

Study Version: 3.0 30 MAY 2022
Short name: MBOP
IRAS: 255446

Full Title	Mechanism of beta-blockade on bacterial translocation in portal hypertension (MBOP) study.
Sponsor	King's College Hospital NHS Foundation Trust (KCH)
Funder	King's College Hospital Charity
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STUDY SYNOPSIS

Title of Study	Mechanism of beta-blockade on bacterial translocation in portal hypertension (MBOP) study.
Protocol Short Title/Acronym	Beta-blockers Or Placebo for Primary Prophylaxis of oesophageal varices (BOPPP) Trial
Sponsor name	King's College Hospital NHS Foundation Trust
Chief Scientific Investigator	Dr Mark McPhail
Eudract number	2018-002509-78
IRAS number	255446
ClinicalTrials.gov	NCT03776955
Medical condition or disease under investigation	Cirrhosis of the liver with small oesophageal varices which have not bled
Purpose of Study	To determine if Carvedilol reduces gut bacterial translocation in patients with cirrhosis preventing all cause decompensation
Primary objective	To characterise bacterial translocation and the microbial-immuno-metabolic response in patients with cirrhosis and small varices receiving carvedilol or placebo with the aim of demonstrating a reduction in bacterial translocation and inflammatory response due to carvedilol resulting in reduced all cause decompensation.
Study Design	Multicentre, blinded, randomised controlled trial (RCT) of NSBB (i.e. carvedilol) v placebo in patients with small oesophageal varices (OVs) will be recruited as part of the NIHR HTA funded BOPPP study. In a subset of this cohort whole blood, plasma, peripheral blood mononuclear cells and stool will be collected at baseline, 1, 2 and 3 years.
Endpoints	<p><u>PRIMARY ENDPOINTS</u></p> <ol style="list-style-type: none"> 1. All cause decompensation* (including liver related death) <p><u>SECONDARY ENDPOINTS</u></p> <ol style="list-style-type: none"> 1. Reduction in circulating bacterial DNA* 2. Reduction in gut permeability by D-lactate and FABP-2* 3. Reduction in inflammatory response by cytokine analysis* 4. Acute on chronic liver failure (ACLF)*

Study Version: 3.0 30 MAY 2022
Short name: MBOP
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	<p>5. Infection*</p> <p>6. Adverse events and Hospitalisations</p> <p>7. Quality of life</p> <p>*MBOP specific outcomes</p>
Sample Size	600 patients
Summary of eligibility criteria	Participants that have already consented to take part in BOPPP and randomised to receive IMP (as below)
BOPPP IMP, dosage and route of administration	Oral Carvedilol 6.25 mg to 12.5 mg OD. 12.5 mg can be taken as 6.25 mg BD if preferred by patient.
BOPPP Active comparator product(s)	Oral Placebo 1 to 2 tablets
Duration of Study	<p>Each patient will receive three years of trial medication as part of BOPPP. (Carvedilol, or Placebo). We anticipate a 24 month* (2 year) recruitment phase, and a projected further 39 months (3.25 year) of outcome assessment. BOPPP is a 69 month (5.75 years) trial.</p> <p>*COVID-19 dependent</p>
Version and date of final study protocol	3.0, 30 MAY 2022

Version Control

Version	Date of version	Reason for change
1.0	22 November 2019	NA - First version
2.0	18 June 2021	1) Updated Sponsor contact information 2) Updated expected duration of study 3) Addition of secondary outcome and secondary objective 4) Removal of dietary data requirements 5) Change in sample size for saliva and faecal samples
2.1	30 May 2022	1) Updated duration of study to align with BOPPP 2) Updated sample volumes

Contents

Study Synopsis	2
1. Background and Rationale	7
2. Study Objectives and Design	9
2.1 Study Objectives	9
2.2 Study Outcomes.....	10
2.2.1 Feasibility Outcomes:.....	10
2.2.2 Primary outcome and clinical endpoints.....	10
2.2.3 Secondary outcome	10
2.3 Study Design	10
2.4 Future Research	11
3. Selection and Withdrawal of Participants	11
3.1 Inclusion Criteria	11
3.2 Exclusion Criteria.....	11
3.3 Selection of Participants	11
3.4 Permanent Withdrawal from the Study.....	12
3.5 Expected Duration of Study	12
4. Study Visits and Procedures.....	12
4.1 Informed Consent	12
4.2 Randomisation	12
4.3 Study Visits.....	12
4.4 Sample Collection	13
4.4.1 Blood.....	13

