-cause mortality, and liver-related mortality.
9. Change in depression and anxiety score using HADS at 3, 6, 12 and 24 months post-randomisation
10. Change in alcohol use disorder-related events in patients enrolled with alcohol-related cirrhosis as assessed by the alcohol-use disorders identification test (AUDIT score) [33] at 3, 6, 12 and 24 months post-randomisation, and urinary ethyl glucuronide/ethyl sulphate levels if tested as part of the standard of care.
11. Safety based on safety assessments including physical examination, clinical laboratory evaluation, vital signs and reported as adverse events (AEs) or serious adverse events (SAEs) over 24 month follow-up period.

Mechanistic endpoints:

1. **Matched faecal and plasma biomarkers** (D-lactate, fatty acid- binding protein 2 and ammonia) measured by enzyme-linked immunosorbent assay (ELISA).
2. **Faecal proteases** as markers of DNA replication, tissue re-modelling and immune response as a proxy of gut mucosal barrier integrity.
3. Matched **plasma and faecal cytokine profiles** using a combination of ELISA and by electrochemiluminescence using the Mesoscale discovery platform (MSD) [Tumour necrosis factor-alpha, interferon-gamma, interleukin (IL)-1-beta, IL-6, IL-8, IL-10, IL-17A, IL-17E, IL-17F, IL-21 and IL-22].
4. **Plasma, faecal and urinary metabonomics**. Short–chain fatty acids measured by proton magnetic resonance spectroscopy (^{1}NMR). Tryptophan metabolites and primary, secondary and tertiary bile acids measured with liquid chromatography tandem mass spectrometry (LC-MS) [Butyrate and Succinate].
5. **Faecal proteomics** will identify protein changes using quantitative high-resolution mass spectrometry coupled with liquid chromatography LC-MS/MS analysis.

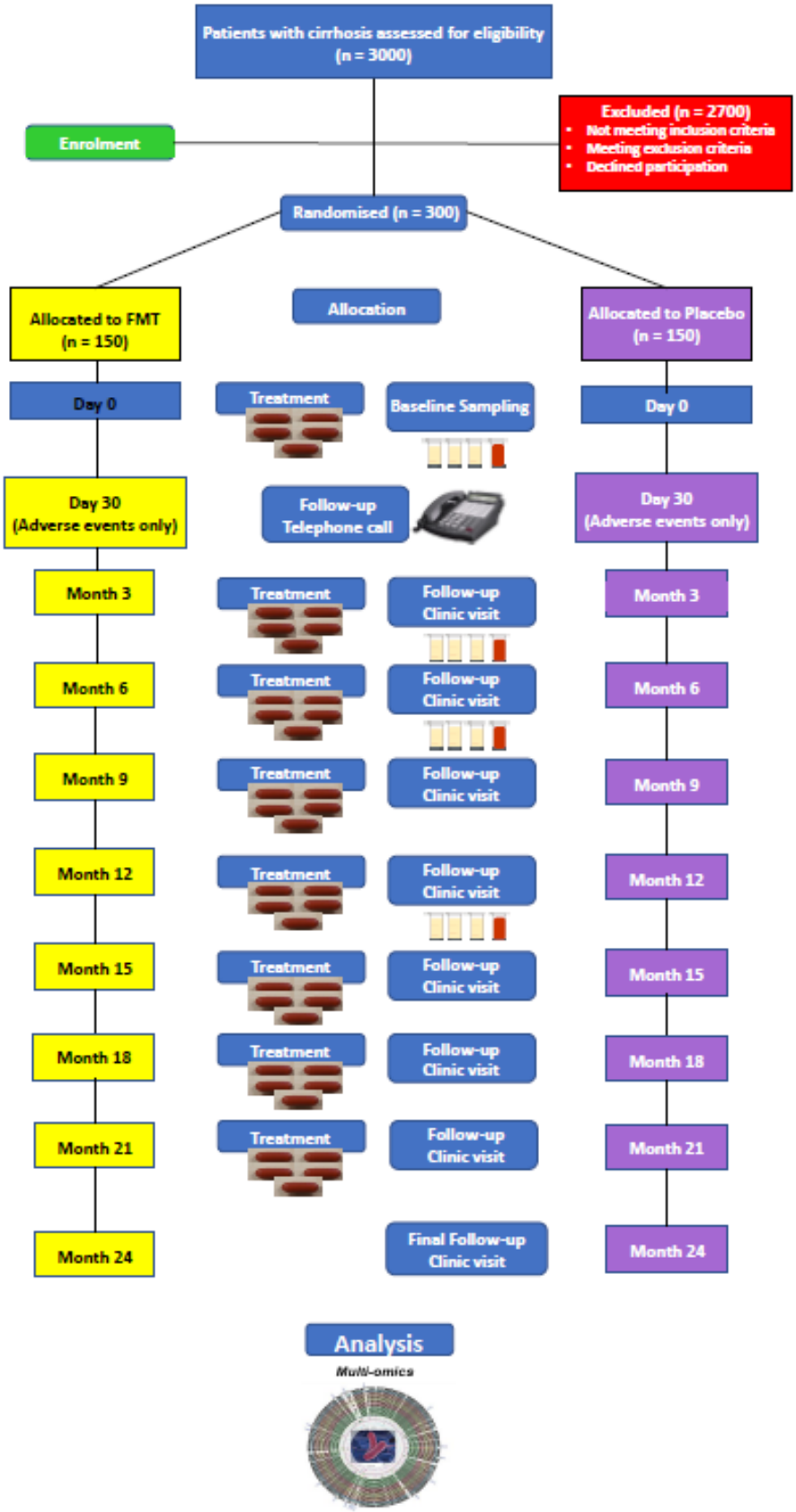
6. Matched **faecal and blood microbiome composition** (including mycome, virome and phagome) and **microbial diversity** by metagenomic sequencing [20 million reads using NovaSeq as a marker of bacterial translocation].
7. **Faecal PCR for pathogenic species of interest** including copy number of DNA of cytolysin positive and negative *[E. faecalis,* enteropathogenic *E. coli and Candida albicans]*.
8. Analysis of the presence and dynamics of the **microbial 'resistome'** (ensemble of genes encoding AMR in a given microbiome using the comprehensive antibiotic resistance database [CARD]).
9. The effect on FMT in cirrhosis on the human circulating immune profile, including **monocyte phenotype and function**. To investigate the potential immunomodulatory properties of FMT, monocyte surface expression of CD16, HLA-DR, MerTK, Axl, CD163, PD-1, PD-L1, bile acid receptors TGR5 and FXR, transcriptomic profile and functional capacity (LPS-induced TNF-alpha, IL-6 and IL-10 production, phagocytosis and oxidative burst) will be explored in stored peripheral blood mononuclear cells (PBMCs).
10. To investigate whether FMT restores **mucosa-associated invariant T (MAIT) cell numbers, phenotype and function** isolated from PBMCs. MAIT cells are an innate-like T cell population that are abundant in the liver (where they comprise 20-50% of the hepatic T cell population) and peripheral circulation. MAIT cells represent a fundamental sentinel system for the homeostatic sensing of the gut flora and exert essential and non-redundant roles in the control of bacterial infections.
11. Change in lipid profile (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol) in those with Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD) and MASLD-ALD Overlap cirrhosis, and HbA1c for diabetic patients only.
12. To interpret the faecal microbiome sequencing and metabolome analyses and how it impacts the patients gut microbiome composition in relation to dietary habits using a dietary questionnaire.

5.4 Trial Design

This is a phase III multicentre, randomised, double-blind parallel group placebo-controlled trial evaluating encapsulated FMT *versus* matched placebo for 2 years. Trial participants will be randomised 1:1 to FMT or placebo. Participants will receive the investigational medicinal product (IMP) every 91 days +/- 14 days for 21 months and will be evaluated at baseline before the IMP is administered and at 30-days (nurse-led telephone interview), 3-months, 6-months, 1 and 2 years. Blood, stool and urine will be collected at baseline, 3-months, 6-months and 1 year.

Please refer to Consort Trial Participant Flow Diagram (Section 4.5).

5.5 Trial Flowchart (CONSORT Diagram)



5.6 Study Schedule of Events

Table 2: The table below presents the summary of the efficacy parameters measured at scheduled visits

	Screening (Day -28 to Day 0)	Baseline (Day -7 to Day 0)	**Screening & Baseline Repeat****	Randomisation (Day 0)	Post-randomisation IMP dispensing (Day 0 + 1 day)	Day 30 +/- 7 days	Month 3 +/- 14 days	Month 6 +/- 14 days	Month 9 +/- 14 days	Month 12 +/- 14 days	Month 15 +/- 14 days	Month 18 +/- 14 days	Month 21 +/- 14 days	Month 24 +/-14 days	**First hospitalisation for Infection**
Informed consent	X														
Eligibility confirmation	X		X												
Urinary b-HCG test (females of childbearing potential) *	X	X	X												
BMI		X													
Randomisation				X											
IMP dispensing					X		X	X	X	X	X	X	X		
IMP accountability					X		X	X	X	X	X	X	X		
IMP administration					X		X	X	X	X	X	X	X		
IMP compliance					X		X	X	X	X	X	X	X		
Nurse-led telephone call (safety)						X									
Adverse event monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination		X					X	X	X	X	X	X	X	X	X
Index Hospitalisation (Primary Endpoint)															X
Concomitant medications check		X	X			X	X	X	X	X	X	X	X	X	
Dietary Questionnaire		X													
Routine haematology, biochemistry, glucose, clotting (INR) and C-reactive protein (Standard of care)		X	X				X	X		X		X		X	
Study blood sampling		X					X	X		X					
Study urine sampling		X					X	X		X					
Study stool sampling		X					X	X		X					
Serum sample (for archiving)		X													

HbA1C (for diabetic participants only)		X					X	X		X		X		X	X
Lipid Profile (for MASLD and MASLD-ALD Overlap Cirrhosis participants only)		X					X	X		X		X		X	X
Liver disease severity score (MELD, Child Pugh, UKELD)		X	X				X	X		X		X		X	X
Quality of life questionnaire (EQ-5D-5L)		X					X	X		X				X	
AUDIT questionnaire (for alcohol-related and MASLD-ALD Overlap cirrhosis participants only)		X					X	X		X				X	X
U**rinary ethyl glucuronide / E**thyl sulphate test (For alcohol-related and MASLD-ALD Overlap cirrhosis participants only) (Only if part of standard of care) ***		X					X	X		X				X	X
Hospital and Anxiety Depression Scale (HADS)		X					X	X		X				X	

* When screening and baseline visits are performed on the same day, the Urinary b-HCG test (females of childbearing potential) will only need to be performed once for eligibility confirmation.

