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CARBALIVE

Clinical evaluation of nanoporous carbon adsorbent with tailored porosity (Yaq-001) as a new therapeutic for the treatment of liver cirrhosis and non-alcoholic fatty liver disease

**Grant agreement no. 634579**

**H2020 PHC-13-2014**

# D1.4 Submission for ethics approval of the first clinical trial

**Work Package Number**: 1

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**Actual submission date:** 31/01/2017

**Start date of project:** 01/05/2015 **End date of project**: 31/10/2019 **Duration:** 54

**Lead beneficiary for this deliverable:** *Alpha Bioresearch*
**Contributors:** *University College London*

| Project co-funded by the European Commission within the H2020 Programme (2014-2020) | | |
|---|---|---|
| **Dissemination Level** | | |
| **PU** | Public | |
| **CO** | Confidential, only for members of the consortium (including the Commission Services) | X |
| **CI** | Classified, as referred to in Commission Decision 2001/844/EC | |



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Grant Agreement No 634579"*

# Carbalive Safety Study Documentation

The CARBALIVE Safety Study documentation is now complete for submission to the Research Ethics Committee in the United Kingdom and Switzerland. The final version of the following clinical trials documents have been developed within the CARBALIVE consortium and follow several meetings of the CARBALIVE clinical trials committee. The Patient Information Sheet has also been developed within the consortium, and following consultation with the UK national patient body, the British Liver Trust.

Please find attached the final documents required for ethics l approval of the CARBALIVE safety study:

**Documentation**

- **Clinical Investigation Plan**
  - Clinical investigation plan synopsis
  - Scheduled of visits & procedures
  - Introduction, background and rationale
  - Risks and benefits of the investigational device and clinical investigation
  - Objectives and outcome measures
  - Investigational plan
  - Patient population
  - Investigational product
  - Study procedures by visit
  - Details of study procedures
  - Adverse events, serious adverse events: recording and reporting
  - Statistical methods
  - Obligations of the sponsor and the investigator

- **Patient Information Sheet**

- **Informed Consent**

- **Investigator's Brochure**
  - Introduction
  - Investigational Device Information
  - Nonclinical Studies
  - Clinical Data
  - Risk Management
  - Summary Of Data And Guidance For The Investigator

- **List of participating investigators / sites**

- **Clinical investigation plan synopsis**



*"This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Grant Agreement No 634579"*

**CLINICAL INVESTIGATION PLAN:**

**SAFETY AND TOLERABILITY OF YAQ-001 IN PATIENTS WITH CIRRHOSIS**

**Short name: CARBALIVE-SAFETY**

| | |
|---|---|
| **CIP Number:** | **Yaq001-S-001** |
| **Clinical phase:** | **First-in-human clinical investigation** |
| **Sponsor:** | **Yaqrit Ltd**<br>Interchange Triangle, 3rd floor,<br>Triangle building Stables Market,<br>Chalk Farm Rd, London, NW1 8AB, United Kingdom<br><br>Phone: +44 207 433 2795; Fax: +44 207 433 2775<br><br>CEO: **Daniel Green**<br>Medical Expert/ Project Coordinator: **Rajiv Jalan**<br>Medical Monitor: **Jane Macnaughtan** |
| **Lead Statistician:** | **Marco Pavesi**<br>EF-CLIF Consortium Data Centre<br>Travessera de Gracia, 11; 7th floor; 08021 Barcelona, Spain<br><br>Phone: +34 93 227 18 27 |
| **Sponsor contact (CRO):** | **Maria-Jose Estefano**<br>Alpha Bioresearch, S.L.<br>Aribau, 112 – 5 – 1; 08036 Barcelona, Spain<br><br>Phone: +34 93 323 34 72; Fax: +34 93 451 66 09 |

# SPONSOR SIGNATURE PAGE

Daniel Green
CEO, Yaqrit Ltd

Signature and date

_____

Rajiv Jalan
Medical Expert/ Project coordinator, Yaqrit Ltd

Signature and date

_____

Jane Macnaughtan
Medical monitor, Yaqrit Ltd

Signature and date

_____

Marco Pavesi
Lead Statistician, EF-CLIF

Signature and date

_____

Maria-Jose Estefano
Sponsor contact (Alpha Bioresearch, CRO)

Signature and date

_____

# INVESTIGATOR STATEMENT OF AGREEMENT

I agree to:

- Implement and conduct this study diligently and in strict compliance with this clinical investigation plan, the Declaration of Helsinki, the Medical Devices Directive 93/42/EEC, ISO14155:2011 standards, the Principles of Good Clinical Practice, and all applicable laws and regulations.
- Maintain all information supplied by Yaqrit Ltd in confidence and, when this information is submitted to an Independent Ethics Committee (IEC) or another group, it will be submitted with a designation that the material is confidential.

I have read this clinical investigation plan in its entirety and I agree to all aspects.

Principal Investigator:

(printed name) _____

Institution name: _____

Principal Investigator signature and date:

_____

## PARTICIPATING CLINICAL INVESTIGATORS

This trial will be conducted at investigational sites from European countries with liver units specialized in the management of patients with liver cirrhosis. The list of involved sites is provided in the attached document "List of Participant Sites".

## OTHER INSTITUTIONS INVOLVED IN THE CLINICAL INVESTIGATION

**Laboratory of Institute of Liver and Digestive Health – University College London**

Upper Third Floor, UCL Medical School,

Rowland Hill Street, Hampstead, London NW3 2PF, United Kingdom

**King's College Hospital Reference Biochemistry Laboratories**

King's College Hospital

Bessemer Road, London, SE5 9RS, United Kingdom

**Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)**

Research Institute

Carrer del Rosselló, 149, 08036 Barcelona, Spain

**Liver Unit Laboratories - Hospital Clínic**

Villarroel, 170, 08036 Barcelona, Spain

**Laboratory of Department of Medical and Surgical Sciences – University of Bologna**

U.O Semeiotica Medica, Alma Mater Studiorum University of Bologna

Via Albertoni 15, 40138 Bologna, Italy

**UVCM - Laboratory**

Department of Gastroenterology, University Clinic for Visceral Surgery and Medicine

Inselhospital, CH-3010 Bern, Switzerland

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# LIST OF ABBREVIATIONS

| Abbreviation | |
|---|---|
| ACLF | Acute on chronic liver failure |
| ADE | Adverse device effect |
| ADL | Activities of daily living |
| AE | Adverse event |
| ALT | Alanine aminotransferase |
| APTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| BCS | Biopharmaceutics Classification System |
| BDL | Bile duct ligated |
| BDRM | Blind Data Review Meeting |
| BMI | Body mass index |
| BP | Blood pressure |
| ºC | Degrees Celsius |
| CA | Competent Authority |
| CIP | Clinical Investigation Plan |
| CK-18 | Creatine kinase- 18 |
| CRA | Clinical Research Associate |
| CRF | Case Report Form |
| CRO | Contract Research Organization |
| CTCAE | Common toxicity criteria for adverse events |
| d | Day |
| dL | Decilitre |
| DM | Data Manager |
| DMC | Data Management Centre |
| DMP | Data Management Plan |
| DNA | Deoxyribonucleic acid |
| DSMB | Data Safety Monitoring Board |
| EAA | Endotoxin Activity Assay |
| EASL | European Association for the Study of the Liver |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| EU | European Union |
| FDA | Food and Drug Administration (USA) |
| g | Gramme |
| GCP | Good Clinical Practice |
| GGT | Gamma-glutamyltransferase |
| Hb | Haemoglobin |
| HCT | Haematocrit |
| Hg | Mercury |
| hrs | Hours |
| ICF | Informed Consent Form |

| Abbreviation | |
|---|---|
| ICH | International Conference on Harmonization |
| Ig | Immunoglobulin |
| INR | International Normalized Ratio |
| ISF | Investigator Site File |
| ISO | International Organization for Standardization |
| kcal | Kilocalorie |
| kg | Kilogram |
| L | Litre |
| LBP | Lipopolysaccharide binding protein |
| LDH | Lactate dehydrogenase |
| Ln | Natural Logarithm |
| LPS | Lipopolysaccharide |
| m | Metre |
| MAC | Mid-arm Circumference |
| MAMC | Mid-arm Muscle Circumference |
| MCD | Methionine Choline Deficient |
| MCV | Mean Corpuscular Volume |
| MedDRA | Medical Dictionary for Regulatory Authorities |
| MELD | Model for End Stage Liver Disease |
| mg | Miligram |
| min | Minute |
| µL | Microlitre |
| mL | Mililitre |
| mm | Milimetre |
| MRI | Magnetic Resonance Imaging |
| msec | Milisecond |
| N | Total number of subjects in a population or analysis group |
| NAFLD | Non-alcoholic Fatty Liver Disease |
| nm | Nanometre |
| NMR | Nuclear Magnetic Resonance |
| NOAEL | No-observed Adverse Effect Level |
| OTC | Over-the-counter |
| PHI | Protected health information |
| PP | Per-protocol population |
| PT | Prothrombin time |
| qds | Quater die sumendus (Latin): four times a day |
| QTcB | QT interval using Bazett's correction |
| QTcF | QT interval using Fridericia's correction |
| RFH-GA | Royal Free Hospital – Subjective Global Assessment |
| SADE | Serious Adverse Device Effect |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| Scr | Serum creatinine |
| SD | Standard Deviation |
| SOP | Standard Operating Procedure |

| Abbreviation | |
|---|---|
| SP | Safety Population |
| Tbil | Total bilirubin |
| TIPSS | Transjugular Intrahepatic Portosystemic Shunt |
| TMF | Trial Master File |
| TSF | Triceps Skinfold Thickness |
| UCL | University College of London |
| ULN | Upper Limit Normal |
| uNGAL | Urinary neutrophil gelatinase-associated lipocalin |
| USADE | Unanticipated Serious Adverse Device Effect |
| uTLR4 | Urinary toll-like receptor 4 |
| UVCM | Universitätsklinik für Viszerale Chirurgie und Medizin |
| WBC | White Blood Cell |
| WHO | World Health Organization |



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# 1. CLINICAL INVESTIGATION PLAN SYNOPSIS

| | |
|---|---|
| **CIP Number:** | Yaq001-S-001 |
| **Title:** | Safety and tolerability of Yaq-001 in patients with cirrhosis |
| **Short name:** | CARBALIVE-SAFETY study |
| **Clinical phase:** | First-in-human clinical investigation |
| **Purpose:** | To ascertain the safety and tolerability of oral administration of Yaq-001 in patients with cirrhosis |
| **Study design:** | Multicentre, randomized, double blinded, placebo controlled trial to evaluate safety and tolerability of oral administration of Yaq-001 therapy in two dosing cohorts.<br>56 cirrhotic patients with diuretic-responsive ascites will be enrolled. Patients will be randomized to two dosing cohorts:<br>• Cohort 1 (1:1 randomization)<br>  ○ Standard medical treatment + Yaq-001 (4 g/ day) – n= 14.<br>  ○ Standard medical treatment + placebo-control (placebo for 4 g of Yaq-001/ day) – n= 14.<br>• Cohort 2 (1:1 randomization)<br>  ○ Standard medical treatment + Yaq-001 (8 g/ day) – n= 14.<br>  ○ Standard medical treatment + placebo-control (placebo for 8 g of Yaq-001/ day) – n= 14.<br>Study patients will be dosed daily with Yaq-001 for 12 weeks. Assessments of DSMB will take place after 4 and 12 weeks. Investigational centres specialized in the management of patients with liver cirrhosis will participate in the study.<br>For each patient, the study duration will be up to 15 weeks, including the screening (up to 2 weeks), treatment (12 weeks) and 7-day follow up period.<br>The total study duration is estimated to be approximately 12 months from screening of first patient until study completion of the last patient. |
| **Investigational product:** | Yaq-001: highly adsorbent activated carbon with tailored pore size distribution<br>Placebo control: methylcellulose dyed to match Yaq-001 |

| | |
|---|---|
| **Objectives:** | **Primary objective**:<br>Assessment of the safety and tolerability of Yaq-001<br>**Secondary objectives**:<br>• Assessment of changes in blood endotoxin activity<br>• Assessment of changes in organ function<br>• Assessment of changes in nutritional status |
| **Inclusion criteria:** | 1. Male and female patients<br>2. Age ≥ 18 years at screening<br>3. Clinical diagnosis of cirrhosis for any cause. Liver biopsy is not required<br>4. Cirrhotic patients with diuretic-responsive ascites and Child-Pugh score = 7-11 inclusive<br>5. Abstinence from alcohol for at least 4 weeks prior to screening |
| **Exclusion criteria:** | 1. Refusal or inability (lack of capacity) to give informed consent<br>2. Prohibited medication within 4 weeks before the start of the study treatment: all oral antibiotics, immunosuppressants, long acting benzodiazepines or barbiturates and antiviral medication<br>3. Change in dose of proton pump inhibitor therapy within 4 weeks before the start of the study treatment<br>4. Patients with once daily medications in which orocaecal transit time is greater than 10 hours<br>5. Patients requiring medication in which the dosing schedule is three times per day or greater<br>6. Antiviral therapy for hepatitis C within 3 months prior to screening<br>7. Hospital admission for liver-related indication for at least 4 weeks (except paracentesis)<br>8. BMI > 35 or BMI < 18<br>9. *Clostridium Difficile* diarrhoea within 4 weeks before the start of the study treatment<br>10. Uncontrolled infection (chronic viral hepatitis is not an exclusion criterion)<br>11. Human immunodeficiency virus<br>12. Presence of a transjugular intrahepatic portosystemic shunt (TIPSS)<br>13. Participation in any clinical study of an investigational medicinal product within 30 days of five half-lives of the investigational product, whichever is longer<br>14. Presence of clinically relevant cardiovascular, pulmonary, gastro-intestinal, renal, hepatic, metabolic, haematological, neurological, psychiatric, systemic, ocular, gynaecologic or any acute infection disease or signs of acute illness that, in the opinion of the investigator, might compromise the patient's safe participation in the trial.<br>15. Presence of the history of cancer within the past 5 years with exception of hepatocellular carcinoma within Milan criteria, adequately treated localised basal cell carcinoma of the skin, in situ cervical carcinoma or solid malignancy surgical excised in total without recurrence for five years.<br>16. Women of child bearing potential. Only postmenopausal women or with surgical sterilization will be included. |
| **Primary endpoints:** | Safety assessments will be performed at Day 1, Weeks 1, 4, 8 and 12<br>Safety assessment will include:<br>• Physical examination<br>• Vital signs: blood pressure, pulse rate, respiratory rate and body temperature<br>• Clinical laboratory tests: haematology, coagulation, clinical chemistry and urinalysis |

| | |
|---|---|
| | • 12-lead ECG<br>• Reported and observed adverse events. |
| **Prohibited concomitant medication:** | • Once daily medications in patients with orocaecal transit time greater than 10 hours<br>• Medications in which the dosing schedule is three times per day or greater<br>• Antiviral therapy for hepatitis C |
| **Timing of concomitant medications:** | Morning doses should be taken at least 10 hours after carbon therapy<br>Evening doses should be taken at least 4 hours before carbon therapy |
| **Statistical considerations:** | This study is not powered for statistical significance. The clinical safety and all other data generated will be described using descriptive statistics |

## 2. SCHEDULED OF VISITS & PROCEDURES

| | SCREENING (Up to 14 days before Randomization) | RANDOMIZATION (D1) | 1-WEEK VISIT (D7±3 days) | 4-WEEK VISIT (D28±5 days) | 8-WEEK VISIT (D56±5 days) | 12-WEEK VISIT end of treatment (D84±5 days) or WITHDRAWAL VISIT | TERMINATION VISIT (7 days after end of treatment or after withdrawal visit) |
|---|---|---|---|---|---|---|---|
| Informed Consent | X | | | | | | |
| Medical history | X | | | | | | |
| Physical examination | X | X | X | X | X | X | X |
| Vital signs | X | X | X | X | X | X | X |
| Blood samples for haematology, coagulation and clinical chemistry (fasting) and urine samples (Local lab) | X | X | X | X | X | X | |
| Orocaecal Transit Time Test | X | | | | | | |
| 12-lead safety ECG | X | X | X | X | X | X | |
| Child Pugh Score | X | X | X | X | X | X | |

| | SCREENING (Up to 14 days before Randomization) | RANDOMIZATION (D1) | 1-WEEK VISIT (D7±3 days) | 4-WEEK VISIT (D28±5 days) | 8-WEEK VISIT (D56±5 days) | 12-WEEK VISIT end of treatment (D84±5 days) or WITHDRAWAL VISIT | TERMINATION VISIT (7 days after end of treatment or after withdrawal visit) |
|---|---|---|---|---|---|---|---|
| MELD Score | X | X | X | X | X | X | |
| Core labs: sample collection | | X | | X | | X | |
| Endotoxin activity assay | | X | X | X | X | X | |
| Dispensation of investigational product | | X | X | X | X | | |
| Accountability of investigational product | | | X | X | X | X | |
| Assessment of patient's compliance | | | X | X | X | X | |
| Concomitant medication | | X | X | X | X | X | X |
| Nutritional Assessment: Dietary intake, anthropometric validation and global assessment (RFH-GA) | | X | X | X | X | X | X |
| Adverse events | X | X | X | X | X | X | X |

# 3. INTRODUCTION, BACKGROUND AND RATIONALE

## 3.1. Name and description of investigational product

Yaq-001 is a carbon adsorbent with tailored porosity capable of removing bacterial endotoxin and other metabolic toxins relevant to the progression of bacterial translocation and endotoxaemia in patients with cirrhosis and NAFLD.

Yaq-001 will be regulated as medical device given that the mode of action appears to be mechanical in nature, by physically binding the bacterial metabolites within the intestinal tract. Yaq-001 is not systemically absorbed: does not appear to be a direct pharmacological, immunological or metabolic effect (see section 3.3.2 for summary of results of mass balance study).

## 3.2. Unmet medical need and rationale for use of Yaq-001 in the treatment of cirrhosis and NAFLD

The WHO estimates that over 650 million people globally and more than 29 million European citizens suffer from a chronic liver condition with in excess of 170.000 European deaths from cirrhosis alone each year[1]. Liver disease remains the 5th most common cause of death across Europe in individuals aged 45-65 years and costs more than €15.8bn per annum in total health costs and loss in economic productivity[2].

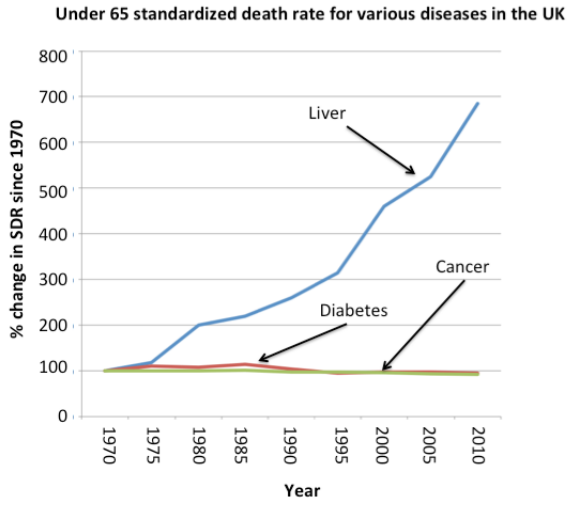


**Figure 1:** This figure shows that standardized mortality rates for patients with liver disease has increased exponentially in the UK whereas death rates in other chronic non-communicable diseases such as diabetes and cancer are falling (British Association for the Study of Liver: A time to act).

Alarming increases in obesity rates and an ageing population mean that liver disease will become an even greater health concern for Europe over the next decade. The three major causes of liver disease, i.e. alcoholic liver disease, NAFLD and viral hepatitis are amenable to prevention and treatment, providing an opportunity to reduce the burden of liver disease in Europe and saving lives[3]. Gut-derived endotoxaemia and bacterial translocation play a central role in the pathogenesis of NAFLD[4], the development of cirrhosis and its

[1] Blachier et al; Journal of Hepatology; 2013
[2] Lozano et al; Lancet; 2013
[3] Olson et al; 2011
[4] Henao-Mejia et al; Nature; 2012

complications[5] [6]. Targeting increased bacterial translocation with poorly absorbed antibiotics is the basis for current therapeutic intervention in patients with very advanced liver disease[7] [8]. However, this approach has the problem of infection with resistant organisms, emergence of resistant strains of bacteria and limits its use to "treat" patients with advanced disease rather than as a preventive strategy[9] [10] [11]. Minimal progress has been made with developing alternatives to this current interventional regime and a non-antibiotic strategy to prevent bacterial endotoxaemia and bacterial translocation is an unmet need.

Recent advances in synthetic activated carbon technology have resulted in the development of a carbon with a uniquely tailored porosity conferring a high absorptive capacity for gut-derived bacterial metabolites and toxins relevant to pathogenesis in liver disease (Yaq-001). The academic-industrial consortium CARBALIVE has developed a novel strategy using nanoporous carbons of tailored porosity, originally developed by MAST (US 2010/0029795) in a FP5 project-BRPR-CT97-0561, which is a safe approach that has been shown to reduce bacterial translocation.

### 3.3. Physical and biological characteristics of Yaq-001

Background information on Yaq-001 is summarized below. More detailed information is provided in the investigator's brochure.

#### *3.3.1. Physical properties of Yaq-001*

Yaq-001 is highly absorbent, synthetically derived spherical carbon with a pore size distribution relevant to the adsorption of gut-derived pathogenic factors relevant to pathogenesis of liver disease. The bimodal porosity is within meso/ macroporous (> 2 nm) and microporous (< 2 nm) range. The larger meso/macropores allow removal of larger biological relevant molecules such as bacterial endo- and exo-toxins together with inflammatory cytokines. The micropores adsorb other pathogenic mediators such as acetaldehyde and indoles (exact composition by intellectual property protected). The large surface area allows for favourable adsorption kinetics.

Details concerning the manufacture of the investigational device are provided in the investigator's brochure.

#### *3.3.2. Summary of mass balance study*

Greater than 95% of Yaq-001 carbon spheres were recoverable from faeces within 48 hours of Yaq-001 dosing in a healthy rat model. The difference between the recovered and reference materials is most likely due to carbon beads being lost during the extended washing cycles.

#### *3.3.3. Summary of studies in animal models of cirrhosis*

UCL has performed a range of preclinical investigations that have demonstrated the proof of concept and validated the therapeutic potential of the product in clinically relevant animal models of advanced chronic liver disease and NAFLD. The results of these studies confirm that Yaq-001 has several beneficial effects on pathophysiological parameters of liver disease. The studies were performed in models of chronic liver failure

[5] Wiest et al; Journal of Hepatology; 2014
[6] Seki et al; Nat Med; 2007
[7] Bass et al; New England J Med; 2007
[8] Fernandez et al; Gastroenterology; 2007
[9] Bass et al; New England J Med; 2010
[10] Fernandez et al; Gastroenterology; 2007
[11] Jalan et al; Journal of Hepatology; 2014



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and NAFLD. The salient points of the results of the nonclinical studies to support the clinical use of Yaq-001 in the treatment of cirrhosis and NAFLD are:

***Rate bile duct ligated (BDL) model of cirrhosis***

- Yaq-001 treated animals had significantly lower ALT, creatinine and portal pressure.
- Endotoxin-induced deterioration of liver function (ALT), renal function (creatinine) and portal pressure was significantly reduced in Yaq-001 treated rats, compared with control animals (LPS was administered to mimic the development of ACLF).
- Yaq-001 normalised endotoxin-induced organ injury associated with a significant improvement in liver oxidative stress.
- Reductions in markers of bacterial translocation were observed with Yaq-001 therapy associated with a significant improvement in intestinal permeability.
- BDL-induced cachexia in BDL rats was prevented in the Yaq-001 treated rats. No significant differences in electrolyte or plasma micronutrient levels were observed in Yaq-001 treated rats.

***Models of non-alcoholic fatty liver disease***

- Oral Yaq-001 therapy resulted in a significant attenuation of liver injury in genetically obese mice and non-alcoholic steatohepatitis induced by a methionine choline deficient diet (MCD).
- A significant normalisation in endotoxin-induced liver oxidative stress was observed with Yaq-001 treated genetically obese mice.
- A trend towards reduction in body weight was observed with Yaq-001 treated genetically obese mice. No reduction was observed in Yaq-001 treated heterozygote controls despite pair-feeding.
- Yaq-001 was associated with a significant reduction in glycosuria in genetically obese mice suggestive of an improvement in insulin resistance.
- Significant normalization of urinary metabolomics profile was observed with Yaq-001 therapy.

### *3.3.4. Toxicology*

Oral administration of Yaq-001 for 13 weeks was well tolerated in rats at dosages of up to 2.05 g/kg/day. Yaq-001 was cleared from the gastrointestinal tract within 48 hours. There was no evidence of toxicity. There were no treatment-related clinical observations: body weights, food consumption or ophthalmic examination. No adverse events were seen in laboratory evaluations (clinical pathology, haematology, coagulation, clinical chemistry, and urinalysis) or in histopathology or organ weights. Based on these results, the NOAEL is considered to be ≥ 2.05 g/kg/day in the rat (15x the expected maximum human dose).

Please, refer to investigator's brochure for a summary of the nonclinical safety program.

## 3.4. Rationale for the study

### *3.4.1. Rationale for the study design*

This first-in-human clinical investigation will provide data on the safety and tolerability for two doses of Yaq-001 in cirrhotic patients. It is anticipated that these data will provide sufficient information to allow for the design of subsequent performance and safety studies.

Two dose cohorts in cirrhotic patients with diuretic-responsive ascites will be employed in this first-in-human clinical investigation. Cirrhotic patients with diuretic-responsive ascites are the target population for Yaq-001, making it desirable to understand the safety of the device in this population to more optimally determine the

dose. Obtaining safety in healthy volunteers may not be relevant given that pathological bacterial translocation and intestinal dysbiosis does not occur in healthy patients with normal liver function.

Inclusion of a placebo group in the context of a double-blind, randomized, controlled trial will provide the most rigorous test of safety in order to evaluate the oral effects of Yaq-001 allowing for inclusion of fewer patients than for non-placebo control arm. Participants in the placebo control group will benefit from the attention that they receive from the study investigators and staff as well as from the ancillary treatments and diagnostics they receive as part of a clinical investigation.

#### *3.4.2. Rationale for dose selection*

As this is a first-in-human clinical investigation with this particular formulation of carbon, the sponsor will take extra precaution and proceed with the next dose cohort only if the lower dose is found to be safe and well tolerated. At month 1 (lower dose cohort), the DSMB will assess the data and determine whether the study can proceed to the higher dose cohort.

*In vitro* experiments and *in vivo* animal feasibility studies revealed that the intended effect of Yaq-001 could be achieved for rats at a dose of 4g/kg body weight (see section 3.3.3). The equivalent daily human dosing would be 0.65 /kg body weight. For a 70 kg human, the equivalent daily dose would be 45.16 g. If, however the equivalent total dose for the 14-day treatment schedule in rats is considered and administered over 90 days instead as per safety study protocol, the calculated daily dosing for human safety study is 7.03g. From previous studies with AST-120 in cirrhotic patients, a favourable safety profile has been demonstrated with a dose of 3 g qds[12]. The maximum recommended starting dose for Yaq-001 was calculated according to FDA's Guidance for Industry Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (July 2005). The scheduled dosing has been selected as 4g per day for Cohort 1 and 8g per day for Cohort 2.

#### *3.4.3. Rationale for key evaluations*

The safety profile of Yaq-001 is not known. This will be the first time to be administered to humans. The safety and tolerability of two dose levels of Yaq-001, dosed daily for 12 weeks, will be evaluated in sequential groups of patients. Doses of 4 or 8 g/day will be tested. Patients will be followed 7 days post-last dose.

# 4. RISKS AND BENEFITS OF THE INVESTIGATIONAL DEVICE AND CLINICAL INVESTIGATION

## 4.1. Expected clinical benefits

Pre-clinical investigations have confirmed that Yaq-001 has several beneficial effects on pathophysiological parameters of liver disease:

a) Yaq-001 had beneficial effects on nutrition, the liver, kidney and portal hypertension in cirrhosis.
b) Yaq-001 prevents the development of ACLF in chronic liver disease.

[12] Pockros et al; EASL; 2009

c) No adverse effects of Yaq-001 were seen on the gut histology.
d) Yaq-001 had significant beneficial effect on gut translocation through restoration of gut permeability and microbiome composition and function.
e) Yaq-001 had significant beneficial effect on systemic and innate immune populations along the gut-liver axis.
f) Yaq-001 attenuates inflammation by reduced inflammasome activation.

Based on pre-clinical data, the expected clinical benefits for cirrhotic patients are:

- An improvement in organ function: kidney, liver, brain, intestinal and immune.
- A reduction in blood endotoxin activity.
- A reduction in episodes of hepatic decompensation.
- An improvement in nutritional status.

### 4.2. Expected adverse device effects

No major safety issues are anticipated with the investigational product but all patients will be closely monitored during the scheduled study visits. In the unlikely event that a patient becomes unwell and concern is expressed possibly for the safety of the patient it will of course be possible to break the randomization code and ascertain if the patient is receiving investigational product or placebo.

Based on the labelling of another carbon product (Kremezin®, AST-120) anticipated device-related events are: nausea, vomiting, constipation and diarrhoea.

### 4.3. Risks and expected adverse device effects that are to be assessed

The objective of this study is to assess the safety profile of Yaq-001. The safety parameters to be assessed in this clinical investigation are presented in section 5.

### 4.4. Risks associated with participation in the clinical investigation

Patient visit schedule has been designed to be minimally intrusive whilst retaining sufficient assessment visits to allow for safety monitoring. Small risks associated with venepuncture may include pain and temporary dizziness. Assessment of orocaecal transit time involves exposure to a small radiation dose in the form of a plain abdominal X-ray. If inconclusive, either a capsule endoscopy test or MRI scan will be performed. Capsule endoscopy is associated with a small risk of capsule retention. MRI scan may be associated with claustrophobia.

### 4.5. Possible interactions with concomitant medical treatments

Previous studies with AST-120 have demonstrated that administration of AST-120 at least 1.5 hours after administration of concomitant medication does not significantly impact drug pharmacokinetic profiles. Interval between Yaq-001 and concomitant medication administration must therefore be 4 hours. Patients on dosing regimens for concomitant medications of three or more times per day will be excluded.

Intestinal transit, even if prolonged, may be assumed to be identical for both drug and device and therefore dosing interval is the key parameter to determine drug-device interactions. Nonetheless, in light of the fact that some cirrhotic patients have shown to have prolonged orocaecal intestinal transit, exclusion of patients with very prolonged orocaecal transit times with the potential to functionally impact on drug-device interactions is included in the clinical investigation plan as additional safety measure. The thresholds for exclusion are defined in the exclusion criteria.

### 4.6. Steps that will be taken to control or mitigate the risks

The utilization of a DSMB for this clinical investigation is discussed in section 6.2.

Cohort 2 cannot commence until the DSMB has reviewed the safety data in an unblinded manner and recommends advancement to the next cohort. Because of the known symptomatology for this disease state, the DSMB can confirm that the incidence of AEs in the patients treated with Yaq-001 medical device (active) does not exceed the incidence of AEs in placebo group for each dose cohort.

### 4.7. Risk-to-benefit rationale

Yaq-001 has a favourable safety profile in preclinical studies. Extensive clinical experience with a similar compound AST-120 in renal patient cohorts has shown a favourable safety profile. Given the central role of bacterial toxins and metabolites in the pathogenesis of cirrhosis and its complications, the potential clinical benefit of binding and eliminating these factors with Yaq-001, offsets the risks of the trial. Furthermore, both treatment and placebo groups would benefit from the close medical follow-up provided as part of the assessment visit schedule.

## 5. OBJECTIVES AND OUTCOME MEASURES

### 5.1. Primary objective

The main objective of this clinical investigation is to assess the safety and tolerability of Yaq-001 throughout the three months' treatment period.

To that end, the following outcome measures at randomization, 1-week, 4-weeks, 8-weeks and 12-weeks will be considered:

- Physical examination
- Vital signs: blood pressure, pulse rate, respiratory rate and body temperature
- Clinical laboratory tests: haematology, coagulation, clinical chemistry and urinalysis (see the list of parameters in section 10.7)
- 12-lead ECG
- Reported and observed adverse events.

### 5.2. Secondary objectives

The secondary objectives of this clinical investigation are the following:

- Assessment of changes in blood endotoxin activity
- Assessment of changes in organ function
- Assessment of changes in nutritional status

#### *5.2.1. Assessment of changes in blood endotoxin activity*

The changes in blood endotoxin activity, measured by the EAA, will be used as device-related performance indicator.

The EAA will be performed at randomization, 1-week, 4-week, 8-week and 12-week visits.