4.4.2	Saliva	13
4.4.3	Stool.....	13
4.5	Splenic Stiffness	13
4.6	Associated data from BOPPP	13
5.	Statistics.....	14
5.1	Estimation of all cause decompensation rate	14
5.2	Sample size calculation	14
5.3	Sample size calculation sensitivity scenarios.....	14
5.3.1	Variations in placebo arm event rate	14
5.3.2	Reduced recruitment.....	14
5.3.3	Variations in Hazard Ratio (HR).....	14
5.4	Statistical analysis	14
5.4.1	Primary outcome	14
5.4.2	Secondary outcomes.....	15
5.4.3	Missing data and population under investigation.....	15
5.4.4	All cause decompensation rate.....	15
5.4.5	Adverse Event (AE), and Serious AE reporting	15
5.4.6	Causal Mediation Analysis (No mediation analysis is planned for BOPPP Trial).....	15
5.4.7	Moderators and Subgroups	16
5.4.8	Data Management	16
6.	Dissemination output and anticipated impact.....	16
7.	Ethics/Regulatory Approvals	17
8.	Study Oversight.....	17

Abbreviations

ACLF	Acute on chronic liver failure
BT	Bacterial Translocation
CSPH	Clinically Significant Portal Hypertension
GWAS	Genome Wide Association Studies
HRA	Health Research Authority
HTA	Human Tissue Authority
HVPG	Hepatic Venous Portal Gradient
ITT	intention to treat population
KCH	King's College Hospital NHS Foundation Trust
LPLV	Last Patient Last Visit
MBOP	Mechanism of beta-blockade on bacterial translocation in portal hypertension study
MELD	The model for end stage liver disease
MHRA	Medicines and Healthcare products Regulatory Agency
NO	Nitric Oxide
NSBB	Non Selective Beta-Blockers
OV	Oesophageal Varices
PCR	polymerase chain reaction
REC	Research Ethics Committee
SAP	Statistical Analysis Plan
SOPs	Standard Operating Procedures
TLR	Toll-Like Receptor
TNF- α	Tumor Necrosis Factor-alpha
VCTE	Vibration Controlled Transient Elastography
VH	Variceal Haemorrhage

1. Background and Rationale

Non selective beta-blockers (NSBB) are widely used to treat or prevent variceal haemorrhage (VH) but their use and underlying mechanism for preventing other decompensation phenotypes is disputed⁷. While reducing portal pressure is likely to prevent bleeding there are potential benefits linked to, or even independent, of the effect of portal pressure. These include reducing bacterial translocation (BT) from the gut into the portal system, which itself is associated with worsening liver failure and risk of sepsis-induced decompensation⁸. Whether this is related to modifications of the underlying gut bacterial populations or in reducing BT itself requires mechanistic exploration to optimise therapies. The deleterious effect of increased BT includes induction of pro-inflammatory states (which lead to vasoplegic shock) and repeated activation and subsequent exhaustion of the immune system such that future bacterial challenge results in hypo-reactive responses and increased susceptibility to infection or mortality⁹. While observational studies suggest these links exist the mechanistic role of beta-blockers in modulating these responses is poorly understood.

A recent study in patients with compensated cirrhosis and clinically significant portal hypertension (CSPH, with or without esophageal varices) demonstrated that beta-blockers were associated with a reduced incidence of all cause decompensation, particularly related to the development of ascites¹⁰. In this study CSPH was defined as a hepatic venous portal gradient (HVPG) of >10mmHg defined by invasive measurement. Patients with varices have CSPH by definition even if HVPG is not measured. The beneficial effect of NSBB was not necessarily associated with a reduction in portal pressure and so suggests other mechanisms may be more relevant. Also patients received propranolol or carvedilol and in those with small varices the hazard ratio for reducing the incidence of decompensation or death was 0.45 (0.2-0.98). The authors themselves note that carvedilol was better tolerated and the measurement of HVPG response could have been omitted. However, whether the effect of on all-cause decompensation is related to non-hemodynamic effects is not known.