**These procedures must be repeated if time between baseline visit and randomisation is more than 7 days. If the time between baseline and randomisation elapses over 28 days, the full screening and baseline visit procedures must be repeated.

***Only performed if this is a part of the participant's standard of care routine.

6 Trial Medication

6.1 Investigational Medicinal Product (IMP)

Investigational Medicinal Product – Active Intervention (FMT):

Faecal Microbiota Transplant (FMT) contains lyophilised faecal product, encapsulated in size 0, Swedish Orange, Delayed-Release methylcellulose capsules (DRcaps™, Capsugel®. The capsule colour and opaque appearance of the capsules have been chosen to maintain blinding. The product is non-sterile and contains human faeces, in addition to sodium chloride 0.9% w/v and 5% trehalose dihydrate as excipients.

The FMT capsules will be manufactured, packaged, labelled and QP certified at the FMT Laboratory at St Thomas' Hospital, London, SE1 7EH, in accordance with current Good Manufacturing Practice (cGMP) principles, under licence number MIA(IMP) 11387.

The drug substance is derived from a donated healthy human faecal sample. Each donated faecal sample is a unique and novel product, therefore no testing on the composition of the donated faecal sample is performed. The only testing that is performed is that for pathogen exclusion. All donors are selected and screened to ensure absence of risk factors for transmissible diseases and donated material is tested to ensure absence of pathogenic organisms.

Stability/viability testing of the FMT capsules has been performed and confirms stability and viability for 24 weeks at -20°C and 8 months at -80°C. The testing showed that the capsules contained viable bacteria with no significant loss in viability or change in composition compared to fresh stool over the respective timeframes.

FMT material will be fully traceable from donor to recipient. Each batch of FMT will have a code allowing identification of the donor. Aliquots of donor stool will be kept for 30 years to allow for future testing if required.

The manufacturer of the IMP is:
FMT Laboratory
Centre for Clinical Infection and Diagnostics Research (CIDR)
5th Floor North Wing
St Thomas' Hospital
Westminster Bridge Road
London
SE1 7EH
Manufacturer's Licence No: MIA(IMP) 11387

Investigational Medicinal Product – Placebo Intervention:

The PROMISE trial is using a placebo as a comparator arm as there is no available alternative specific therapy to reduce mortality and improve outcome in cirrhosis.

The placebo product contains microcrystalline methylcellulose. It is supplied as a size 0, Swedish Orange Delayed-Release capsule (DRCap) and provides a complete match with regards to the appearance (*e.g.,* dimensions, colour) to the FMT capsules.

The placebo capsules will be manufactured, packaged, labelled and QP certified at the Royal Free London NHS Foundation Trust, Clinical Trial Manufacturing and Supplies Unit, Pond Street, London, NW3 2QG, under licence number MIA(IMP) 11149.

6.2 Dose, Packaging and Labelling

The interventions will be supplied to sites in HDPE DUMA bottles with child-resistant, tamper evident caps. Each bottle will contain 5 capsules. The information presented on the IMP labels will be annex 13 compliant.

Trial participants will receive five capsules of the Investigational Medicinal Product (IMP) to be taken orally at a hospital clinic every 91 days +/- 14 days for a total of 21 months.

Trial participants are recommended to fast for 4-hours prior to treatment. Light snack is allowed if medically advised and fasting requirements will be under the PI's discretion. At each treatment dose, the participant will be able to fast prior to their hospital visit and the fasting duration will be captured on the database. The fast is to facilitate absorption of the IMP and reduces the likelihood of participant vomiting after IMP administration. They will be requested to ingest all capsules within 30 minutes and are permitted unlimited water or squash.

Trial participants who are unable or are unwilling to ingest the IMP will be withdrawn from the study and the reasons will be recorded.

Trial participants who are unable to swallow all 5 capsules or are only able to swallow 1 or more of the capsules but not all 5 will continue to be followed-up as per the study schedule but this will be recorded in the eCRF.

Trial participants with alcohol-related or MASLD-ALD Overlap cirrhosis must be abstinent for a minimum of 4 weeks prior to randomisation and are encouraged to remain abstinent from alcohol for the duration of the trial (abstinence from alcohol in this group of patients is considered standard of care regardless of enrolment into a clinical trial). If the participant relapse back to drinking alcohol, they will still be included in the trial and continue to receive IMP as per schedule. Relapse to drinking alcohol will be assessed as a secondary endpoint with the AUDIT questionnaire. [33]

Trial participants must not have received antibiotics within 7-days prior to randomisation and at each dosing visit. The routine prescription of prophylactic antibiotics including ciprofloxacin and co-trimoxazole (prophylaxis of spontaneous bacterial peritonitis) and rifaximin (hepatic encephalopathy) will not be permitted at randomisation as they will reduce the efficacy of the FMT and will exclude a patient from the trial if the treating physician deems ongoing treatment is necessary. If at randomisation a participant is deemed likely to need to remain on the long-term antibiotics, then they should be excluded as this will impede the efficacy of the IMP. **If any antibiotic is later clinically indicated during the course of the study for the treatment of suspected or confirmed infection, or rifaximin for the treatment of recurrent overt hepatic encephalopathy, then these will be permitted to be**

given as standard of care. If the participant is receiving short term antibiotic treatment, there will be a 7-day washout period before continuing IMP per dosing schedule. If the participant is receiving long-term antibiotic treatment, the participant will continue to receive IMP as per the dosing schedule. The participant will no longer receive further IMP doses if they have been hospitalised for an infection and have met the primary endpoint.

The development of infection resulting in hospitalisation will meet the primary endpoint and the participant will no longer receive further IMP doses, although they will continue to be followed up for the duration of the trial.

6.3 IMP Risks

In 2015, the UK Human Tissue Authority (HTA) published an opinion that FMT does not fall within the scope of the UK Human Tissue (Quality and Safety for Human Application) Regulations 2007, but also recommended that establishments conducting FMT should act in accordance with the HTA "Guide to Quality and Safety Assurance for Tissues and Cells" for patient treatment. The Medicine and Healthcare Products Regulatory Agency (MHRA) classifies FMT as a medicinal product. All medicinal products should be produced according to the principles of GMP under MHRA licence.

FMT is a heterogenous product, whose mechanism of action remains to be ascertained. As such it is difficult to assess the risks associated with FMT administration. The main concern relating to FMT is the potential for transmissible infection.

The majority of reported side effects from FMT are gastro-intestinal and short-lived/self-limiting. [20] The side effects also relate to route of administration *e.g.* endoscopy. As a result of the encapsulated formulation, there is no need for endoscopic administration of FMT. Two cases of extended spectrum beta-lactamase (ESBL) producing *E. coli* bacteremia have been reported in immunocompromised patients (one with hepatitis C cirrhosis and one with myelodysplastic syndrome, post stem cell transplant) in the USA, linked to a single donor FMT. The patient with myelodysplastic syndrome sadly died as a result of his infection. [40] Prior to this, FMT was not screened for ESBL-producing organisms in the USA. The Food and Drug Administration (FDA) halted FMT trials temporarily as a result and ESBL screening in the USA is now mandatory. **Our FMT is routinely screened for ESBL and carbapenemase producing organisms and aliquots of donor stool are stored for 30-years for full traceability.**

To minimise risk, the FMT laboratory at GSTT will be used to manufacture FMT, which holds a MHRA Manufacturer's Authorisation for Investigational Medicinal Products (MIA(IMP)) and complies with MHRA rules and guidance for Pharmaceutical Manufacturers and Distributors (Orange Guide, 2017), in accordance with GMP.

FMT is endorsed by NICE [23] for the treatment of recurrent *C. difficile* infection and has been successfully used to treat over 250 patients at GSTT. **15 participants with cirrhosis received FMT as part of the PROFIT trial (NCT02862249). [27] There were no IMP-related serious adverse events.**

Please refer to the Investigators Brochure, which contains the reference safety information for the FMT product**.**

6.4 Drug Accountability

The pharmacy clinical trials team must maintain accurate accountability records of the IMP, which will be reviewed by the trial CRA for monitoring purposes.

Following participant administration with the IMP, the IMP bottle and any unused capsules must be disposed of in the clinic room in clinical waste bins/bags. The packaging and unused capsules do not need to be returned to pharmacy for reconciliation and disposal. The number of capsules administered, and number of capsules discarded will be recorded and verified for monitoring purposes.

The disposal of unused IMP stored in pharmacy (*i.e.*, damaged/ expired stock, or stock remaining at the end of the trial) is only permitted with written Sponsor approval and CI authorisation. Do not destroy unused study drug without prior authorisation from the Sponsor. Once authorised, destruction can be conducted at site in accordance with the site IMP destruction SOP and disposed of in accordance with the hospitals clinical waste procedures.