#### *5.2.2. Assessment of changes in organ function*

Changes in kidney, liver, brain, intestinal and immune functions will be assessed by means of:

- CHILD-PUGH score
- MELD score
- Clinical laboratory tests (see the list of parameters in section 10.7)

CHILD-PUGH and MELD scores will be calculated at screening, randomization, 1-week, 4-week, 8-week and 12-week visits.

Clinical laboratory tests will be performed at randomization, 4-week and 12-week visits.

#### *5.2.3. Assessment of changes in nutritional status*

Whilst Yaq-001 had a favourable safety profile in nonclinical studies, assessment of changes in nutritional status will be performed as an important secondary objective to ensure Yaq-001 therapy does not result in depletion of micronutrients with longer term treatment. This is of particular importance as carbons act as non-selective adsorbents and characterization of impact on micronutrient status in a longer term context is required. In the event of micronutrient deficiencies, appropriate supplementation will be prescribed as part of standard medical care.

Clinical assessment of nutritional status will include:

- Global assessment (RFH-GA)
- Clinical laboratory tests (see the list of parameters in section 10.7)

Global assessment will be performed at randomization, 1-week, 4-week, 8-week and 12-week visits.

Clinical laboratory tests will be performed at randomization, 4-week and 12-week visits.

# 6. INVESTIGATIONAL PLAN

## 6.1. Study design

This is an international, multicentre European study. In order to avoid bias, the study will utilize randomization, double-blinding and placebo as the control group. To mitigate risk, two dose levels will be utilized in sequential groups of patients, with oversight by DSMB (see Figure 2: **Carbalive Safety Study Algorithm** and section 6.2).

Cirrhotic patients with diuretic-responsive ascites will be randomized to:

- Standard medical treatment + Yaq-001 or
- Standard medical treatment + placebo of Yaq-001

Two cohorts, with 28 patients per cohort, will receive either Yaq-001 or matching placebo. 56 cirrhotic patients with diuretic-responsive ascites will be enrolled (randomized to treatment). Study patients will be dosed for 12 weeks. Investigational sites specialized in the management of patients with liver cirrhosis will participate in this trial. See section 9 for study procedures by visit.

- Cohort 1 (1:1 randomization)
  - Standard medical treatment + Yaq-001 (4 g/ day) – n= 14.
  - Standard medical treatment + placebo-control (placebo for 4 g of Yaq-001/ day) – n= 14.
- Cohort 2 (1:1 randomization)
  - Standard medical treatment + Yaq-001 (8 g/ day) – n= 14.
  - Standard medical treatment + placebo-control (placebo for 8 g of Yaq-001/ day) – n= 14.

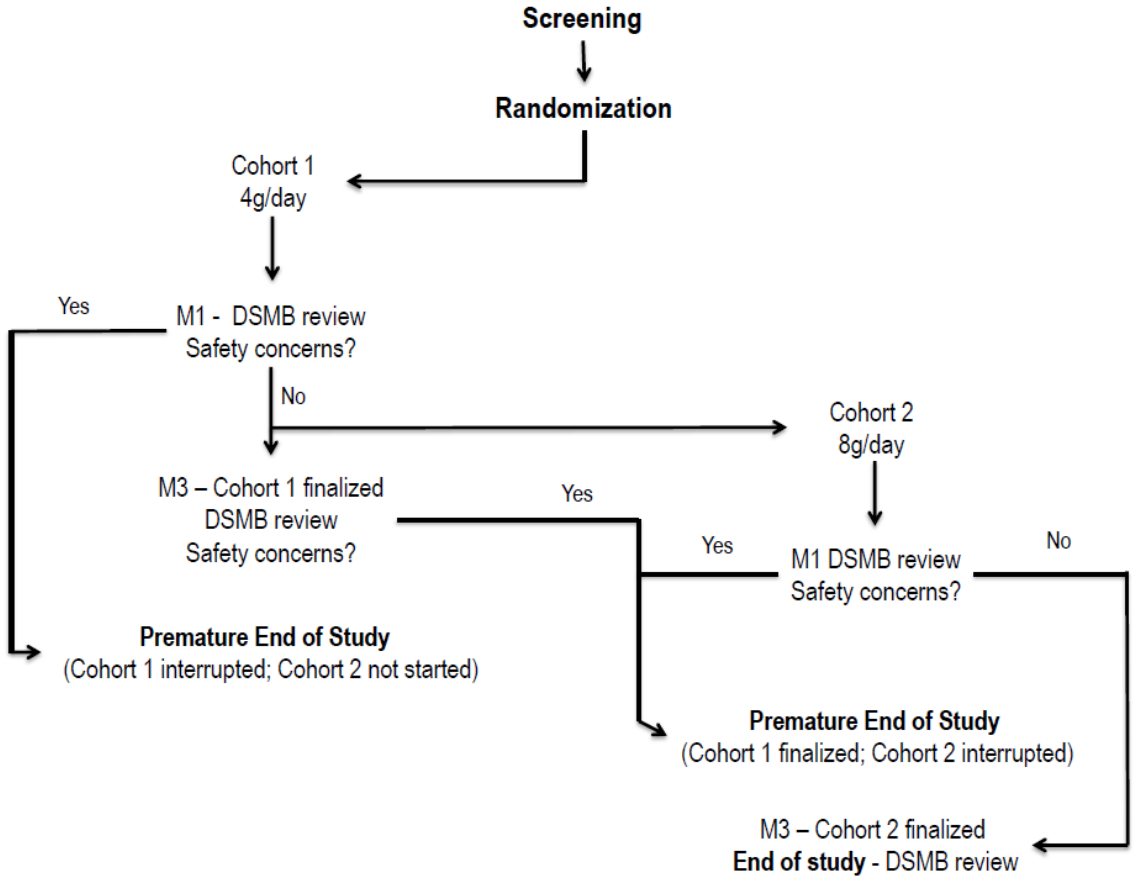


**Figure 2:** Carbalive Safety Study Algorithm

### 6.2. Initiation of sequential dose levels

As outlined in Figure 2: **Carbalive Safety Study Algorithm**, the DSMB will review the unblinded safety and tolerability data from the Cohort 1 after completion of 4 weeks of therapy. The review will be based on the assessment of reported and observed AEs, routine clinical laboratory results, vital signs, ECG findings and physical examination.

The DSMB will then make a recommendation as to whether the Cohort 2 can commence. In general, the safety and tolerability information from all 28 patients in the first cohort (14 Yaq-001: 14 placebo) will be known before decisions are made about starting the Cohort 2. Both the sponsor and the participating investigators will remain blinded. The criteria for recommending proceeding to the next dosing cohort or stopping the study will be defined in the DSMB charter.

The DSMB will be composed of an independent statistician and two hepatologists. Their roles, organization and procedures to follow in the review of the study data will be described in the DSMB charter.

### 6.3. Suspension or early termination of the clinical investigation

Sponsor reserves the right to discontinue this clinical investigation at any stage with suitable written notice to the investigators. Possible reasons include:

- USADE occurs and it presents an unreasonable risk to the patients (see section 11)

- DSMB recommendation.
- Unsatisfactory rate of subject recruitment.
- Unsatisfactory compliance with eligibility criteria.

If early termination of the clinical investigation occurs patients will perform a withdrawal visit as soon as possible and a termination visit, 7 days after the withdrawal visit.

#### *6.3.1. Early termination due to toxicity criteria*

Investigational device toxicity will be assessed on the basis of the criteria presented in the Table 1A (APPENDIX B), including causality assessments.

Medical monitor may request unblinding of the randomization code for patient(s) with any of the toxicity criteria listed in APPENDIX B.

# 7. PATIENT POPULATION

## 7.1. Number of sites and patients

The study will be conducted at approximately 8 investigation sites in Europe, on average 7 patients per site. A total of 56 patients will be enrolled (randomized to treatment). Each patient may participate in only one cohort. For each patient, the study duration will be up to 15 weeks; including the screening (up to 2 weeks), treatment (12 weeks) and 7-day follow up periods.

The total duration of the study is estimated to be approximately 12 months from screening of first patients until study completion of the last patient.

## 7.2. Selection criteria

### *7.2.1. Inclusion criteria*

Patients accepted for inclusion into the study must meet all of the following criteria:

1. Male and female patients
2. Age ≥ 18 years at screening
3. Clinical diagnosis of cirrhosis for any cause. Liver biopsy is not required
4. Cirrhotic patients with diuretic-responsive ascites and Child-Pugh score = 7-11 inclusive
5. Abstinence from alcohol for at least 4 weeks prior to screening

### *7.2.2. Exclusion criteria*

Patients with any of the following criteria are to be excluded:

1. Refusal or inability (lack of capacity) to give informed consent
2. Prohibited medication within 4 weeks before the start of the study treatment: all oral antibiotics, immunosuppressants, long acting benzodiazepines or barbiturates and antiviral medication
3. Change in dose of proton pump inhibitor therapy within 4 weeks before the start of the study treatment
4. Patients with once daily medications in which orocaecal transit time is greater than 10 hours
5. Patients requiring medication in which the dosing schedule is three times per day or greater
6. Antiviral therapy for hepatitis C within 3 months prior to screening
7. Hospital admission for liver-related indication for at least 4 weeks (except paracentesis)
8. BMI > 35 or BMI < 18

9. Clostridium Difficile diarrhoea within 4 weeks before the start of the study treatment
10. Uncontrolled infection (chronic viral hepatitis is not an exclusion criterion)
11. Human immunodeficiency virus
12. Presence of a transjugular intrahepatic portosystemic shunt (TIPSS)
13. Participation in any clinical study of an investigational medicinal product within 30 days of five half-lives of the investigational product, whichever is longer
14. Presence of clinically relevant cardiovascular, pulmonary, gastro-intestinal, renal, hepatic, metabolic, haematological, neurological, psychiatric, systemic, ocular, gynaecologic or any acute infection disease or signs of acute illness that, in the opinion of the investigator, might compromise the patient's safe participation in the trial.
15. Presence of the history of cancer within the past 5 years with exception of hepatocellular carcinoma within Milan criteria, adequately treated localised basal cell carcinoma of the skin, in situ cervical carcinoma or solid malignancy surgical excised in total without recurrence for five years.
16. Women of child bearing potential. Only postmenopausal women or with surgical sterilization will be included.

## 7.3. Patient enrolment and randomization

### *7.3.1. Informed consent*

The investigator will choose patients in accordance with eligibility criteria detailed in section 7.2. Prior to any procedure of the study, patients will receive a comprehensive explanation of the nature, scope and possible consequences of the study. The following information will be given in both oral and written form, in the patient's native language, by a member of the study staff designated to conduct the consent process:

- Purpose of the clinical investigation
- Investigational status of the product
- Voluntary participation in the study
- Methodology: nature and extent of any procedures, how long it lasts, number of visits and additional tests
- A 50-50 chance of receiving standard of care plus placebo
- Benefits expected for the subject and society, adding that participation in the study does not necessarily mean any benefit for the patient's health
- Possible adverse events, including those related to investigational product and those related to any study procedure
- Other effective therapeutic alternatives, if any
- Insurance coverage to provide them any compensation in the case of harm to their health that might occur as result of their participation in the study
- Local ethics committee approval
- Right to withdraw consent at any time without affecting patient's medical treatment
- Confidentiality and privacy arrangements. Patients will receive a detailed explanation of the proposed use and disclosure of their PHI. The information will be used in accordance with the local data protection law
- Arrangements for access to information. Patient must be informed that his/ her medical records may be examined by authorized monitors or quality assurance auditors appointed by sponsor, by appropriate EC members and by inspectors from regulatory authorities
- Compensation arrangements

- Any foreseen future uses of the research results, data or materials, including commercial uses
- Sources of co-funding, if any, for the project

After having the nature and risks of the study explained to them, patients will be allowed sufficient time to consider participation in the study.

Each patient must provide written informed consent to participate in the study. The informed consent shall be obtained as per protocol and in compliance with applicable EC requirements. Written informed consent must be obtained from each patient prior to any study-related activities being conducted. The investigator must retain all original signed and personally dated ICFs, together with any subsequent EC-approved amended versions in the ISF. One copy of the original signed and dated ICF, and any amendments, must be given to the patient.

By signing the ICF, the patient agrees to complete all evaluations required by the clinical investigation plan, unless the patient withdraws voluntarily or is terminated from the study for any reason.

If any new information becomes available that significantly affects the patient's risk by participating in the clinical investigation, the new information will be communicated by the investigator to patients who consented to participate in the study, in accordance with EC requirements. The ICF will be updated and patients will be re-consented if necessary. Patients unable to provide valid informed consent will be excluded from the study.

The patient's ability to provide informed consent will be based on the patient's capability to:

- Understand the information relevant to decision
- Retain that information
- Use or weigh that information as part of the process of making the decision
- Communicate his/ her decision (whether by talking, using sign language or any other means)

### *7.3.2. Enrolment and randomization*

Patients will be considered included into the study once informed consent is obtained. Patients screening numbers will be assigned to patients following signature of the informed consent.

A log of patients having given informed consent (screening log) will be maintained in the ISF at the investigational site independent of whether they are or are not enrolled (randomized to treatment).

Patients who drop out during screening phase prior to randomization will be considered a screening failure. In this case, the reason for screening failure will be documented in the patient's medical records, the eCRF and the screening log.

Patients completing the screening period (eligible patients) will be randomized to treatment (Yaq-001/ Placebo) in a 1:1 randomization ration for both cohorts. A unique randomization number will be assigned to the patient by eCRF. Patients assigned a randomization number (consented, screened and found to be eligible) are considered enrolled in the study.

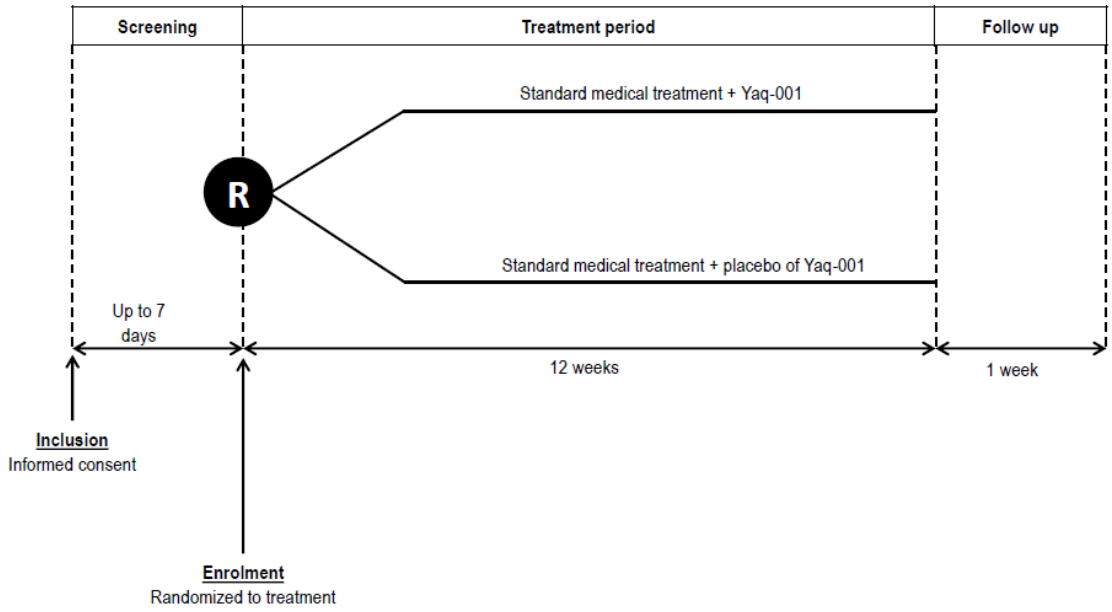


**Figure 3:** Timeline for patient involvement

A computer-generated randomization schedule will be provided to the unblinded clinical packaging organization (independent of the clinical study) by independent statistician prior to the start of the study. The person responsible for generating the randomization list will not be involved in the trial conduct and statistical analysis to preserve double blind condition until database is completed and locked.

## 7.4. Withdrawal of individual patients

### *7.4.1. Reasons for withdrawal*

By withdrawing their informed consent, patients are free to discontinue their participation in the study at any time and without prejudice to further treatment. The investigator must withdraw any patient from the study if that patient requests to be withdrawn. Although a subject is not obliged to give his/ her reason for withdrawing prematurely from the study, the investigator should make a reasonable effort to ascertain the reason, while fully respecting the subject's rights.

Reasons that subjects may be withdrawn by the investigator include, but are not limited to the following:

- Eligibility criteria are being violated
- Patient does not follow requirements for the study (misses study visits, does not take the investigational product, etc)
- Intolerable AEs (serious and non-serious), including the clinically significant safety laboratory results
- Non-compliance with the treatment (less than 70% or more than 120%)

#### *7.4.2. Handling of withdrawals*

##### *7.4.2.1. Withdrawal of consent*

In the event that the patient withdraws from the study early for whatever reason, the investigator must inform the sponsor immediately via eCRF.

The primary reason for discontinuation and the date should be documented in the source records and reported on the applicable page of the eCRF.

All data collected prior to withdrawal will be retained for subsequent analysis. No additional follow up will be required or data recorded once withdrawn from the study.

##### *7.4.2.2. Premature treatment discontinuation*

Treatment with investigational product should be discontinued if:

- The patient needs to take concomitant drugs that interfere with the investigational product
- The patient is no longer able to participate for other medical reasons (e.g. surgery, AEs or other diseases)

The following signals (change from baseline) should be considered for referral to the DSMB:

- 50% increase in MELD score
- 100% increase in creatinine
- Device-related acute decompensation or episode of ACLF
- Unexplained 50% reduction in BMI

If a patient fulfils any of the above criteria, the case should be referred to the DSMB. The investigator should decide the relationship of the event to the medical device and suspend treatment until DSMB review.

Patients prematurely withdrawn from the treatment will be followed up until the end of the study. They will be monitored carefully, which includes the assessments according to the schedule of visits and procedures. Other additional assessments can be agreed upon by the investigator and sponsor's medical monitor.

#### *7.4.3. Replacement of patients*

Patients who are included in the study and do not fulfil the eligibility criteria will be replaced. Patients who discontinue from the study at any time after randomization and intake at least one dose of the investigational product will not be replaced.

# 8. INVESTIGATIONAL PRODUCT

## 8.1. Clinical supplies

#### *8.1.1. Identity of investigational products*

Yaq-001 – highly adsorbent activated carbon with tailored pore size distribution

Placebo – methylcellulose beads dyed to match Yaq-001 beads

### *8.1.2. Packaging of investigational products*

Yaq-001 and the placebo for the Yaq-001 will be supplied as sachets for oral administration. Yaq-001 contains no excipients. The placebo will be indistinguishable from Yaq-001 in appearance, taste and smell.

### *8.1.3. Supply of investigational products*

Both Yaq-001 and placebo sachets will be supplied in kits, containing enough investigational product for dosing between visits + 5 days.

Cohort 1

- The kit dispensed at randomization visit will contain 12 (7+5) day's supply (24 sachets)
- The kit dispensed at 1-week visit will contain 26 (21+5) day's supply (52 sachets)
- The kit dispensed at 4-week and 8-week visits will contain 33 (28+5) day's supply (66 sachets at 4-week visit and 66 sachets at 8-week visit)

Cohort 2

- The kit dispensed at randomization visit will contain 12 (7+5) day's supply (48 sachets)
- The kit dispensed at 1-week visit will contain 26 (21+5) day's supply (104 sachets)
- The kit dispensed at 4-week and 8-week visits will contain 33 (28+5) day's supply (132 sachets at 4-week visit and 132 sachets at 8-week visit)

### *8.1.4. Labelling*

Investigational product will be labelled with the following information:

- CIP number
- Visit number and patient number
- Lot number and use-by-date
- Storage conditions
- Sponsor name and address
- "FOR CLINICAL INVESTIGATION USE ONLY"
- "KEEP OUT OF REACH OF CHILDREN"

Packaging and labelling will be performed in accordance with all applicable regulatory requirements. Copies of the labels will be stored in the ISF and the sponsor's TMF. The investigational device and the placebo will be labelled in the respective local languages.

### *8.1.5. Blinding of study product*

Study product and placebo will be packaged and labelled to ensure blinding is maintained.

### *8.1.6. Storage conditions*

Clinical supplies will be stored in a secure area at the investigational sites to prevent unauthorized access. The supplies should be stored at temperature less than 25º C. Study product must be kept in its closed original packaging.

The minimum/ maximum storage temperature must be measured and documented by investigator/ pharmacist weekly.

### *8.1.7. Shipment, inventory and return of clinical supplies*

Clinical supplies of Yaq-001/ placebo will be sent to the investigational site using standard and monitored shipping methods.

Accountability for clinical supplies must be maintained. For this purpose, the investigator/ pharmacist must maintain records of the investigational product received by the investigational site, the inventory at site, the dispensing of each kit to the patient, the patient's return of unused supplies to the site, and the return to the sponsor's packager of the unused supplies.

These records must be kept updated and will contain, at minimum, the following information:

- Date of receipt of clinical supplies
- Lot numbers of clinical supplies received
- Use-by-date
- The identification of the patient to whom the investigational product was dispensed, including the randomization number
- The date and the quantity of investigational product dispensed to the patient
- Any product accidentally or deliberately destroyed
- Date of return and quantity of unused investigational product

These records must be available for review during the study by the sponsor's designated monitor. All investigational products will be accounted for by a Yaqrit monitor/ contractor.

At the end of the study, after reconciliation by a sponsor's representative, remaining supplies must be returned to the sponsor's contract packager. The sponsor (or sponsor's representative) will provide written instructions to the investigational site regarding return of unused supplies. Destruction of study products at investigational site is not allowed.

## 8.2. Dosage and administration of study product

### *8.2.1. Method of assigning patients to treatment groups*

Treatment assigned is determined by the randomization code, generated by the independent statistician. Patients will be randomly assigned to Yaq-001 or placebo arms in a balanced 1:1 ratio. The clinical supplies will be packaged according to the randomization code.

The assigned patient study kit number will be recorded in the source documents and accountability log and the corresponding visit kit should be given to the patient. By adopting this procedure, the relevant parties will be blinded to the treatment assignment.

### *8.2.2. Dose*

The Yaq-001 device or placebo will be administered orally.

Cohort 1 (n= 28): Yaq-001 or placebo will be administered daily (4g of Yaq-001 or placebo for 4g of Yaq-001 nocte) for 12 weeks.

Cohort 2 (n= 28): Yaq-001 or placebo will be administered daily (8g of Yaq-001 or placebo for 8g of Yaq-001 nocte) for 12 weeks.

Interim analysis will be performed at 4 weeks into cohort 1 and the DSMB will review the data and will advise as to whether cohort 2 can begin. In this way, the study will be concertinaed into a shorter time frame. The DSMB

will also monitor safety end-points at 12 weeks for each cohort. The analysis of safety data by the DSMS will take approximately 21 days.

### *8.2.3. Investigational product assignment and administration for each patient*

Dispensing of investigational product for the double-blind treatment period will begin at randomization visit (day 1). Dispensing will occur on 3 occasions over the treatment period: 1-week, 4-weeks and 8-week visits.

Before starting the treatment, patients should be instructed to take the investigational product once daily at night after dinner. Yaq-001/ placebo must be taken placing the content of the sachet on the back of the tongue and swallowed with a glass of water.

To ensure a dose interval of about 24 hours, the treatment should be taken at the same time every day.

No double doses should be taken and half doses or reductions are not permitted.

If a patient accidentally takes more than the prescribed dose, he/ she should immediately consult the investigator.

If patients are taking any medicines during the treatment period of this study, they must not take them at the same time as Yaq-001/ placebo.

Morning doses of any medicine should be taken at least 10 hours after Yaq-001/ placebo doses. Evening doses of any study medication should be taken at least 4 hours before Yaq-001/ placebo.

### *8.2.4. Accountability procedures and treatment compliance*

The investigator must maintain records documenting the dispensation to patients and the return of used and unused supplies. Patients must be instructed to return empty sachets, as well as unused sachets.

Patient compliance should be checked at each patient visit by the investigator or investigational site staff and reported on accountability records. Because Yaq-001 is not systematically absorbed, pharmacokinetics cannot be employed in this study in order to monitor patient's treatment compliance.

Compliance will be determined by monitoring of returned supplies throughout the study and is defined as patient consumption of between 70-120% of total dose. Patients will be asked to bring all product containers (used and unused) with them to each study visit. Treatment compliance greater than 120% or less than 70% will constitute a major non-compliance and result in withdrawal from the study.

### *8.2.5. Blinding and procedures for unblinding*

#### *8.2.5.1. Unblinded personnel*

The study will be conducted in a double-blind manner. Investigators and everyone involved in analysing data or involved in the conduct of this clinical investigation will remain blinded to the randomised treatment assignments until after database lock. However, rules regarding breaking the code for an individual in emergency situation are provided in the next section.

The members of the DSMB will be unblinded.

#### *8.2.5.2. Unblinding of individual patients (procedures for emergency unblinding)*

Unblinding of an individual patient treatment code is to be executed only on emergency basis, when the investigator needs to know the treatment assignment for a patient in order to properly care for the patient.

Sealed emergency envelopes containing the randomization code will be provided to the investigational site separate from the clinical supplies and will be stored in the ISF. The emergency envelope may only be opened in emergency situations when the identity of the investigational product must be known to the investigator in order to provide appropriate medical treatment or if required to assure safety of trial participant.

The investigator will immediately report unblinding to the sponsor, outlining the reasons for unblinding, the date and the person who broke the code. The event will also be registered on the eCRF.

Whenever possible, the sponsor should be contacted before breaking the blinded emergency code. Should any code be broken, the respective patient will be withdrawn from further participation in the study and a written explanation must be given by the investigator.

### 8.3. Prior and concomitant medications

Throughout the study, the investigator may prescribe concomitant therapy deemed necessary to provide adequate supportive care. Medications taken within 30 days of randomization and all concomitant therapy must be recorded in the source documents and transcribed onto the appropriate pages of the eCRF, including details of the reason for use as well as the administered dose, route and treatment duration.

#### *8.3.1. Prohibited concomitant medication*

- Once daily medications in patients with orocaecal transit time greater than 10 hours.
- Medications in which the dosing schedule is three times per day or greater.
- Antiviral therapy for hepatitis C.

#### *8.3.2. Timing of allowed concomitant medications*

If patients are taking any medicines during the treatment period, they must not take them at the same time as Yaq-001/ placebo:

- Morning doses of any medications should be taken at least 10 hrs after Yaq-001/ placebo.
- Evening doses of medications should be taken at least 4 hrs before Yaq-001/ placebo.

### 8.4. Precautions, contraindications and warnings

Adverse events should be treated in accordance with standard medical practice.

At each scheduled visit, patients will be evaluated to determine if there are any clinically significant changes from baseline and from the previous visit. Patients with significant findings must remain under close follow-up as clinically indicated. Any patient who develops an AE will be followed up until a probable diagnosis and the relationship to study device have been determined, and until the AE has resolved, no longer requires further follow-up, or a new chronic baseline condition has been established.

Upon completion of the study, sole medical care will be transferred to the responsible physician. The exception to this will be management of a SADE resulting from the study until clinical resolution.

## 9. STUDY PROCEDURES BY VISIT

No study procedures may be initiated prior to the patient signing the informed consent in compliance with the principles of the GCP.

A summary of visits and procedures is found in the schedule of visits & procedures provided in the section 2. The procedures and evaluations at each of the visits are outlined below. Additional details of study procedures are provided in section 10.

### 9.1. Screening visit (up to 14 days before randomization)

Prior to any study procedure, potential patients will be fully informed about the study (see section 7.3.1). Patients who wish to participate in the study must read and understand the patient information sheet and sign and personally date the ICF after the investigator or investigator staff has answered all questions to the candidate's satisfaction. Screening procedures can begin only after the ICF has been signed.

Screening will occur within up 14 days before administration of investigational product. Potential study patients will be evaluated for entry into the study according to the stated inclusion and exclusion criteria. Patients who drop out during screening phase prior to randomization (identified as not eligible) will be considered screening failure. In this case, the reason for screening failure will be documented on patient's medical records, eCRF and the log of patients included.

The following procedures will be performed at screening visit:

- Signed, written informed consent
- Demographic information including gender, age
- Medical history
- Child-Pugh classification
- Medication history over the past 30 days, including prescription and non-prescription OTC medications, herbal mediations, vitamins and minerals.
- Physical examination, including calculation of BMI
- Vital signs
- 12-lead ECG
- Local lab: blood samples for haematology, coagulation and clinical chemistry (fasting) and urine sample. All results should be available and reviewed prior to randomization. The time of review must be noted in the source to assure review occurred prior to randomization
- MELD score
- Review inclusion and exclusion criteria
- Orocaecal transit time test: result should be available and reviewed prior to randomization. The time of review must be noted in the source to assure review occurred prior to randomization

### 9.2. Randomization visit (day 1)

The following evaluations will be conducted on day 1:

- Review medical history
- Review of concomitant medications
- Physical examination
- Vital signs
- 12-lead ECG
- Local lab: samples for haematology, coagulation and clinical chemistry (fasting) and urine sample
- Core labs: samples collection
- EAA
- Child-Pugh classification
- MELD score

- Nutritional assessment: dietary intake, anthropometric validation and global assessment (RFH-GA)
- Confirm eligibility
- Randomization
- Investigational product dispensation
- Instruct the patient on how to take the investigational product starting the same day of the visit at night

### 9.3. 1-week visit (day 7 ± 3 days)

The following evaluations will be conducted on day 7:

- Update of medical history, including any changes in medical status since the previous visit (AEs)
- Update of concomitant medications
- Physical examination
- Vital signs
- 12-lead ECG
- Local lab: samples for haematology, coagulation and clinical chemistry (fasting) and urine sample
- Child-Pugh classification
- MELD score
- EAA
- Nutritional assessment: dietary intake, anthropometric validation and global assessment (RFH-GA)
- Accountability of investigational product
- Assessment of patient's compliance
- Dispensation of investigational product

### 9.4. 4-week visit (day 28 ± 5 days)

The following evaluations will be conducted on day 28:

- Update of medical history, including any changes in medical status since the previous visit (AEs)
- Update of concomitant medications
- Physical examination
- Vital signs
- 12-lead ECG
- Local lab: samples for haematology, coagulation and clinical chemistry (fasting) and urine sample
- Core labs: samples collection
- Child-Pugh classification
- MELD score
- EAA
- Nutritional assessment: dietary intake, anthropometric validation and global assessment (RFH-GA)
- Accountability of investigational product
- Assessment of patient's compliance
- Dispensation of investigational product

### 9.5. 8-week visit (day 56 ± 5 days)

The following evaluations will be conducted on day 56:

- Update of medical history, including any changes in medical status since the previous visit (AEs)
- Update of concomitant medications
- Physical examination

- Vital signs
- 12-lead ECG
- Local lab: samples for haematology, coagulation and clinical chemistry (fasting) and urine sample
- Child-Pugh classification
- MELD score
- EAA
- Nutritional assessment: dietary intake, anthropometric validation and global assessment (RFH-GA)
- Accountability of investigational product
- Assessment of patient's compliance
- Dispensation of investigational product

### 9.6. 12-week visit, end of treatment (day 84 ± 5 days) or withdrawal visit

The following evaluations will be conducted on day 84 (or at any time if the patient is prematurely withdrawn from the study):

- Update of medical history, including any changes in medical status since the previous visit (AEs)
- Update of concomitant medications
- Physical examination
- Vital signs
- 12-lead ECG
- Local lab: samples for haematology, coagulation and clinical chemistry (fasting) and urine sample
- Core labs: samples collection
- Child-Pugh classification
- MELD score
- EAA
- Nutritional assessment: dietary intake, anthropometric validation and global assessment (RFH-GA)
- Accountability of investigational product
- Assessment of patient's compliance

### 9.7. Termination visit (7 days after end of treatment)

The following evaluations will be conducted at termination visit:

- Update of medical history, including any changes in medical status since the previous visit (AEs)
- Update of concomitant medications
- Physical examination
- Vital signs

### 9.8. Priority of timing of study evaluations

When multiple evaluations are scheduled in the same visit, the priority of each will be as follows:

- Vital signs (BP and pulse rate) should be measured before blood samples are collected
- Blood for clinical laboratory tests (haematology and clinical chemistry) should be collected at the same time that blood samples for additional tests to be analysed in the core labs.
- Physical examination can be conducted either before or after other evaluations.

# 10. DETAILS OF STUDY PROCEDURES

The procedures conducted during the study are outlined in this section and summarized in section 2. Study procedures by study visit are provided in section 9.