Patients with cirrhosis, particularly during decompensation have higher levels of circulating bacterial DNA even in the absence of overt infection (known as 'sterile inflammation'¹¹). The *cause* of this is related to a combination of factors including an alteration in the gut microbial profile, an overgrowth of bacteria within the small intestine and increased gut permeability due to intestinal barrier dysfunction (in part driven by portal hypertension-related vascular shear stress), all leading to pathological BT from the gut¹². The *consequence* of excess bacterial DNA in the circulation is postulated to be persistent low-grade stimulation of the immune system *via* toll-like receptor (TLR) signaling, leading to eventual exhaustion of monocytes and hypo reactivity to further microbial challenge, most evident in the dysfunction in myeloid cell of the innate immune system. The result is increased expression of pro-regulatory proteins on monocytes and reduced secretion of pro-inflammatory cytokines. This could increase the risk of decompensation and severity when it occurs. Bacterial DNA measured by polymerase chain reaction (PCR) in both serum and ascites was increased in 1/3 of cirrhotic patients in the absence of overt

infection^{13 14}. Bacterial DNA was positively correlated with Tumor Necrosis Factor-alpha (TNF- α) and nitric oxide (NO)^{14 15}. Bacterial DNA has been associated with a higher risk of decompensation although this was outside the context of a prospective, randomized controlled trial. It is not known definitively if circulating bacterial DNA load can be decreased by NSBB.

Does the oral microbiota play a role in BT?

Oral microbiota – like gut microbiota - can have beneficial or pathogenic effects, with the balance dependent to an extent on natural defense mechanisms within the oral cavity³⁵. These effects can be localised within the oral cavity itself, or more systemic due to BT of microbes and/or their products and thus affecting other organ systems. Salivary dysbiosis has been increasingly linked to not only local tissue effects affecting the mouth but all also a multitude of systemic conditions, primarily due to BT³⁶. Periodontitis has for example been associated with an increased risk of diabetes mellitus³⁷, cardiovascular³⁸, and respiratory diseases³⁹, pregnancy-related complications⁴⁰, cancer⁴¹, and chronic liver disease⁴².

Oral dysbiosis is also linked to the pathogenesis of cirrhosis and the progression to advanced liver disease. Utilising quantitative metagenomics, it has been shown that 75,245 microbial genes differ in abundance between patients with cirrhosis and healthy individuals and, of relevance, that over 50% of these taxonomically assigned bacterial species are of buccal origin, suggesting an invasion of the gut by oral bacteria from the mouth in cirrhosis⁴³. Salivary dysbiosis, represented by a reduction in commensal bacteria, has been shown to occur in cirrhosis, but only partially reflects the changes observed in the fecal microbiota in cirrhosis, with associated systemic and salivary-specific inflammation⁴⁴. Accordingly, the oral-gut-liver axis in cirrhosis may impact important pathobiological functions⁴⁵, and understanding how NSBB therapy impacts upon these interrelated processes is key to better interrogating underlying mechanisms of disease and identifying potential therapeutic targets. However, given the difficulty in assigning causation it would be important to assess effects during a high quality randomized controlled trial. The MBOP study aims to address this unmet scientific need.

Are the beneficial effects of carvedilol mediated by portal pressure reduction?

The gold standard measurement of portal pressure was previously thought to be invasive measurements of the hepatic venous portal gradient. Here a wire is passed via the jugular vein into the hepatic vein (where a pressure measurement taken) and then the wire wedged in the venous sinusoids to determine the wedge pressure (as a proxy for portal pressure). The difference is the HVPG and determines risk of future complications of portal hypertension. This measurement is not only invasive but is not available in all centers. Furthermore, there is variability in measurement technique and even international experts accept that it is unlikely to be required in large interventional studies and is certainly not required longitudinally as these do not predict other beneficial effects of NSBB¹⁰. The optimal noninvasive proxy is splenic Fibroscan™ whereby acoustic elastic shear stress is

Study Version: 3.0 30 MAY 2022
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IRAS: 255446

quantified ultrasonography. It correlates strongly with HVPG and with subsequent portal hypertension related complications. Specific Fibroscan™ technology exists (EchoSens Fibroscan 630) to measure splenic stiffness and will be used in MBOP to obtain a measure of portal hypertension.