6.5 Storage and Supplies

6.5.1 FMT:

Store the FMT product in a locked freezer at:

- -20°C (range -25°C to -15°C),

An automated electronic temperature monitoring system/device must be used at sites for temperature monitoring and reporting.

In the event of a temperature excursion, quarantine the supplies and notify the Sponsor immediately following the instructions in the Pharmacy Manual.

Supplies that are in quarantine, must be clearly segregated from stock that is available for allocation.

FMT capsules thaw quickly once they are removed from the freezer and come to room temperature within 5-minutes. Once the product is removed from frozen storage at the point of dispensing, it must be either administered within 6 hours or quarantined (in pharmacy) or disposed of (in clinic); it cannot be refrozen.

6.5.2 Placebo:

Store the placebo product in a secure location at room temperature maintaining the temperature at less than 30°C.

Temperature monitoring and reporting is required. In the event of a temperature excursion, quarantine the supplies and notify the Sponsor immediately following the instructions in the Pharmacy Manual.

6.5.3 Supplies:

The site pharmacy teams will be unblind to a participant's treatment allocation. The trial centre pharmacy will dispense the correct treatment arm in accordance with the unblinded

randomisation notification on receipt of a trial-specific prescription which has been signed by a prescriber on the site delegation log.

The Kings Clinical Trials Unit (KCTU) Intervention Management System will be used to manage supplies centrally and at study sites. The KCTU Trials Pharmacist will place the initial order and subsequent orders to restock sites with the interventions.

6.6 Participant Compliance

Participants will take their IMP at their hospital outpatient visits so adherence will be directly observed. The number of capsules taken by the participant will be recorded in the CRF. If the participant is unable to take the full dose or capsules are damaged/dropped the reason for the deviation from the protocol will be recorded in the CRF and on a deviation log which is filed in the ISF. If a participant misses their visit or is outside the window for the visit, we will still give them the dose and record it as a protocol deviation.

6.6 Concomitant Medication

Specific concomitant medications including antibiotics (as part of standard of care), beta blockers (Carvedilol and Propranolol), Lactulose (prebiotic), probiotics, anti-depressants/anti-anxiety/psychiatric medication and proton pump inhibitors (PPI) received during the treatment phase will be recorded in the relevant CRF for this trial.

Trial participant must not have received antibiotics within 7 days prior to randomisation and at each dosing visit.

The routine prescription of prophylactic antibiotics including ciprofloxacin and co-trimoxazole (prophylaxis of spontaneous bacterial peritonitis), rifaximin (hepatic encephalopathy) and probiotics (other than foods)* will not be permitted at randomisation as they may reduce the efficacy of FMT and will exclude a participant from the trial if the treating physician deems ongoing treatment is necessary. If any antibiotics is later indicated during the course of the study for the treatment of suspected or confirmed infection, or rifaximin for the treatment of recurrent overt hepatic encephalopathy, then these will be permitted to be given as standard of care.

If a participant has been treated with antibiotics for an acute infection following commencement of IMP which did not necessitate hospital admission *i.e.,* did not meet primary endpoint, then repeat dosing will be delayed until 7 days following finishing the antibiotic course. If, however the participant has been commenced on long-term prophylactic antibiotics as part of their standard clinical care that will continue for the remaining duration of the trial including ciprofloxacin and co-trimoxazole (prophylaxis of spontaneous bacterial peritonitis) and rifaximin (hepatic encephalopathy), then participants will be permitted to receive their IMP as per dosing schedule. If antibiotics are given during hospital admission due to infection then the participant has met the primary endpoint. This would result in discontinuation of IMP but the participant would remain in the study for follow-up.

Specifically, participants will not be required to follow a specific diet, nor will they be required to use contraception (FMT is not known to be teratogenic). Please refer to the Investigators Brochure for further safety data.

*There are no universally accepted definitions of probiotics, and there is a plethora of products available. These substances are often contained in several commonly consumed foodstuffs *e.g.,* yoghurt, cheese, beer etc. For the purposes of this study participants who consume foodstuffs containing probiotics will not be excluded from the trial, other products will be reviewed on a case-by-case basis. A rule of thumb to aid decision making is to allow products that can be found in the food aisles of supermarkets and exclude products that can found in the pharmacy/health products or vitamins aisle.

7 Selection and Withdrawal of Participants

Eligibility screening of the patients will be carried out by a research nurse at each clinical site who will evaluate the following inclusion and exclusion criteria. This will then be reviewed and approved by the Site Principal Investigator (PI).

7.1 Inclusion Criteria

1. Aged ≥ 18 years
2. Confirmed Alcohol-related (ALD) or Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD) or MASLD-ALD Overlap cirrhosis based on clinical, radiological and/or histological criteria.
3. MELD score 8-16 [28]
4. Patients with alcohol-related cirrhosis must have been abstinent for a minimum of 4 weeks prior to randomisation.
5. Patients must be deemed to have the capacity to provide written informed consent to participate.

7.2 Exclusion Criteria

1. Severe or life-threatening food allergy (*e.g.,* peanut allergy)
2. Pregnancy or planned pregnancy*. Urine testing will be performed at screening to rule out pregnancy in females.
3. Breast-feeding
4. Patients treated for acute variceal bleeding, infection, overt hepatic encephalopathy, bacterial peritonitis or ACLF within 14 days prior to randomisation.
5. Active alcohol consumption of >20 grams/day [1 unit of alcohol contains 10mLs or 8g of alcohol]
6. Had a previous liver transplant
7. Patients with inflammatory bowel disease.
8. Patients with coeliac disease.
9. Patients with a history of prior gastrointestinal resection or surgery that could change the gut microbiome or result in bacterial overgrowth *e.g.* gastric bypass
10. Active malignancy including hepatocellular carcinoma
11. Patients with an expected life expectancy <6 months or listed for liver transplantation
12. Infected with HIV, hepatitis B or C [patients who have undetectable hepatitis B or C DNA/RNA can be recruited].
13. Patients who have received antibiotics or probiotics (excluding food stuffs containing 'live bacteria' such as live yoghurts, kefir, fermented vegetables such as sauerkraut/kombucha or cheese) within 7 days prior to randomisation.

14. Swallowing disorder, oral-motor dyscoordination or likely inability/unwillingness to ingest study medication.
15. Patients who have received another investigational drug or device within 4 months prior to randomisation.

16. Patients, who in the opinion of the PI, have a medical condition, or other relevant psychological, familial, or social factor that may jeopardise their health, compliance, or influence the trial integrity in any way.

* A pregnancy test (urine) will be performed for all females of childbearing potential. A woman is considered of childbearing potential *i.e.*, fertile, following menarche and until becoming port-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

7.3 Selection of Participants

We plan to recruit from sixteen UK centres serving populations where rates of Alcohol-related Liver Disease (ALD), MASLD and MASLD-ALD Overlap cirrhosis patients are amongst the highest in the UK. This encompasses North-East and North-West England, the Midlands, London, Wales and Scotland. Trial participants will be enrolled from gastroenterology/hepatology outpatient clinics but GP and specialist referrals to the nearest recruiting PROMISE trial centre may also be eligible to be recruited. Trial participants will be pre-identified from hospital databases according to their MELD score calculated in the previous 3 months. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

No inducement to healthy donors or participants will be offered. Reimbursement of travel expenses for donor and participants will be permitted (up to a maximum of £30 return for 4 follow-up visits for participants and £20 per visit for donors). If recruiting centres require additional support with travel reimbursements, the team can contact the trial coordinating centre.

7.4 Donor Screening

Donors for the PROMISE Trial are registered as patients at Guy's and St Thomas' NHS Foundation Trust. They will receive written and verbal information about the screening and donation processes and the use of FMT in clinical service and research studies by a registered medical practitioner.

In brief donors must:

1. Be aged between 18 and 60 years of age
2. Have a body mass index between 18 and 30
3. Not have received any antimicrobials within the past 3 months
4. Not have any known prior exposure to HIV and/or viral hepatitis, and known previous or latent tuberculosis
5. Not have any risk factors for blood borne viruses, including high risk sexual behaviours, use of illicit drugs, any tattoo/body piercing/needlestick injury/blood transfusion/acupuncture, all within the previous 6 months
6. Not received a live attenuated vaccine within the past 6 months

7. Not have any underlying gastrointestinal conditions/symptoms (*e.g.,* history of IBD, irritable bowel syndrome (IBS), chronic diarrhoea, chronic constipation, coeliac disease, bowel resection or bariatric surgery),
8. Not have any acute diarrhoea/gastrointestinal symptoms within the 2 weeks prior to donating
9. Not have any signs or symptoms consistent with Covid-19 for 4 weeks prior to their donation but prior Covid-19 infection will not preclude donation.
10. Not have any family history of any significant gastrointestinal conditions (*e.g.,* family history of Inflammatory Bowel Disease (IBD) or colorectal cancer)
11. Not have any history of atopy (*e.g.,* asthma, eosinophilic disorders)
12. Not have any systemic autoimmune conditions
13. Not have any metabolic conditions, including diabetes and obesity
14. Not have any neurological or psychiatric conditions, or known risk of prion disease
15. Not have any history of chronic pain syndromes, including chronic fatigue syndrome and fibromyalgia
16. No have any history of any malignancy
17. Not currently be taking any regular medications or have had any antibiotics within the past 3 months.
18. No history of receiving growth hormone, insulin from cows or clotting factor concentrates.
19. No history of receiving an experimental medicine or vaccine within the past 6 months
20. No history of travel to tropical countries within the past 6 months

Interested donors will provide written informed consent to undergo the screening process and donate faecal samples to be used in the study. They will be assessed by a registered medical practitioner to determine if they meet the specified inclusion and exclusion criteria as defined by the local Standard Operating Procedure (SOP).