## 10.1. Demographic information

- Gender
- Age

## 10.2. Child-Pugh classification

| Parameter | Points assigned | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| Ascites | Absent | Slight | Moderate |
| Bilirubin, mg/dL | ≤ 2 | 2 – 3 | > 3 |
| Albumin, g/dL | > 3.5 | 2.8 – 3.5 | < 2.8 |
| Prothrombin time<br><br>* Seconds over control<br>* INR | <br><br>1 – 3<br>< 1.8 | <br><br>4 – 6<br>1.8 – 2.3 | <br><br>> 6<br>> 2.3 |
| Encephalopathy (according to West Haven criteria for assessment of hepatic encephalopathy) | None | Grade 1 – 2 | Grade 3 – 4 |

A total score of 5-6 is considered grade A (well-compensated disease); 7-9 is grade B (significant functional compromise); and 10-15 is grade C (decompensated disease). These grades correlate with one- and two-year patient survival.

| Grade | Points | One-year patient survival (%) | Two-year patient survival (%) |
|---|---|---|---|
| A: well-compensated disease | 5 – 6 | 100 | 85 |

| | | | |
|---|---|---|---|
| B: significant functional compromise | 7 – 9 | 80 | 60 |
| C: decompensated disease | 10 – 15 | 45 | 35 |

**West Haven Criteria for assessment of hepatic encephalopathy**

- **Grade 1** - Trivial lack of awareness; euphoria or anxiety; shortened attention span; impaired performance of addition or subtraction
- **Grade 2** - Lethargy or apathy; minimal disorientation for time or place; subtle personality change; inappropriate behaviour
- **Grade 3** - Somnolence to semi-stupor, but responsive to verbal stimuli; confusion; gross disorientation
- **Grade 4** - Coma (unresponsive to verbal or noxious stimuli)

### 10.3. Orocaecal transit time

Standardised radio-opaque markers such as the SITZMARKS capsules will be used to determine orocaecal transit time. Patients will ingest a single capsule at midnight prior to investigation. A single plain abdominal X-ray will be taken at 10:00 am the following day to assess the extent of elimination of the radio-opaque markers. Patients with < 20% retention of radio-opaque markers proximal to the ileocaecal valve will be eligible for the study.

If the result from the above exam is indeterminate, a second assessment will be performed. This will either be Cine-MRI or smart pill method, depending on the local availability. With both methods, the data will be transmitted remotely and analysed by a single operator in the UCL within a maximum 5-day turnaround time.

**Cine-MRI with gadolinium contrast – GIQUANT**

The dynamic series can be acquired on any modern MRI scanner providing an excellent physiological view of the small bowel motility and orocaecal transit time. MRI is safe, well tolerated by patients and non-invasive making it an excellent modality for physiological investigations of the bowel. The patient will swallow a contrast pill and undergo a 20 min MRI scan 10 hrs later.

**Smart Pill**

The Smart Pill GI Monitoring System measures whole gut and regional gut (stomach, small bowel and colon) transit times and thus provide a measure of orocaecal transit time. The smart pill is swallowed by the patient and data collected from a data receiver attached to the patient. This method will be used to assess orocaecal transit time in instances in which MRI is unavailable or contraindicated.

### 10.4. Physical examination

Physical examination will be performed by medical qualified personnel. Documentation of and findings from the physical examination must be part of the source documents available at investigational site.

New abnormal findings will be recorded as AEs on medical records and eCRF if judge clinically relevant by the investigator. In the event that clinically relevant findings are observed in the physical examination at the end of the treatment, the patient should be followed by the investigator until resolution of the event, or an agreement has been made with the sponsor that follow-up is sufficient on that patient.

At each study visit, physical examination includes:

- Height (only at screening)
- Weight. BMI should be calculated at screening in order to check the exclusion criteria no. 8 using the following formula:

$$BMI = \frac{Weight\ (Kg)}{Height^2\ (m^2)}$$

- General appearance
- Head
- Eyes
- Ears
- Nose
- Throat
- Neck
- Skin
- Cardiovascular system
- Respiratory system
- Abdominal system
- Nervous system

### 10.5. Vital signs

Vital signs will be measured at each study visit, including:

- Supine blood pressure (systolic and diastolic) and radial pulse rate will be evaluated after at least 5 minutes' rest
- Also, BP (systolic and diastolic) and pulse rate will be evaluated after 2 min of standing still (after the supine measurement) in order to assess for postural hypotension
- Body temperature oral or aural (to be the same throughout the study)
- Respiratory rate

Measurements will be made in a quiet environment at a comfortable temperature.

### 10.6. 12-lead ECG

A 12-lead safety ECG will be recorded for at least 1 min in the supine position. A qualified physician will review the ECG.

Blinded ECG tracing will be scanned and uploaded to the eCRF. ECG intervals/ durations (RR, PR, QT, QTcF, and QTcB intervals and QRS duration) will be measured for individual patients and entered in the corresponding page of the eCRF.

### 10.7. Clinical laboratory tests

#### *10.7.1. Local laboratory*

Haematology (including coagulation), clinical chemistry and urinalysis testing as shown in Table 1 will be evaluated throughout the study to assess the safety and tolerability of the study product at each study visit.

Laboratory samples will be requested and drawn per site requirements and the analysis run at the local laboratory of the participating centres.



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Laboratory normal ranges, certifications and licenses will be provided to the sponsor prior the first patient being included. Laboratory normal ranges of each investigational centre will be sent to DMC and entered into the eCRF.

| | **Parameters** |
|---|---|
| **Haematology**<br>(EDTA blood) | ▪ Haemoglobin<br>▪ Haematocrit (HCT)<br>▪ Mean corpuscular volume (MCV)<br>▪ White blood cell (WBC) total count<br>▪ WBC absolute differential count: neutrophils, lymphocytes, monocytes, eosinophils, basophils<br>▪ Platelet count<br>▪ Erythrocyte sedimentation rate (ESR) |
| **Coagulation**<br>(plasma) | ▪ Activated partial thromboplastin time (APTT)<br>▪ Prothrombin time (PT) reported as INR (International Normalized Ratio)<br>▪ Fibrinogen |
| **Clinical chemistry**<br>(plasma) | ▪ Renal function:<br>o Sodium<br>o Potassium<br>o Urea<br>o Creatinine; creatinine clearance will be estimated from plasma creatinine using the Cockcroft-Gault equation<br>▪ Liver function:<br>o Alanine aminotransferase (ALT)<br>o Aspartate aminotransferase (AST)<br>o Total bilirubin, conjugated (direct) bilirubin, unconjugated (indirect) bilirubin<br>o Alkaline phosphatase<br>o Gamma-glutamyltransferase (GGT)<br>o Lactate dehydrogenase (LDH)<br>o Albumin<br>▪ Other:<br>o Total protein<br>o Calcium<br>o Inorganic phosphate<br>o Uric acid<br>o Lactate<br>o Vitamin B9<br>o Vitamin B12<br>o Vitamin D<br>o C-reactive protein (CRP)<br>o Cortisol |

| | |
|---|---|
| **Urinalysis**<br>(first morning urine) | ▪ Blood<br>▪ Protein<br>▪ Glucose<br>▪ Ketones<br>▪ Leukocyte esterase<br>▪ pH (by dipstick)<br>▪ Microscopic examination of the sediment if blood, protein and/or leukocyte esterase are positive in dipstick. |

**Table 1** Local Laboratory Tests

### *10.7.2. Core labs*

The sponsor will provide the required materials for processing the samples to be sent to the core labs and will also provide instructions for obtaining, processing, storage and shipment of samples.

| | **Parameters and function assessment**<br>Keys for assessment of function:<br>**L** = measure of liver injury/function<br>**K** = measure of kidney injury/function<br>**B** = measure of cerebral function<br>**I** = Measure of Intestinal function<br>**Imm** = Measure of Immune function |
|---|---|
| **Plasma** | ▪ Zinc (Zn) - **(I)**<br>▪ Copper (Cu) - **(I)**<br>▪ Selenium (Se) - **(I)**<br>▪ sCD14 – **(Imm)**<br>▪ Lipopolysaccharide (LPS) - **(I)**, *LAL assay*<br>▪ Intestinal-fatty-acid-binding protein (IFABP) - **(I)**<br>▪ Bactericidal permeability increasing protein (BPI) - **(I)**<br>▪ Cytokine/ chemokine profiling – **(Imm)**<br>▪ ICAM-1 – **(Imm)**<br>▪ VCAM - **(L)**<br>▪ ADMA - **(L)**<br>▪ SDMA - **(L)**<br>▪ IMA - **(L)**<br>▪ NPY - **(I)**<br>▪ Albumin function - **(L)** |
| **Serum** | ▪ Vitamin B1 - **(I)**<br>▪ Vitamin B2 - **(I)**<br>▪ Vitamin B3 - **(I)**<br>▪ Vitamin B6 - **(I)**<br>▪ Vitamin A - **(I)**<br>▪ Vitamin E - **(I)**<br>▪ Vitamin K - **(I)**<br>▪ Cytokeratin-18 (CK-18 M30/M65) - **(L)**<br>▪ Serum bacterial DNA - **(I)** |

| | |
|---|---|
| | ▪ Lipopolysaccharide binding protein (LBP) - **(I)**<br>▪ sCD163 (cytokine/ chemokine profiling) - **(Imm)** |
| **Stool** | ▪ Metabolomic profiling - **(I)**, *1-NMR spectroscopy*<br>▪ Microbiome profiling - **(I)**, *next generation DNA sequencing*<br>▪ Bile acid profiling - **(I, L)**, *liquid-chromatography-mass spectroscopy*<br>▪ Albumin function - **(L)** |
| **Urine**<br>**(morning urine and 5h-urine volume)** | ▪ Metabolomic profiling - **(I)**, *1-NMR spectroscopy*<br>▪ Bile acid profiling - **(I, L)**, *liquid-chromatography-mass spectroscopy*<br>▪ Lactulose - **(I)**<br>▪ Rhamnose - **(I)**<br>▪ Xylose - **(I)**<br>▪ uNGAL - **(K)**<br>▪ TFF3 - **(K)**<br>▪ u-LFABP - **(K)**<br>▪ u-IL18 - **(K)**<br>▪ u-TLR4 - **(K)**<br>▪ GST-p - **(K)** |

**Table 2 -** Core Lab Tests

A total of 20 ml of blood, 9 ml of morning urine, 5-hour urine volume and a sample of stool will be required for the above assays. After collecting the morning urine, the patient should drink a sugary drink which will be provided by the investigator. All the urine the patient passes in the following 5 hours should be collected in a special container provided by the investigator and brought to the visit.

### 10.8. Endotoxin activity assay

The EAA uses a highly specific IgM antibody for the most preserved structural component of endotoxin, Lipid-A. This allows the EAA specifically measure endotoxin activity in human whole blood. The EAA is a rapid assay (approximately 30 min from sample to result).

### 10.9. MELD score

The model for end stage liver disease (MELD) score is a disease severity scoring system applied to adult liver patients calculated using a formula that relies on three objective variables:

- Serum creatinine (Scr; mg/dL)
- Total bilirubin (Tbil; mg/dL)
- INR

The following rules must be observed when using this formula:

- 1 is the minimum acceptable value for any of the three variables
- The maximum acceptable value for serum creatinine is 4
- The maximum value for the MELD score is 40

Formula for the MELD score

MELD score = 10 {0.957 Ln (Scr) + 0.378 Ln (Tbil) + 1.12 Ln (INR) + 0.643}

The MELD score will be automatically calculated by the eCRF.

### 10.10. Clinical assessment of the nutritional status

Assessment of the nutritional status will be performed by the RFH-GA (Morgan 2006), including:

#### *10.10.1. Dietary intake*

Appetite will be assessed as good, fair or poor. Recent dietary intake will be assessed from dietary history. Details of dietary restrictions and nutritional support will be recorded and used to provide an overall impression of the adequacy of the diet in relation to estimated daily requirements, assessed using Schofield's modification nutritional assessment: dietary intake, anthropometric validation and global assessment (RFH-GA) of the Harris-Benedict equation. Intakes will be categorized as adequate if the met estimated requirements, inadequate if they failed to meet estimated requirements but exceeded 500 kcal/d or negligible if they provided fewer than 500 kcal/d.

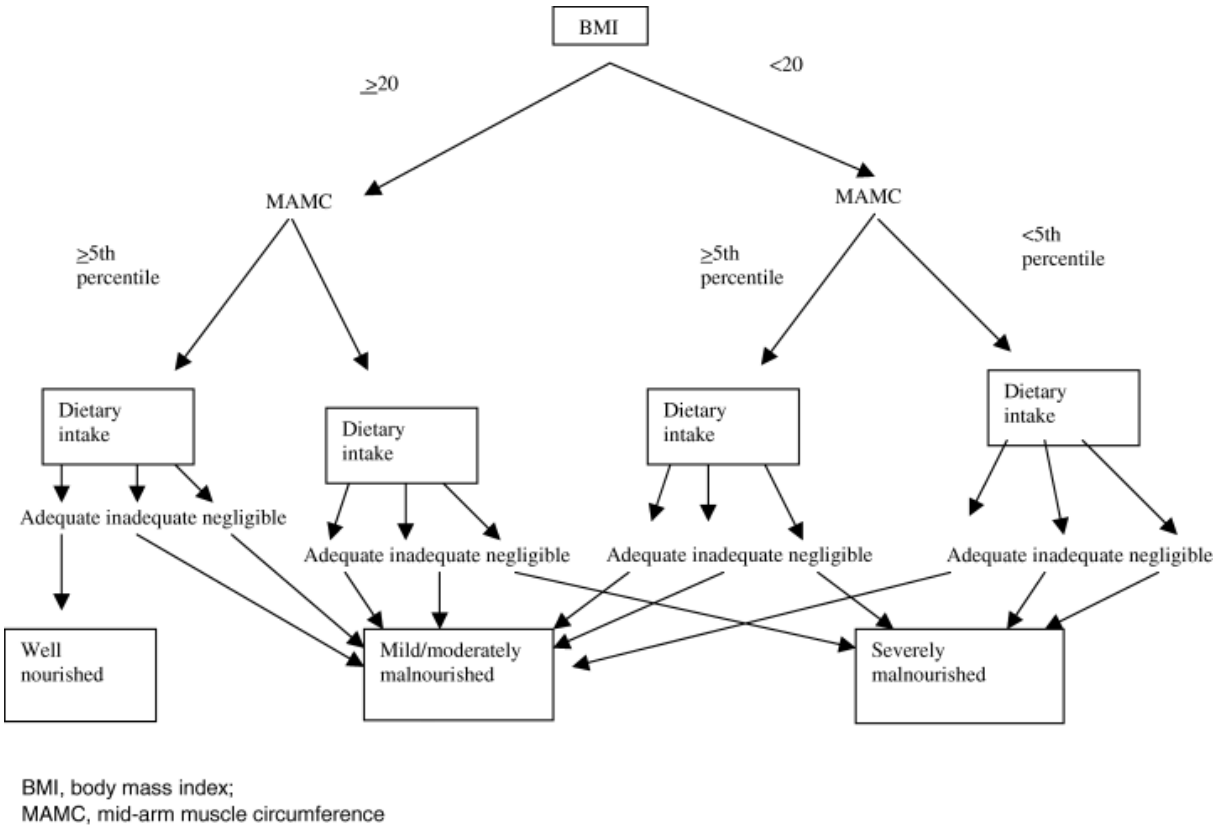
#### *10.10.2. Anthropometric validation*

An estimated dry weight will be determined by deducting a weight for ascites and/ or oedema from the measured weight. BMI will be calculated for the estimated dry weight and height. MAC and TSF will be measured on the non-dominant side of the body. MAMC will then be calculated and MACM and TSF measurements compared with published standards, expressed both in relation to the 5th percentile and as a percentage of the 50th percentile (relative MAMC) for the appropriate age and gender category.

#### *10.10.3. Global assessment*

The patients will be categorized on the basis of the collected data according global assessment scheme as:

(1) Adequately nourished;
(2) Moderately malnourished (or suspected to be); or
(3) Severely malnourished.




BMI, body mass index;
MAMC, mid-arm muscle circumference

**Figure 4:** Royal Free Hospital – Subjective Global Assessment (RFG-SGA)

# 11. ADVERSE EVENTS, SERIOUS ADVERSE EVENTS: RECORDING AND REPORTING

## 11.1. Definitions from MEDDEV 2.7/3 in line with ISO14155 (Clinical Investigations: Serious Adverse Events Reporting under Directives 90/385/EEC and 93/42/EEC)

### *11.1.1. Investigational medical device*

Medical device being assessed for safety or performance in a clinical investigation

### *11.1.2. Adverse event (AE)*

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including any abnormal laboratory finding) in subjects, users or other persons **whether or not related to the investigational medical device**.

NOTE 1: This definition includes events related to the investigational device or the comparator

NOTE 2: This definition includes events related to the procedures involved

NOTE 3: For users or other persons this is restricted to the events related to the investigational medical devices

### *11.1.3. Serious adverse event (SAE)*

Adverse event that led to

a) Death.
b) A serious deterioration in health of the subject, that either resulted in:
    1) A life-threatening illness or injury, or
    2) A permanent impairment of a body structure or a body function, or
    3) In-patient hospitalization or prolongation of existing hospitalization, or
    4) Medical or surgical intervention to prevent life threatening illness or
    5) Injury or permanent impairment to a body structure or a body function.
c) Foetal distress, foetal death or a congenital abnormality or birth defect.

NOTE 1: SAEs include device deficiencies that might have led to a serious adverse event if a suitable action had not been taken or an intervention had not been made.

NOTE 2: Planned hospitalization for pre-existing condition, or a procedure required by the clinical investigation plan, without a serious deterioration in health, is not considered a serious adverse event.

#### *11.1.4. Device deficiency*

Inadequacy of an investigational medical device related to its identity, quality, durability, reliability, safety or performance. This may include malfunctions, use error, or inadequacy in the information supplied by the manufacturer.

#### *11.1.5. Adverse device effect (ADE)*

Adverse event related to the use of an investigational medical device.

NOTE 1: This includes any adverse event resulting from insufficiencies or inadequacies in the instructions for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational medical device.

NOTE 2: This includes any event that is a result of a use error or intentional abnormal use of the investigational medical device.

#### *11.1.6. Serious adverse device effect (SADE)*

Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event

#### *11.1.7. Unanticipated serious adverse device effect (USADE)*

Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report

NOTE: anticipated SADE is an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report.

### 11.2. Collecting and recording of adverse events by clinical investigator

The investigators will monitor the occurrence of adverse events for each patient during the course of the clinical investigation.

All adverse events (AEs) reported by the subject, observed by the investigator or documented in the medical records will be recorded on the respective eCRF page, whether believed by the investigator to be related or unrelated to the investigational product.

Any relevant change in safety parameters (vital signs, laboratory values or ECG parameters) should be reported as AE. Patients will be asked at study visits about the occurrence of any adverse events.

AEs must be reported from the date the patient is included (signature of informed consent) to the completion of the study (termination visit) and will be monitored through the course of the clinical investigation. Additional information related to a previously reported AE should be updated in the appropriate eCRF page.

All AEs will be graded according to the criteria in section 11.6. Additionally, the investigator will determine the relationship of the AE to the administration of the investigational device as described in the section 11.7.

### 11.3. Serious adverse event reporting

#### *11.3.1. Reportable events*

- Any SAE, as defined in section 11.1
- Any device deficiency, as defined in section 11.1, that might have led to a SAE if:
  (a) Suitable action had not been taken or
  (b) Intervention had not been made or
  (c) If circumstances had been less fortunate
- New findings/ updates in relation to already reported events.

#### *11.3.2. Reporting by the investigator to the sponsor*

Any reportable event that occurs during this clinical investigation must be reported to the sponsor's medical monitor via eCRF immediately (within 24 hours after investigational site personnel's awareness of the event). The date the site staff become aware of the reportable event must be recorded in the source documents.

Notification of the SAEs to the sponsor will be performed via eCRF. If there is a technical failure of the eCRF, other modalities such an email of faxed transmission may be used until such a time that the eCRF system is functional. However, the SAE or device deficiency should be entered in the eCRF as soon as the system is available.

Follow up information (new findings or updates to already reported events) must be submitted within 24 hours after investigational site personnel's awareness by updating the appropriate eCRF with any new information collected (or by faxing or e-mailing the updated SAE reporting forms to the medical monitor).

The medical monitor is available to provide medical guidance regarding SAEs. Contact information for the medical monitor is as follows:

Dr Jane Macnaughtan

Mobile 24h: +44 07973 145918

Phone: +44 207 433 2795

Fax: +44 207 433 2775

E-mail address: jane.macnaughtan@yaqrit.com

### 11.4. SAEs: reporting to Ethics Committee

Any submission of reportable events to the sponsor must also be reported to the clinical site's EC, according to the governing rules of the EC. It is the investigator's responsibility to be informed of the EC's requirements for reporting all reportable events.

### 11.5. Expected adverse events

Given the underlying disease state for the study population for this trial, there are multiple expected adverse events.

The following events are considered expected adverse events. The list given below is intended to clarify the most appropriate categorization of the event for reporting purposes:

| *Category* | *Adverse event* |
|---|---|
| ***Hepatobiliary disorders*** | ▪ Acute on chronic liver failure and decompensated cirrhosis<br>▪ Variceal haemorrhage<br>▪ Spontaneous bacterial peritonitis<br>▪ Hepatorenal syndrome<br>▪ Ascites<br>▪ Hepatic hydrothorax<br>▪ Hepatic encephalopathy<br>▪ Jaundice<br>▪ Oedema<br>▪ Bruising |
| ***Renal and urinary disorders*** | ▪ Pre-renal failure |
| ***Cardiac disorders*** | ▪ Cardiovascular insufficiency<br>▪ Hypotension |
| ***Respiratory, thoracic and mediastinal disorders*** | ▪ Hepatopulmonary syndrome<br>▪ Portopulmonary hypertension |
| ***Infections and infestations*** | ▪ Increased risk of infection<br>▪ Bacteraemia<br>▪ Fungaemia |
| ***Endocrine disorders*** | ▪ Osteoporosis/ Pathological fractures<br>▪ Hypoadrenalism<br>▪ Infertility<br>▪ Gynaecomastia<br>▪ Hair loss<br>▪ Amenorrhoea |
| ***Investigations*** | ▪ Anaemia<br>▪ Thrombocytopaenia<br>▪ Leucopaenia<br>▪ Coagulopathy<br>▪ Elevated aminotransferases<br>▪ Elevated GGT<br>▪ Hypoalbuminaemia<br>▪ Hyperammonaemia<br>▪ Hyperlactatemia |
| ***Metabolism and nutrition disorders*** | ▪ Abnormalities of serum sodium, potassium, calcium, magnesium, phosphate |

| | |
|---|---|
| ***Nervous system disorders*** | ▪ Peripheral neuropathy<br>▪ Autonomic neuropathy<br>▪ Headache |
| ***Neoplasms benign, malignant and unspecified (including cysts and polyps)*** | ▪ Hepatocellular carcinoma (HCC)<br>▪ All non-HCC cancer |
| ***Multiple organ failure*** | ▪ Death may occur as an outcome of the complications of the underlying disease state |
| ***Expected device – related adverse events: gastrointestinal disorders*** | ▪ Nausea<br>▪ Vomiting<br>▪ Constipation<br>▪ Diarrhoea |

### 11.6. Severity grading for adverse events

The investigator will assess all AEs according to the criteria described below. The severity of an event should be graded into one of the three classes which describe the clinical severity of the event as it occurred.

The three classes of severity are:

- Grade 1 (Mild): does not interfere with daily living and no medications are needed to relieve the event
- Grade 2 (Moderate): somewhat interferes with daily living or medications needed to relieve event
- Grade 3 (Severe): incapacitating, activities of daily living are prohibited because of the event

### 11.7. Causality assessment

The causality assessment of the AEs will be conducted following the guideline on medical devices MEDDEV 2.7/3 entitled "Guidelines on medical devices – Clinical investigations: SAE reporting under Directives 90/385/EEC and 93/42/EEC.

The relationship between the use of the investigational device (including the medical – surgical procedure) and the occurrence of each AE shall be assessed and categorized. During causality assessment activity, clinical judgement shall be used and the relevant documents, such as the investigator's brochure, the CIP or the risk analysis report shall be consulted, as all the foreseeable SAEs and the potential risks are listed and assessed there. The presence of confounding factors, such as concomitant medication/ treatment, the natural history of underlying disease, other concurrent illness or risks factors shall also be considered.

The above considerations apply also to the SAEs occurring in the comparison group.

For the purpose of harmonising reports, each SAE will be classified according to five different levels of causality. The sponsor and the investigators will use the following definitions to assess the relationship of the SAEs to the investigational medical device or procedures:

**(1) Not related**

Relationship to the device can be excluded when:

- The event is not a known side effect of the product category the device belongs to or of similar devices and procedures
- The event has no temporal relationship with the use of the investigational device or the procedures

- The SAE does not follow a known response pattern to the medical device (if response pattern is previously known) and is biologically implausible
- The discontinuation of medical device application or the reduction of the level of activation/ exposure – when clinically feasible – and reintroduction of its use (or increase of the level of activation/ exposure) do not impact on the serious event
- The event involves a body-site or an organ not expected to be affected by the device or procedure
- The serious event can be attributed to another cause (e.g. an underlying or concurrent illness/ clinical condition, an effect of another device, drug, treatment or other risks factors)
- The event does not depend on a false result given by the investigational device used for diagnosis when applicable
- Harms of the subject are not clearly due to use error
- In order to stablish the non-relatedness, not all criteria listed above might be met at the same time, depending on the type of device/ procedures and the serious event

**(2) Unlikely**

The relationship with the use of the device seems not relevant and/ or the event can be reasonably explained by another cause, but additional information may be obtained.

**(3) Possible**

The relationship with the use of the investigational device is weak but cannot be ruled out completely. Alternative causes are also possible (e.g. an underlying or concu*rrent* illness/ clinical condition or/ and an effect of another device, drug or treatment). Cases were relatedness cannot be assessed or no information has been obtained should also be classified as possible.

**(4) Probable**

The relationship with the use of the investigational device seems relevant and/ or the event cannot reasonably explained by another cause, but additional information may be obtained.

**(5) Causal relationship**

The serious event is associated with the investigational device or with the procedures beyond reasonable doubt when:

- The event is a known side effect of the product category the device belongs to or of similar devices and procedures
- The event has a temporal relationship with the investigational device use/ application or procedures
- The event involves a body-site or organ that
    - The investigational device or procedures are applied to
    - The investigational device or procedures have an effect on
- The serious event follows a known response pattern to the medical device (if the response pattern is previously known)
- The discontinuation of medical device application (or reduction of the level of activation/ exposure) and the reintroduction of its use (or increase of the level of activation/ exposure) impact on the serious event (when clinically feasible)
- Other possible causes (e.g. an underlying or concurrent illness/ clinical condition or/ and an effect of another device, drug or treatment) have been adequately ruled out
- Harm to the subject is due to error in use
- The event depends on a false result given by the investigational device used for diagnosis, when applicable
- In order to stablish the relatedness, not all the criteria listed above might be met at the same time, depending on the type of device/ procedures and the serious event.



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The sponsor and the investigators will distinguish between the SAEs related to the investigational device and those related to the procedures (any procedure specific to the clinical investigation). An AE can be related both to procedures and investigational device. Complications of procedures are considered not related if the said procedures would have been applied to the patients also in absence of the investigational device use/ application.

In some particular cases, the event may be not adequately assessed because information is insufficient or contradictory and/ or the data cannot be verified or supplemented. The sponsor and the investigators will make the maximum effort to define and categorize the event and avoid these situations. Where the sponsor remains uncertain about classifying the serious event, it should not exclude the relatedness and classify the event as "possible".

Particular attention shall be given to the causality evaluation of unanticipated serious adverse (device) events. The occurrence of unanticipated events related to the use of the device (USADE) could suggest that the clinical investigation places subjects at increased risk of harm that was to be expected beforehand.

### 11.8. Recording clinical laboratory abnormalities and other abnormal assessments as adverse events

Any laboratory abnormality that is considered by the investigator to be clinically significant for the disease population being studied will be recorded as an AE on the appropriate eCRF page if:

- It is not associated with an already reported AE, diagnosis or pre-existing condition;
- There is a change in concomitant medication in direct response to the abnormal laboratory result.

Any laboratory abnormality assessed by the investigator as meeting the regulatory definition of a serious adverse event will be reported as an SAE following protocol requirements (see section 11.3).

### 11.9. Intercurrent illness and pre-existing diseases

Intercurrent illness, including signs/ symptoms of a pre-existing disease state (medical history), that are present at or before investigational product administration (including those that manifest between signature of the informed consent and administration of investigational product) are to be recorded as medical history/ pre-existing conditions. Those events that manifest with the same severity, frequency, or duration subsequent to investigational product administration need not be recorded as AEs. However, incidents for which there is an increase in severity or duration of the intercurrent illness or pre-existing disease must be reported on the AE eCRF page. Pre-existing conditions should be reported on the appropriate eCRF page as medical history.

### 11.10. Data safety monitoring board

The role of the DSMB in overseeing the safety profile of the investigational medical device is discussed in sections 3.4.2, 6.1 and 6.2. The DSMB will review the safety data after all patients in cohort 1 have completed one month of therapy. The results of the 1-month therapy in cohort 1 will be used to determine the safety of dose escalation for cohort 2. Safety data will also be reviewed by the DSMB for:

- Cohort 1 at month 3.
- Cohort 2 at month 1.
- Cohort 2 at month 3.

See Figure 2: **Carbalive Safety Study Algorithm**.

In addition, details regarding the process to be utilized by the DSMB are presented in the DSMB charter.

# 12. STATISTICAL METHODS

## 12.1. Sample size

56 patients (28 patients per cohort) will be included in this clinical investigation. Since this is the first-in-human study to evaluate the safety and tolerability of Yaq-001, this sample size is considered to meet the study objectives.

## 12.2. Structure and methodology of statistical analysis

All statistical analyses of study data will be carried out by the Data Centre of the EF-CLIF Consortium.

A statistical analysis plan (SAP) including the list of all tables, listings and graphs will be issued before the study database lock. The sponsor's medical expert will explicitly approve this SAP and any further amendments.

SAS v 9.3 or later will be the statistical software used to analyse the data sets. A complete set of raw data listings will be appended to the final study report. All tables, figures and listings will be presented in word and pdf formats without any manual editing, i.e. they will appear as they have been programmed using the statistical package.

## 12.3. Analysis populations

There will be two analysis populations for this trial:

- The safety population (SP) will include all the enrolled (randomized to treatment) patients who take at least one dose of the investigational product.
- The per-protocol population (PP) will include all the patients who satisfied the eligibility criteria, took at least 70% of the treatment and did not present major protocol non-compliances. The precise reasons for excluding patients form the PP will be fully defined and documented in the Data Review Meeting Report.

The SP will be the main study population. All the statistical analyses of laboratory tests and additional parameters will be carried out also on the PP.

## 12.4. Blind data review

No interim analyses are planned for this study; however, two different reviews of blind data will be carried out by the DMC:

(1) A blind data review meeting (BDRM) will be held at the end of the study and before opening the randomization code, in order to assign the study patients to each analysis population. The DMC will summarise the blind information regarding each patient's compliance with eligibility criteria and treatment administration and all protocol non-compliances. This information will be reviewed by the international project coordinator, a representative of the sponsor, a representative of the CRO in charge of study monitoring, the study statistician and the data manager. A BDRM report will be issued with the final assignment of study patients to each analysis population and a detailed justification for each exclusion

(2) A blind data review of all those data needed to evaluate the safety and tolerability of study product will be carried out by the study statistician at DMC. For each patient, all AE's and laboratory abnormalities, laboratory results, vital signs, ECG findings and physical examination will be listed together with the corresponding changes for baseline (when needed). These blinded listings will be sent to the DSMB to evaluate the safety and tolerability of the investigational device according to the stopping rules defined in this study protocol (see section 6.3). After each meeting, the DSMB will issue a report including the results

of this evaluation and the corresponding recommendation to continue or to stop the study. There will be 4 of these DSMB meetings (see section 11.10).

## 12.5. Statistical analysis

### *12.5.1. Descriptive statistics*

Categorical variable will be summarized by means of counts and percentages. Continuous variable will be summarized by means of the number of non-missing observations, the mean and its 95% confidence interval, the standard deviation (SD), the median and the corresponding inter-quartile range, the minimum and the maximum of each variable distribution.

Where applicable, these summaries will be reported by treatment and visit.

### *12.5.2. Demographics and baseline characteristics*

All demographic and baseline characteristics (age at screening, gender, weight, height, BMI, medical history, physical examination, prior medications) will be listed for individual patients and summarized using the appropriate summary statistics.