Rationale

BOPPP is an efficacy study and as such does not have the inbuilt capability to undertake biological sampling. The MBOP study will therefore leverage participants already recruited to the BOPPP Trial and receiving IMP and give them the opportunity to provide research focused biological samples to answer the hypothesis on the effect of beta-blockers on bacterial translocation.

MBOP has a different primary outcome to BOPPP and will provide new important scientific and mechanistic knowledge in its own right that could extend to other indications that beta-blockade is used for. There are also important future gains to accrue from obtaining such a large set of biological samples contemporaneously from the BOPPP cohort of participants that can be utilised for future research. Circulating bacterial DNA reduction will be the primary mechanistic outcome. Further comprehensive examination of blood and faecal signatures from patients using samples obtained over 3 years participation will provide unique additional evidence of mechanism of effect. We will collect details on lifestyle (alcohol/smoking/recreational drug use) to address potential confounders.

This opportunity is unlikely to be available again in the volume of patients with cirrhosis at an early stage of their disease where intervention is likely to alter the natural history and prevent progression. As the BOPPP Trial has started it is important to start bio-fluid collection early to maximise the scientific gain of this study.

2. Study Objectives and Design

2.1 Study Objectives

This study will address the question whether primary prophylaxis with a NSBB, namely carvedilol, is mediated by reduced rates of BT in patients with cirrhosis and small varices, and if this reduces all-cause decompensation. By measuring bacterial DNA, markers of gut permeability, phenotyping the subsequent immune response and gut microbiome the underlying mechanism of the benefit of beta-blockade will be characterised.

Secondary benefits include collection of samples for future research in a highly characterised cohort where more detailed immune, metabolic and microbial phenotyping can be analysed in the future. In the future a more wide ranging microbial, metabolic and immune characterisation is possible but this will require funding via another source, alongside a long term follow up using routine data.

Specific objectives are:

1. To determine it is feasible to undertake the MBOP study.
2. To determine the clinical effectiveness of the reduction in all cause decompensation in patients treated with carvedilol versus placebo after 3 years.
3. To determine if circulating bacterial DNA levels are reduced by treatment with carvedilol.
4. To confirm that the gut microbiome itself is not modulated by carvedilol but that gut permeability itself is reduced.
5. To demonstrate that pro-inflammatory responses and monocyte phenotype and function are mediated with carvedilol *via* reduction in circulating bacterial DNA.
6. To evaluate the performance of spleen stiffness by VCTE to assess oesophageal varices at baseline as well as during treatment by carvedilol.

2.2 Study Outcomes

2.2.1 FEASIBILITY OUTCOMES:

Stage 1 will determine that MBOP is feasible against the following Go/No Go criteria at 1 year of initiation of MBOP: This will be analysed using King's College Hospital as the pilot site.

- 1) To have randomised > 33% of BOPPP patients
- 2) To have followed up > 70% of patients eligible for follow up

2.2.2 PRIMARY OUTCOME AND CLINICAL ENDPOINTS

- All cause decompensation as defined by any one of:
 - *Variceal haemorrhage
 - *New or worsening ascites
 - *New or worsening overt hepatic encephalopathy
 - *Increase in Child Pugh by one grade
 - *Increase in MELD score
 - *Liver-related death

2.2.3 SECONDARY OUTCOME

- Reduction in circulating bacterial DNA
- Reduction in gut permeability by D-lactate and FABP-2
- Reduction in inflammatory response by cytokine analysis
- Oesophageal varices grade by gastroscopy
- *Adverse Events (AE)
- *Serious AEs
 - Acute on chronic liver failure (ACLF)
 - Death (Liver-related, non-liver-related, all cause)
- *Infection
- *Quality of life

* obtained via analysis of BOPPP Trial data

2.3 Study Design

On the back of BOPPP, participants consenting to the MBOP study will provide blood, saliva and stool samples at baseline, 1, 2 and 3 years.