The screening process will be documented using a donor questionnaire for risk factors and testing for a range of infectious agents and in accordance with national guidelines published jointly by the British Society of Gastroenterology and the Healthcare Infection Society in the UK. [35] Blood and stool samples will be tested according to a local SOP and in accordance with National guidelines. All diagnostic testing will be undertaken by ViaPath Analytics LLP at Guy's and St Thomas' NHS Foundation Trust. This laboratory is accredited to ISO 15189:2012 by the United Kingdom Accreditation Service (accreditation reference 8640).

If any tests are positive the donor is informed and referral to their GP is offered (with the donor's consent). If the donor is eligible to proceed to donation, a unique donor number will be assigned which is recorded in the FMT donor master file and electronic database. A record of the unique donor number will also be made on the donors Electronic Patient Record (EPR).

Results of all donor testing will be recorded on the EPR and are reviewed and deemed as satisfactory by a registered medical practitioner prior to release of FMT product. As stool samples from donors will have metagenomic and metabolomic analyses performed (which would not ordinarily be undertaken as part of standard of care), donors will also be recruited and consented as part of the PROMISE trial.

7.5 Consent

All trial investigators seeking consent must have received specific training in the taking of consent and be up-to-date on their Good Clinical Practice (GCP) training. For each trial participant, an Investigator delegated to take consent must obtain written informed consent prior to conducting a trial-related procedure involving the participant. If a prospective recipient has signed informed consent more than three months prior to being enrolled into the study, it is recommended to obtain a second signed consent form to ensure that the participant still agrees to study participation. The investigator must always provide the patient with a copy of the completed consent form and participant information sheet and store the original in the Trial Master File (TMF). Signed, original consent forms must be retained in the Investigator's Site File (ISF) at all times and made available (for review) to study monitors, auditors and inspectors, upon request. It is recommended that copies of the signed participant consent form(s) should also be kept in the patient notes. A comprehensive verbal explanation by the Investigator will accompany the written information sheet given to potential trial participants.

Potential participants will be identified by their clinical team responsible for their care and approached to see whether they would be interested in participating in this research study. If agreeable, they will be given the relevant participant information sheet (PIS) to read. Participants should be given at least 24 hours the information sheet thoroughly and the opportunity to clarify any points that they do not understand. At the end of the discussion, the participant should be granted as long as they feel necessary to digest the information provided and to consider their involvement in the trial. They should be free to discuss their participation with others outside the clinical trial team (*e.g.,* family, friends, general practitioner) and must not feel pressured to provide an immediate decision. Participants should also be allowed a second opportunity to ask the investigator and/or research nurse questions regarding their participation, after the initial interview. All queries or concerns about the trial should be answered to the satisfaction of the subject. When obtaining informed consent, the investigator should respect the free will of the individual and must not exert undue influence on the subject or enforce compulsory enrolment into the study.

Informed consent forms will be in compliance with applicable regulations and will have been reviewed and approved by a Research Ethics Committee prior (REC) to initiation of the study. The participant information sheet will contain a full explanation of the purpose and nature of the study, a description of the procedures, the possible advantages and risks, alternate treatment options, a statement of confidentiality of participant study records, an explanation of whom to contact about the research, the participant's rights, and notification that participation is voluntary, and refusal will involve no penalty or loss of medical benefits. These requirements are in accordance with the most current revision of the Declaration of Helsinki 1996.

The Principal Investigator (PI) retains overall responsibility for the conduct of research at their site, this includes the taking of informed consent of participants at their site. They must ensure that any person delegated responsibility to participate in the informed consent process is duly authorised, trained and competent to participate according to the ethically approved protocol, principles of Good Clinical Practice (GCP) and Declaration of Helsinki.

7.6 Randomisation Procedure / Code Break

7.6.1 . Randomisation

Randomisation will be undertaken by the local research team at each site once written informed consent has been obtained, eligibility confirmed, and baseline data collected.

Trial participants will be randomised on a 1:1 basis to either FMT or placebo.

A web-based randomisation system has been designed, using the bespoke King's Clinical Trials Unit (KCTU) randomisation system. The randomisation system has been created in collaboration with the trial analyst/s and the CI and maintained by the King's Clinical Trials Unit for the duration of the project. It will be hosted on a dedicated server within KCL.

Randomisation will be at the level of the individual using the method of block randomisation with varying block sizes, stratified by site.

Data entry

Randomisation will be undertaken by recruiting site staff, by authorised staff onto the randomisation system by going to www.ctu.co.uk and clicking the link to access the randomisation system. A full audit trail of data entry will be automatically date and time stamped, alongside information about the user making the entry within the system.

Security

The CI or delegate will request usernames and passwords from the KCTU. System access will be strictly restricted through user-specific passwords to the authorised research team members. It is a legal requirement that passwords to the randomisation system are not shared, and that only those authorised to access the system are allowed to do so. If new staff members join the study, a user-specific username and password must be requested via the CI or delegate *(e.g.*, Trial Manager) from the KCTU team and a request for access to be revoked must be requested when staff members leave the project. Study site staff experiencing issues with system access or functionality should contact the CI or delegate (*e.g.*, Trial Manager) in the first instance.

Participant initials and date of birth will be entered on the randomisation system. Whereas NHS number, email addresses, participant names and addresses and full postcodes will not be entered into the randomisation system. No data will be entered onto the randomisation system unless a participant has signed a consent form to participate in the trial.

Data Quality Processes

The CI team will undertake appropriate reviews of the entered data, [in consultation with the project analyst] for the purpose of data cleaning. No data can be amended in the system, however CI or delegate (e.g., Trial Manager) may request King's Clinical Trials Unit to add notes against individual subject entries to clarify data entry errors.

Database Lock

Upon request, KCTU will provide a copy of the final exported dataset to the CI in .csv format and the CI will onward distribute as appropriate.

7.6.2 Blinding

The Chief Investigator, site Principal Investigators, participants and outcome assessors will be blinded to the treatment allocation.

The IMP packs cannot be fully blinded due to a difference in the temperature storage conditions, expiry dates and batch number configurations for the FMT and placebo products. For this reason, the site pharmacy trials team and the nurse administering the intervention will be unblinded. Research nurses administering the intervention will only be delegated this role and will not be permitted to assess outcomes or collect data. This is because there is the possibility that the research nurse administering the IMP could become unblind to the treatment arm due to the storage conditions and labelling of the IMP and placebo. This arrangement will mitigate the risk of unblinding participants and assessment bias.

The senior statistician will also be fully blinded, but the junior statistician will be unblinded from the 1st DMC meeting with data onwards.

6.6.3 Unblinding

Whilst the safety of participants in the trial must always take priority, maintenance of blinding is crucial to the integrity of the trial. Investigators should only break the blinding when information about the -participant's trial treatment is clearly necessary for the appropriate medical management of the participant and where stopping the blinded medication is not sufficient.

Should an alternative to unblinding not be identified, and if unblinding is required to optimise medical management of the participant, investigators should follow the unblinding process below.

Each randomised participant will be provided with a card containing the contact numbers for the trial team and emergency contact details. Participants will be requested to carry this card with them at all times whilst participating in the trial.

7.6.3 Emergency Unblinding

A 24-hour telephone-based emergency code break service, Emergency Scientific Medical Services (eSMS), has been commissioned for this study. Participants will be asked to carry emergency alert cards during the study, which will have details of the code break telephone number. The caller can request code break from eSMS using the participant's study PIN, which is allocated by the MACRO EDC system after consent.

Investigators should only request emergency code break when information about the participant's trial treatment is necessary for the appropriate medical management of the participant and where stopping the blinded medication is not sufficient. The treating physician or investigator will have the primary right to break the blind in any moment in case of emergency and they will be able to unblind immediately and without delay.

All FMT batches will be fully traceable with aliquots of all donor stools stored for 30-years for future testing if deemed necessary.

24-hour emergency code break telephone number: 020 3282 0458

7.7 Withdrawal of Participants

A trial participant has the liberty to withdraw their consent at any time and for any reason, without penalty or loss of benefits to which the individual would otherwise be entitled. Participants who withdraw consent will discontinue their future participation in the trial, but we will request to collect future routine outcome data from the patient notes (*e.g.*, hospitalisation due to infection). Prior to giving consent, recipients will be informed that they are able to request the destruction of stored biological samples (*e.g.,* blood/stool) upon withdrawal, and that this will only be possible for samples that have not been tested at the time of withdrawal. Participants will not be able to request the deletion of data generated from tested samples.

Participants have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw participants from the study drug in the event of intercurrent illness, AEs, SAEs, SUSARs, protocol violations, cure, administrative reasons or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of participants should be avoided. Should a participant decide to withdraw from the study, all efforts will be made by the investigator to report the reason for withdrawal as thoroughly as possible.

The investigator will maintain a record of all participants who discontinue from the trial prior to completion: the reason(s) for trial discontinuation will be documented. In the event, a participant wishes to discontinue the treatment (i.e., withdraw from the study drug only), efforts will be made to continue to obtain follow-up data by encouraging participants to attend the follow-up visits.

Participants who wish to withdraw from trial medication (IMP) only will be asked to confirm whether they are still willing to provide the following:

- trial-specific data at follow-up visits including safety and adverse event monitoring.
- data collected as per routine clinical practice at visits.
- Withdrawn participants will not be replaced.