### *12.5.3. Analysis populations*

Patient's compliance with eligibility criteria and treatment administration, major protocol non-compliances, patient's withdrawals and the reason of withdrawal (e.g. AE, protocol non-compliance, requirement for an unacceptable concomitant medication, lost to follow up, failed to return, consent withdrawal and other reasons) and assignment to each analysis population will be summarized by means of the appropriate statistics.

### *12.5.4. Prior and concomitants medications*

All prior and concomitant medications will be assigned a generic name and a drug class based on the WHO dictionary. Coding will be performed by the sponsor's medical monitor or designee. Prior and concomitant medications will be listed and summarized by means of the appropriate statistics.

### *12.5.5. Safety and tolerability variables*

The following variables will be analysed to assess the safety and tolerability of the investigational device:

**Adverse events**

Adverse events will be classified into standard terminology using the MedDRA. Coding will be performed by the sponsor's medical monitor or designee. Adverse events will be listed by reported verbatim term and MedDRA-preferred term, start and stop date and time, study day, duration, severity, relationship with study device and seriousness. Additionally, data may be grouped for analysis by different levels of the MedDRA hierarchy. The incidence of AEs, the incidence of treatment-related AEs, and the severity of AEs will be tabulated by last treatment and visit.

The number and percentage of patients with SAEs and treatment-related SAEs and patients who withdraw due to an AE will be tabulated by treatment and visit.

**Clinical laboratory tests**

Lab parameters will be listed for individual patients. Baseline for clinical laboratory parameters will be defined as the last evaluation before dosing with investigational product (this will be on Day 1). Summary statistics for each lab parameter and the corresponding changes from baseline will be calculated for each parameter except

urinalysis and summarized by study treatment; urinalysis results will be listed but not summarized. Each clinical laboratory test result will be classified according to the normal reference range provided by the clinical laboratory. The number of patients showing clinically significant deviations from normal ranges will be summarized by study treatment and visit.

**Vital signs**

All results will be listed for individual patients. Each vital sign measure will be tabulated by visit and last treatment taken at the time of the visit. Vital signs results (including supine and standing systolic and diastolic BP, pulse rate, and body temperature) will be listed for individual patients. Baseline for vital sign measurements will be defined as the last evaluation before dosing with study product (which will be Day 1). Summary statistics will be reported for each visit and for the corresponding changes from baseline by study treatment.

All vital signs and the corresponding changes from baseline will be summarized by study treatment and visit.

For assessment of the potential orthostatic hypotension, for each patient at each time-point, the change in standing BP from supine BP (both systolic and diastolic) will be calculated. The mean change on standing for systolic and diastolic BP at each time-point will be calculated. To evaluate the potential for Yaq-001 to cause orthostatic hypotension, the number and percentage of patients with > 20 mm Hg fall in systolic BP or > 10 mm Hg fall in diastolic BP after 2 minutes of standing will be summarized by study treatment and visit.

**ECG**

Means of the triplicate ECG intervals/ durations (RR, PR, QT, QTcF and QTcB intervals and QRS duration) will be listed for individual patients. The appropriate summary statistics, including mean absolute change from baseline, will be reported for each measure by study treatment and visit.

**Additional information to collect**

The analysis of individual organ-function parameters, immune function parameters, gut microbiome assessment, markers of bacterial translocation, intestinal permeability assays, bile acid profiling, mRNA studies and urinary biomarkers will be carried out in central laboratories (see APPENDIX A: CORE LABS). These data, blood endotoxin activity parameters, organ system function and nutritional status variables, together with the corresponding changes from baseline (when applicable), will be listed for each patient and summarized using the appropriate statistics by treatment and visit.

Any additional analyses will be detailed in the SAP. Any deviation from the planned analyses will be indicated and justified in the final study report.

### 12.6. Data management

The processing, reporting and transfer of personal data from all participating patients will comply with the provisions in Data Protection Act (EU Data Privacy Directive 95/46/EC).

#### *12.6.1. Completion of the eCRFs*

The completion of the eCRF will be made by authorised site personnel. The investigator must ensure the accuracy, completeness and timeliness of data reported in the eCRF. Any change or correction in the eCRF will be tracked through an audit trail procedure.

#### *12.6.2. Data validation, data review and query management*

The eCRF will include electronic validation checks for data verification. Validation procedures (edit checks) will run on new or modified patient data.

The program will test all data for completeness, consistency, and plausibility as described in the DMP and produce queries for erroneous, incomplete and missing data.

Any necessary queries are sent to the investigator by the CRA or DM via eCRF. Resolved queries will be returned to the CRA or DM (depending on who raised the query) via eCRF. If answers are incomplete or discrepant with other data, re-queries may be necessary.

#### *12.6.3. SAE reconciliation*

At the end of the study before database lock, the sponsor will provide the final SAE database to DM for reconciliation. If any discrepancies are found, appropriate queries will be generated for clarification.

#### *12.6.4. Database lock*

Before locking the database, the following activities must be finalized:

- All data have been processed;
- All queries have been solved;
- The coding list has been reviewed for completeness and consistency.

## 13. OBLIGATIONS OF THE SPONSOR AND THE INVESTIGATOR

The sponsor and the investigator must comply with all applicable regulations. In addition, the investigator must follow local and institutional requirements pertaining, but not limited, to investigational device, clinical investigation, informed consent (including the use and disclosure of protected health information) and ethics committee requirements. The sponsor will provide notification to the investigator of clinical investigation plan and amendment approvals by regulatory authorities, if applicable.

The investigator will notify to the sponsor, in advance, of any inspection scheduled with at regulatory authority. The investigator will be available to respond to reasonable requests and inspection queries made by the authorized regulatory authority representatives. The investigator will also provide the sponsor advanced notification of their relocation to another institution in order to assure the transfer of the study to an agreed upon qualified person.

Delegation of duties of the investigator to his/her collaborators will be documented in a Delegation of Responsibilities/ Signature Log provided by the sponsor and in accordance with its SOPs. The investigator is ultimately responsible for the conduct of all aspects of the study.

### 13.1. Compliance with good clinical practices

This clinical investigation will be conducted in accordance with ISO 14155:2011, "*Clinical Investigation of Medical Devices for Human Subjects – Good Clinical Practice",* the ICH Guideline on Good Clinical Practice (E6) and the current version of the Declaration of Helsinki. The investigator at each clinical site must sign the Investigator Statement of Agreement (see INVESTIGATOR STATEMENT OF AGREEMENT).

### 13.2. Clinical investigation termination and premature close-out of an investigational site

The sponsor has the right to terminate this clinical investigation at any time. Reasons for terminating the clinical investigation plan are provided in section 6.3.

Lack of compliance with current GCP requirements or other applicable regulatory requirements may result in premature close-out of an investigational site.

### 13.3. Ethics committee

EC approval for the CIP, the ICF and any patient recruitment materials will be obtained at each investigational site prior to inclusion of patients in this clinical investigation.

Documentation of the EC favourable opinion of the CIP, ICF and any patient recruitment materials must be submitted to the sponsor prior to the start of the clinical investigation. A membership roster or other description of the EC's composition will also be provided to the sponsor.

Any necessary amendments to the CIP and/or ICF and the investigator's brochure will be prepared by the sponsor and provided to the investigator for submission to the EC.

### 13.4. Informed consent process

The principal investigator and the sponsor must agree upon the format and content of the ICF before it is submitted to the EC for approval. The consent form must comply with the requirements of ISO 14155:2011 and provide authorization to sponsor designated CRA/ auditor to access to patient's medical records and personal information.

The sponsor will provide to the investigator, in writing, any new information that significantly bears on the patient's risk to receive the investigational device. This new information will be communicated by the investigator to patients who consent to participate in the study in accordance with EC requirements. The ICF will be updated and patients will be re-consented if necessary.

### 13.5. Data acquisition

CRFs will be completed electronically and managed at a centralized database. CRA will review the CRFs for completeness and accuracy by comparing them to the source documents at the clinical investigation site.

Data management personnel will manage the database and will utilize the inter- and intra-CRF validation and logic edit checks for quality control purposes. Queries will be generated, and query responses will be entered into the database.

Instructions for CRF completion and query resolution will be detailed in the reference manual.

### 13.6. Source documents

Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations, and other activities during a clinical investigation. Source documentation serves to substantiate the integrity of study data, confirms the observations that are recorded, and confirms the existence of study participants. Source documentation also serves to ensure data quality by creating audit trails and enabling verification that data is represented completely and accurately. Data collection is the responsibility of the clinical study staff at the site under the supervision of the site investigator. The investigator will maintain complete and accurate documentation for the study.

As defined in the section 1.52 of the ICG Guideline for Good Clinical Practice (ICH E6), source documents may include: original documents, data, and records (e.g. hospital records; clinical and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; pharmacy dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification of accuracy and completeness; microfiches; photographic negatives; microfilm or magnetic media; X-rays; and subject files and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical study). All source

documents and laboratory reports will be reviewed by the clinical team to ensure that they are accurate and complete.

### 13.7. Retention of documentation

Investigators are required to maintain all study documentation, including copies of the CRFs, ICFs and adequate records for receipt and disposition of all investigational device supplies, for a period of 10 years following the regulatory approval date of the device, or until 10 years after the device investigational program is discontinued, unless a longer period is required by applicable law or regulation. Subject identity information will be maintained for 10 years unless applicable law or regulation requires a longer period.

The investigator will obtain written permission from the sponsor before destroying any study records, and the sponsor will notify the investigator in writing when the study records can be destroyed.

### 13.8. Confidentiality

The study protocol and other written materials provided by Yaqrit Ltd, as well as documentation, data and all other information generated as part of this clinical investigation will be held in strict confidence by the investigator and site staff. No information concerning the study or the data will be released to any unauthorized third part without written approval of the sponsor.

### 13.9. Protocol non-compliances

The instructions and the procedures specified in this clinical investigation plan require diligent attention to their execution. Principal investigators and site personnel should avoid protocol non-compliances.

Under emergency circumstances, non-compliances of the CIP to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the EC. Such non-compliances shall be documented and reported to the sponsor and the EC as soon as possible.

### 13.10. Study monitoring

Prior to the initiation of the clinical investigation, a designated CRA will visit the site at which the clinical investigation is to be conducted according to Yaqrit SOPs. The CRA shall ensure that the investigator understands the investigational status of the device, all requirements of the CIP and his/ her regulatory responsibilities as an investigator.

The CRA will visit the clinical study site at appropriate intervals to ensure compliance with the CIP, to verify accuracy, completeness and correctness of data and to perform accountability of investigational product supplies.

The CRA or other authorized representatives of the sponsor may review all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic or hospital) and pharmacy records for the patients in the study. The clinical study sites will permit access to such records.

Sponsor and/ or designee will monitor the clinical trial over its duration according to the pre-specified monitoring plan, which include the planned extent of the source data verification.

### 13.11. Quality assurance and quality control

In addition to the routine monitoring procedures, audits of clinical research activities in accordance with Yaqrit SOPs may be performed to evaluate compliance with the principles of the GCP.

A regulatory authority may also wish to conduct an inspection during the study or after its completion. If an inspection is requested by a regulatory authority, the investigator must inform Yaqrit immediately of the request. The clinical study site will permit access to all necessary records (see section 13.10).

The CIP, each step of the data-recording process, and the handling of the data, as well as the study report will be subject to independent review by Yaqrit or its representatives.

### 13.12. Publication

Yaqrit Ltd, as the sponsor, has proprietary interest in the study. Authorship and manuscript composition will reflect joint cooperation between the investigator and Yaqrit personnel. Authorship will be established prior to the writing of the manuscript. Clinical study agreements contain additional information regarding timing, right to publish, and manuscript review prior to presentation or publication.

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# APPENDIX A: CORE LABS

***Laboratory of Institute of Liver and Digestive Health – University College London***

| Specimen | Parameters |
|---|---|
| **Plasma** | ▪ Zinc (Zn)<br>▪ Copper (Cu)<br>▪ Selenium (Se)<br>▪ sCD14<br>▪ Lipopolysaccharide (LPS)<br>▪ Intestinal-fatty-acid-binding protein (IFABP)<br>▪ Bactericidal permeability increasing protein (BPI)<br>▪ Cytokine/ chemokine profiling<br>▪ ICAM-1<br>▪ VCAM<br>▪ ADMA<br>▪ SDMA<br>▪ IMA<br>▪ NPY |
| **Serum** | ▪ Vitamin B1<br>▪ Vitamin B2<br>▪ Vitamin B3<br>▪ Vitamin B6<br>▪ Vitamin A<br>▪ Vitamin E<br>▪ Vitamin K<br>▪ Cytokeratin-18 (CK-18 M30/M65)<br>▪ Serum bacterial DNA<br>▪ Lipopolysaccharide binding protein (LBP)<br>▪ sCD163 (cytokine/ chemokine profiling) |
| **Stool** | ▪ Metabolomic profiling |
| **Urine** | ▪ Metabolomic profiling<br>▪ u-LFABP<br>▪ u-TLR4 |

***King's College Hospital Reference Biochemistry Laboratories***

| Specimen | Parameters |
|---|---|
| **Stool** | ▪ Bile acid profiling |
| **Urine**<br>**(morning urine and 5h-urine volume)** | ▪ Bile acid profiling<br>▪ Lactulose<br>▪ Rhamnose<br>▪ Xylose |

***Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS)***

All samples to be analysed at core labs will be sent at the end of the study to IDIBAPS – Biobanking Services. This site will manage the distribution of the samples received to each core lab.

***Liver Unit Laboratories- Hospital Clínic***

| Specimen | Parameters |
|---|---|
| **Urine** | ▪ uNGAL<br>▪ TFF3<br>▪ u-IL18<br>▪ GST-p |

***Laboratory of Department of Medical and Surgical Sciences – University of Bologna***

| Specimen | Parameters |
|---|---|
| **Plasma** | ▪ Albumin function |
| **Stool** | ▪ Albumin function |

***UVCM - Laboratory***

| Specimen | Parameters |
|---|---|
| **Stool** | ▪ Microbiome profiling |



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# APPENDIX B

**Common toxicity criteria for adverse events grade definition**

| | |
|---|---|
| **Grade 1** | Mild, asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated |
| **Grade 2** | Moderate, minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental ADL |
| **Grade 3** | Severe or medically significant but not immediately life-threating; hospitalization or prolongation of hospitalization indicated; disabling; liming self-care ADL |
| **Grade 4** | Life-threating consequences; urgent intervention indicated |
| **Grade 5** | Death related to AE |

**Table 1A: toxicity criteria – SAE and laboratory abnormalities**

| Parameter | Definition |
|---|---|
| Serious Adverse Device Effect[1] | Device-related serious adverse event |
| Increased liver transaminases | Serum AST and ALT are both increased to > 5 x ULN (i.e. CTCAE Grade ≥ 3) **AND** 2 x baseline value |
| Decreased total leukocyte count | Leukopenia to < 2000/ µL [< 2.0 x $10^9$ / L] (i.e. CTCAE Grade ≥ 3) **AND** 25% decrease from baseline value |
| Decreased total neutrophil count | Neutropenia to < 500/ µL [< 0.5 x $10^9$ / L] (i.e. CTCAE Grade ≥ 4) **AND** 50% reduction from baseline value |
| Decreased platelet count | Thrombocytopenia to < 25.000/ µL [< 25 x $10^9$ / L] **AND** 50% reduction from baseline value |
| Increased serum creatinine | Serum creatinine to 1.5 x ULN **AND** 50% increase from baseline value |
| Prolongation of QTcF interval | QTcF > 500 msec (i.e. CTCAE Grade ≥ 3) **OR** increase from baseline of ≥ 60 msec |

[1] Unless the SAE is an event described in the table 1B

[2] To be confirmed by repeated test

[3] ULN= upper limit of normal reference range of the clinical laboratory

[4] CTCAE = Common toxicity criteria for adverse events, version 4.2 (see Study Reference Manual for full CTCAE listing) available at: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)

[5] QTcF= QT interval Fridericia's correction

**Table 1B: toxicity criteria – Neurology and Gastroenterology**

| | |
|---|---|
| Somnolence | > CTCAE Grade 2 [Somnolence or sedation interfering with function] |
| Confusion | > CTCAE Grade 2 [Confusion, disorientation, or attention deficit interfering with function] |
| Dizziness | > CTCAE Grade 2 [Dizziness interfering with function] |
| Memory impairment | > CTCAE Grade 2 [Memory impairment Interfering with function] |
| Cranial neuropathy | > CTCAE Grade 2 [Symptomatic e.g. loss of hearing or taste] |
| Seizure | > CTCAE Grade 2 [One brief generalized seizure; seizure(s) well controlled by anticonvulsants or motor seizures not interfering with activities of daily living] |
| Vomiting | > CTCAE Grade 2 [2-5 episodes in 24 hrs; intravenous fluids indicated] |
| Unspecified | If considered appropriate by the medical monitor and investigator |

# PATIENT INFORMATION SHEET

Title of Project: “Safety and tolerability of Yaq-001 in patients with cirrhosis”;

Short name: CARBALIVE-SAFETY

The Institute of Liver and Digestive Disease at Royal Free Hospital is conducting a clinical trial in which you are being invited to participate.

Before you decide whether to take part in it, you need to understand why this clinical trial is being done and what it would involve for you. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide if you want to participate in the study.

## What is the purpose of the study?

We all have bacteria in our gut, but in healthy people these bacteria normally remain confined there in the gut. However, in patients with cirrhosis (scarring of the liver) these bacteria, or their fragments called endotoxins, leak from the gut into the blood and cause harm, particularly infection. When this happens, patients can become very ill.

At the moment the only way we can treat this problem is with antibiotics. The drawback with antibiotics is that bacteria become resistant to them and are no longer effective. Antibiotics can also upset the normal balance of bacteria in the gut causing diarrhoea. Therefore, the finding of other products, different from antibiotics, able to treat this leakage problem is a current topic of research in patients with cirrhosis.

This clinical trial is looking into a new way to lower the leakage of bacteria into the blood with a new product called Yaq-001. This new product works by binding these bacteria or their fragments in the gut, and in this way, confining them to the gut.

Yaq-001 is a new type of carbon which is being developed by the company Yaqrit Ltd. in cooperation with researchers of the University College of London. In previous laboratory experiments the researchers have shown that Yaq-001 can bind harmful bacterial toxins which together are eliminated with stool without being absorbed. This has been shown to have beneficial effects in a laboratory model of cirrhosis. We now want to see if the same effect is seen in humans. This is the purpose of this clinical trial: to test for the first time the product Yaq-001in patients with cirrhosis.

This clinical trial has been designed to assess if the treatment with Yaq-001 is safe for you and is well tolerated by you. Additionally we will be looking to see if Yaq-001 helps to improve your overall health status.

A total of 56 patients from 8 hospitals in the UK, France, Italy, Spain and Switzerland will participate in this clinical trial.

**Why have I been invited to participate?**

Your doctor has explained to you that you suffer from cirrhosis. This study is about a new approach to reduce the complications of cirrhosis.

**Do I have to take part?**

Your decision to participate or not will affect your further treatment by your doctor in no way. You are under no obligation to participate in this study.

**What will happen to me if I take part?**

If you decide to participate, you will be given this information sheet to keep and you will be asked to sign and date a consent form. You will also be given a copy of the consent form to keep.

The treatment and procedures that you will have to undergo during the study are described below.

***Screening visit:***

After having signed the informed consent, you will undergo a screening visit up to two weeks prior to start of the study treatment.

On this first visit we will perform a general evaluation including:

- review of your medical history and medication history
- physical examination
- vital signs: blood pressure, pulse rate, respiratory rate, and body temperature
- electrocardiogram (to measure the electrical activity of your heart)
- blood and urine tests

These tests above are part of the normal management of a patient with your disease.

In order to determine your suitability for the study, it will be necessary to make an additional test to assess your bowel transit time: you will be asked to swallow a small tracer pill at night and 10 hours later the following day an X-ray will be performed to see how far it has moved. The tracer pill is opaque to the radiation of X-ray, so it is visible in the X-ray image.

Your doctor may arrange for you take to another test if there is any doubt on the X-ray based test. One option will be to swallow a small pill which will take pictures of your intestine. The other option would be to have an MRI (Magnetic Resonance Imaging) scan after swallowing a contrast pill. Neither of these options involves radiation.

### *Randomisation and Blinding*

If you are eligible for the study, you will then be grouped to receive one of the following two treatments:

- your standard treatment plus Yaq-001
  or
- your standard treatment plus placebo

Placebo is a non-active treatment; it is a pill similar to Yaq-001 in appearance, taste and smell but which has no effect at all. The use of placebo is needed to help understand better how safe and tolerable Yaq-001 really is.

The treatment group in which you will be included is decided by means of a process called randomisation, by which the treatment for each patient is randomly assigned by a central computer, that is, a process similar to making a choice by tossing a coin. This means that you have an equal chance of 50% of being treated with Yaq-001 or with placebo.

The treatment you are receiving will be unknown throughout the study, meaning that no one involved in the study (doctors, nurses, patients, or families) will know what each patient is receiving.

The first 28 patients included in the study will receive a daily dose of 4g Yaq-001 or placebo, and the second 28 patients will receive a daily dose of 8g Yaq-001 or placebo. In this way, the safety and tolerability of Yaq-001 will be assessed at two different dosage levels. We will only start patients on the higher dose once we have information about the 4g dose group.

### *Treatment Period (Day 1, 1 week, 4 weeks, 8 weeks and 12 weeks)*

You will be treated for 12 weeks. During this period, you will be assessed by a study doctor.

The following assessments will be performed at Day 1, 1 week, 4 weeks, 8 weeks and 12 weeks:

- physical examination
- vital signs: blood pressure, pulse rate, respiratory rate, and body temperature
- electrocardiogram (to measure the activity of your heart)
- collection of blood and urine samples for analysis at the hospital laboratory
- review of changes in your medical status since the previous visit
- review of changes in your medications taken since the previous visit

On three occasions (day 1, 4 weeks, 12 weeks) you will be asked to provide additional samples for analysis outside the hospital. These will include a total of 20 ml of blood, 9 ml of morning urine, a 5 hour urine collection and a sample of stool. You will be asked to provide a first morning urine sample. After you have collected this, you should drink a sugary drink which will be provided by your doctor. All the urine you pass in the following 5

hours should be collected in a special container (which we will provide you with) and brought to your visit.

The samples will be collected and processed at your hospital , and will then be frozen and shipped to specialised laboratories in London, Barcelona, Bern and Bologna where the analysis for all the patients' samples will be done .
At each visit during the treatment period, your appetite will be assessed by the study doctor. Furthermore, your weight, mid-arm circumference and triceps skinfold thickness will be measured in the non-dominant side of your body to assess your nutritional status.

The study treatment will be supplied to you as sachets for oral administration. At day 1, 1-week, 4-weeks and 8-week visits you will receive a kit containing enough treatment for you to take it between visits. The study doctor will instruct you to take the study treatment once at night after dinner. You will be asked to carefully keep all the used and unused sachets and to return them in the same box they came in at each visit.

***Termination visit***

7 days after the end of the study treatment, you will perform the last visit of the study. The following assessments will be done:

- physical examination
- vital signs: blood pressure, pulse rate, respiratory rate, and body temperature
- review of changes in your medical status or medications taken since the previous visit

**Expenses**

There will be no charge to you for any of the procedures or treatment products associated with this study. You will not incur any additional costs as a participant of this study. Any travel expenses (cost to travel back and forth to the clinical study site) incurred during the study will be reimbursed.

**Will I be paid if I take part in this clinical trial?**

Other than reimbursement for travel expenses, you will not be paid to take part in this study.

**What are the alternatives for treatment?**

At present only antibiotics such as ciprofloxacin can be used to treat the leakage of bacteria from the gut into the blood. The drawback with antibiotics is that bacteria become resistant to them and are no longer effective. Antibiotics can also upset the normal balance of bacteria in the gut causing a form of infectious diarrhoea called *Clostridium Difficile* diarrhoea. This can be particularly dangerous in patients with weaker immune systems such as those with cirrhosis.

**What are the possible disadvantages and risks of taking part in the study?**

Oral carbons have been used safely in humans for centuries. Previous large studies have demonstrated that oral carbons are safe in patients with cirrhosis and on dialysis.

There is a possibility that the carbons may bind and affect other medications you may be taking. Taking Yaq-001 and your other medications at the exact times specified by your doctor should circumvent this problem.

In large quantities, Yaq-001 may cause the stool to turn black but we do not expect that to happen with the doses used in this study. Because Yaq-001 is a very good adsorbent, there is a small possibility that some of your vitamin levels will go down slightly and you may need to take supplements during the study. We will monitor your vitamin levels throughout the study to make sure you get the right supplements you need.

Your doctor will also instruct you exactly when to take your other medications to prevent any possible interactions with Yaq-001. There is also a small possibility that you may be slightly more constipated when you first use Yaq-001. Your doctor will prescribe the laxatives you may need to maintain your normal bowel habit.

**What are the side effects of any treatment received when taking part?**

Nausea, vomiting, constipation and diarrhoea have been reported with activated carbon products. Your doctor will prescribe you appropriate medications to counter this if this becomes a problem.

**Other Risks**

There may possibly be other side effects and risks that are unknown at this time. If you are concerned about other, unknown side effects, please discuss this with the researchers.

**How will risks be minimized or prevented?**

Regardless of participation in this clinical trial, you will be treated and monitored according to standard of care. Every effort will be made to minimize or eliminate all potential risks and discomforts.

**What are the possible benefits of taking part?**

This study is being undertaken to confirm that Yaq-001 is safe and well tolerated in patients with cirrhosis. It may also have the benefit of the reduction in bacteria and bacterial toxins into the blood, and the improvement of organ function (liver, brain, kidney, intestine and immune system) and nutritional status.

If you agree to take part in this clinical trial, there may not be direct benefits for you. The study doctor cannot guarantee that you will benefit from participation in this clinical trial.

However, the information gained from this study may lead to better management of the complications of cirrhosis in the future.

**What will my responsibilities be during the study?**

It is your responsibility to:

- Ask questions about anything that is not understood.
- Follow the researchers' instructions, including timing of taking the study treatment, and timing of taking your other medications.
- Return supplies of used and unused study product in the box that it came in.
- Let the researchers know if there are telephone number or address changes.
- Report to the study researchers any injury or illnesses while you are in the study even if you do not think it is related.

**If I agree to take part in this clinical trial, will I be told of any new risks that may be found during the study?**

Yes. You will be told if any new information becomes available during the study that could cause you to change your mind about continued participation or that is important to the patient's health or safety. You may be asked to sign a revised consent form if this occurs.

**Can I stop taking part in this clinical trial?**

Yes. If you decide to participate in the clinical trial and later change your mind, you are free to stop participation in the study at any time.

If you decide to stop taking part in this clinical trial, this will not affect your relationship with staff or doctors. Whether you participate or not in the study will have no effect on your legal rights or the quality of your health care.

**Are there procedures I should follow after stopping my participation in this study?**

You will need to let the study doctor know immediately that you wish to withdraw from the study. You will continue your regular standard of care visits with your treating doctor/healthcare team.

**If I agree to take part in this study, can I be removed from it without my consent?**

Yes. The doctors may decide to take you off this study if:

- the health authorities order to stop the clinical trial
- they believe that participation in the study is no longer safe for you
- you do not take your trial treatment as directed by your doctor

**What happens when the clinical trial has ended?**

The treatment period is for 12 weeks only with a follow-up period of one week. We will not offer further Yaq-001 therapy after the treatment period is complete.

**Will my taking part in the study be kept confidential?**

All information which is collected about you during the course of the study is personal data and will be kept strictly confidential. However, by signing the Informed Consent Form, you are authorising individuals appointed by the sponsoring company, and members of the NHS Trust and from Ethics Committee who approved this study, to read the medical information about you in connection with your participation in the study.

The monitors and auditors employed or contracted by the company sponsoring the study (Yaqrit Ltd) will have access to your medical records and study data pertinent to you in order to verify that your rights and welfare are being respected and to verify the validity of the study data that is being collected.

In addition, regulatory authorities may review your records while conducting inspections of the study to confirm that the sponsor and the investigator are carrying out the study correctly and to assess the validity of the data generated from the study.

The study data that the sponsor requests from the study doctors does not include your name, address or other personal details. Instead, the doctor assigns a code number to your records that are sent to the sponsor. Your name will be never used for study information or to label your blood or urine or stool samples. There will be only one list which links the study number to your name, and this list will be safely kept in a locked cabinet in the hospital.

The sponsor will add all the collected study data into a research database. If you stop your participation in the study, we will stop collecting any new data but the sponsor will use the data that has already been collected.

We can assure you that all the data acquired from the study will be kept confidential in keeping with the Data Protection Act.

**Involvement of the GP/family doctor**

Your GP will be informed of your participation in this study only if you agree. However, if you prefer that we do not contact your GP, please inform your study doctor who will ensure that this does not happen.

**What will happen to the biological samples (e.g., blood, urine, stool) I give?**
All samples will be given a coded number before being shipped from your hospital for analysis in the central laboratories abroad. This ensures that your samples remain anonymized. All samples will be only used to obtain the results of this study and will be destroyed once they have been analysed.

The samples will be processed for analyses to provide study data. If additional samples are taken for any other tests than specified in this informed consent, we will inform you at the time. You would need to sign an additional consent form at the time.

**What will happen to the results of the clinical trial?**

The results of this study will be submitted for publication to a clinical journal. The authors will be the study doctors of this study. The results of the study may be presented at local, national or international conferences. A letter will also be sent out to all participants informing them of the general outcome of the study. Your identification as a study participant will not be disclosed in any publication.

**Who is organising and funding this clinical trial?**

Funding for the study is being provided for by a European Horizon 2020 grant. The study is being organized by sponsoring company, Yaqrit Ltd.

**Who has reviewed the study?**

This study has been evaluated and approved by the Research Ethics Committee *REC NAME* on *APPROVAL DATE*

**What if there is a problem?**

In the unlikely event that something goes wrong or if you have a concern about any aspect of this study, please speak to your study doctor or ask to speak with the doctor in charge of the study at the hospital, Dr. R. Mookerjee (020 7433 2793).

If you remain unhappy and wish to complain formally, you can do this via the hospital's Patient Advice and Liason Service (PALS). Contact details are as follows:

RFH PALS office, Royal Free Hospital, Pond Street, London, NW3 2QG
Tel: 020 7472 6445/6/7; email: pals@royalfree.nhs.uk

**Harm**

If there is a problem or adverse event during the study, participants will be treated according to standard clinical strategies and will be followed up until the adverse event is resolved or stabilized or until a plausible explanation of the cause of the event has been found. In the unlikely event of injury or illness resulting from this study, emergency medical treatment is available and will be provided to you.

While it is not expected that you will suffer any health problems by taking part in this study, in the event that something does go wrong and you are harmed during the research, the study sponsor has an insurance policy that provides compensation for impairment of health or injuries that may occur in connection with your participation in the study. What is

not covered by the insurance policy is if the impairment of health is related to your current medical condition or is a natural consequence of your medical condition.

If the harm is due to someone's negligence you may have ground for legal action for compensation against Royal Free London NHS Foundation Trust but you may have to pay your legal costs.

In addition, the normal National Health Service complaints mechanisms will still be available to you.

**Whom do I call if I have questions or problems?**

For questions about the study, do not hesitate to contact your study doctor:

Dr: ……

Phone: …….. during regular business hours

Phone: …….. after hours and on weekends and holidays.

*(Form to be on headed paper)*

Centre Number: 01

Study Number: Yaq001-S-001

Participant Identification Number for this trial:

**CONSENT FORM**

Title of Project: "Safety and tolerability of Yaq-001 in patients with cirrhosis"; Short name: CARBALIVE-SAFETY

Name of Researcher: **Dr. Raj Mookerjee;** Institute of Liver and Digestive Disease; Royal Free Hospital.

Please initial box

1. I confirm that I have read the information sheet dated 31 Jan 2017 (version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant section of my medical notes and data collected during the study may be looked at by individuals from the sponsor company Yaqrit Ltd, from regulatory authorities or from NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers. ☐

5. I agree to my General Practitioner being informed of my participation in the study. ☐

6. I understand that the information held and maintained by the Health and Social Care Information Centre (or amend as appropriate) and other central UK NHS bodies may be used to help contact me or provide information about my health status. ☐

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

7. I agree to take part in the above study ☐

| Name of participant | Date | Signature |
|---|---|---|
| | | |

| Name of person taking consent | Date | Signature |
|---|---|---|
| | | |

When completed: 1 for participant; 1 for researcher site file; 1 (original) to be kept in medical notes.