At King's College Hospital NHS Foundation Trust only:

- Spleen stiffness will be measured baseline, 1, 2 and 3 years.

2.4 Future Research

Biological samples will be stored within a biobank for future analysis, subsequent to funding from other sources during or after the lifetime of sample collection. These analyses are not central to the current hypothesis being tested. However, they represent a unique sample set for exploration of other metabolic and genetic determinants of decompensation in patients with cirrhosis. These other analyses will not add to the cost of this research study. The samples and attendant protocols for samples storage will allow the following techniques to be used in future:

- Metabonomics (lipids, biogenic amines, bile acids)
- Proteomics
- RNAseq
- Genomics/GWAS
- Faecal and salivary metagenomics
- More extensive circulatory and faecal/salivary cytokine multiplex profiling
- FACS/CytOF of PBMCs and cell subtypes

Given BOPPP will provide clinical and MBOP will provide mechanistic parameters and be linked to hospital admission statistics there is the future opportunity to use artificial intelligence (AI) and machine learning methods to find novel methods to predict decompensation. There is expertise within KCL to perform this task within a liver AI group (Dr Mark McPhail, Dr Zina Ibrahim) but this will be subject to future funding. Nevertheless, this is an important secondary gain of this project.

3. Selection and Withdrawal of Participants

3.1 Inclusion Criteria

- To have consented to the BOPPP trial and randomised to receive IMP.

3.2 Exclusion Criteria

- Inflammatory bowel disease.
- Previous or planned gastric surgery.

3.3 Selection of Participants

MBOP will recruit up to 600 BOPPP participants following REC / HRA, MHRA and local approval and Sponsor green light to approach BOPPP participants. Recruitment will be focused at KCH as part of the Stage 1 assessment. When further peer-reviewed funding is obtained, MBOP will be offered to all open BOPPP sites that have the relevant facilities to store the biological samples required for the study.

3.4 Permanent Withdrawal from the Study

Participants have the right to withdraw from MBOP at any time and stop providing biological samples and/or the Fibroscan measurement. This can be done at the same time as BOPPP withdrawal or separately as only withdrawing from MBOP.

3.5 Expected Duration of Study

As MBOP is dependent on recruitment to BOPPP, recruitment to MBOP will end when recruitment to the BOPPP trial ends.

4. Study Visits and Procedures

4.1 Informed Consent

An MBOP study specific Patient Information Sheet (PIS) will be supplied to participants that are interested and who's BOPPP-Baseline appointment has not taken place. Participants will have the opportunity to read the PIS and ask questions regarding the study. The risks and benefits will be written in the PIS and discussed at recruitment. Participants approached will already be eligible for MBOP. No additional screening is necessary. A separate MBOP consent form will be used.

4.2 Randomisation

Participants would have been already been randomised as part of the BOPPP trial so there is no additional randomisation as part of MBOP. The BOPPP randomisation sequence is generated by a King's College London Clinical Trials Unit online randomisation system using varying permuted blocks of size 4 and 8, within each site. The allocation sequence will be concealed from participants, clinicians, researchers, and analysts. As there is no control over which participants consent to BOPPP within randomisation block, there is a chance of unequal variance to active or inactive IMP. As such, an MBOP Data Monitoring Committee (DMC) will be unblinded to the treatment allocation for each MBOP participant to assess and provide advice on unequal distribution.

4.3 Study Visits

Prior to the BOPPP participant's baseline visit, the research nurse will confirm eligibility (no gastro-intestinal disorders / planned or past gastric surgeries). At the BOPPP baseline visit the participant will be consented to MBOP and the biological samples will be collected, along with the Fibroscan (KCH only). Subsequent biological samples and Fibroscans performed (KCH only) will be collected annually up to 3 years.

Trial procedures	Baseline	Month 12 (+/-) 6weeks	Month 24 (+/-) 6weeks	Month 36 (+/-) 6weeks	At trial completion Or decompensation
Informed consent	X				
Eligibility criteria	X				
Blood Sample*	X	X	X	X	
Saliva Sample*	X	X	X	X	
Stool Sample*	X	X	X	X	
Fibroscan**	X	X	X	X	
Outcome Recording		X	X	X	X

Table 2: Study visits and procedures. * Centres with experience in handling and storage of these bio-samples only. ** King's College Hospital only.