7.8 Expected Duration of Trial

Recruitment will be carried out for approximately 18-months and follow-up will be carried out for at least 2-years, and in the last 6-months, we plan to analyse, report, and disseminate. At the end of one year after the first site being opened to recruitment, we will assess recruitment rates and the feasibility of continuing the PROMISE trial. Each individual participant will remain on trial for 24 months from the date of treatment.

7.9 End of trial definition

The end of trial will be deemed to occur after database lock following completion of monitoring of the last patient last visit undergoing the PROMISE trial.

8 Trial Procedures

8.1 By Visit:

8.1.1 Screening Visit (Day -28 to Day 0):

During this visit, participants will sign the informed consent form, having read the PIS and having had sufficient time to discuss this with their family, friends and clinicians. Eligibility screening of the participants will be carried out by a research nurse and confirmed by a delegated medical investigator or a sub-investigator at this visit. Screening may be done on the same day as baseline or prior to baseline up to Day -28. When screening and baseline visit are performed on the same day, the Urinary b-HCG test (female of childbearing potential) will only need to be performed for eligibility confirmation.

8.1.2 Baseline Visit (Day -7 to Day 0):

Adverse events and concomitant medications will be recorded and a physical examination (including height, weight, blood pressure, heart rate, temperature and oxygen saturation) will be performed to ensure that the participant is clinically stable. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the database. Medical history of liver cirrhosis, diabetes and current or past history of psychiatric disorders will be recorded. Body Mass Index (BMI) will be calculated. A pregnancy test (urine) will be performed for all females of childbearing potential.

Bloods will be taken for haematology (full blood count), clotting (INR), biochemistry (sodium, potassium, urea, creatinine, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma-glutamyl transferase), glucose and C-reactive protein. A serum sample will also be collected and stored.

Blood will also be taken for HbA1c (for participants with diabetes only) and a lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) for patients with MASLD or MASLD-ALD Overlap cirrhosis only. Liver disease severity score (MELD, Child Pugh, UKELD) will be measured at this timepoint. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

Additional samples of blood, urine and stool will be obtained as per Trial Sampling Schedule of Events (**refer 8.2.2**). We must have a baseline blood, stool and urine samples before randomisation (Participants will be provided with a urine and stool collection pot to take home, once participant has been introduced to the study information. Participants may collect the urine and stool sample at home and bring them to their next visit, if this is easier for them. The sample will only be collected by the site team once the trial participant has consented to the trial.) The sample should be produced preferably within 24 hours but must be within 48 hours of their baseline visit.

A baseline dietary questionnaire quality of life questionnaire (EQ-5D-5L) [38] and Hospital Anxiety and Depression Scale (HADS) questionnaire will also be completed. For those participants with ALD or MASLD-ALD Overlap cirrhosis, an AUDIT questionnaire will be completed. [33] If an urinary ethyl glucuronide/ethyl sulphate test has been performed as part of the standard of care, the result will be recorded in the CRF.

8.1.3 Randomisation (Day 0):

The formal inclusion and exclusion criteria will be verified, and the participant entered into the randomisation system to generate the treatment allocation. The IMP will then be dispensed following randomisation. If the investigator deems the participant fit to go ahead, the IMP will be administered to the participant and the number of capsules successfully ingested will be recorded.

Randomisation should happen once the trial participant arrives at the clinic for IMP administration. Where a participant cannot be treated on the same day, they can receive treatment up to 24 hours post randomisation. However, if randomisation cannot happen within 7 days of baseline visit, to ensure the patient meets the eligibility criteria, they will need to be rescreened and reassessed prior to randomisation as per Schedule of Events.

8.1.4 Day 30 (+/- 7 days) Telephone Visit:

Trial participants will receive a telephone call from the research nurse to check for adverse events and to record concomitant medications.

8.1.5 Month 3 (+/- 14 days) Visit:

Bloods will be taken for haematology (full blood count), clotting (INR), biochemistry (sodium, potassium, urea, creatinine, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma-glutamyl transferase), glucose and C-reactive protein.

Blood will also be taken for HbA1c (for participants with diabetes only) and a lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) for participants with MASLD or MASLD-ALD Overlap Cirrhosis only. Liver disease severity score (MELD, Child Pugh, UKELD) will be measured at this timepoint. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

Additional samples of blood, urine and stool will be obtained as per Trial Sampling Schedule of Events **(refer 8.2.2).** We must have a baseline blood, stool and urine samples before the FMT is administered (participants may collect urine and stool samples at home and bring them to their appointment if this is easier for them). The sample should be produced within 48 hours before the visit.

A quality of life questionnaire (EQ-5D-5L)[38], Hospital Anxiety and Depression Scale (HADS) questionnaire will also be completed and for those participants with ALD or MASLD-ALD Overlap cirrhosis, an AUDIT questionnaire will be completed. [33] If an

urinary ethyl glucuronide/ethyl sulphate test has been performed as part of the standard of care, the result will be recorded in the CRF.

Adverse events and concomitant medications will be recorded and a physical examination (including weight, blood pressure, heart rate, temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database. If the investigator deems the participant fit to go ahead, the IMP will be administered to the participant and the number of capsules successfully ingested will be recorded. These will be repeated for all subsequent visits *i.e.,* at 6, 9, 12, 15, 18, 21 and 24 months.

8.1.6 Month 6 (+/- 14 days) Visit:

Bloods will be taken for haematology (full blood count), clotting (INR), biochemistry (sodium, potassium, urea, creatinine, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma-glutamyl transferase), glucose and C-reactive protein.

Blood will also be taken for HbA1c (for participants with diabetes only) and a lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) for participants with MASLD or MASLD-ALD Overlap cirrhosis only. Liver disease severity score (MELD, Child Pugh, UKELD) will be measured at this timepoint. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

Additional samples of blood, urine and stool will be obtained as per Trial Sampling Schedule of Events **(refer 8.2.2)**. We must have a blood, stool and urine samples before the FMT is administered (participants may collect urine and stool samples at home and bring them to their appointment if this is easier for them). The sample should be produced preferably within 24 hours but must be within 48 hours of the visit.

A quality of life questionnaire (EQ-5D-5L), [38] Hospital Anxiety and Depression Scale (HADS) questionnaire will also be completed and for those participants with alcohol-related or MASLD-ALD Overlap cirrhosis, an AUDIT questionnaire will be completed. [33] If an urinary ethyl glucuronide/ethyl sulphate test has been performed as part of the standard of care, the result will be recorded in the CRF.

Adverse events and concomitant medications will be recorded and a physical examination (including weight, blood pressure, heart rate, temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database. If the investigator deems the participant fit to go ahead, the IMP will be administered to the participant and the number of capsules successfully ingested will be recorded.

8.1.7 Month 9 (+/- 14 days) Visit:

Adverse events and concomitant medications will be recorded and a physical examination (including weight, blood pressure, heart rate temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database. If the investigator deems the participant fit to go ahead, the IMP will be administered to the participant and the number of capsules successfully ingested will be recorded.

8.1.8 Month 12 (+/- 14 days) Visit:

Bloods will be taken for haematology (full blood count), clotting (INR), biochemistry (sodium, potassium, urea, creatinine, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma glutamyl-transferase), glucose and C-reactive protein.

Blood will also be taken for HbA1c (for participants with diabetes only) and a lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) for participants with MASLD or MASLD-ALD Overlap Cirrhosis only. Liver disease severity score (MELD, Child Pugh, UKELD) will be measured at this timepoint. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

Additional samples of blood, urine and stool will be obtained as per Trial Sampling Schedule of Events **(refer 8.2.2)**. We must have a blood, stool and urine samples before the FMT is administered (participants may collect urine and stool samples at home and bring them to their appointment if this is easier for them). The sample should be produced preferably within 24 hours but must be within 48 hours of the visit.

A quality of life questionnaire (EQ-5D-5L) [38] Hospital Anxiety and Depression Scale (HADS) questionnaire will also be completed and for those participants with alcohol-related or MASLD-ALD Overlap cirrhosis, an AUDIT questionnaire will be completed. [33] If an urinary ethyl glucuronide/ethyl sulphate test has been performed as part of the standard of care, the result will be recorded in the CRF.

Adverse events and concomitant medications will be recorded and a physical examination (including weight, blood pressure, heart rate, temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database. If the investigator deems the participant fit to go ahead, the IMP will be administered to the participant and the number of capsules successfully ingested will be recorded.

8.1.9 Month 15 (+/- 14 days) Visit:

Adverse events and concomitant medications will be recorded and a physical examination (including weight, blood pressure heart rate, temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database. If the investigator deems the participant fit to go ahead, the IMP will be administered to the participant and the number of capsules successfully ingested will be recorded.

8.1.10 Month 18 (+/- 14 days) Visit:

Bloods will be taken for haematology (full blood count), clotting (INR), biochemistry (sodium, potassium, urea, creatinine, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma-glutamyl transferase), glucose and C-reactive protein.

Blood will also be taken for HbA1c (for participants with diabetes only) and a lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) for participants with MASLD or MASLD-ALD Overlap Cirrhosis only. Liver disease severity score (MELD, Child Pugh, UKELD) will be measured at this timepoint. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

Adverse events and concomitant medications will be recorded and a physical examination (including weight, blood pressure, heart rate, temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database. If the investigator deems the participant fit to go ahead, the IMP will be administered to the patient and the number of capsules successfully ingested will be recorded.

8.1.11 Month 21 (+/- 14 days) Visit:

Adverse events and concomitant medications will be recorded and a physical examination (including weight, blood pressure heart rate, temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database. If the investigator deems the particpant fit to go ahead, the IMP will be administered to the participant and the number of capsules successfully ingested will be recorded.