# Investigator's Brochure

# Yaq-001
# *(Bimodal Porosity Carbon Beads)*
# For Oral Administration

**Document Edition Number: 00**

**Release Date: 28 January 2017**

**Yaqrit Ltd,**
**Interchange Triangle, 3rd Floor**
**Triangle Building Stables Market**
**Chalk Farm Road**
**London NW1 8AB United Kingdom**

**CONFIDENTIAL**
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## 1.0 INTRODUCTION

The World Health Organisation estimates that over 650 million people globally and more than 29 million European citizens suffer from a chronic liver condition, with greater than 170,000 European deaths from cirrhosis alone each year[1]. Liver disease remains the fifth most common cause of death across Europe in individuals aged 45-65 years and costs more than €15.8bn per annum in total health costs and loss in economic productivity[2].

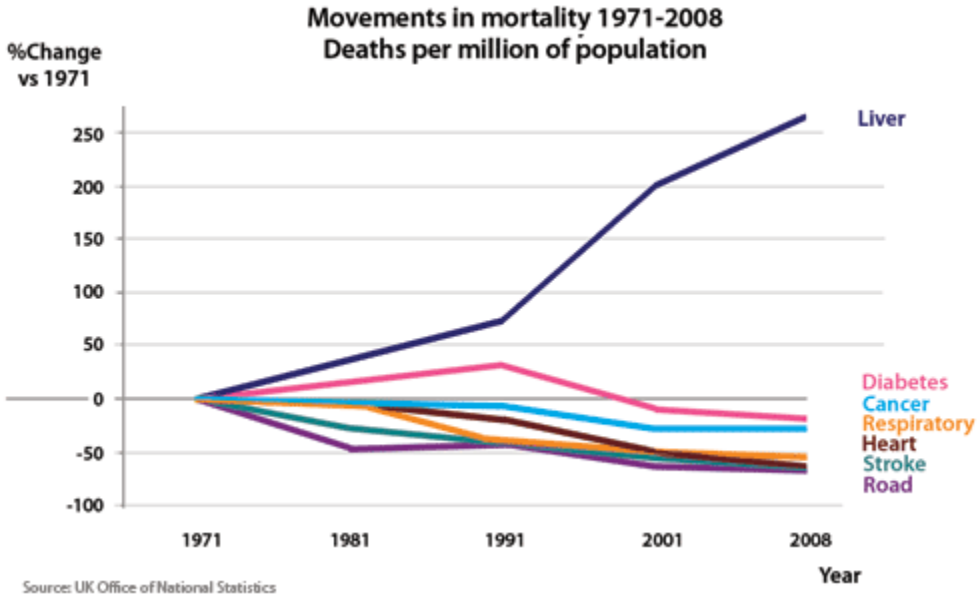



Figure 1.This figure shows that standardized mortality rates for patients with liver disease has increased exponentially in the UK whereas death rates in other chronic non-communicable diseases such as diabetes and cancer are falling.

Deaths related to liver disease worldwide have almost doubled over the last 20 years to more than one million per year[3], in contrast to other major diseases such as cancers and heart disease where death rates are stable or declining. Alarming increases in obesity rates and an ageing population mean that liver disease will become an even greater health concern for Europe over the next decade. The three major causes of liver disease, i.e. alcoholic liver disease, non-alcoholic fatty liver disease (NAFLD) and viral hepatitis, are amenable to prevention and treatment, providing an opportunity to reduce the burden of liver disease in Europe and to save lives.[4] Gut-derived endotoxaemia and bacterial translocation play a central role in the pathogenesis of NAFLD[5], and the development of cirrhosis and its complications.[6,7]

Targeting increased bacterial translocation with poorly absorbed antibiotics is the basis for current therapeutic intervention in patients with very advanced liver disease.[8,9] Selective intestinal decontamination using antibiotics is currently the option of choice but is only a short-term solution to reduce the complications associated with cirrhosis such as hepatic encephalopathy, hepatorenal syndrome and spontaneous bacteria peritonitis. However, this approach has the risk of infection with resistant organisms, and emergence of resistant strains of bacteria. This limits its use to 'treat' patients with advanced disease rather than providing a preventative strategy.[8,9.10] Minimal progress has been made in developing

alternatives to the current interventional regime and a non-antibiotic strategy to prevent bacterial endotoxaemia and bacterial translocation is an unmet medical need.

Patients are already presenting and dying with liver disease at an earlier age with a 5-fold increase in the development of cirrhosis in 35-55 yr olds over the last decade.[2] There is now a critical clinical need to address this disease by targeting the most effective means of preventing complications, enhancing quality of life and reducing deaths among the European population.

Development of Yaq-001 is supported in part by a grant award from the European Commission, Horizon 2020 programme to develop a new treatment for liver disease.

## 2.0 INVESTIGATIONAL DEVICE INFORMATION

### 2.1 General description of the device

Carbalive (Yaq-001) is an engineered adsorbent carbon of bimodal porosity. It is capable of removing bacterial endotoxin and other metabolic toxins relevant to the progression of bacterial translocation and endotoxaemia in patients with cirrhosis and NAFLD. Yaq-001 selectively removes toxins – mainly bacterial products and inflammatory molecules that damage the liver and other organs – without interacting with the mucous membrane of the gut.

Yaq-001 is a black microspherical carbon bead with a diameter of approximately 0.1-0.5 mm and a surface area of ≈1500 $m^2$/g. The large surface area and large tri-modal pore volume are thought to result in the superior adsorptive capacity of the carbons for biomolecules ranging from low to high molecular weight. The preliminary elemental analysis of Yaq-001 is:

| Elements | Contents |
|---|---|
| Carbon | ≥ 95 % |
| Oxygen | ≤ 5 % |

The manufacturing process for Yaq-001 limits release of dust microparticle irritants and incorporates a superior micro-, meso- and macroporous capacity for targeted adsorption of low molecular weight, protein bound and high molecular weight species known to be responsible for dysregulated inflammation and infection. Micro, -, meso, - and macroporosity terms are used according to the IUPAC definition of pores <2 nm, 2-50 nm and >50 nm, respectively. It is this tailored nanoporosity which defines a broader adsorption profile and an improved efficiency for biotoxins not removed by other microporous carbon preparations.

Yaq-001 has been shown to adsorb small, middle and high molecular weight biological molecules in non-clinical studies including bacterial toxins, lipopolysaccharide, staphylococcus enterotoxin B, staphylococcus enterotoxin A, TSST-1, and bacterial metabolite toxins.

Yaq-001 is not systemically absorbed. Greater than 95% of Yaq-001 was recovered in animal faeces; the balance was most likely due to carbon beads being lost during the extended washing cycles called for in the study methodology.

### 2.1.1 Manufacturing Process

The MAST TE7 beads are prepared from a commercially available (ISO standard) high purity novolak phenolic resin polymer. The production involves a patented polymer bead process that, via a solvent-pore-forming method, produces the meso/macroporous resin beads. These polymer beads are converted to the dual pore structure (micro +meso/ macro pores) carbon beads via a patented two stage -- pyrolysis followed by activation -- process. The carbon beads undergo a rigorous quality control analyses prior to release.

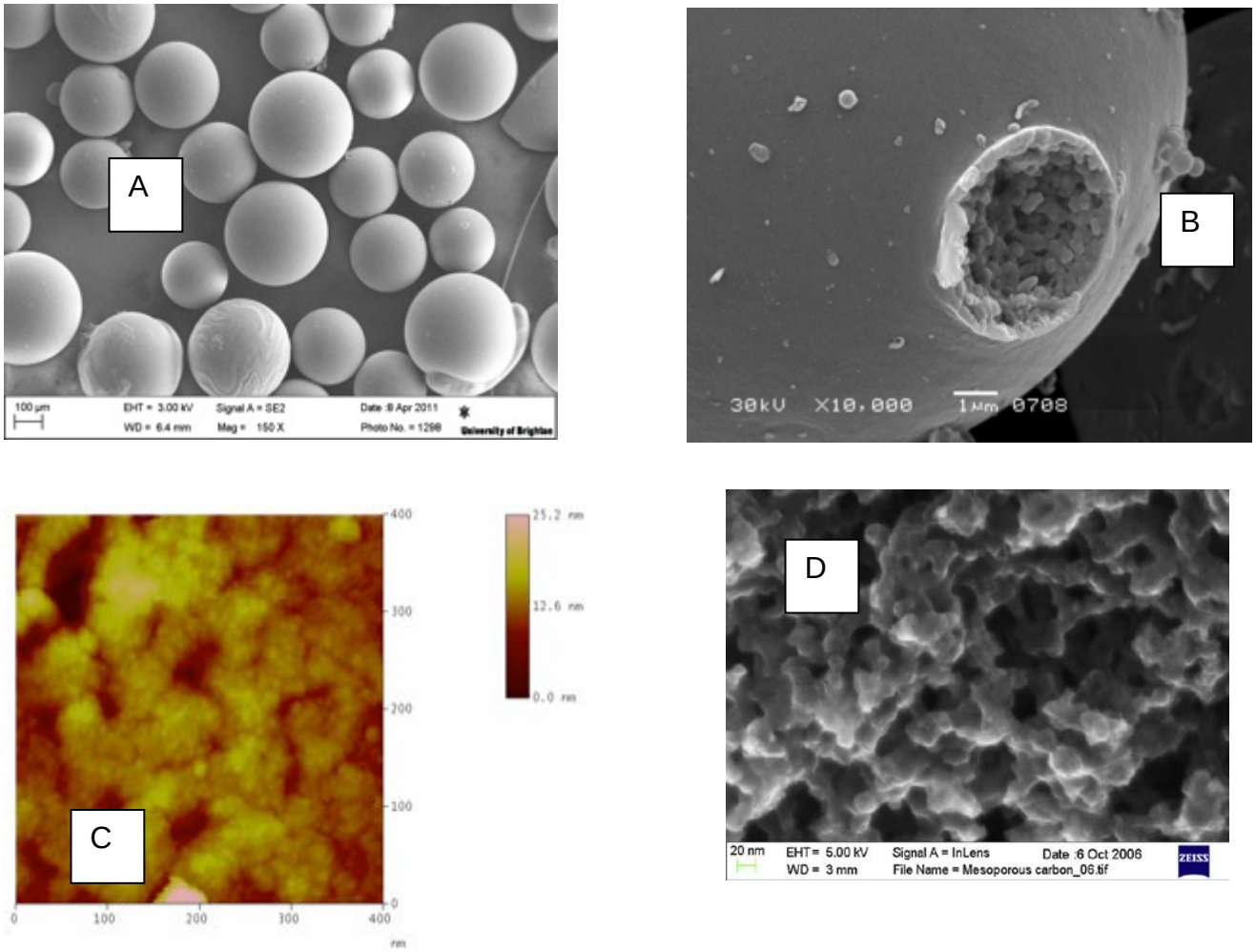



Figure 2. The spherical form of the particles is shown in (A); (B) shows the external skin that forms during the dispersion process. The internal pore structures are shown in more detail in (C) and (D).

### 2.1.2 Stability

Stability testing for the current clinical trial material is ongoing and continuously monitored. If any lots of the investigational device fall out of specification, the clinical trial material will be replaced with a new lot.

### 2.1.3 Yaq-001 Handling and Storage

Yaq-001 must be stored in a dry, ventilated area at controlled room temperature (15 – 25$^{o}$ C; 59 – 77$^{o}$ F), under 60% relative humidity. Protect the carbon beads against physical damage and keep away from moisture. Yaq-001 will absorb water at relative humidities above ≈ 45%.

## 2.2 Mechanism of action / rationale for clinical trial

Recent advances in synthetic carbon technology, combined with the understanding that translocation of bacterial metabolites and toxins in liver disease, have led to the development of synthetic nanoporous carbons with a uniquely tailored porosity, acquired during synthesis and activation, to address issues associated with microbial translocation in the treatment of liver disease (Yaq-001) [Patent: Priority application: PCT/GB/1204696.7]. Yaq-001 is an engineered carbon with defined pore sizes critical to its functionality in binding and removal of endotoxin and inflammatory molecules.

Yaq-001 is not absorbed through the wall of the alimentary tract into the body. The mechanism of action of Yaq-001 is mechanical in nature; it physically binds bacterial metabolites within the intestinal tract. There does not appear to be a direct pharmacological, immunological or metabolic effect, given that Yaq-001 is not systemically adsorbed.

Several clinical studies have demonstrated increased intestinal permeability in chronic liver disease, which increases with the severity of the disease, allows the transfer of bacterial toxins and toxic metabolites to the portal vein and then to the liver. Multiple factors such as intestinal bacterial overgrowth and alterations in the flora, increased intestinal permeability, impaired immunity, intestinal inflammatory response, toxic metabolites such as acetaldehyde and reduced liver function contribute to increased bacterial translocation leading to fibrosis and cirrhosis and its complications. When combined, these factors cause rapid progression of the severity of the disease, exposing an already-damaged liver to excessive stress and toxic injury further complicating the disease.

### 2.2.1 The role of gut integrity and its alterations in liver disease

Barriers separating the gut microflora from the host include secretory components (e.g. bile, mucus and antimicrobial peptides) and mechanical anatomic structures (epithelial cells and cell-cell-contacts). The former limits direct contact between the intestinal bacteria and the epithelial cell surface, decreasing the likelihood of direct bacterial contact with epithelial cell surfaces. The latter interacts closely with antigen-presenting cells, beneath, and inter-epithelial cells, within the lamina propria to maintain intestinal integrity. Finally, the so-called gut-associated lymphatic tissue (GALT), representing the largest immunological organ in the human body, provides immune protection involving the rapid detection and killing of bacteria. In chronic liver diseases, alterations in these three barriers are present.

In, non-alcoholic fatty liver disease (NAFLD), as well as in liver cirrhosis, increased intestinal permeability has been demonstrated and linked to increased inflow of bacterial products into the liver via pathological bacterial translocation. In liver cirrhosis, contributing factors include reduced bile flow, diminished levels of antimicrobial peptides and changes in expression and function of tight junction proteins (determining cell-cell-contacts) in the intestine. [6]

### 2.2.2 The microbiome, it's importance and alterations in cirrhosis and NAFLD

Humans harbour nearly 100 trillion intestinal bacteria, which represent around ten times more microbial cells than we have in our own eukaryotic cells. This microbiome, on one hand, plays a key role for metabolism and energy harvest which relates to obesity and NAFLD and on the other hand represents the largest reservoir of antigens and bacterial products which pre-disposes to hepatic inflammation/injury mediated by increased bacterial translocation from the gut into the liver. In fact, germ-free mice are protected from high-fat diet–induced weight gain, obesity and NAFLD.[11]

Quantitative alterations (bacterial overgrowth), as well as qualitative changes in intestinal microbiome (taxonomic differences in microbial communities), have been reported in NAFLD and cirrhosis.[12] Using culture-independent techniques such as quantitative polymerase chain reaction (PCR) and deep sequencing of a conserved region in the bacterial 16S ribosomal RNA (rRNA) gene, distinct modulations in these entities have been shown:

- Patients suffering from NASH have a lower proportion of members of the *Ruminococcaceae* family than healthy subjects;
- *Escherichia* is the only abundant genus of bacteria in the intestinal microbiota that is significantly disproportionate between obese children and paediatric patients with NASH;
- Patients with cirrhosis display a more profound intestinal dysbiosis than do those with NAFLD. [13]

Compared with healthy controls, patients with cirrhosis have fewer *Bacteroidetes* and higher abundance of *Proteobacteria* and *Fusobacteria* at the phylum level. At the genus level, these patients have low abundance of *Lachnospiracaceae* and *Ruminococcaceae*, and mainly an abundance of *Veillonella* and *Streptococcu*s, which includes species of oral origin. Overall, oral commensals invade the gut in patients with cirrhosis, and the severity of these changes parallels that of the disease state itself.

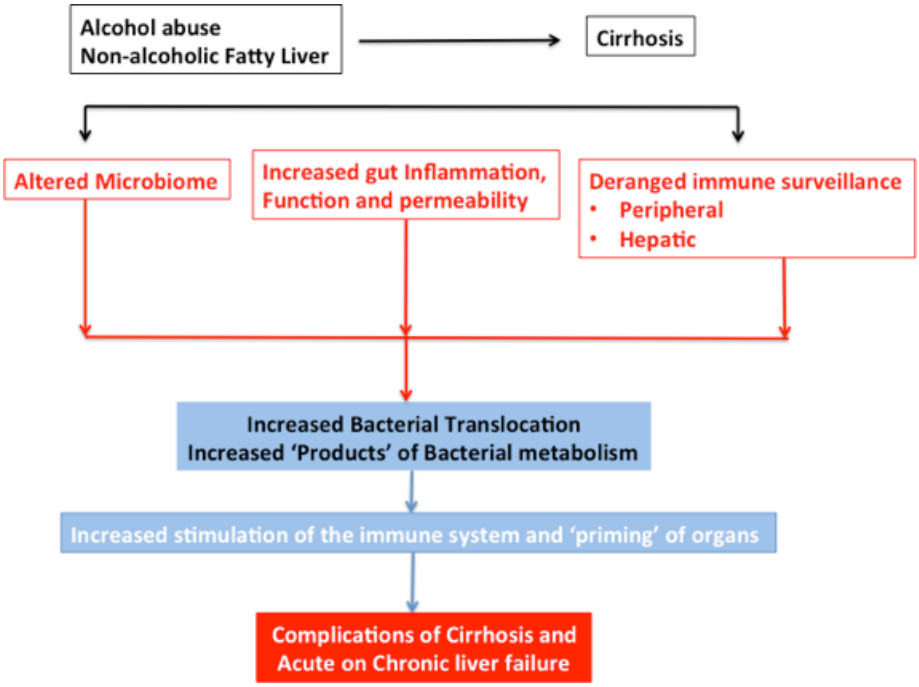



Figure 3. Role of bacterial translocation in disease progression in chronic liver disease. This figure shows that the progression of liver injury to cirrhosis and from cirrhosis to its primary complications, including acute on chronic liver failure, is modulated by increased translocation of bacteria or its products.

One of several examples on the impact of qualitative changes on the translocation of microbial products from the gut comes from studies in obese mice.[14] An increase in *Bifidobacterium* spp. has been shown to modulate inflammation by increasing production of GLP-2, which reduces intestinal permeability and thus translocation of endotoxin (lipopolysaccharide (LPS). Moreover, NAFLD as well as liver cirrhosis are frequently associated by small intestinal bacterial overgrowth (SIBO). Factors contributing to SIBO in cirrhosis include impaired motility of the small intestine, reduced bile flow, and altered secretion of immunoglobulin A and antimicrobial molecules.[6] In both entities SIBO has been linked to increased intestinal permeability and translocation of bacteria and bacterial products, which ultimately reach the liver. Therefore, not surprising the microbiome impacts vastly on the development and progress of NAFLD as well as liver cirrhosis.

### 2.2.3 The important role of bacterial translocation in the pathogenesis of NAFLD and complications of cirrhosis

Gut dysbiosis and bacterial translocation play an increasing recognized role in the progression of advanced cirrhosis and in the transition from NAFLD to non-alcoholic steatohepatitis (NASH). In advanced cirrhosis, bacterial translocation constitutes the pathophysiological hallmark of bacterial infections, such as spontaneous bacterial peritonitis (SBP), which is a major determinant of mortality. In this setting, bacterial translocation importantly contributes to clinical complications of cirrhosis by worsening the hemodynamic derangement of portal hypertension through nitric oxide overproduction. In fact, the severity of peripheral vasodilation is greater in animal models of cirrhosis and in patients with ascites and bacterial translocation. These models show the most severe systemic inflammation. [15] Most of the evidence supporting the relationship between microbial translocation and cirrhosis progression comes from studies showing that selective bowel decontamination with non- or poorly absorbed antibiotics in patients with decompensated cirrhosis - because of variceal bleeding, SBP,

ascites with renal insufficiency or encephalopathy - reduces the 1) rate of bacterial infections, including SBP, 2) the rate of complications of portal hypertension and 3) more importantly improves survival. The beneficial effect of bowel decontamination in the clinical outcomes of cirrhosis is associated with an improvement in hemodynamic abnormalities with attenuation of endothelial activation and activation of endogenous vasoactive systems, and improvement in peripheral vasodilation in those patients with advanced cirrhosis and bacterial translocation. [15]

The available evidence also indicates a role for the gut microbiota in the etiology of NAFLD and especially in the progression to its more advanced state, NASH (non-alcoholic steatohepatitis). The progression of simple steatosis to steatohepatitis is primarily an inflammatory, rather than a metabolic process, in which inflammation provoked by gut dysbiosis plays a pre-eminent role. SIBO of Gram-negative organisms promotes insulin resistance, increases endogenous ethanol production and induces choline deficiency, as detailed below, all factors strongly linked to NAFLD and NASH. [12, 16] More specifically, strong evidence, mostly experimental, supports the pathogenetic involvement of LPS-driven liver inflammation in NASH, including:

- presence of endotoxaemia and up-regulation of liver TNF gene expression in patients and animal models;
- requirement of signalling via the receptor for LPS, TLR4, in monocytes/macrophages for liver steatosis development;
- resistance to NASH in mice deficient in sensing PAMPS;
- increased sensitivity to endotoxin induced hepatotoxicity and steatohepatitis of genetically obese mice.

Moreover, intact bacteria or bacterial products derived from the intestine trigger TLR4 and TLR9 activation, which results in an increased rate of chronic liver disease progression in mice that house a colitogenic gut microbiota associated with inflammasomal deficiency.[5] The contribution of LPS to the pathogenesis of NAFLD, inflammation and the related dysmetabolic abnormalities is supported by various interventional studies in animal models, using antibiotics or probiotics. In these studies, modulation of gut microbiota reduces endotoxaemia, insulin resistance, body weight gain and oxidative stress in visceral adipose tissue in mice models of obesity. [17]

### 2.2.4 Products of bacterial metabolism may be more important than the bacteria itself

It is important to point out that the contribution of intestinal microflora to the progression of chronic liver disease is beyond the simple translocation of viable bacteria as a pathogenetic factor. Bacterial translocation is defined as the penetration of viable commensal bacteria across the intestinal gut barrier to the mesenteric lymph nodes (MLN) with bacteraemia, seeding in extraintestinal sites and eventual infection. However, the concept of microbial translocation should be extended to:

- translocated bacterial components, such as endotoxin and its active component, LPS, and fragments of bacterial DNA[18], and
- absorbed products derived from bacterial metabolism and/or from the interaction of the microbiota with the diet, such as ethanol, acetaldehyde and trimethylamine, and short-chain and free fatty acids. [19]

The mechanisms by which the components or the metabolic products of gut bacteria contribute to the progression of cirrhosis and NAFLD include:

- LPS stimulates monocytes/macrophages to produce the proinflammatory cytokines, TNF, IL-1 and IL-6. In the liver. LPS activates TLR4 on Kupffer and hepatic stellate cells triggering an essential inflammatory cascade, which results in the production of proinflammatory and profibrogenic mediators in the splanchnic and peripheral circulation. LPS-driven cytokine release promotes endothelial activation and nitric oxide induction. [20]
- Acetaldehyde and its metabolites lead to the formation of reactive oxygen species, which increase oxidative stress and, ultimately, induce liver injury. Both ethanol and LPS also promote the production of reactive oxygen species by parenchymal and non-parenchymal liver cells. [19,12]
- Microbial modification of bile acids impairs their bacteriostatic effect, favours bacterial overgrowth and changes signalling pathways involved in energy and lipid metabolism with modifications in lipid peroxidation and liver storage of fatty acids. [12]

### 2.2.5 Role of Yaq-001 in the treatment of cirrhosis and NAFLD

As detailed above, chronic liver disease is associated with a loss of the symbiotic relationship between the microflora and the intestine of the host. [21] The quantitative and qualitative microbiome changes present in cirrhosis and NAFLD promote bacterial translocation. In turn, inflammatory mediators contribute to disrupt the tight junctions between intestinal epithelial cells leading to translocation of intraluminal contents into the systemic circulation. The pathophysiology, which drives liver disease and its complications, is strongly associated with changes in gut barrier function, which allows bacteria and bacterial products to move into the mesenteric lymph nodes and on to other sites. Whilst the exact mechanisms, which promote bacterial translocation, are still poorly understood, removal of LPS and other mediators, which promote sustained enteric, hepatic and systemic inflammation, is a promising approach for the treatment of liver disease.

The recent studies suggesting that alterations in the microbiome and gut permeability in patients with NAFLD led to the investigation of the potential of Yaq-001 in treating this condition in animal models. These studies confirmed that Yaq-001 reduces liver injury and prevents steatosis in these animal models.

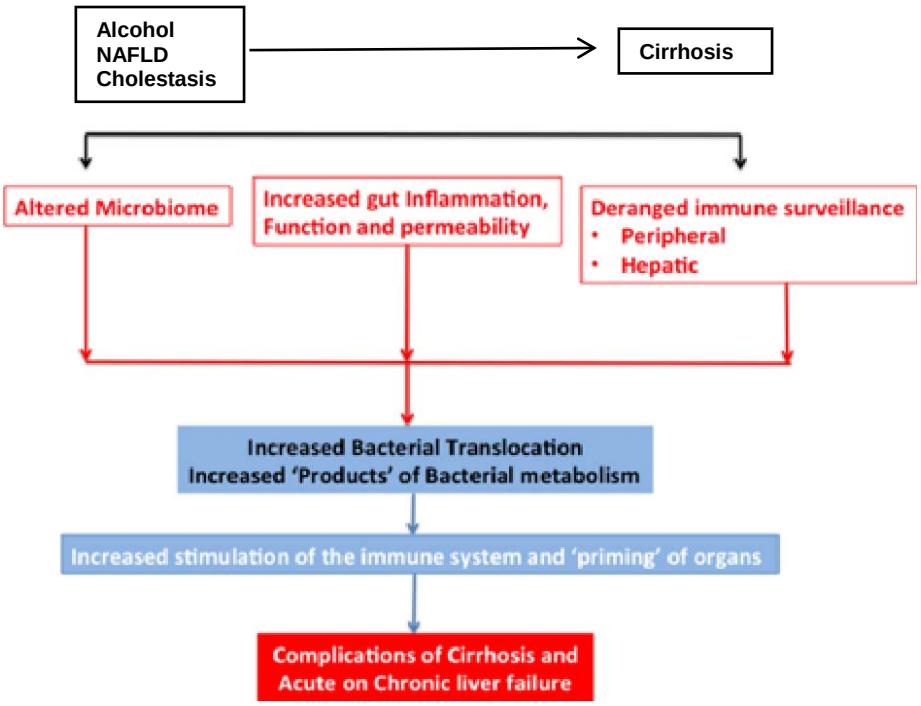



Figure 4. Pathogenesis of bacterial translocation and role in the natural history of cirrhosis

Please refer to **Section 3** for a summary of the mechanism of action of Yaq-001.

### 2.3 Regulatory Classification of Yaq-001

Yaq-001 is invasive through a body orifice and intended for long-term use (continuous use for >30 days). Thus, in accordance with the Medical Device Directive 93/42/EEC, Annex IX, it is anticipated that Yaq-001 will be classified as a Class IIb medical device.

## 3.0 NONCLINICAL STUDIES

### 3.1 Overview

Yaq-001 is a highly adsorbent, synthetically derived spherical carbon with a pore size distribution extending into the larger nanoporous range to allow removal of larger biotoxins such as bacterial LPS, and enterotoxin B.

University College London has performed a series of nonclinical investigations that demonstrate the **proof of concept, the mechanism of action, and the therapeutic potentia**l of Yaq-001 in clinically-relevant animal models of advanced chronic liver disease and NAFLD. The salient points of the results of the nonclinical studies to support the clinical use of Yaq-001 in the treatment of cirrhosis and NAFLD are as follows:

### 3.1.1 Rat bile duct ligated (BDL) model of cirrhosis

- No adverse effect of Yaq-001 was detected on gut mucosa. [22]
- Yaq-001 treated animals had significantly lower ALT, creatinine and portal pressure. [23]
- Endotoxin-induced deterioration of liver function (ALT), renal function (creatine), and portal pressure was significantly reduced in Yaq-001 treated rats, compared with control animals. (LPS was administered to mimic the development of acute on chronic liver failure (ACLF). [24]
- Yaq-001 normalised endotoxin-induced organ injury associated with a significant improvement in liver oxidative stress. [24]
- Reductions in markers of bacterial translocation were observed with Yaq-001 therapy associated with a significant improvement in intestinal permeability. [25]
- BDL-induced cachexia in BDL rats were prevented in the Yaq-001 rats. No significant differences in electrolyte of plasma micronutrient levels were observed in Yaq-001 treated rats. [24]
- Taken together, Yaq-001 diminishes organ dysfunction in a model of cirrhosis by conferring a phenotype of diminished endotoxin sensitivity.

### 3.1.2 Other models of cirrhosis

- Metabolomic Profiling: A significant difference in urinary $^{1}$NMR analysis metabolomic profile was observed in cirrhotic rats compared to sham controls. Yaq-001 modulates the metabolomic profile away from disease in the BDL model but does not exert a significant effect in control rats. In tissue metabolomic profiling, Yaq-001 did not exert any significant effect on ileal or colonic tissue.
- Changes in Gene Expression: Significant reductions in gene expression in oxidative stress, inflammation, fibrosis, and cell death pathways was reduced in Yaq-001-treated BDL rats. Most of the genes involved in pathways relating to response to bacterial infection in the terminal ileum were down-regulated by Yaq-001 therapy. Yaq-001 inhibited neuronal cell death in cirrhotic rats. A reduction in cognitive impairment was also observed with Yaq-001 treatment.

### 3.1.3 Models of Nonalcoholic Fatty Liver Disease (NAFLD)

- Oral Yaq-001 therapy resulted in a significant attenuation of liver injury in genetically obese mice and non-alcoholic steatohepatitis induced by a methionine choline deficient diet (MCD). [24]
- A significant normalisation in endotoxin-induced liver oxidative stress was observed in Yaq-001 treated genetically obese mice. [26]
- A trend towards reduction in body weight was observed with Yaq-001 treated genetically obese mice. No reduction was observed in Yaq-001 treated

heterozygote controls despite pair-feeding. [24]

- Yaq-001 was associated with a significant reduction in glycosuria in genetically obese mice, suggestive of an improvement in insulin resistance. [24]

- Significant normalization of urinary metabolomics profile was observed with Yaq-001 therapy.

Oral Yaq-001 administration results in an attenuation of the heightened endotoxin sensitivity observed in cirrhosis and NAFLD. This results in an improvement in organ injury and haemodynamic status and is associated with a normalization of innate immune function. At the level of the gut barrier interface, Yaq-001 is associated with a distinct alteration in microbiome composition and function with an improvement in intestinal permeability and nutritional status observed.

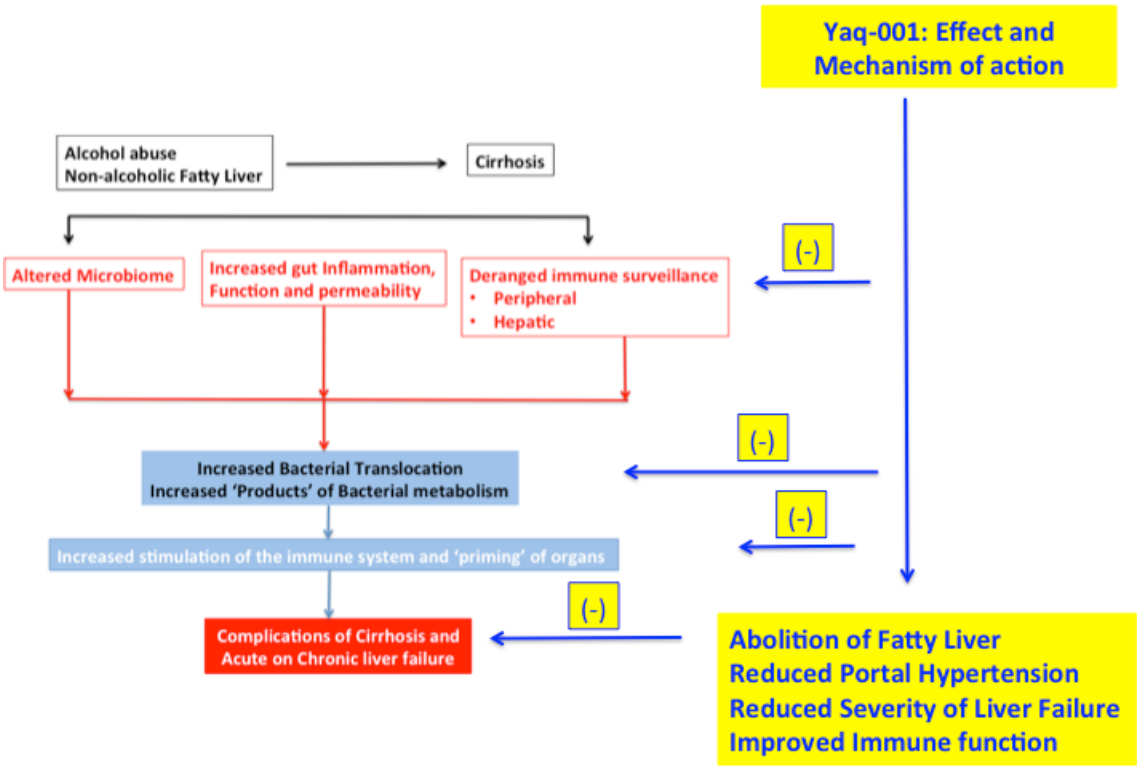



Figure 5. Mechanism of action of Yaq-001: The data presented in the following sections of this IB support the mechanisms by which Yaq-001 prevents the development of NAFLD and complications of cirrhosis. The data show that Yaq-001 modulates the microbiome, increases in gut inflammation and deranged immune function, which impacts translocation of bacterial products. This reduces the mechanisms that sensitise the organs, thereby preventing the development of NAFLD and complications of cirrhosis.