4.4 *Sample Collection*

To maximise internal consistency for the processing and subsequent measurements, samples will be shipped from sites for central processing at King's College Hospital. Samples will be storage at -80°C (or in liquid nitrogen for PBMC) will allow high quality retrieval of cells, RNA, DNA, plasma and serum for subsequent analysis.

A more detailed description of the technical requirements for MBOP will be provided in a Separate Laboratory Manual. A summary is provided below.

4.4.1 BLOOD

We will take approximately 65ml of blood per patient. This will be taken as:

- 4 ml EDTA tube whole blood for bacterial and host DNA quantification and profiling
- 6 ml plain serum tube for cytokine profiling and future metabolic profiling
- 2.5 ml PAXgene tube whole blood for RNAseq
- 36 - 52 ml lithium heparin for PBMC isolation and plasma aliquot storage

4.4.2 SALIVA

- 8 ml of saliva by passive drool

Saliva samples will be obtained following a one hour abstinence from eating, drinking or smoking. 8mLs of saliva will be obtained by unstimulated whole mouth passive drooling with the head tilted forward, into a standard clinical collection specimen container. All salivary samples will be divided into aliquots (minimal 1ml) without any additional storage medium or preservative and stored for future DNA isolation and molecular microbiological analysis at -80°C. This will be done after centrifugation to generate a pellet with the supernatant stored separately.

4.4.3 STOOL

- Two 20 ml universal container to obtain 20 g of faeces

Faecal samples will be obtained as close to the time of study visits as possible. Faecal samples have optimal preservation characteristics for molecular analysis when kept at room temperature and brought to the laboratory within 24 hours after collection and stored at -80°C until DNA extraction can be performed. Faecal samples will be divided into aliquots (~1-2grams) without any additional storage medium or preservative and stored for future DNA isolation and molecular microbiological analysis. It has previously been shown that the composition of the microbiota⁴⁶ and protease activity⁴⁷ is stable in faecal samples for up to 24hrs.

Future analysis will be conducted in Quadram Institute Bioscience, Norwich (BBSRC strategically funded research Institute focused on gut microbes and health) using fully anonymised samples.

4.5 *Splenic Stiffness*

Spleen stiffness will be measured by a specialist ultrasound technique on the Fibroscan™ 630 loaned by Echosens (Paris, France) and this will be performed at King's College Hospital only at annual intervals to track changes in portal pressure during the study in both arms.

4.6 *Associated data from BOPPP*

All of the data collected from MBOP will be analysed alongside decompensation data that is being collected at corresponding follow up intervals from the BOPPP Trial.

5. Statistics

5.1 *Estimation of all cause decompensation rate*

The PREDESCI study randomised 201 patients with no or small varices, and the rate of decompensation was 27% at 3 years in the placebo group. Given this included patients with no varices we are likely to see a higher rate in the BOPPP study. Therefore, we assume a rate of 30% at 3 years. In the PREDESCI study the HR for beta-blockers was 0.50 (95%CI 0.26 to 0.97). To allow a more conservative estimate of the treatment effect we assume a HR of 0.60 given that we will not be undertaking HVPG measurements (which may artificially select patients with higher subsequent event rates) but this rate and other parameters are explored by undertaking sample size sensitivity scenarios below.

5.2 *Sample size calculation*

Assuming a rate of all cause decompensation at 3 years of 30% and a HR of 0.60, with a power of 90% and alpha of 0.05 then a total sample size of 540 would be required. To account for dropout (loss to follow up or transplantation) 600 patients would be required and is the planned sample size for MBOP.

5.3 *Sample size calculation sensitivity scenarios*

5.3.1 VARIATIONS IN PLACEBO ARM EVENT RATE

We also performed a sensitivity analysis given the variation in event rate reported in Section 1. If the three-year control arm event rate in the study is 40%, the study will retain 90% power with 364 patients. If the three-year event rate is 25% we will retain 85% power with the 600 patients planned.