8.1.12 First hospitalisation for infection (primary end point)

Site Principal Investigators will determine if participant have met the primary end point *i.e.,* the date participant was admitted to the hospital for infection as defined in **Section 5.2**. If this is not clear, they will defer to the Chief Investigator for a final opinion. Patients will then be reviewed for adverse event monitoring and concomitant medications recorded. A physical examination (including weight, blood pressure, heart rate, temperature and oxygen saturation) will be performed or the findings recorded from the medical records if the participant is admitted to a hospital other than their recruiting trial centre.

Results of the standard of care blood tests taken on admission will be documented in the CRF including haematology (full blood count), clotting (INR), biochemistry (sodium, potassium, urea, creatinine, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma-glutamyl transferase), glucose and C-reactive protein. Blood, urine, stool, sputum, skin and/or ascitic culture results, as applicable, will be documented in the CRF along with the report of a chest radiograph if performed. Liver disease severity score (MELD, Child Pugh, UKELD) will be measured at this timepoint. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

For those participants with alcohol-related or MASLD-ALD cirrhosis, an AUDIT questionnaire will be completed which may be performed up to 14-days following discharge. This can be undertaken over the telephone if the admission is to a hospital other than the recruiting hospital. [34] If an urinary ethyl glucuronide/ethyl sulphate test has been performed as part of the standard of care, the result will be recorded in the CRF.

8.1.13 Month 24 (+/- 14 days) Visit:

Trial participants will attend for one final visit for adverse event monitoring and recording of concomitant medications. A physical examination (including weight, blood pressure heart rate, temperature and oxygen saturation) will be performed. Any abnormal vital signs data, which is deemed clinically significant by the investigator, taking into account medical history and concomitant medications, will be reported as an Adverse Event (AE) and recorded on the Adverse Event Log. The non-clinically significant vital signs data will not be recorded on the database.

Bloods will be taken for haematology (full blood count), clotting (INR), biochemistry (sodium, potassium, urea, creatinine, albumin, bilirubin, aspartate transaminase, alanine transaminase, alkaline phosphatase and gamma-glutamyl transferase), glucose and C-reactive protein.

Blood will also be taken for HbA1c (for participants with diabetes only) and a lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) for participants with MASLD or MASLD-ALD Overlap cirrhosis only. Liver disease severity score (MELD, Child Pugh, UKELD) will be measured at this timepoint. It is recommended to use the MELD Na (UNOS/OPTN) Score Calculator from MD Calc, linked here: MELD Na (UNOS/OPTN) (mdcalc.com).

A final quality of life questionnaire (EQ-5D-5L) [38], Hospital Anxiety and Depression Scale (HADS) questionnaire will also be completed and for those participants with alcohol-related or MASLD-ALD Overlap cirrhosis, an AUDIT questionnaire will be

completed. [33] If an urinary ethyl glucuronide/ethyl sulphate test has been performed as part of standard of care, the result will be recorded in the CRF.

8.2 Laboratory Tests

8.2.1 Standard of Care:

The following routine blood tests will be performed at baseline (day 0) and at 3, 6, 12 and 18 and 24-months. These will form part of the safety assessment and adverse event monitoring and are anticipated to be taken as part of routine clinical care assuming participants with cirrhosis will be seen at least every 6-months in the outpatient clinic. These tests will be performed by the local laboratory.

- Full Blood Count (FBC) – to include haemoglobin, neutrophils, platelets, WBC (lymphocytes) and monocytes.
- International Normalised Ratio (INR)
- Sodium
- Potassium
- Urea
- Creatinine
- Albumin
- Aspartate transaminase (AST)
- Alanine transaminase (ALT)
- Alkaline phosphatase (ALP)
- Gamma-glutamyl transferase (GGT)
- Glucose
- C-Reactive Protein (CRP)
- Bilirubin
- * HbA1c
- ** Lipid profile (Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides)
- ***Urinary ethyl glucuronide/ethyl sulphate test if tested locally as part of standard of care routine.

*For participants with diabetes only

**For participants with MASLD or MASLD-ALD Overlap Cirrhosis only

***For participants with ALD or MASLD-ALD Overlap Cirrhosis only

8.2.2 Study-Specific Tests:

Centres will be supplied visit-specific blood, urine and faeces collection kits with detailed instructions on collection, processing, storage and shipping. Blood will be collected in the supplied BD vacutainer tubes, centrifuged within 1-hour and stored/biobanked at -80 °C until shipping to the central laboratory at the James Black Centre, King's College London. Urine and stool samples will be collected in sterile universal containers and stored at -80 °C until shipping to the central laboratory at the James Black Centre, King's College London. Frequency for the collection of the samples will be decided by the Trial Manager after liaising with individual sites and will be dependent on the number of participants recruited per month per site. At the end of the trial, blood and surplus samples collected will be stored at the King's Liver Research Tissue Bank for use in future research programmes..

Table 3: Summary of the study specific tests

Study-specific laboratory analyses	**Baseline**	**Month 3 (**+/- 14 days)	**Month 6** (+/- 14 days)	**1 year** (+/- 14 days)
Blood* (EDTA purple) 9mL x2 for bacterial DNA quantification and immunological marker assays	X	X	X	X
Blood* (Clotted Gold) 5mL x1 for storage	X	X	X	X
Blood* (Clotted red 9mL x2 for metabonomic profiling (serum) and bile acid profiling (LCMS)	X	X	X	X
London sites: Imperial, St. George's and King's College Hospital Blood* (Lithium heparinised green 9mL x8, for PBMC, monocyte phenotype and function assays and metabonomics (plasma) **Non-London sites** Blood* (Lithium heparinised green 9mL x1, for metabonomics (plasma))	X	X	X	X
Urine for metabolic profiling (20 mL sterile universal container) (metabonomic analysis with LCMS)	X	X	X	X
Faeces for biomarker, metabonomic, proteomic and cytokine analysis (20 mL sterile stool collection container)	X	X	X	X

Faeces for metagenomic sequencing (20 mL sterile stool collection container) (microbiome composition including mycome and virome diversity, pathogenic species of interest and resistome)	X	X	X	X

*To screen, we will look at SoC bloods no longer than 3 months. If it is older than 3 months, it will need to be updated.

9 Assessment of Efficacy

9.1 Efficacy Parameters

The following efficacy parameters will be recorded in the eCRF at the scheduled visits as per **Table 2**:

9.1.1 Primary Efficacy Parameters

To evaluate the efficacy of encapsulated FMT **to reduce the susceptibility of infection** in participants with cirrhosis measured by **the time to first infection resulting in hospitalisation (primary endpoint)**.

9.1.2 Secondary Efficacy Parameters

- To evaluate the efficacy of encapsulated FMT to reduce **decompensating cirrhotic events**: (i) acute variceal bleeding, (ii) new onset ascites, (iii) spontaneous bacterial peritonitis, (iv) hepatic encephalopathy and (v) development of acute-on-chronic liver failure (the development of one or more organ failures). [33]

- To evaluate the impact of encapsulated FMT on **all-cause hospitalisation** documenting **(i) length of stay** and **(ii) requirement for high dependency and intensive care.**

- To assess the impact of encapsulated FMT on (i) **antibiotic usage episodes** and (ii) **development of antimicrobial resistance** as measured by colonisation with methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant *Enterococci* (VRE), extended spectrum beta-lactamase producing bacteria (ESBL), linezolid-resistant *Enterococci* (LRE) and fluoroquinolone-resistant Gram negative and carbapenem-resistant Enterobacterales (CRE). [8]

- Change in **liver disease severity scores** (Child Pugh, [39] MELD [28] and UKELD [40]).

- **All-cause** and **liver-related mortality**.

- Change in **quality of life** (EQ-5D-5L questionnaire).

- Change in HADS score.

- Change in the **alcohol-use disorders identification test (AUDIT score)** [33] and **urinary ethyl glucuronide/ethyl sulphate levels** recorded if tested as part of the standard of care in participants with alcohol-related or MASLD-ALD Overlap cirrhosis.

10 Assessment of Safety

10.1 Specification, Timing and Recording of Safety Parameters

The following safety parameters will be recorded in the eCRF at the scheduled visits as per **Table 2:**

- Adverse events (as detailed in 10.2)
- Episodes of infection not requiring hospital admission
- Antibiotic usage (number of episodes)
- Nausea for >72 hours following IMP administration.
- Abdominal pain or discomfort for >72 hours following IMP administration.
- Diarrhoea >7 days following IMP administration.

**Diarrhoea is defined in this study according to the World Health Organisation as the passage of three or more loose or liquid stools per day (or more frequent passage than is normal for the individual). Frequent passing of formed stools is not diarrhoea. If participants are taking lactulose or other aperients, these must have been stopped for 72-hours before diarrhoea can be diagnosed.*

The following liver-related events, if leading to hospitalisation, will require expedited reporting to KHP-CTO:

- Liver disease progression
- Any known complication of cirrhosis including variceal bleeding, new-onset ascites, hepatic encephalopathy, spontaneous bacterial peritonitis, hepatorenal syndrome, acute-on-chronic liver failure and sepsis.
- Recurrence of ascites or jaundice
- Hepatocellular carcinoma
- Death as a result of a known complication of cirrhosis or disease progression.

10.2 Procedures for Recording and Reporting Adverse Events

Any Adverse Event (AE) or Serious Adverse Event (SAE) will be reported from the time of consent.

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

1. **Adverse Event (AE)**: Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.

2. **Adverse Reaction (AR):** Any untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant.

3. **Unexpected Adverse Reaction (UAR)**: An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in the Investigator's Brochure (IB).

4. **Serious Adverse Event (SAE), Serious Adverse Reaction (SAR) or Suspected Unexpected Serious Adverse Reaction (SUSAR):** Any adverse event, adverse reaction or unexpected adverse reaction, respectively, 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.