### 3.1.4 Mass Balance Study

Greater than 95% of Yaq-001 carbon spheres were recoverable from faeces within 48 hrs of Yaq-001 dosing in a healthy rat model. The difference between the recovered and reference materials is most likely due to carbon beads being lost during the extended washing cycles. This study demonstrates the lack of systemic absorption of Yaq-001.

### 3.1.5 13-week rat toxicity study

The in vivo study included histopathological examination throughout the entire GI tract. No abnormalities and/or indications of cell damage were noted by independent, certified pathologists. No visible lesions in any gut tissue, including cecum, colon, rectum, duodenum, ileum, and jejunum were seen upon macroscopic examination in the 13-week rat toxicity study. Nor were any effects on mandibular or mesenteric lymph nodes seen after prolonged exposure to Yaq-001.

The pathology results showed no gross or microscopic findings or organ weight changes associated with dietary administration of Yaq-001 in the diet at doses of 0.7, 1.4 and 2 g/kg/day (equivalent to 5x, 10x, and 15x the projected maximum clinical dose).

### 3.1.6 In vitro drug-drug interaction studies

The in vitro adsorption kinetics of Yaq-001 for a range of commonly prescribed medications in cirrhosis was determined. Yaq-001 is a non-absorbed, non-selective adsorbent which, like other non-selective adsorbents such as AST-120 and cholestyramine, has an adsorptive capacity for commonly used drugs. Yaq-001 increased the adsorption of all medications tested in vitro. Yaq-001 had a higher adsorption capacity for amoxicillin and rifaximin than the control microporous carbon and an equivalent absorption for tenofovir.

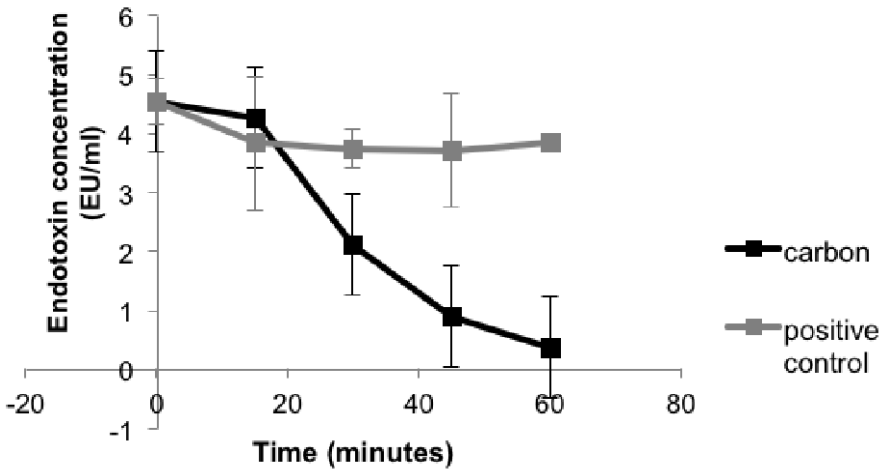
The clinical significance of the in vitro data is unknown. Until in vivo pharmacokinetic DDI studies are conducted, the conventional means to mitigate the risk of drug-device interactions is to employ a dosing interval of up to 4 hours based on the Biopharmaceutical Classification System (BCS) recommendations. As additional precautionary measure, patients will be screened for orocaecal time and exclusion criteria for this parameter are established in the protocol. See Sections 3.7 and 6 of this IB and the Clinical Investigational Plan for further discussion.

## 3.2 In vitro studies

In vitro studies demonstrated that nanoporous carbons in the TE6-TE8 range (Yaq-001) have rapid adsorption kinetics for endotoxin, acetaldehyde and formylmethionine-leucyl-phenylalanine with no significant impact on bacterial growth kinetics. They therefore exhibit optimal properties for oral prevention/treatment in liver disease, i.e. binding of important intraluminal factors, which drive pathogenesis without exerting an antibiotic effect on the resident bacterial flora with the attendant risks of dysbiosis.

### 3.2.1 Endotoxin Adsorption

The concentration of endotoxin detected in the simulated intestinal fluid (SIF) solution initially spiked with 10 EU ml-1 declined from a detected value of 4.5 EU ml-1 at time 0 to 0.4 EU ml-1 following 60 minutes' incubation with the test carbons. The control solution maintained a steady 4.5 EU ml-1 concentration over time. These findings demonstrate rapid endotoxin adsorption kinetics in an appropriate pathophysiological context (Figure 8).




### 3.2.2 *Figure 6. Adsorption kinetics of carbon for* E. coli *endotoxin* **Acetaldehyde Adsorption**

The kinetics of acetaldehyde removal by the TE7 carbon was very rapid. The majority of the acetaldehyde was removed after an incubation time of 1 minute at room temperature following a spike of acetaldehyde in simulated intestinal fluid. Incubation of carbon with acetaldehyde solution an equilibrium adsorptive capacity of 0.16 g per gram of carbon was reached, within 3 minutes, using a 400 uM acetaldehyde spike (Figure 9).

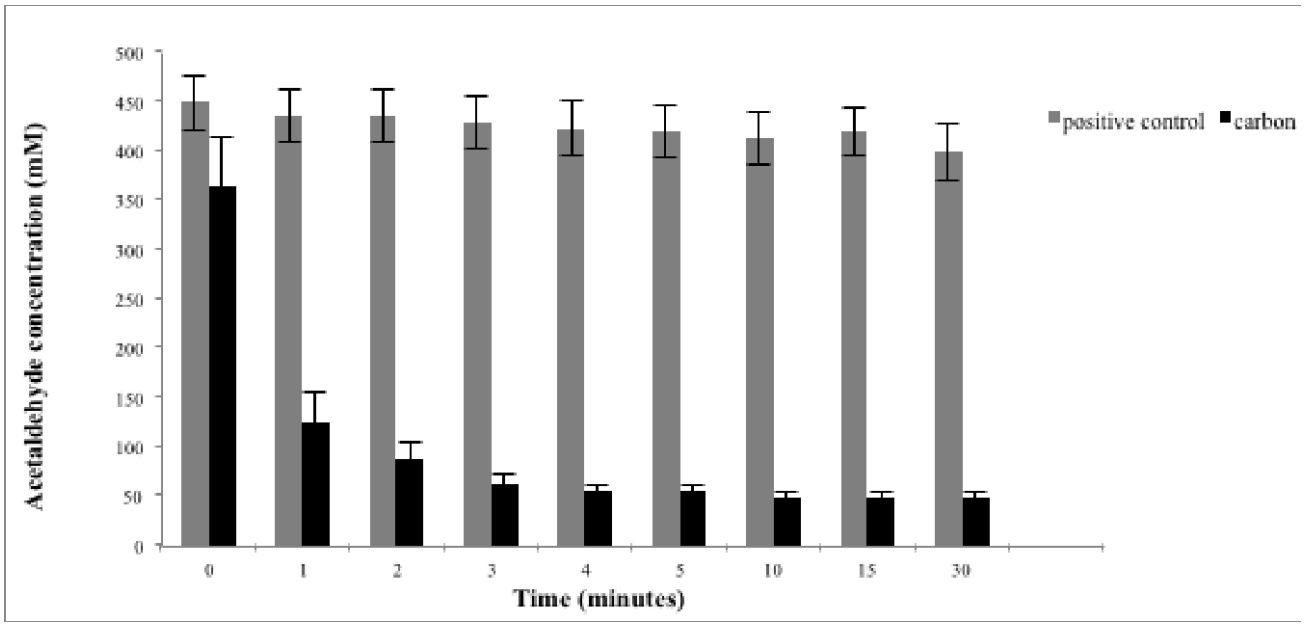



Figure 7. Acetaldehyde removal over time by TE 7 carbon compared to no carbon control

### 3.2.3 fMLP (Formylmethionine-leucyl-phenylalanine) adsorption

fMLP is a potent polymorphonuclear leukocyte (PMN) chemotactic factor and is the major peptide chemotactic factor produced by E. coli. This pro-inflammatory peptide is produced by enteric aerobic and anaerobic bacteria resulting in potent production of reactive oxidant species (ROS). Colonic instillation of fMLP has been used to induce an animal model of mucosal inflammation. Figure 10 demonstrates that Yaq-001 exhibits rapid adsorption kinetics for fMLP.

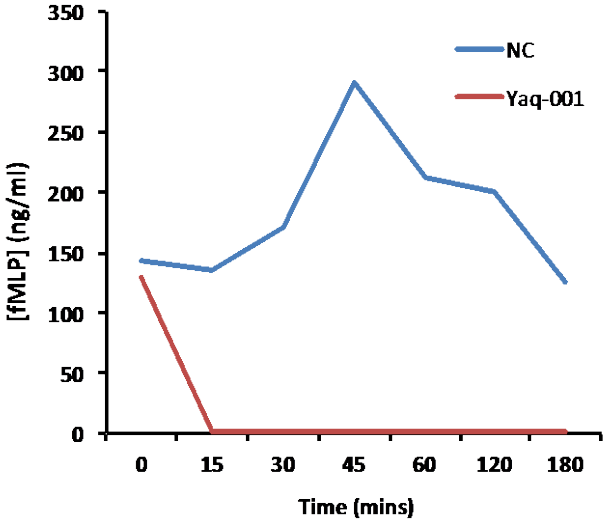



Figure 8. Adsorption kinetics of Yaq-001 compared to no carbon (NC) for fMLP as determined by ELISA assay

### 3.2.4 Studies to determine effects of carbon on bacterial growth kinetics: Investigating the effect of direct contact incubation of test carbons on bacterial metabolism

The direct incubation of test carbon with bacterial suspensions of either *E. coli* or *S. aureus* in culture medium indicated that the TE7 and TE8 carbon does not affect bacterial growth for either species following direct contact of 4 to 6 hours. The luminescence measurement is an indirect measure of cell viability and cell number via the determination of bacterial ATP. Levels of luminescence, reflective of bacterial metabolism on sample contact, were comparable to the no adsorbent control for both species over the time course of the experiment. In contrast, the commercial oral carbon control greatly reduced the luminescence signal after only 30 minutes' incubation of bacteria with the carbon (Figure 9 and Figure 10).

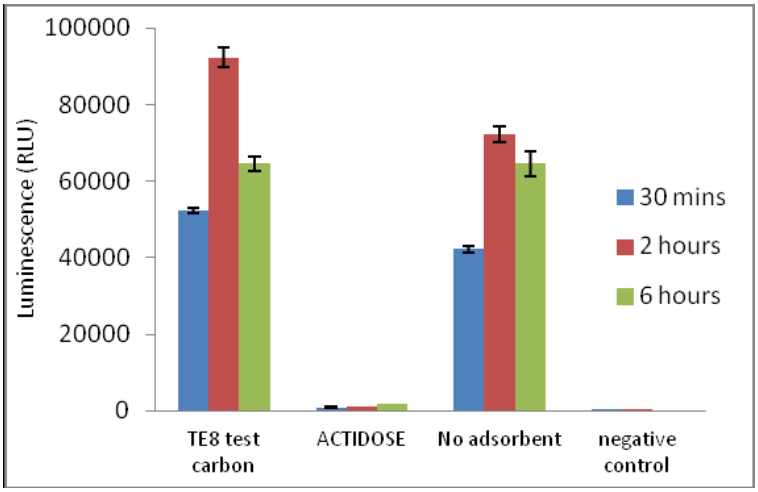



Figure 9. Effect of carbon on *E. coli* growth kinetics (BacTitre-Glo microbial cell viability assay)

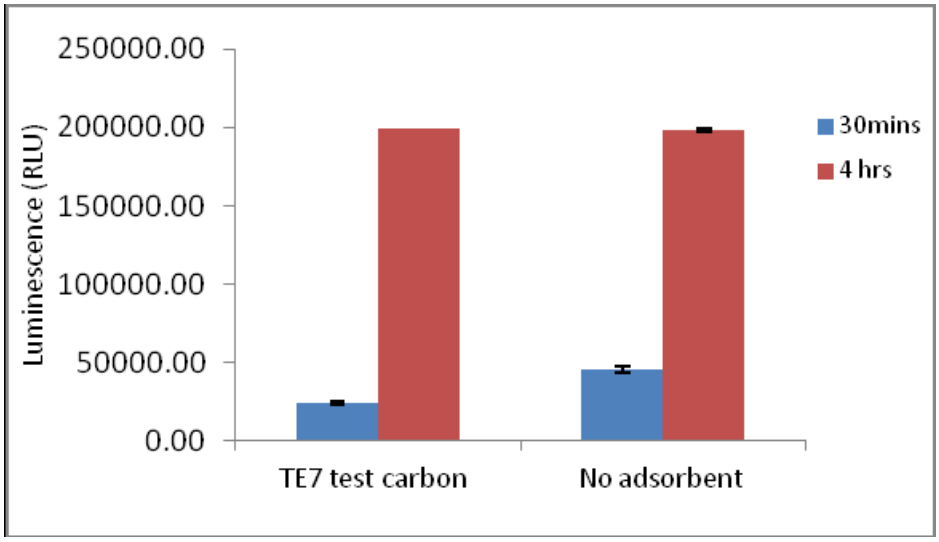



Figure 10. Effect of carbon on *S. aureus* growth kinetics (BacTitre-Glo microbial cell viability assay)

### 3.2.5 Investigating the effect of carbon leachate on bacterial metabolism

The direct incubation of TE7 carbon leachate with bacterial suspensions of either *E. coli* or *Bacillus subtilis* in culture media indicated that the TE7 carbon leachate does not affect bacterial growth for either species for up to 72 hrs incubation. The optical density (OD) measurement is an indirect measure of bacterial number. OD values for the TE7 sample were comparable to the no adsorbent control for both species over the time course of the experiment (Figure 11 and Figure 12).

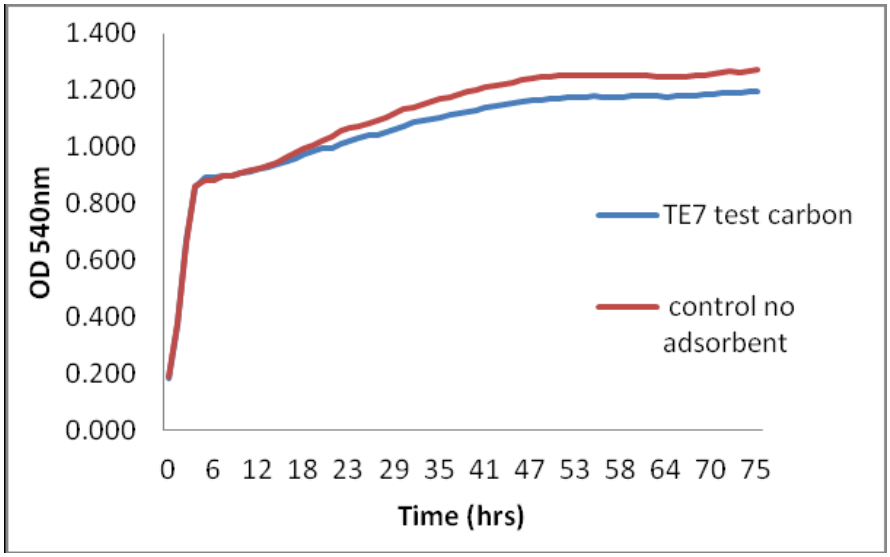



Figure 11. Effect of carbon leachate on *E. coli* growth kinetics (Bioscreen turbidometric analyser)

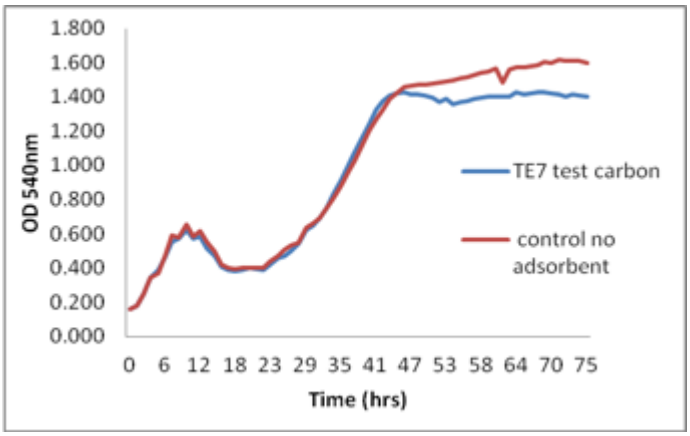



*Figure 12. The effect of carbon leachate on the* B. subtilis *growth kinetics (Bioscreen turbidometric analyser)*

### 3.3 Studies of Yaq-001 in animal models of cirrhosis

About 200 animals were studied in the experimental protocols. In general, the animals were culled 4 weeks after either bile-duct ligation (BDL) or Sham operation. They were randomised to treatment with up to 4g/kg of Yaq-001 or placebo for 2-weeks prior to being culled.

#### 3.3.1 Effect of Yaq-001 on liver and end organ function in BDL cirrhotic rats.

Yaq-001 treated animals had significantly lower ALT, creatinine, brain water and portal pressure. There were no significant differences in severity of fibrosis and mean arterial pressure between treated and placebo groups. Bile-duct ligation (BDL)-induced cachexia in the BDL animals was prevented in the Yaq-001 treated animals. Yaq-001 has beneficial effects on nutrition, the liver, kidney and portal hypertension in an animal model of cirrhosis. **Figure 13**.

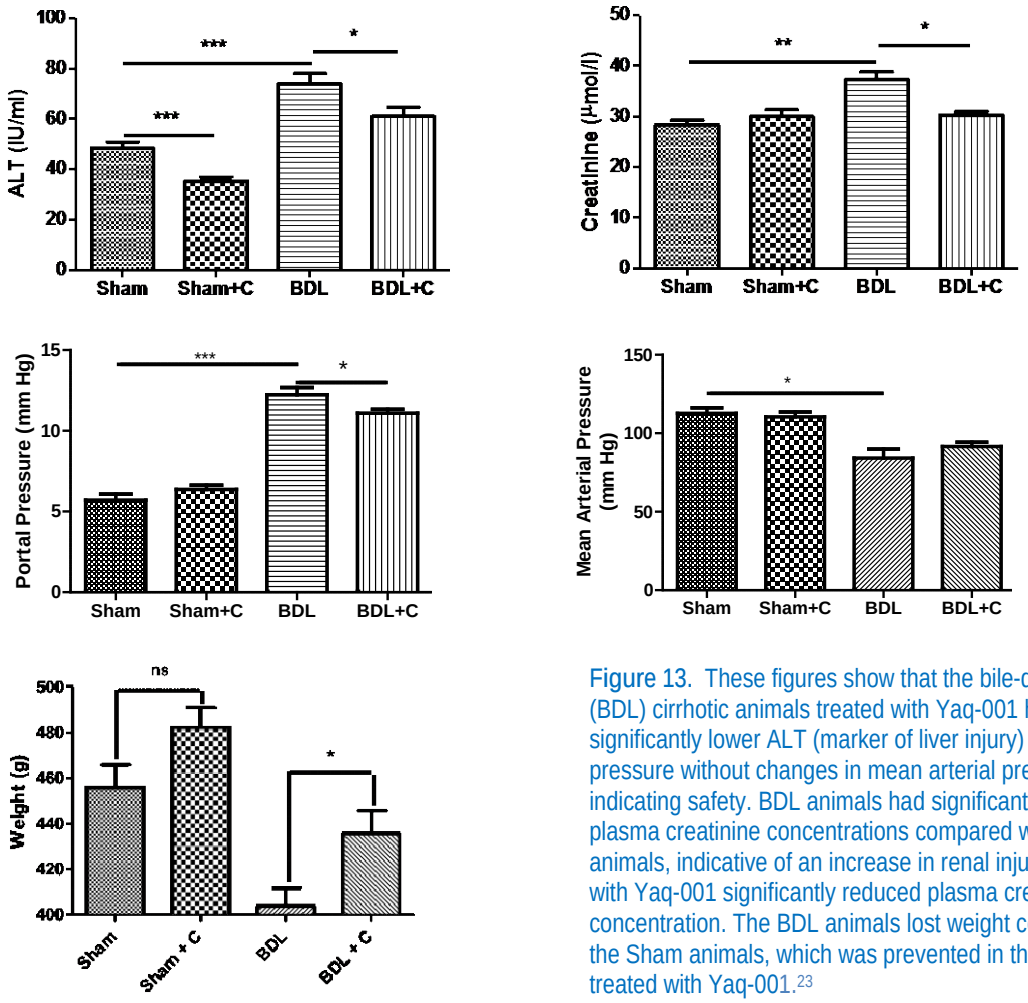



Figure 13. These figures show that the bile-duct ligated (BDL) cirrhotic animals treated with Yaq-001 have significantly lower ALT (marker of liver injury) and portal pressure without changes in mean arterial pressure indicating safety. BDL animals had significantly higher plasma creatinine concentrations compared with the Sham animals, indicative of an increase in renal injury. Treatment with Yaq-001 significantly reduced plasma creatinine concentration. The BDL animals lost weight compared with the Sham animals, which was prevented in the BDL animals treated with Yaq-001.[23]

* denotes p<0.05 ** denotes p<0.005 *** denotes p<0.001

### 3.3.2 Effect of Yaq-001 on LPS - induced acute on chronic liver failure (ACLF)

In these experiments, BDL rats were administered LPS to mimic the development of acute on chronic liver failure (ACLF). The treatment groups were Yaq-001 or placebo. LPS-induced deterioration of liver function (ALT), renal function (creatinine), brain water and portal pressure observed in the placebo-treated group was significantly reduced in the animals treated with Yaq-001.

Yaq-001 prevents the development of liver injury and portal pressure in models of ACLF.

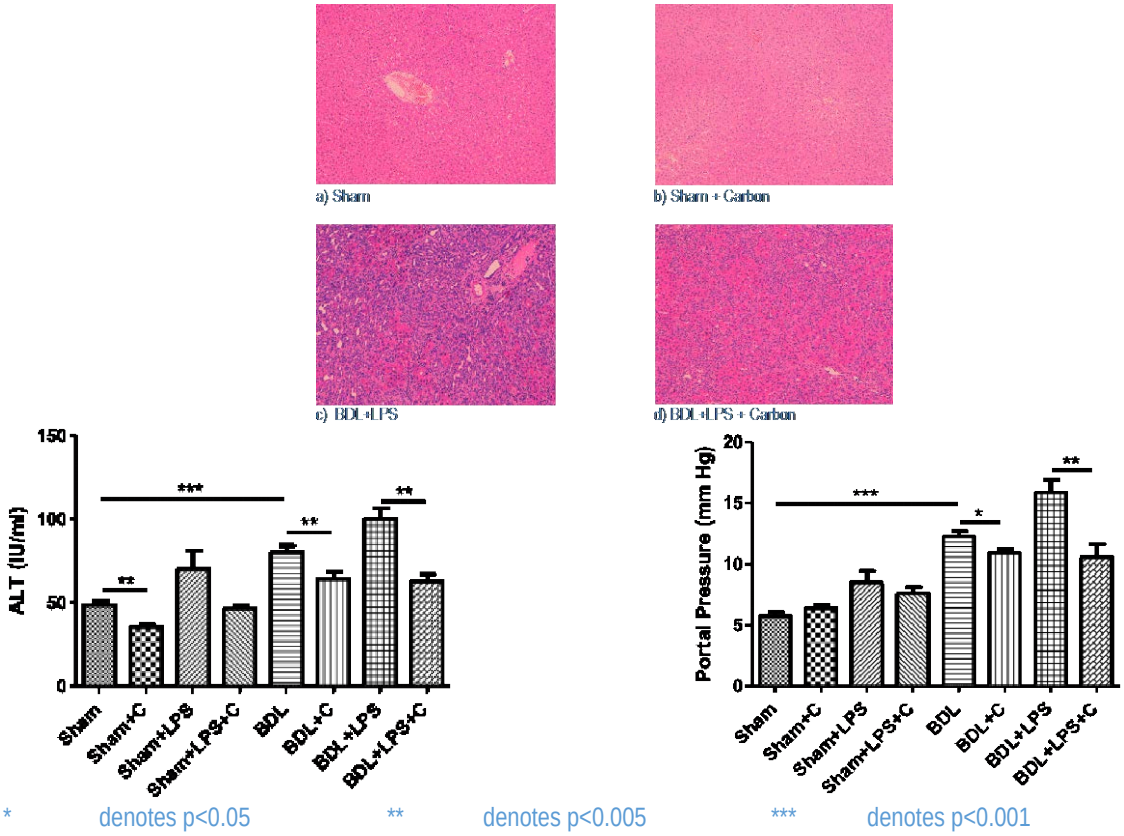
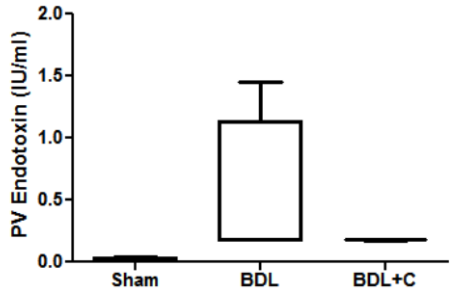


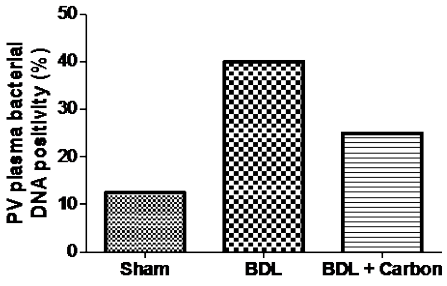

Figure 14. The above figures show that Yaq-001 prevents the occurrence of acute on chronic liver failure in animals treated with LPS. Administration of LPS to BDL animals leads to an increase in histological liver injury (see above panel (Haematoxylon and Eosin staining of liver tissue). This was manifested biochemically by an increase in ALT and associated with increase in portal pressure. These changes were attenuated in animals treated with Yaq-001 indicating that Yaq-001 may prevent the occurrence of complications of cirrhosis and acute on chronic liver failure. [22]

### 3.3.3 Effect of Yaq-001 on gut permeability and bacterial translocation in BDL rat model

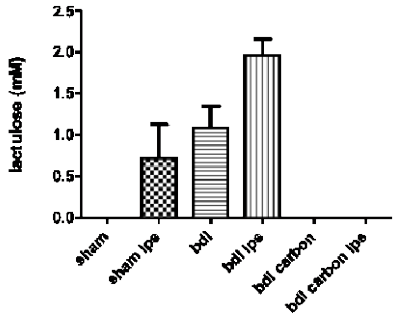
Reductions in translocated bacterial products were observed in the group treated with Yaq-001, compared with controls. The pathologically increased intestinal permeability observed in BDL rats was found to be improved with oral Yaq-001 therapy. Significant improvements in stool microbiome composition were observed in Yaq-001 fed BDL rats with greater representation of more beneficial autochthonous bacteria. No adverse changes were observed in mucosal histology with Yaq-001 therapy. These data indicate that Yaq-001 had significant beneficial effect on gut translocation through restoration of gut permeability and shift in stool microbiome composition towards a relative expansion of Firmicutes phyla and a reciprocal contraction in bacteroides populations. **Figure 15**



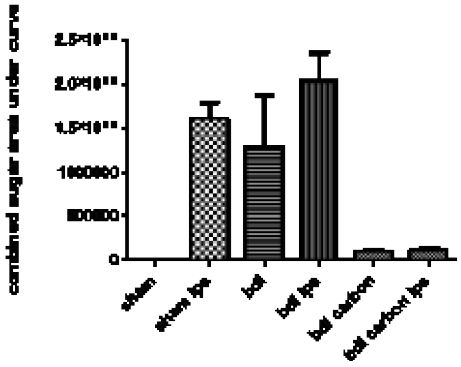

a) Portal endotoxin concentrations



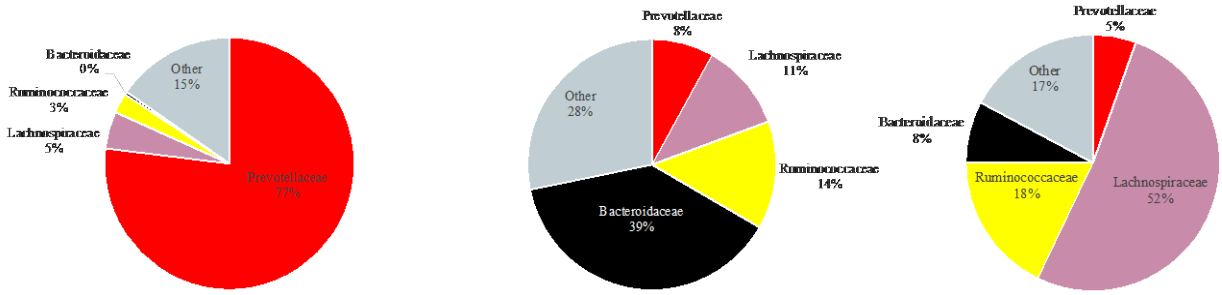

b) Portal venous bacterial DNA positivity




c) Urinary lactulose excretion

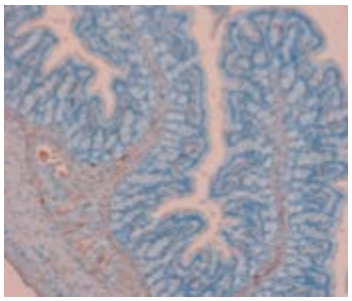



d) Urinary excretion of rhamnose and 3-o-methyl-glucose

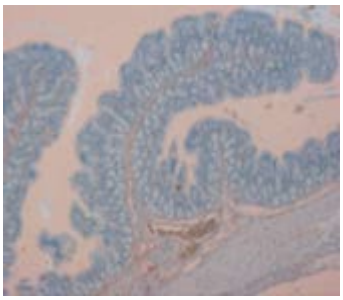



Sham BDL BDL+Yaq-001

e) Stool microbiome composition



Sham



Sham + Carbon

Figure 15. Effect of Yaq-001 on bacterial translocation, intestinal permeability (urinary lactulose, rhamnose, 3-O-methyl-glucose excretion) and microbiome composition.

Top panel: Yaq-001 treated BDL animals had significantly lower portal venous endotoxin levels and portal venous Bacterial DNA indicating reduced translocation.

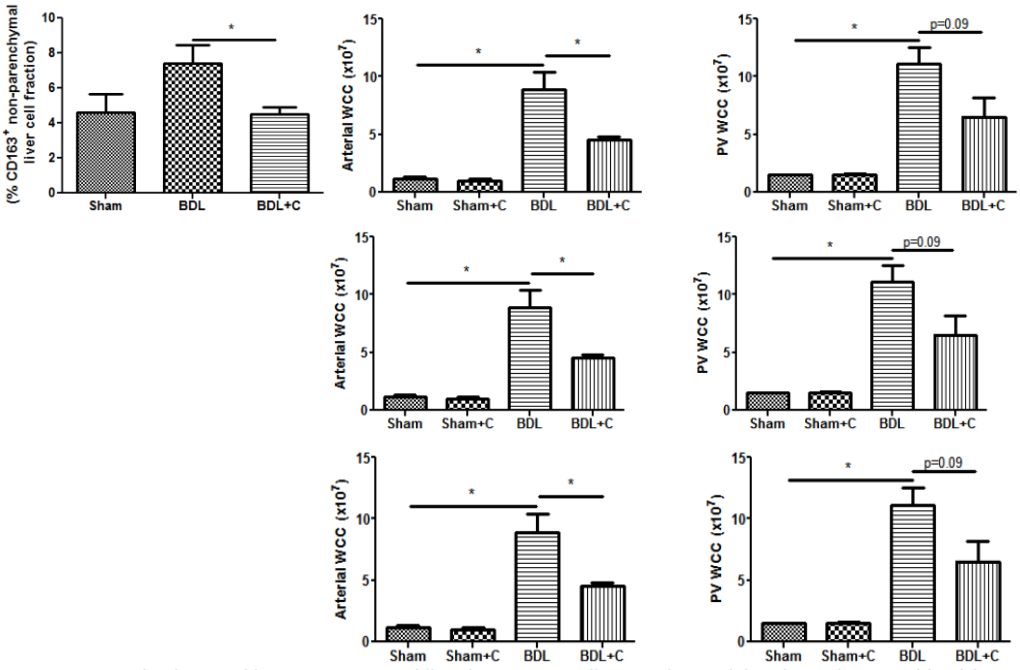
Middle panel: The middle panel shows that the BDL animal had a high urine excretion of lactulose/rhamnose/6-O-methylglucose indicating increased intestinal permeability, which was prevented in the animals treated with Yaq-001.