The MBOP study has a median follow up of 3.5 years, rather than 3 years, with the first patient having 4.5 years of follow up. This will inflate the number of decompensation events.

5.3.2 REDUCED RECRUITMENT

Should recruitment be more difficult than expected then assuming a 30% event rate in the placebo arm, and HR of 0.6 then at 400 patients the study will still retain 80% power (this is 1/3 of the total BOPPP population).

5.3.3 VARIATIONS IN HAZARD RATIO (HR)

If the HR is 0.5 (in keeping with the PREDESCI study) then the study will reach 90% power at 335 patients recruited. If the HR is 0.7 then the study will still have 75% power at 600 patients recruited.

5.4 *Statistical analysis*

Stage 1b: Determining the Feasibility of the MBOP Study. The feasibility outcomes will be summarised and compared to the MBOP Go/No Go criteria (See Table 3).

Stage 2: Evaluating the effectiveness of MBOP

The TSC will approve both the Statistical Analysis Plan, and Causal Mediation Analysis (MBOP)

5.4.1 PRIMARY OUTCOME

The time-till-decompensation for the two allocation groups be analysed using a multi-level Cox's proportional hazards model, and adjusted for: patient age, size of varices, and grade of OV, fitting hospital site as a random intercept. The assumption of proportionality will be visually assessed using a log-log plot and Kaplan Meier Plot, with an at-risk table for the two groups. We will report a hazard ratio, with associated 95% confidence interval and p-value.

5.4.2 SECONDARY OUTCOMES

Continuous data will be analysed using general linear mixed effects model, with random effects for patient, and adjusted for site, patient age, size of varices, use of long-term antibiotics (especially Rifaximin) and lactulose (which may impact D-lactate measurement). The mean difference between allocation group will be presented alongside 95% CI and p-value. Dichotomous outcomes will be analysed using a mixed effects logistic regression, similarly to the above.

5.4.3 MISSING DATA AND POPULATION UNDER INVESTIGATION

Missing covariate data will be explored for patterns of systematic missingness. If there are reasons for missingness, we will consider appropriate methods for imputation that are appropriate, given the reason. Routine data completeness checks will be presented to the DMC/TSC. Missing outcome data will be reviewed and patient notes throughout and checked at the end of study for decompensation, and mortality. The population will be analysed using a modified intention to treat analysis, including all patients that are randomised, receive the intervention, and provide at least one post baseline time-point. Population(s) under investigation

Primary/Secondary outcomes:

A modified intention to treat population (ITT) will include all patients in the primary outcome. Patients who experience any decompensation will be described as a treatment failure and event. Those who do not experience a decompensation within follow up will be recorded as a non-event and censored at the last time of follow up. A sensitivity analysis of the primary outcome will be carried out with the per-protocol population accounting for adherence.

Mortality will be analysed with a time-to-event analysis and using an intention to treat population (ITT; as per the primary outcome). An ITT principle will be used. Where a patient is determined a treatment failure as per the primary outcome

5.4.4 ALL CAUSE DECOMPENSATION RATE

Patients censored prior to 1 year will be imputed as not having an event.

5.4.5 ADVERSE EVENT (AE), AND SERIOUS AE REPORTING

These will be reported to the Sponsor, REC/HRA and MHRA following the process outlined in the BOPPP study.

5.4.6 CAUSAL MEDIATION ANALYSIS (NO MEDIATION ANALYSIS IS PLANNED FOR BOPPP TRIAL)

A key objective of MBOP is to gain insight of the impact of portal pressure (non-invasively assessed by splenic stiffness) and BT on the primary and secondary outcomes. The mechanism study will investigate the mediation process in this model and, through that, illuminate key basic knowledge about generalisation of the pathway. Some of the pathways of interest are illustrated in Figure. 1. If the efficacy analysis shows significant between group difference (direct effect), in which NSBB leading to a significant clinical improvement, then we will use semi-parametric Cox regression models to test for mediation of the intervention on primary symptom outcome decompensation through BT.