5. **Important Medical Events (IME) & Pregnancy: Consideration for Liver Transplantation will be deemed as an IME and will require expedited reporting to KHP-CTO.** Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious. Although not a serious adverse event, any unplanned pregnancy will also be reported *via* the SAE reporting system.

Reporting Responsibilities

King's College Hospital NHS Foundation Trust and King's College London have delegated the delivery of the Sponsor's responsibility for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004 to the King's Health Partners Clinical Trials Office (KHP-CTO).

All SAEs, SARs and SUSARs (excepting those specified in this protocol as not requiring reporting) will be reported immediately (and certainly no later than 24 hours) by the Investigator to the KHP-CTO and Chief Investigator for review in accordance with the current Pharmacovigilance Policy. The KHP-CTO will make the expectedness assessment against the Reference Safety Information section of each Investigational Medicinal Product. If the sponsor or the CI does not agree with the investigator's causality assessment the opinion of both the investigator and the Sponsor will be provided with the report. The adverse event log (AEL) will be reviewed at each trial assessment clinic visit and at follow-ups and following any occurrences in between study visits and follow-ups.

Death as a result of disease progression and other events that are primary or secondary outcome measures are not considered to be SAEs and should be reported in the normal way, on the appropriate CRF.

On a scheduled basis, the Data Monitoring Committee (DMC) will review adverse event information. The KHP-CTO will report SUSARs to the regulatory authorities (MHRA, competent authorities of other EEA (European Economic Area) states in which the trial is taking place.

The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs, which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction. The Chief Investigator and KHP-CTO (on behalf of the co-sponsors) will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually.

10.3 Premature Termination of the Trial

The PROMISE trial may be prematurely discontinued by the Sponsor, Chief Investigator or Regulatory Authority on the basis of new safety information or for other reasons given by the Data Monitoring and Ethics Committee, Trial Steering Committee, Regulatory Authority or Ethics Committee concerned.

If the trial is prematurely discontinued, active participants will be informed, and no further participant data will be collected. The Competent Authority and Research Ethics Committee will be informed within 15 days of the early termination of the trial.

11 Statistics

A Statistical Analysis Plan (SAP) will be drafted during the trial and will be finalised prior to the trial statistician becoming partially blinded (able to view participants and outcome partitioned and coded as A/B). The SAP will cover all of the trial analysis details.

11.1 Sample Size

Moreau *et al*. [41] performed a double-blind trial of the long-term fluoroquinolone antibiotic norfloxacin *versus* placebo in 291 patients with advanced cirrhosis (Child Pugh C) who had not received recent fluoroquinolone therapy. The study was performed at 18 clinical sites in France. Patients were randomly assigned to groups given 400mg norfloxacin (n=144) or placebo (n=147) once daily for 6 months. The primary outcome was 6-month mortality, as estimated by the Kaplan-Meier method. The Kaplan-Meier estimate for 6-month mortality was 14.8% for patients receiving norfloxacin and 19.7% for patients receiving placebo (p=0.21). In competing risk analysis that took liver transplantation into account, the cumulative incidence of death at 6-months was significantly lower in the norfloxacin group than in the placebo group (sub-distribution hazard ratio, 0.59; 95% confidence interval, 0.35-0.99). Norfloxacin significantly decreased the incidence of any and Gram-negative bacterial infections without increasing infections caused by *Clostridioides difficile* or multidrug-resistant bacteria. We have estimated FMT to be at least as effective as norfloxacin to reduce infection (hazard ratio = 0.625). [44]

We have assumed that 65% of patients in the control group experience an infection resulting in hospitalisation within 2-years and that this is reduced to 48.9% in the FMT arm. In order to detect this hazard ratio of 0.625, with 80% power (alpha = 0.047) we would need to analyse 270 patients (to experience 155 events). In order to account for a 10% loss to follow up, we will inflate to 300 patients (1:1 ratio). Note: 203 events would provide 90% power to detect this

effect, and 261 events would provide 80% power to detect a HR = 0.70. Using a group sequential design, an interim analysis will be carried out after 81 events (alpha = 0.006).

11.2 Recruitment

Trial recruitment will be monitored closely by the Trial Manager and reported at the TMG meetings. Anonymised participant pre-screening logs will be requested by sites and assessed on an ongoing basis. Section 4.4 outlines how participants will be identified for inclusion in the trial.

Additionally, the KCTU Standard Operating Procedures (SOPs) will guide the trial statistician's reports outlining recruitment numbers across the trial and at site level to the DMC. The DMC will be consulted to advise on potential strategies where there are recruitment difficulties *i.e.,* including but not limited to; modifications to the inclusion/exclusion criteria, targeted recruitment drives, escalation at site level to PI/R&D, opening additional study centres or site closure.

11.3 Analysis

Primary Outcome: The number of days from randomisation to first infection [32] leading to hospital admission.

Assessment of efficacy:

The main analysis will include all randomized participants (eligibility as defined in section 7) in the arms to which they were randomized, regardless of what treatment they received post-randomisation, with the intent to estimate an intention-to-treat (ITT) type estimand. For the primary analysis, a treatment policy strategy will be used for any intercurrent events (such as discontinuation of treatment or death), that is the occurrence of the intercurrent event will be considered irrelevant in defining the treatment effect of interest. Sensitivity analyses will examine this assumption using different strategies for the intercurrent events. Population definitions, target estimands and handling of intercurrent events will be further described in the Statistical Analysis Plan.

Primary analysis: A Cox baseline proportional hazards model of the time to infection will be fitted with frailty effect to account for site heterogeneity, adjusted for baseline disease severity, participant age, sex and baseline cirrhosis severity. The adjusted hazard ratio will be presented with associated 95% confidence interval. Proportionality will be assessed using a Kaplan Meier survival and log-log plots.

Secondary analysis:

Time to event outcomes including mortality (all-cause; liver-related), hospitalisation, progression to ACLF [33] and hepatic decompensation will be analysed in a manner consistent with the primary analysis.

Binary outcomes (infection rates, AMR incidence and antibiotic usage) will be analysed with a mixed-effects multivariable logistic regression, fitting a random effect for site, and fixed effects for: participant age, sex, baseline disease severity.

Continuous outcomes (liver disease severity scores; AUDIT score; HADS score; EQ-5D-5L score) will be analysed using a mixed effects multivariable regression, adjusted in a consistent manner with the primary outcome analysis including baseline version of the outcome if applicable.

Missing data and population under investigation:

Missing data will be quantified and explored for structure and breaches to the assumption of missing at random. If missing outcome data is greater than 5%, predictors of missing outcomes will be explored and if post-randomisation variables predict missingness, multiple imputation considered.[45] The primary population will include all randomised participants regardless of subsequent events (an "intention to treat" population). A sensitivity analysis of the primary outcome will be carried out to estimate the treatment effect for those participants who complied with the intervention (a "per-protocol" type population), by estimating the complier average causal effect. [46]

Subgroup analyses:

The following subgroups will be used to explore for moderating effect: sex; participant age; baseline disease severity, socioeconomic status (using the index of multiple deprivation), and additional factors if determined as important by the Trial Steering Committee. [45]

Adherence: Participant adherence will be determined by participants taking >70% of their allocated intervention.

11.4 Formal Interim Analysis and Stopping rules

Formal interim analysis for efficacy and futility:

Using an asymmetric two-sided non-binding two-stage group sequential design with 90% power and 2.5% type-1 error, we will apply a lower bound cut-off rule to conclude futility and upper bound cut-off to determine superiority. After a minimum of 81 events, stage 1 will conclude futility if $Z<0.41$ (nominal p-value > 0.6599), favouring FMT, or superiority if $Z>2.75$ (H0: Reject, nominal $p<0.006$) favouring FMT. Applying type 1-error spending using HwangShih-DeCani and Gamma = -4 (one-sided, stage one spent error =0.003). Using this alpha spending approach, at the final analysis, the null hypothesis for efficacy will be rejected if $p<0.047$.

11.5 Internal Pilot 'Go'/'No Go' progression

At the end of one year after the first site has opened for recruitment, we will assess the feasibility of the trial in an internal pilot. The criteria are shown below in the table in green.

At the end of one year after the first site has opened to recruitment, we will assess the recruitment rate and the feasibility of continuing the PROMISE Trial. We will progress to the full trial by meeting the following 'go/'no go' criteria, as indicated by meeting the green criteria: *<80% will result in being flagged as amber and less than 64% red.	*Red	Amber	Green
Open eight sites	<6	6 to 7	8 or more
Randomise 80 participants	<64	65-79	80 or more
Participant adherence (% of patients)	<64	65-79	80 or more

11.6 Understanding the Mechanisms of Action

We will carry out a mediation analysis to assess the indirect and direct effects of potential mediators. We will perform mediation analysis to investigate whether the effect of FMT on reduced infection rate is explained by a third variable/s (mediator/s) that could be also under influence of FMT. The mechanistic endpoints will be fitted as mediators (for example: bacterial translocation, faecal/plasma biomarkers, microbial diversity etc.). Each potential mediating variable will be entered separately in mediation analyses to convey the indirect influence of FMT on the infection rate. If any of the potential mediators are not affected by the FMT, then it will not be considered as a mediator. A multi-level mediation analysis will be conducted with the estimation of various quantities for mediation including average causal mediation effect (indirect effect), average direct effect, and total effect. Subgroups will be investigated as moderating effects.