Bottom panel: These data shows that the administration of Yaq-001 has an effect on the microbiome composition with an increased representation of autochthonous bacterial populations with Yaq-001. [25]

Representative figure showing gut histology obtained from the colon showed no differences in the animals treated with Yaq-001 compared with the untreated animals.

### 3.3.4 Effect of Yaq-001 on the number and the inflammatory phenotype of the migrating cells from the gut to the liver in BDL rat model

BDL rats were found to have an increase in tissue resident and circulating innate immune populations. This is associated phenotypically with a heightened primed state to endotoxin challenge resulting in a significant increase in reactive oxidant species production and heightened organ injury. Yaq-001 therapy resulted in a significant improvement in tissue resident and circulating innate immune populations along the gut-liver axis together with a significant attenuation in LPS-induced ROS production. These data suggest that oral Yaq-001 therapy conditions the innate immune phenotype towards the level of healthy control with a reduced in endotoxin-sensitivity.




Absolute total leucocyte, neutrophil and monocyte cell counts in arterial and portal venous blood (x$10^7$ cells/ml)

* denotes p<0.05 ** denotes p<0.005

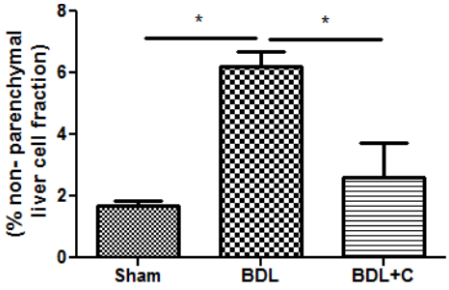
Figure 16. These data provide a potential mechanism for the effect on Yaq-001 in protecting the BDL animals from liver injury by altering the functionality of immune cells along the gut-liver axis. BDL animals have an increase Kupffer cells expressed as %CD163 positive total non-parenchymal cell fraction. Both these were

modulated towards normality. These populations were significantly reduced towards sham controls with oral Yaq-001 therapy[23]

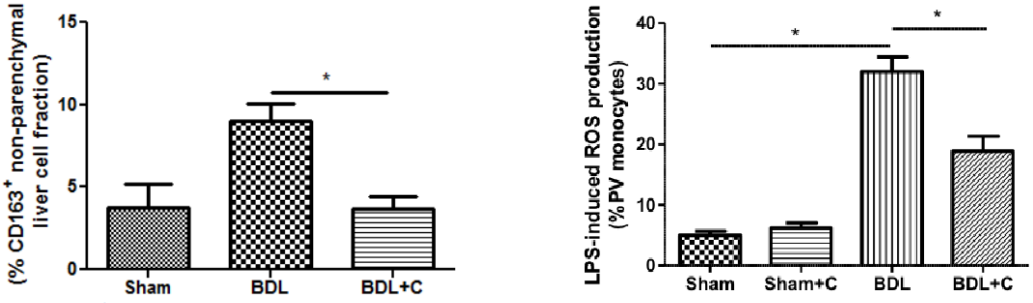
### 3.3.5 Effect of Yaq-001 treatment on production of reactive oxygen species from hepatic Kupffer cells in BDL rat model

Bile duct ligation was observed to be associated with a significant increase in total liver ROS; an effect observed to be attenuated with Yaq-001 treatment. Kupffer cells were subsequently isolated from the Yaq-001 treated and untreated BDL animals. Yaq-001 treated animals retained normal Kupffer cell phenotype with regards to phagocytic function and reactive oxygen species (ROS). ROS production in response to endotoxin challenge was found to be significantly reduced in Yaq-001-treated cirrhotic rats. The data provide a mechanism of action for the beneficial effects of Yaq-001 on liver injury.

Total Liver Production of Reactive Oxidant Species




LPS-induced ROS production in Kupffer cells and Portal Venous Monocytes



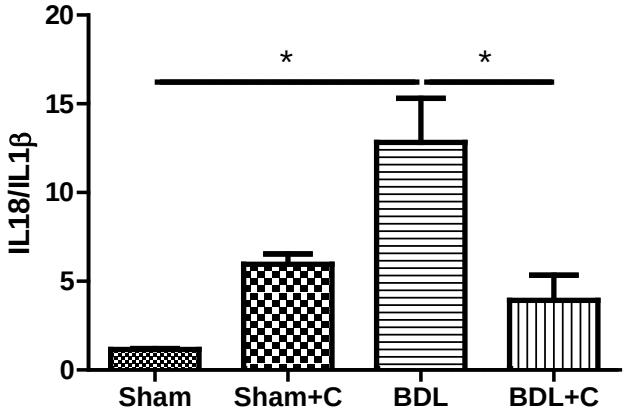

* denotes p<0.05

Figure 17. These figures help define the mechanism of the protective effect of Yaq-001 in BDL animals.

These figures demonstrate that total liver ROS production is significantly attenuated towards sham controls with oral Yaq-001 therapy. BDL rats have an increased ROS production in response to LPS challenge indicative of a heightened primed state both in Kupffer cells and trafficking portal venous monocyte populations. This is attenuated with oral Yaq-001 therapy suggestive that biologically there is a reduction in endotoxin sensitivity in both liver resident and trafficking monocyte populations [25]

### 3.3.6 Effect of Yaq-001 on inflammation by reduced inflammasomal activation in BDL rat model

These data provide evidence that Yaq-001 attenuates inflammation by reducing inflammasomal activation. Bile duct ligation is associated with a significant increase in inflammasomal activation, as evidenced by an increase in IL1b/IL18. This is significantly attenuated with Yaq-001 therapy. As described earlier, a significant reduction in ROS production was observed in Yaq-001-treated cirrhotic rats. This suggests that the predominant mechanism of action of carbon therapy is a reduction in ROS-induced inflammasomal activation. [27]



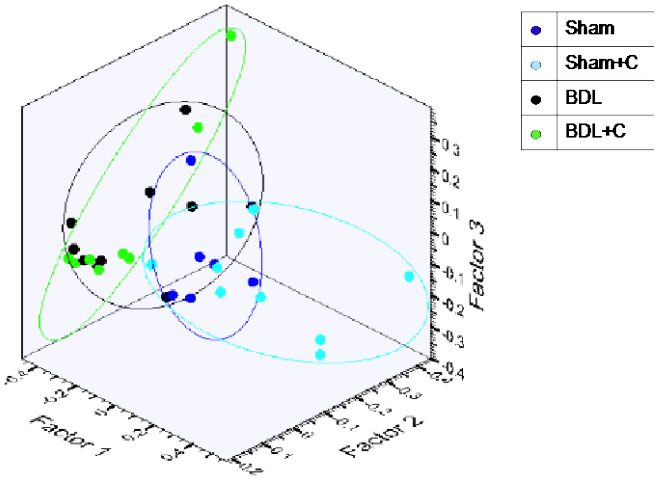

* denotes p<0.05

Figure 18. The data describe the effect of arterial plasma on 'reporter cell lines (HEK cells)' to determine whether Yaq-001 alters inflammasomal activation. The above figure describes the effect of plasma obtained from BDL rats incubated with the IL1B reporter cells and shows that the plasma from BDL animals induces greater activation of the inflammasome and this is significantly reduced in the Yaq-001 treated animals.

### 3.3.7 Metabolomic Profiling

Metabolomic profiling was conducted using $^{1}$NMR spectroscopy and liquid chromatography–mass spectrometry for analysis of urine and tissue respectively. Urine metabolomics analysis provides a composite assessment of host-microbiome metabolic status.

- ***Urinary 1NMR analysis***




| | Sham | Sham+C | BDL | BDL+C |
|---|---|---|---|---|
| **Sham** | | 0.79 | 1.71 | 1.20 |
| **Sham+C** | 0.79 | | 1.36 | 1.64 |
| **BDL** | 1.71 | | | 0.12 |
| **BDL+C** | 1.20 | 1.64 | 0.12 | |

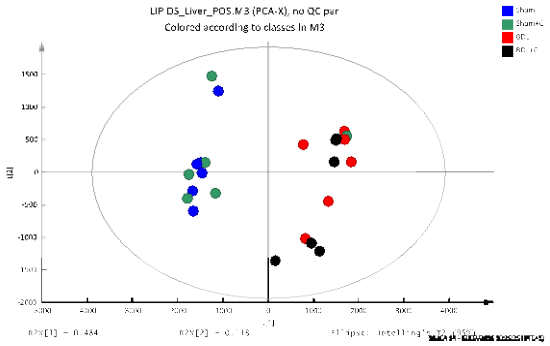
Figure 19. Principal Component Analysis of $^{1}$NMR analysis of urine from BDL and sham rats +/- Yaq-001 therapy

A significant difference in metabolomic profile is observed in cirrhotic rats compared to sham controls. Yaq-001 modulates the metabolomic profile away from disease in BDL model but does not exert a significant effect in control rats. This reflects the efficacy in disease whilst highlighting a favorable safety profile in the control group.

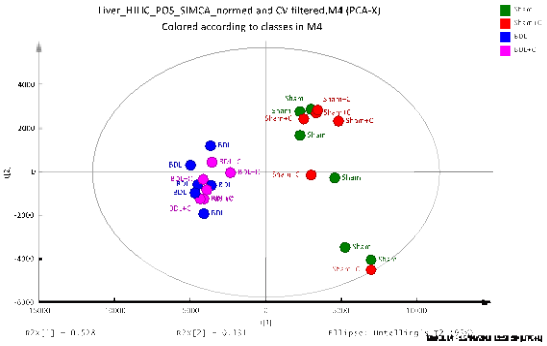
- ***Tissue metabolomic profile***

Tissue metabolomic profiling was determined in liver, ileum and colon using liquid chromatography–mass spectrometry. Significant differences were observed in lipid-soluble and water-soluble fractions in the liver tissue of cirrhotic rats compared to sham controls. Yaq-001 did not exert any significant effect on profile in either cirrhotic or sham rats. No significant differences in lipid or water-soluble fractions of ileal or colonic tissue were observed between BDL and sham rats. Yaq-001 did not exert any significant shift in profile in cirrhotic rats in either the lipid or water-soluble fractions or ileal or colonic tissue.

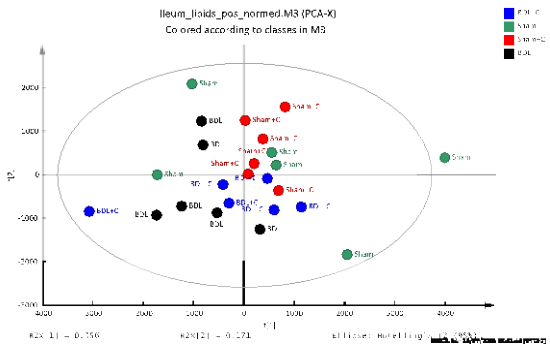
1.a



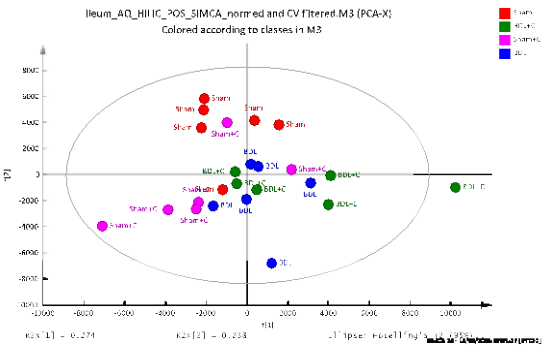

1b



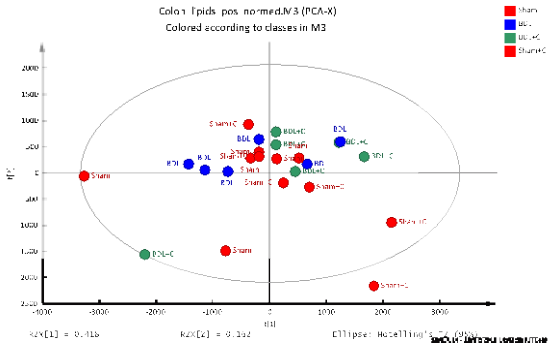

2a




2b




3a




3b

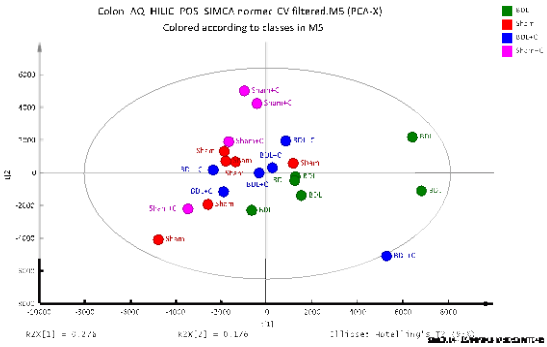



Figure 20. Principal Component Analysis of tissue extract in BDL and sham rats +/- Yaq-001 therapy

20.1a Liver tissue lipid fraction
20.2a Ileal tissue lipid fraction
20.3a Liver tissue lipid fraction

20.1b Liver tissue water-soluble fraction
20.2b Ileal tissue water-soluble fraction
20.3b Liver tissue water-soluble fraction

### 3.3.8 Changes in Gene Expression

RNA-Seq expression analysis was conducted using tissue from liver, terminal ileum and brain of BDL and sham rats with and without Yaq-001 therapy. The data was analysed using Ingenuity Pathway Analysis to identify pathways which are modulated by Yaq-001 therapy in each of these tissues.

- ***Liver***

Significant up-regulation of gene expression relating to pathways in oxidative stress, inflammation, fibrosis and cell death were observed in BDL rats compared to sham controls. Significant reductions in gene expression in each of these four pathways was reduced in BDL rats treated with Yaq-001 therapy:

- oxidative stress (z-score -2.59; p value $2x10^{-5}$
- inflammation (z-score -1.35; p value $4x10^{-3}$
- fibrosis (z-score -1.29; p value $1x10^{-4}$
- cell death (z-score -1.19; p value 1x10-$^{3}$

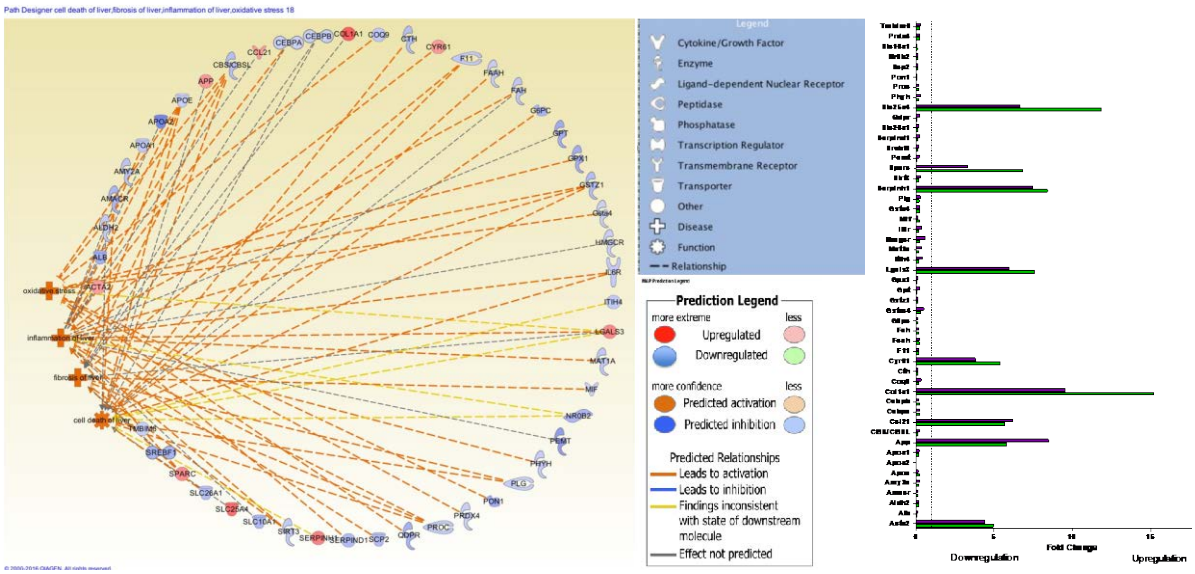



Figure 21. (a) Pathways upregulated in the liver tissue of BDL rats compared to sham controls (b) Relative gene expression in liver tissue of untreated BDL (green) and Yaq-001-treated BDL rats (purple)

- ***Terminal Ileum***

In BDL rats, pathways relating to response to bacterial infection (z-score 1.47; p value $2x10^{-4}$), proliferation of cells (z-score 2.17; p value $1x10^{-6}$) and inhibition of degranulation of lysosomes (z-score -1.49; p value $3x10^{-4}$) are up-regulated. Most of genes involved in these processes were down-regulated by Yaq-001 therapy.

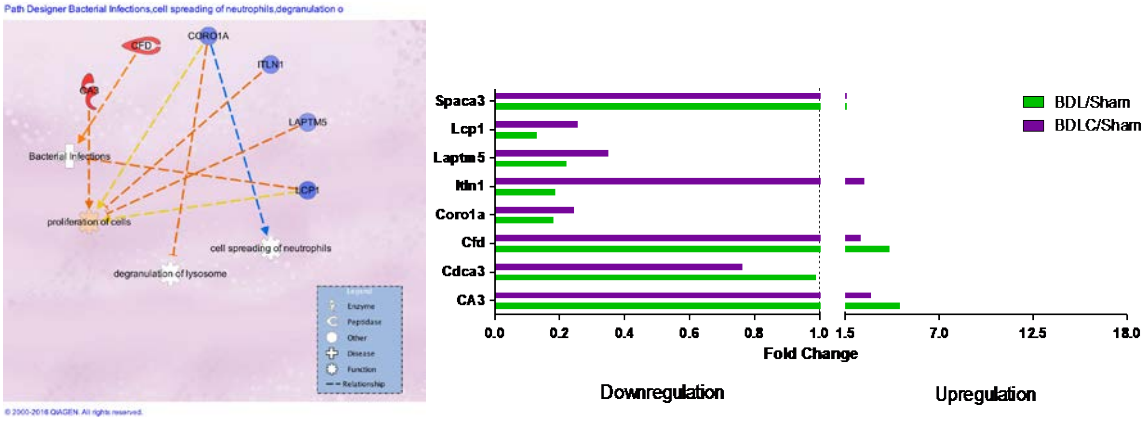



Figure 22. (a) Pathways upregulated in the ileal tissue of BDL rats compared to sham controls (b) Relative gene expression in ileal tissue of untreated BDL (green) and Yaq-001-treated BDL rats (purple)

- ***Brain***

An increase in the gene expression of markers of neuronal cell death was observed in the brain tissue of BDL rats. Yaq-001 inhibited neuronal cell death (z-score -3.45; p value $4x10^{-5}$) in cirrhotic rats. A reduction in cognitive impairment (z-score -3.72; p value $5x10^{-6}$) was also observed with Yaq-001 treatment.

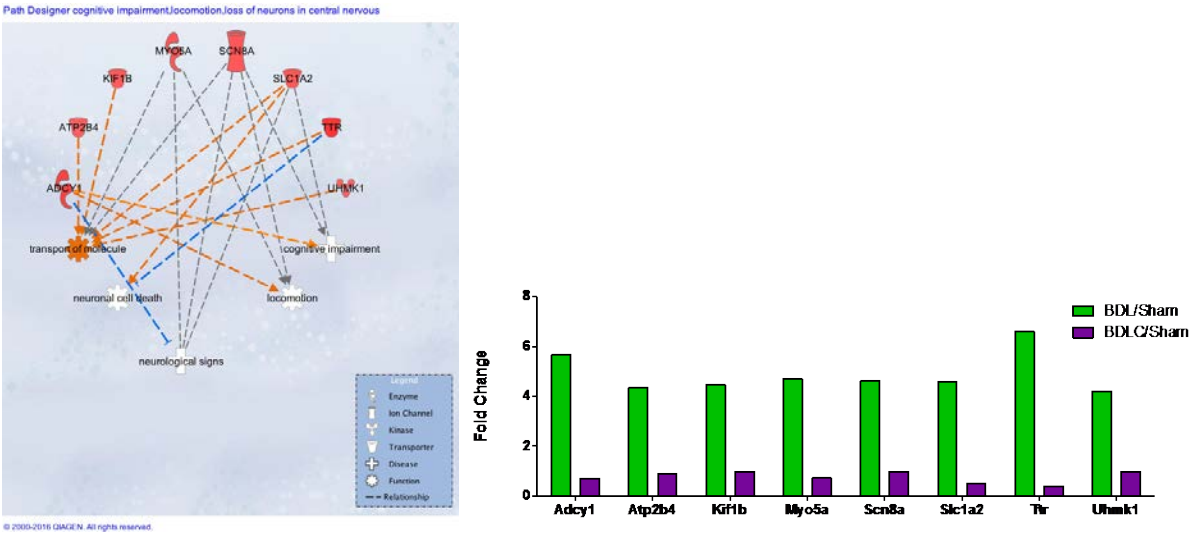



Figure 23. (a) Pathways upregulated in the brain tissue of BDL rats compared to sham controls (b) Relative gene expression in brain tissue of untreated BDL (green) and Yaq-001-treated BDL rats (purple)

### 3.4 Studies of Yaq-001 in animal models of non-alcoholic fatty liver disease (NAFLD)

Two animal models of NAFLD were studied (leptin deficient genetically obese (ob/ob) mice and methionine choline deficient mice (MCD). They were randomised to treatment with up to 0.4 g/Kg of Yaq-001 or placebo for 4-weeks prior to being culled. [26]

#### 3.4.1 *Effect of Yaq-001 on the weight and liver function of genetically* Obese mice (Ob/Ob)

Yaq-001 reduced the weight and severity of liver injury in genetically obese mice. The data showed that the genetically obese, Ob/Ob mice (Leptin deficient) lost weight after a 4-week treatment with Yaq-001; in addition, there was significant reduction in the severity of liver injury.

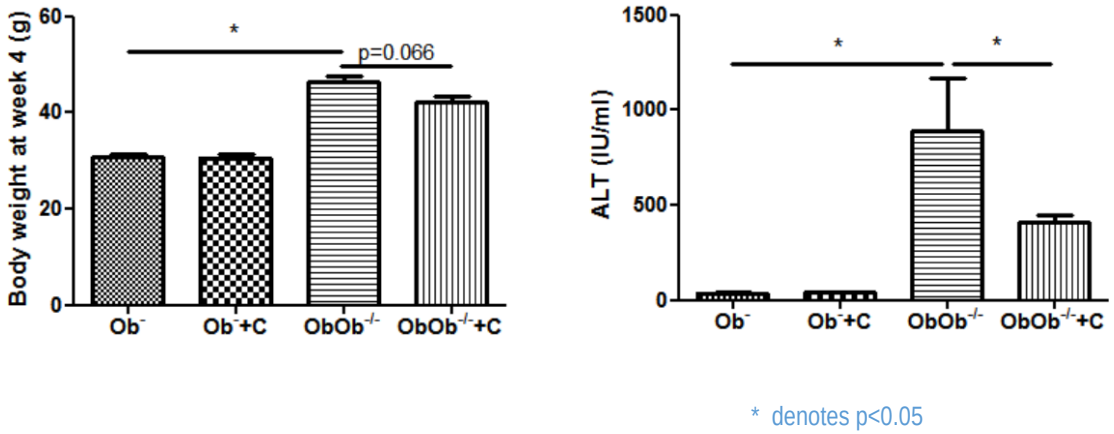



Figure 24. This figure describes the effect of administration of Yaq-001 to Ob/Ob mice or controls for 4 weeks. The Ob/Ob mice had significantly higher ALT compared with controls and this was reduced (halved) in the animals treated with Yaq-001. This was associated with a near significant reduction in weight pointing a possible role of Yaq-001 in treating obesity.[28]

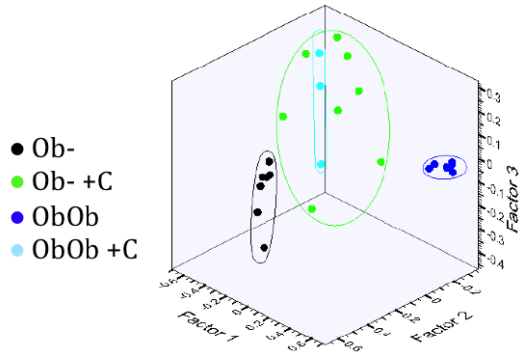
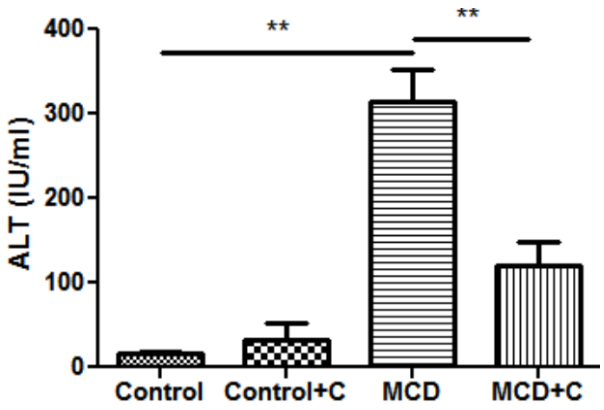



Figure 25. Principal Component Analysis ($^1$NMR spectroscopy of urine in Yaq-001 treated genetically obese mice. This figure demonstrates that Yaq-001 treatment results in a significant shift in metabolomic profile towards control values. A complete loss of glycosuria was observed with carbon-treated obese mice suggestive of a beneficial effect on the metabolic syndrome

### 3.4.2 Effect of Yaq-001 in a diet-induced model of NAFLD

Yaq-001 prevented the development of steatosis. In both MCD-fed mice and genetically obese mice, Yaq-001 prevented the rise in ALT and abrogated the development of fat in the liver.

MCD untreated MCD + carbon




** denotes p<0.005

Figure 26. These figures describe the effect of administration of methionine-choline deficient diet (MCD). Mice fed a MCD diet developed histological features of steatohepatitis (above panel - Haematoxylin and Eosin staining of liver tissue). This liver injury was evidenced biochemically by an increase in ALT which was significantly attenuated by administration of oral Yaq-001 carbon.

### 3.4.3 Effect of Yaq-001 on LPS-induced ROS production by Kupffer cells in genetically Obese mice (Ob/Ob) model [26]

Yaq-001 prevented LPS-induced ROS production by Kupffer cells. A significant reduction in LPS-induced Kupffer cell ROS production was observed in carbon-treated genetically-obese mice compared to control. In addition, a significant reduction in Kupffer cell populations was observed with carbon-treated ob/ob mice.

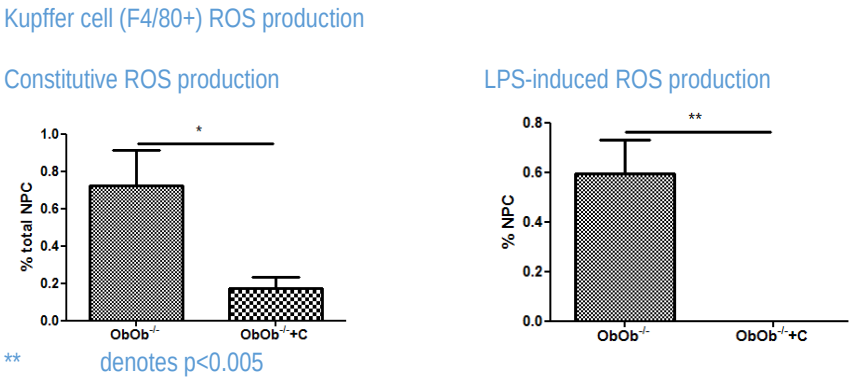



Figure 27. This figure describes the potential mechanism by which Yaq-001 may reduce liver injury in NAFLD. The data show that ROS production from Kupffer cells isolated from the liver of ob/ob mice produced significantly more ROS compared with control animals. Treatment of these animals with Yaq-001 reduced this ROS production from the Kupffer cells indicating a potential mechanism by which injury in these animals is reduced.

### 3.5 Rat Adsorption (Mass Balance) Study

Greater than 95% of Yaq-001 carbon spheres were recoverable from faeces within 48 hrs of Yaq-001 dosing in a healthy rat model. The difference between the recovered and reference materials is most likely due to carbon beads being lost during the extended washing cycles. The recovered carbon material was indistinguishable from the spheres prior to administration to the animals or from the reference samples recovered from the gelatine meal. The carbon material recovered from the rat faeces appears macroscopically unaffected by its transit through the animal intestine. (**Figure 28**)

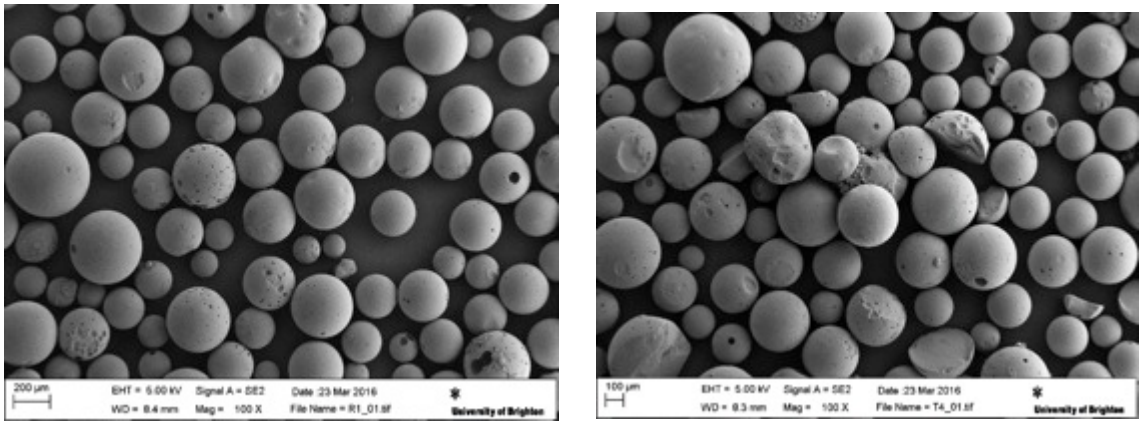


Figure 28. Reference carbon sample v carbon sample isolated from rat faeces

**Reference Sample R1_01:** carbon recovered from the feed mix; never went through the GI tract of animals.

**Test sample T4-01:** Sample isolated from rat faeces after being fed to the animal

As shown in **Figure 29**, there was good agreement between the weight of the carbon recovered from the reference meal samples, and that from the animal faeces. As expected, the weight of the recovered material (0.276 g in reference samples; 0.264 g (±0.036 g SEM) in test samples) was greater than that originally prepared (0.2 g) most likely due to absorbance of material within the carbon pore structure.

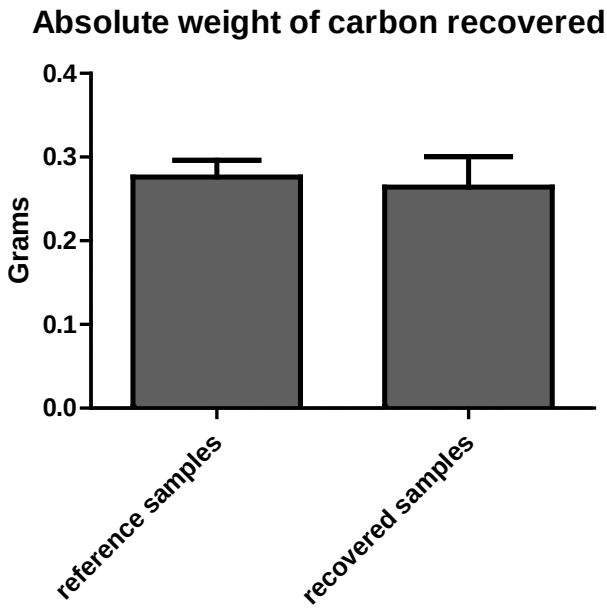



*Figure 29.* Absolute weight recorded for the carbon recovered from the reference samples (n=2) and the animal studies (n=8). Data shown are mean ± SEM

## 3.6 Nonclinical toxicology data

- **13 Week with 4-week recovery period toxicity study in rats**

The objective of this study was to determine the potential toxicity of Yaq-001 as a dietary admix administered for 13 weeks to Wistar rats and to evaluate the potential reversibility of any findings, after a 4-week treatment free period.