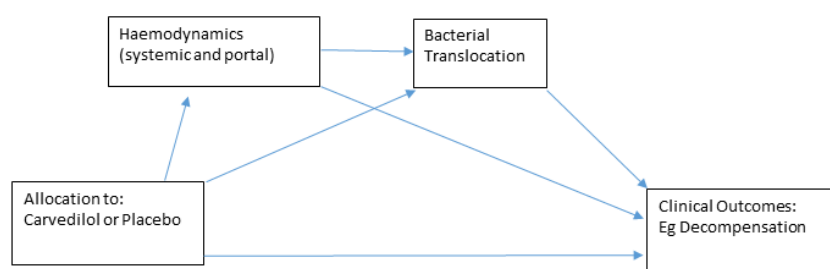


Figure 1: Mediation analysis

As part of the MBOP study, we will perform a mediation analysis to investigate whether the direct effect or the effect of Carvedilol vs Placebo on Decompensation measures (primary and secondary) is explained by a third hemodynamic (portal pressure) or Bacterial Translocation variables (mediator) that was also affected by treatment arm. The mediation model will consist both single and multiple mediator analyses. In the single model each mediator including each hemodynamic or each bacterial translocation variables will be entered separately in mediation analyses to convey the indirect influence of treatment arm on decompensation.

In the multiple mediation model; the linked mediators (hemodynamic variable and bacterial translocation variable) will be entered together in the mediation analysis to evaluate the indirect influence of treatment on decompensation, conditional to mediator-mediator association.

5.4.7 MODERATORS AND SUBGROUPS

We will test whether the mediation analysis is consistent across the age groups by testing for moderation by: age-group; aetiology of liver disease.

5.4.8 DATA MANAGEMENT

Monthly data queries will be generated and forwarded to site PIs to action. The MBOP Statistical Analysis Plan (SAP) will be written and approved following KCTU Standard Operating Procedures (SOPs). The study will be conducted and reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) statement.

6. Dissemination output and anticipated impact

This study will demonstrate the mechanism of effect of carvedilol in preventing decompensation in patients with cirrhosis, potentially extending its indications for use and preventing hospitalisation in patients with advanced liver disease. At present patients with medium or large varices are treated with beta-blockers but not patients with small varices. Furthermore, the proposed use of carvedilol in the BOPPP study is to prevent first variceal haemorrhage. By assessing a different primary outcome of all cause decompensation (favoured by expert consensus) means in the future many more patients may benefit from the use of this medication who at present are denied this due to lack of evidence. If this is demonstrated, then the potential to reduce hospitalisation by up to 1/3 in this patient group exists and to delay the need for subsequent liver transplantation (LT) as decompensation is a defining event to determine the potential requirement for LT. Furthermore, the quality of life of patients is likely to be greatly enhanced through reduced requirement for healthcare usage.

While a number of observational studies have assessed the role of bacterial translocation, gut microbial perturbation or innate immune dysfunction in patients with cirrhosis none have done so in the context of a large randomised controlled trial of a relevant agent. The deliverables will markedly increase our understanding

Study Version: 3.0 30 MAY 2022
Short name: MBOP
IRAS: 255446

of cirrhosis and its natural history. The secondary gains from having such a large biobank of samples will have significant potential for future research.

The findings will be disseminated to a wide range of patients, clinicians, scientists and the general public including; patients in the study, the British Liver Trust, the print, online and television media, twitter, national and international conferences and in high impact scientific journals. We aim to present pre-publication findings at the International Liver Congress and British Association for the Study of the Liver annual meetings. Open access fees will be covered by funding within King's College London.

7. Ethics/Regulatory Approvals

MBOP is an observational study of a double blind randomised controlled trial of an orally delivered IMP. There is considerable evidence of benefit of the IMP in similar populations and this study seeks to provide evidence to extend the indications to those with small OV but equipoise exists for this patient group. The MBOP study will not start without approvals from a research ethics committee (REC) and the Medicine and Healthcare Products Regulations Agency (MHRA).

8. Study Oversight

A Trial Management Group (TMG) will oversee the MBOP as part of managing the BOPPP Trial and will meet monthly. The TMG is responsible for approval of the Study design, reviewing and advising on recruitment, reviewing the final results. The BOPPP TMG is governed by an independent Trial Steering Committee (TSC) and Data Monitoring Committee (DMC) to provide further oversight.