12 Trial Steering Committee (TSC)

An independent Trial Steering Committee (TSC) will be chaired by Professor William Alazawi, an independent hepatologist who chaired the TSC of the safety and feasibility trial of FMT in cirrhosis (PROFIT Trial) which preceded this trial. The TSC will also include Dr David Gillespie, an independent senior statistician, independent microbiologist, joint co-sponsor representative(s), the trial patient advocate, patient/lay representative(s) from the British Liver Trust and clinicians with experience relevant to the trial. The TSC will meet typically between six monthly and annually throughout the period of the trial, and at other times deemed necessary by the chief investigator, the sponsor or by the EME board.

The TSC will provide the overall supervision of the trial. The TSC will be responsible for monitoring trial progress and conduct and advising the NIHR EME over implementation of trial stopping rules on grounds of safety or efficacy. The TSC will approve the PROMISE-TSC terms of reference.

13 Data Monitoring Committee (DMC)

A Data Monitoring and Ethics Committee (DMC) will meet prior (or combined) with the TSC. The DMC will be chaired by Professor Quentin Anstee, an independent hepatologist and include an independent microbiologist and an independent statistician. The DMC, in accordance with ICH GCP guidelines, is responsible for safeguarding the interests of trial participants, monitoring the main outcome measures including safety and efficacy (at interim), and monitoring the overall conduct of the trial. The DMC receives, on a periodical basis, unblinded data on trial recruitment, data quality and safety data. The DMC is required to assess emerging external evidence which might influence the ethical position of the trial. The committee is also expected to advise on any protocol modifications and whether the trial (or specific treatment groups) should be stopped early. The DMC will review recruitment and unblinded safety data per group allocated (and efficacy data at the interim analysis) and report to the TSC regarding any safety or ethical concern raised. The DMC will meet typically between six monthly and annually throughout the period of the trial, and at other times deemed necessary by the chief investigator, the sponsor or by the EME board.

The details of composition of the DMC and terms of reference will be set out in the Data Monitoring Committee charter, which the DMC will approve.

14 Trial Management Group (TMG)

The TMG will be responsible for the day-to-day management of the trial and will meet monthly to oversee the running of the trial. Chaired by the Chief Investigator, the membership will be composed of a chairperson, vice-chair, PROMISE Statisticians, PROMISE Trial Manager, PROMISE Trial Pharmacist and several of the PROMISE co-investigators. The TMG will be responsible for approval of the trial design, reviewing and advising on trial recruitment, reviewing the final results, approving publications and approval of secondary studies.

15 Direct Access to Source Data and Documents

The Investigator(s) will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (*e.g.,* participants' case sheets, blood test reports, X-ray reports, histology reports *etc.*).

16 Ethics & Regulatory Approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents have been submitted and reviewed by the Health Research Authority (HRA), Research Ethics Committee (REC) [East of England Cambridge South], and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation.

Subsequent protocol amendments will be submitted to the REC and Regulatory Authorities for approval. An annual progress report (APR) will be submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended. Pharmacovigilance reporting (*i.e.,* DSUR) will be submitted to MHRA annually on the date of the first anniversary of the first CTA approval.

The Chief Investigator will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the Sponsor) and the REC within the timelines defined in the Regulations. The KHPCTO or delegate will upload the final report to EudraCT on behalf of the Sponsor.

17 Quality Assurance

This trial will be conducted according to the KHP-CTO Standard Operating Procedures as mandated by the KHP-CTO Quality Policy and Collaboration Agreement. Where appropriate, KCTU system SOPs will be followed.

18 Data Handling

The Chief Investigator will act as custodian for the trial data under the General Data Protection Regulations Act 2018 (GDPR Act 2018). The following guidelines will be strictly adhered to:

- All participant data will be pseudo-anonymised.
- All pseudo-anonymised data will be stored on a password-protected computer.
- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act 2018 and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Kings Health Partners Clinical Trials Office Archiving SOP.

19 Data Management

A web based electronic data capture (EDC) system will be designed, using the InferMed Macro 4 system. The EDC will be created in collaboration with the trial analyst/s and the CI and maintained by the King's Clinical Trials Unit (KCTU) for the duration of the project. It will be hosted on a dedicated server within KCL.

The CI or delegate will request usernames and passwords from the KCTU. Database access will be strictly restricted through user-specific passwords to the authorised research team members. It is a legal requirement that passwords to the EDC are not shared, and that only those authorised to access the system are allowed to do so. If new staff members join the study, a user-specific username and password must be requested *via* the CI or delegate (*e.g.*, Trial Manager) from the KCTU team and a request for access to be revoked must be requested when staff members leave the project. Study site staff experiencing issues with system access or functionality should contact the CI or delegate (*e.g.*, Trial Manager) in the first instance.

Participant initials and date of birth will be entered on the EDC. NHS number, email addresses, participant names and addresses and full postcodes will not be entered into the EDC. No data will be entered onto the EDC system unless a participant has signed a consent form to participate in the trial. Source data will be entered by recruiting site staff, typically within 30

days of data collection by authorised staff onto the EDC by going to www.ctu.co.uk and clicking the link to access the MACRO 4 EDC system. A full audit trail of data entry and any subsequent changes to entered data will be automatically date and time-stamped, alongside information about the user making the entry/changes within the system.

The CI team will undertake appropriate reviews of the entered data where appropriate for the purpose of data cleaning and will request amendments as required. No data will be amended independently of the study site responsible for entering the data. Further details will be covered in a separate data management plan.

To ensure completeness of data, participants will be contacted *via* telephone if data is missing *e.g.,* questionnaires, blood results and all efforts will be made to ensure a full and complete record of data has been collected.

The investigator/centres will keep records of all participants (sufficient information to link records *e.g.,* Case Report Form (CRF), hospital records and samples), all original signed informed consent forms and copies of the CRF pages which will then be archived following the trial close out visit.

All data will be entered directly onto the CRF; the CRF would then be considered a source document. If the CRF is then transmitted to the sponsor, the trial site must retain a copy to ensure that the PI has an independent account from the sponsor as to what has occurred during the trial at his/her site.

At the end of the trial, the site PI will review all the data for each participant and provide electronic sign-off to verify that all the data is complete and correct. At this point, all data can be formally locked for analysis.

Upon request, the KCTU will provide a copy of the final exported dataset to the CI in .csv and Excel 2003 format and the CI will onward distribute as appropriate. Trial data will be entered into an electronic CRF database (InferMed MACRO) managed by the KCTU.

20 Publication Policy

The study findings will be disseminated by conventional academic routes, aiming for high impact open access peer-reviewed journal(s), and by involvement with patient support groups, wider media and NHS organisations to ensure a broad readership. In year 1 we will publish the trial protocol. At the end of the trial, the results will be published in one or more high impact open access journals and will include presentations at prominent gastroenterology and hepatology national and international conferences including British Association for the Study of the Liver (BASL), British Society of Gastroenterology (BSG), European Association for the Study of the Liver (EASL) and American Association for the Study of Liver Disease (AASLD) Annual Conferences. Authorship eligibility for publications will be based on the Chief Investigator's judgement on the individual's level of contribution in the design, conduct, and/or analysis of the trial.

We will ensure the research impacts on the management of patients with advanced cirrhosis and shapes policy and guideline development ensuring the data is included in relevant Cochrane reviews and that NICE, BSG and other bodies involved in the development of

national/international guidelines are aware when relevant. As a policy, written dissemination will be in a style that is understandable and useable for all stakeholders including NHS commissioners, clinicians, funding bodies, service users, patient support charities and the general public.

The British Liver Trust (BLT) will be central in leading the dissemination of the trial results and ensuring that the public and patients will be kept fully informed. They will also disseminate relevant findings through their website, newsletters and using social media platforms. At the end of the trial, we will hold a public webinar in conjunction with the BLT as an opportunity to disseminate the study findings and to bring everyone together to look at what was created and share learnings.

Dr Edwards is a scientific advisor to The House of Commons All-Party Parliamentary Group on Liver Health, and Prof Shawcross, Drs Goldenberg, Edwards, Patel and Mullish sit on the Gut Microbiota for Health Expert Panel. We will continue to utilise the links of the Policy Institute at King's College London who have worked with Prof Dame Sally Davies (outgoing Chief Medical Officer) for many years (she is a Visiting Professor and the UK Special Envoy on antimicrobial resistance). We will issue joint press releases coordinated between KCL, King's Health Partners, the NIHR and the British Liver Trust. I will also make myself available for press interviews to discuss study findings with interested media parties. The paradigm shift and the impact that this type of research could have on changing the way that we treat patients with CLD is immense and far-reaching.

To potentially be able to reduce the susceptibility and incidence of infection in this group of individuals that will lead to less prescription of antibiotics, reduced hospitalisations and incidence of antimicrobial resistance would be of huge societal benefit and offer huge cost savings to the NHS. If FMT is shown to have clinical utility and improves outcomes in patients with cirrhosis, this could be integrated into clinical guidelines within the decade and particularly lead to a reduction in the use of prophylactic antibiotics where antimicrobial resistance rapidly develops.

21 Insurance / Indemnity

The Co-Sponsors insurance and indemnity schemes apply. Indemnity is provided by the Clinical Negligence Scheme for the Trust and insurance is provided by King´s College London.

22 Financial Aspects

Funding to conduct the trial is provided by NIHR EME Reference Number: NIHR130730

This study (Grant Reference Number: NIHR130730) is funded by the Efficacy and Mechanism Evaluation (EME) programme, an MRC and NIHR partnership. This funding source had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit the results. The lead sponsor, KCL, will take primary responsibility for ensuring that the design of the study meets appropriate standards and that arrangements are in place to ensure appropriate conduct and reporting.

23 Archiving

At the end of this trial, all trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the 2018 Data Protection Act and archived in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Co-Sponsors Archiving Standard Operating Procedure (SOP).

24 Signatures

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