The study design is shown in Table 1.

Table 1. Design of 13-week toxicity study

| Group No. | Treatment | Dose Level * g/kg/day | Number of Animals | | | |
|---|---|---|---|---|---|---|
| | | | Main Study | | Recovery Period | |
| | | | Males | Females | Males | Females |
| 1 | Control | --- | 10 | 10 | 5 | 5 |
| 2 | Yaq-001 | 0.7 | 10 | 10 | -- | -- |
| 3 | Yaq-001 | 1.4 | 10 | 10 | -- | -- |
| 4 | Yaq-001 | 2.0 | 10 | 10 | 5 | 5 |

*Equivalent to 1000, 2000, and 3000 ppm (5x, 10x, and 15x projected maximum clinical dose)

The following parameters were evaluated: clinical signs, body weights (**Figure 30**), body weight gains, organ weights (***Figure 31***), food consumption, ophthalmology, and clinical pathology parameters (haematologyy, coagulation, clinical chemistry, and urinalysis). Haematological parameters assessed included: haemoglobin, haematocrit, mean corpuscular volume, total white cell, neutrophil, monocyte, lymphocyte, eosinophil or basophil counts, platelet count and prothrombin time. Biochemical parameters assessed included: bilirubin, alanine transaminase, aspartate transaminase, gamma-glutamyl transferase, alkaline phosphatase, albumin, urea, creatinine, sodium, potassium, chloride, calcium, phosphate, glucose, cholesterol, triglycerides, total protein, globulins, creatinine kinase. No significant differences in urine output or specific gravity were observed.

At the end of 13 weeks of treatment, the main study animals were euthanised with gross necropsy findings, organ weights, and subsequent histopathologic examinations conducted. After a 4-week treatment-free period, the recovery animals were euthanised; gross necropsy findings and organ weights were recorded.

There were no deaths or adverse in-life findings that were considered to be related to treatment. Full pathological analysis of 55 organs by independent, certified pathologists revealed no test article-related changes in macro or microscopic appearances or changes in organ weights. No behavioural abnormalities were observed.

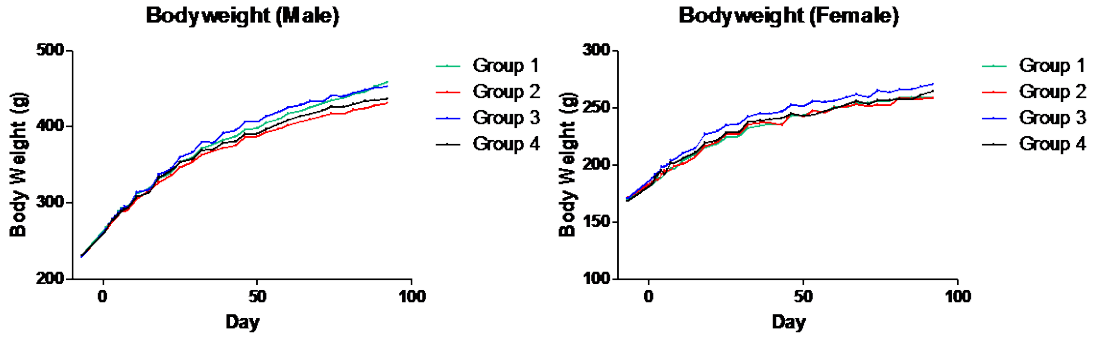



Figure 30. Body weights during study in male and female rats in different doses of Yaq-001 (Group 1 No carbon, Group 2 Yaq-001 0.7g/kg/day, Group 3 1.4g/kg/day, Group 4 2g/kg/day)

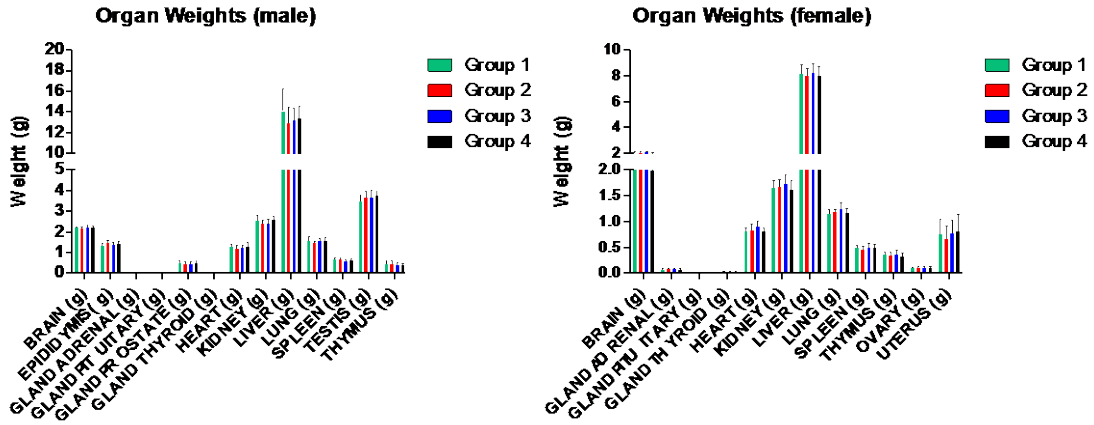



Figure 31. Organ weights during study in male and female rats in different doses of Yaq-001 (Group 1 No carbon, Group 2 Yaq-001 0.7g/kg/day, Group 3 1.4g/kg/day, Group 4 2g/kg/day)

Shortly after treatment started, black faeces were seen in the cages of animals receiving Yaq-001, considered to be due to faecal excretion of the test item.

The conclusion of the independent study officers was that the administration of YAQ-001 orally (in the diet) for 13 weeks was well tolerated in rats at dosages of 0.7, 1.4 and 2g/kg/day (equivalent to 5x, 10x, and 15x the projected maximum clinical dose). There was no evidence of toxicity, and based on these results, the no-observed-effect level (NOEL) was considered to be at least 30000 ppm (the equivalent of 2.05 g/kg/day for males and 2.36 g/kg/day for females), 15 times the anticipated maximum human dose.

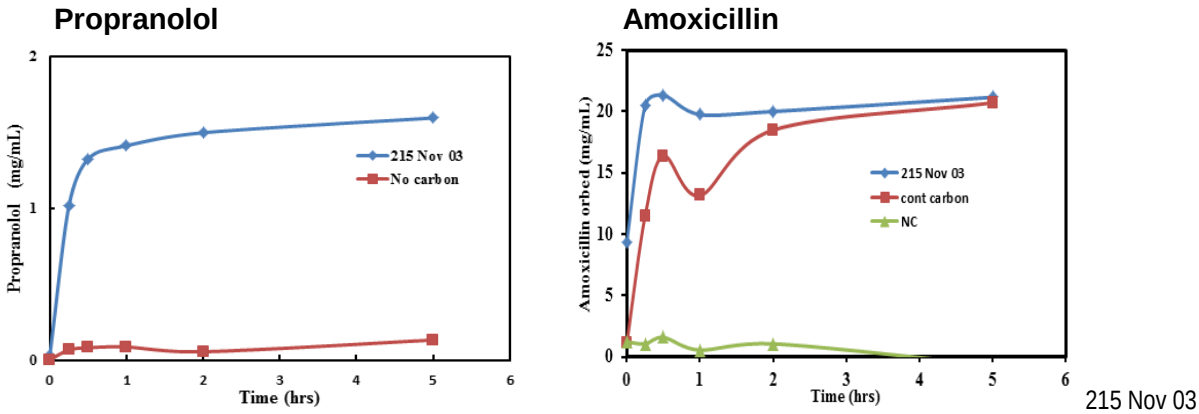
## 3.7 In vitro device-drug interaction data

### 3.7.1 In vitro adsorption kinetics of medications prescribed in cirrhosis + Yaq-001

The in vitro adsorption kinetics of Yaq-001 for a range of commonly prescribed medications in cirrhosis was determined. Drugs from all classes of the Biopharmaceutics Classification System were evaluated as follows:

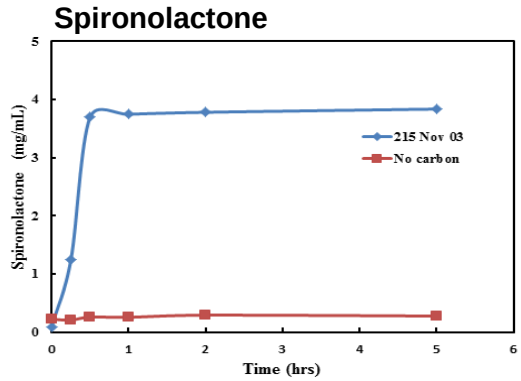
- Class 1 (propranolol, amoxicillin);
- Class 2 (spironolactone);
- Class 3 (metformin, tenofovir);
- Class 4 (frusemide, ciprofloxacin, rifaximin).

Maximal adsorption occurred in the first hour of co-incubation. The comparative microporous carbon was equivalent to Yaq-001 with regard to tenofovir adsorption but inferior to Yaq-001 with regard to amoxicillin and rifaximin adsorption (**Figures 32 - 35**).




215 Nov 03 = Lot 1002 Yaq-001. Cont (control) carbon = commercially available AST-120 NC = No carbon

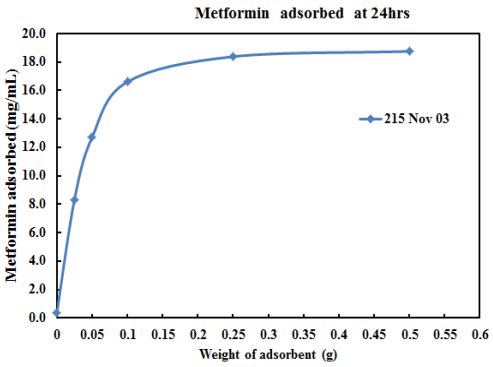
Figure 32. Adsorption kinetics of Yaq-001 for BCS Class 1 drugs



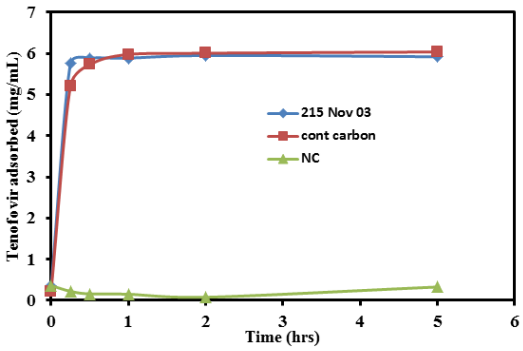

*215 Nov 03 = Lot 1002 Yaq-001. Cont (control) carbon = commercially available AST-120*

*Figure 33. Adsorption kinetics of Yaq-001 for BCS Class 2 drugs*

**Metformin**




**Tenofovir**

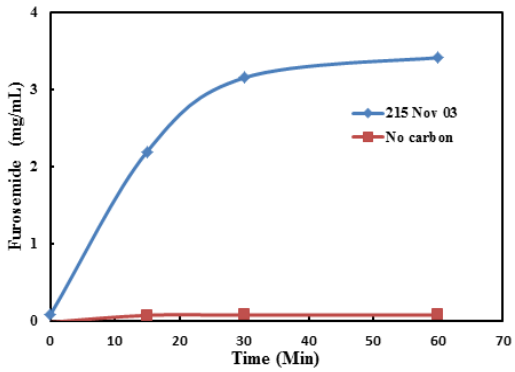



215 Nov 03 = Lot 1002 Yaq-001. Cont (control) carbon = commercially available AST-120.

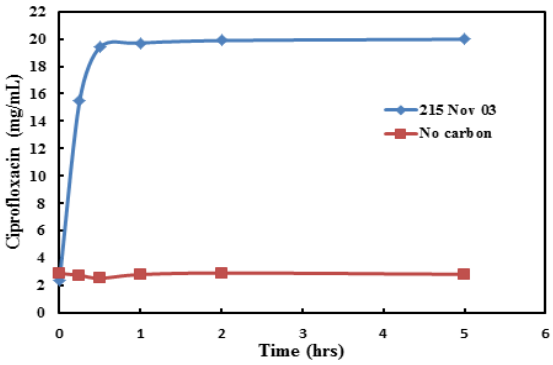
*Note: comparison study for metformin is in progress.*

Figure 34. Adsorption kinetics of Yaq-001 for *BCS Class 3* drugs

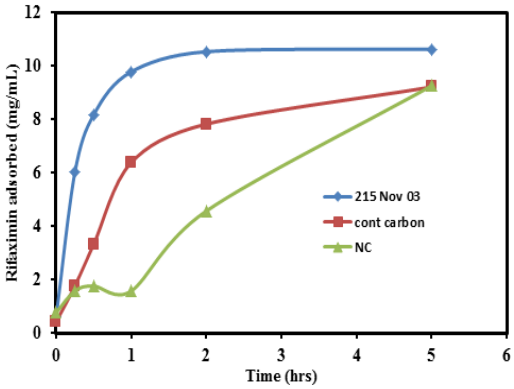
**Frusemide**




**Ciprofloxacin**




**Rifaximin**




*215 Nov 03 = Lot 1002 Yaq-001. Cont (control) carbon = commercially available AST-120*

Figure 35. Adsorption kinetics of Yaq-001 for BCS Class 4 drugs

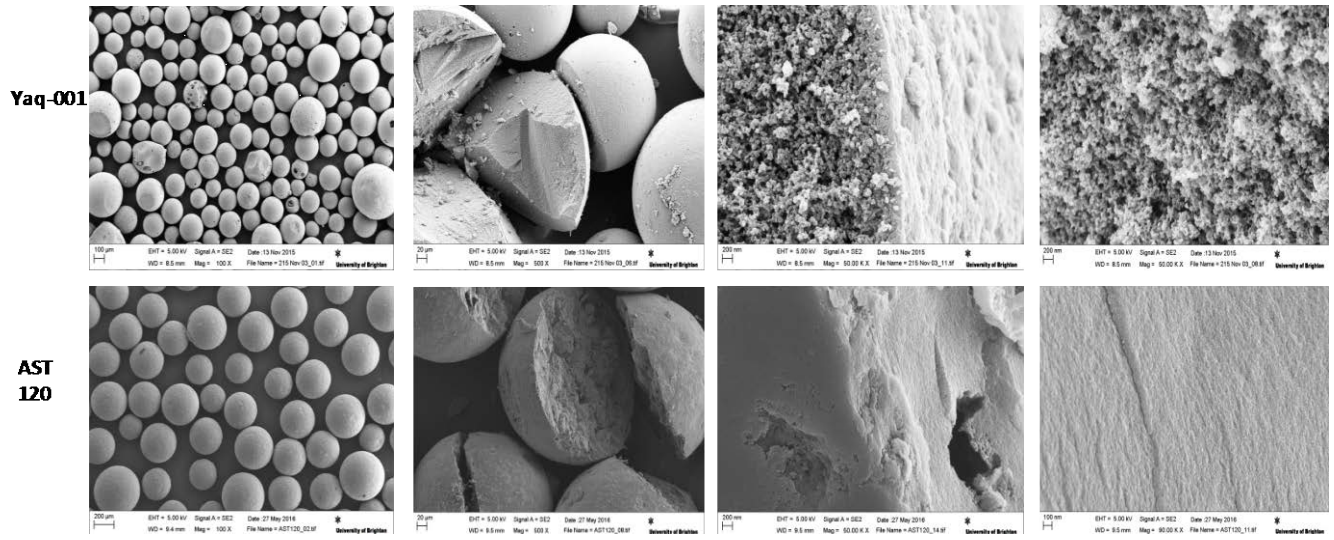



Figure 36. Porous Morphology by Scanning Electron Microscopy (AST-120 vs Yaq-001)

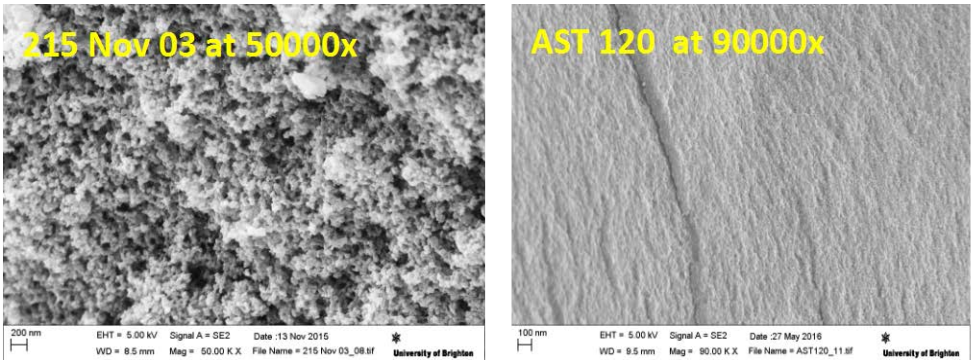



*Figure 37. Internal Porous Structure (AST-120 vs Yaq-001)*

### 3.7.2 Biomolecule Adsorption by AST-120 vs Yaq-001

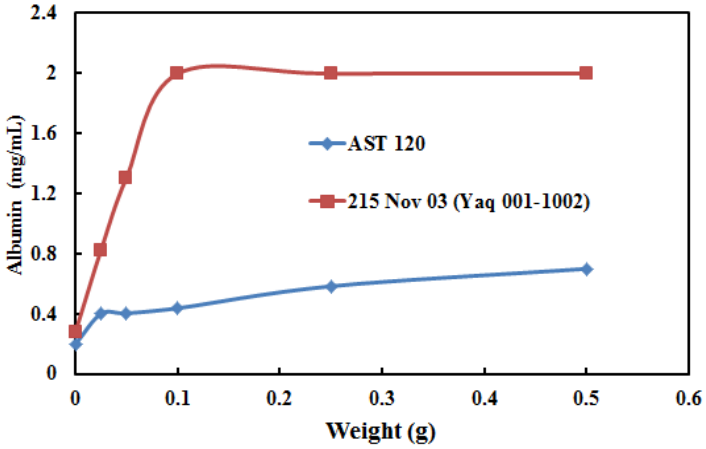



Figure 38. Albumin Adsorption at 24 hours

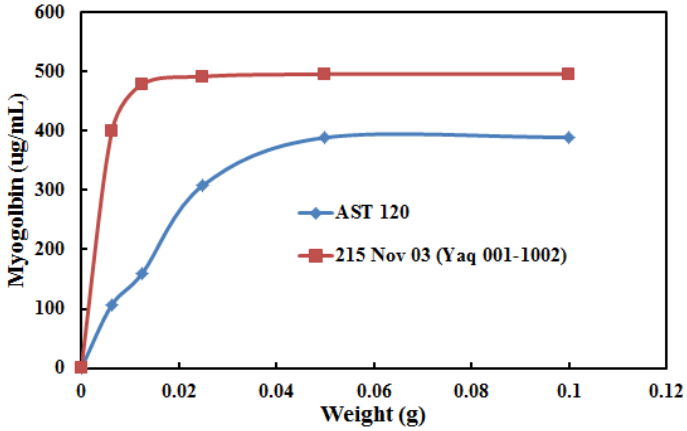



Figure 39. Myoglobin Adsorption at 24 hours

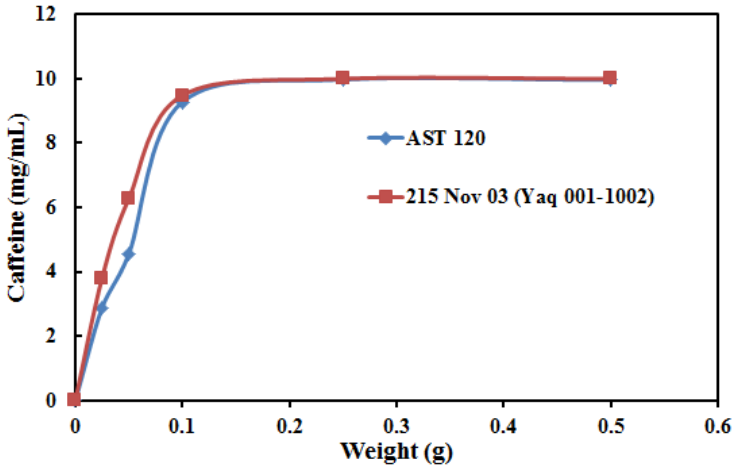



Figure 40. Caffeine Adsorption at 24 hours

### 3.7.3 Mitigation of Risk for Drug-Device Interactions

Yaq-001 is a non-absorbed, non-selective adsorbent which, like other non-selective adsorbents such as AST-120 and cholestyramine, has an adsorptive capacity for commonly used drugs. The conventional means to mitigate the risk of drug-device interactions is to employ a dosing interval of up to 4 hours based on the Biopharmaceutical Classification System (BCS) recommendations. This is a strategy recognised by both the Food and Drug Administration (FDA) and European Medicines Agency (EMA).

According to the Biopharmaceutical Classification System (BCS), drug substances are classified to four classes upon their solubility and permeability:

- Class I agents such as propranolol have a high permeability and solubility. Those compounds are well absorbed and their absorption rate is usually higher than excretion. A dosing interval of +/-1 hour is recommended.
- Class II agents such as spironolactone have a high permeability, low solubility. The bioavailability of these products is limited by their solvation rate. A dosing interval of +/-2 hours is recommended.
- Class III agents such as metformin have a low permeability and high solubility. The absorption is limited by the permeation rate but the drug is solvated very fast. A dosing interval of +/-3 hours is recommended
- Class IV agents such as rifaximin have low permeability and solubility with poor bioavailability. A dosing interval of +/- 4 hours is recommended.

For the purposes the CARBALIVE safety study, a dosing interval of +/- 4 hours will be employed between Yaq-001 and drug administration. Furthermore, only patients with a maximum drug dosing schedule of twice daily will be considered for the study. An orocaecal transit time study will be performed at the screening assessment also to exclude patients with delayed intestinal transit in whom drug-device interaction may constitute a greater risk.

## 4.0 CLINICAL DATA

The Carbalive Safety Study, which is the subject of this Investigator's Brochure, is the first-in-man study for Yaq-001. However, the safe and efficacious use of medicinal carbon is not new. The safety profile of medicinal carbon provides some precedent as to the expected safety of Yaq-001. The medicinal use of carbon dates back many centuries, as an example, for the treatment of malodorous wounds and poisoning. [29] Further medical applications of activated carbon have been developed over many decades:

- for blood purification to treat poisoning and to adsorb toxins in kidney failure[30]
- as microporous charcoal for oral treatment of poisoning,
- in the Gambro Prismaflex Adsorba haemoperfusion systems for severe poisoning and as part of the Gambro MARS liver assist device.

The most rigorously assessed carbon preparation for clinical use is Kremezin® (AST-120, spherical carbon adsorbent) (Class IIa device in EU). AST-120 is a spherical microporous carbon, and, like Yaq-001, it is not systemically absorbed or metabolized. No signals of toxicity were seen in the animal studies nor was a target organ of toxicity identified in chronic toxicity studies or in life-time rodent carcinogenicity studies. (Kremezin labelling). AST-120 has been marketed in Japan since 1991 under the trade name Kremezin®, with an estimated 400,000 patients being exposed to the product to delay time to dialysis in patients with Chronic Kidney

Disease. The most frequent adverse events reported in the labelling are: nausea, vomiting, constipation, and diarrhoea (all with an incidence of <2%). (Kremezin labelling)

AST-120 was evaluated in a multicentre double-blind, placebo-controlled study in 115 subjects with diarrhoea-predominant IBS. [31] The overall incidence of treatment-emergent adverse events (all causalities) in subjects treated with placebo was slightly higher (55.9%) than in those treated with AST-120 (48.4%) in the IBS study AST014. This same pattern was seen in all double-blind, placebo-controlled AST-120 studies conducted, which have shown the incidence of AEs in the placebo group to be the same as, or slightly higher than, the incidence in the AST-120 treatment groups**.** The incidence of serious adverse events was equivocal between the two treatment groups and no serious adverse events in any of the clinical trials were considered to be related to the administration of AST-120. The most commonly reported adverse events in the AST-120 group during the double-blind phase of the IBS-d trial (with an incidence higher than in the placebo group) were: abdominal pain (10.7%), headache (10.7%), nausea 7.1%, constipation 5.4%, vomiting 5.4%, and influenza-like illness (5.4%), Of these events, only abdominal pain, was considered by the investigators as possibly related to the administration of investigational product.

The effects of timing of meals (30 min before or 30 min after AST-120 administration) and the effects of the device on lipid metabolism, calcium metabolism, and blood $\beta_2$-MG levels were investigated. There were no effects of the timing of use, whether before or after a meal, on the efficacy of AST-120. No effects on total cholesterol, triglyceride, calcium and phosphorus levels were identified, thus the authors concluded that there is no effect of AST-120 on lipid or calcium metabolism.[32]

AST-120 has no effects on the lipid-soluble Vitamins 25-OH-D or Vitamin A or the water-soluble Vitamin B6 in patients with renal failure in studies conducted to date,[33] nor does it affect levels of PTH, T3 or T4. **[34]**

The results from AST-120 studies presented above cannot be directly extrapolated to Yaq-001 but are included herein to provide guidance to the clinical investigators.

## 5.0 RISK MANAGEMENT

As this is a first-in-man study with this bimodal porosity carbon bead, extra precaution will be exercised. Administration of the higher cohort dose will occur only if the lower dose is found to be safe and well-tolerated. The Data Safety Monitoring Board (DSMB) will assess the data and determine whether the study can proceed to the next dose. In case of failure of the lower dose, the dose will be halved to determine tolerability.

Yaq-001 is manufactured and controlled under a Quality Management System that is compliant with ISO 13485 (Medical Devices – Quality Management System: Requirements for Regulatory Purposes) and Medical Device Directive 93/42/EEC 14 June 1993 amended by M5 21 September 2007. The clinical trial will be conducted in accord with ISO 14155 (Clinical investigation of medical devices for human subjects – Good clinical practice).

### 5.1 Expected Adverse Events

Given the underlying disease state for the study population for this trial, there are multiple expected serious adverse events. For this trial, foreseeable SAEs are presented below.

- ***Expected Serious Adverse Events***

SOC Hepatobiliary disorders

Acute on chronic liver failure and decompensated cirrhosis
Variceal haemorrhage
Spontaneous bacterial peritonitis
Hepatorenal syndrome
Ascites
Hepatic hydrothorax
Hepatic encephalopathy
Jaundice
Oedema
Bruising

SOC Renal and urinary disorders
Pre-renal failure

SOC Cardiac disorders
Cardiovascular insufficiency
Hypotension

SOC Respiratory, thoracic and mediastinal disorders
Hepatopulmonary syndrome
Portopulmonary hypertension

SOC Infections and infestations
Increased risk of infection
Bacteraemia
Fungaemia

SOC Endocrine disorders
Osteoporosis / Pathological fractures
Hypoadrenalism
Infertility
Gynaecomastia
Hair loss
Amenorrhoea

SOC Investigations
Anaemia
Thrombocytopaenia
Leucopoenia
Coagulopathy
Elevated aminotransferases
Elevated Gamma-glutamyl transpeptidase
Hypoalbuminaemia

Hyperammonaemia
Hyperlactatemia

SOC Metabolism and nutrition disorders
Abnormalities of serum sodium, potassium, calcium, magnesium, phosphate

SOC Nervous system disorders
Peripheral neuropathy
Autonomic neuropathy
Headache

SOC Surgical and medical procedures
Necessity for patients to undergo invasive procedures (e.g. transjugular liver biopsy and haemodynamic assessment)

SOC Neoplasms benign, malignant and unspecified (including cysts and polyps)
Hepatocellular carcinoma (HCC)
All non-HCC cancer

Multiple organ failure
Death

- ***Expected Adverse Events, which may possibly be related to Yaq-001***

SOC Gastrointestinal disorders
Nausea
Vomiting
Constipation
Diarrhoea

Stopping (withdrawal) criteria are presented in the protocol for this study.

## 6.0 SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

Yaq-001 is novel nanoporous carbon adsorbent of endotoxin and bacterial metabolites. Yaq-001 carbon is engineered to selectively remove toxins – mainly bacterial products and inflammatory molecules that damage the liver and other organs – without interacting with the mucous membrane of the gut.

The Yaq-001 device product contains engineered carbons of bimodal porosity as described in **Section 2.** Yaq-001 for clinical use is an oral preparation supplied as 2 gram sachets. No excipients are present in the carbon beads or sachets.

The salient points of the results of the nonclinical studies to support the clinical use of Yaq-001 in the treatment of cirrhosis and NAFLD are:

***Rat bile duct ligated (BDL) model of cirrhosis***

- No adverse effect of Yaq-001 was detected on gut mucosa.

- Yaq-001 treated animals had significantly lower ALT, creatinine and portal pressure.

- Endotoxin-induced deterioration of liver function (ALT), renal function (creatine), and portal pressure was significantly reduced in Yaq-001 treated rats, compared with control animals. (LPS was administered to mimic the development of acute on chronic liver failure (ACLF).

- Yaq-001 normalised endotoxin-induced organ injury associated with a significant improvement in liver oxidative stress.

- Reductions in markers of bacterial translocation were observed with Yaq-001 therapy associated with a significant improvement in intestinal permeability.

- BDL-induced cachexia in BDL rats were prevented in the Yaq-001 rats. No significant differences in electrolyte of plasma micronutrient levels were observed in Yaq-001 treated rats.

- Taken together, Yaq-001 diminishes organ dysfunction in a model of cirrhosis by conferring a phenotype of diminished endotoxin sensitivity.

***Models of Nonalcoholic Fatty Liver Disease (NAFLD)***

- Oral Yaq-001 therapy resulted in a significant attenuation of liver injury in genetically obese mice and non-alcoholic steatohepatitis induced by a methionine choline deficient diet (MCD).

- A significant normalisation in endotoxin-induced liver oxidative stress was observed in Yaq-001 treated genetically obese mice.

- A trend towards reduction in body weight was observed with Yaq-001 treated genetically obese mice. No reduction was observed in Yaq-001 treated heterozygote controls despite pair-feeding.

- Yaq-001 was associated with a significant reduction in glycosuria in genetically obese mice, suggestive of an improvement in insulin resistance.

- Significant normalization of urinary metabolomics profile was observed with Yaq-001 therapy.

Administration of Yaq-001 results in an attenuation of the heightened endotoxin sensitivity observed in cirrhosis and NAFLD. This results in an improvement in organ injury and haemodynamic status and associated with a normalization of innate immune function. At the level of the gut barrier interface, Yaq-001 is associated with a distinct alteration in microbiome composition and function with an improvement in intestinal permeability and nutritional status observed.

The nonclinical safety of Yaq-001 was evaluated in in vivo studies: mass balance study, and a 13-week toxicity study (including histopath examination of gut mucosa). Greater than 95% of Yaq-001 carbon spheres were recoverable from faeces within 48 hrs of Yaq-001 dosing in

a healthy rat model. The difference between the recovered and reference materials is most likely due to carbon beads being lost during the extended washing cycles.
Oral administration of YAQ-001 for 13 weeks was well tolerated in rats at dosages of up to 2 g/kg/day. There was no evidence of toxicity, and based on these results, the no-observed-effect level (NOEL) was considered to be at least (2 g/kg/day), 15 times the anticipated maximum human dose.

The Carbalive Safety Study is the first trial conducted in man. Given that Yaq-001 is not systemically absorbed, serious adverse events related to the administration of Yaq-001 are not anticipated. However, given the underlying disease state for the study population for this trial, there are anticipated SAEs, presented in **Section 5.** Stopping (withdrawal) criteria are presented in the protocol for this study.

The following guidance is provided to clinical investigators:

- Yaq-001 should only be administered in accordance with the individual study protocol being conducted;

- Yaq-001 should not be administered to pregnant or lactating women.

- **Potential interactions with concomitant medical treatments**

Previous studies with AST-120, have demonstrated that administration of AST-120 at least 1.5 hours after administration of concomitant medication does not significantly impact drug pharmacokinetic profiles. Interval between Yaq-001 and drug administration must therefore be at least 2 hours and, also, in accordance with Biopharmaceutics Classification System (BCS) Guidance[35], whichever is longer. Patients on dosing regimens for concomitant mediations of three or more times per day will be excluded.

Intestinal transit, even if prolonged, may be assumed to be identical for both drug and device and therefore dosing interval is the key parameter to determine drug-device interactions. Nonetheless, in light of the fact that some cirrhotic patients have been shown to have prolonged orocaecal intestinal transit, exclusion of patients with very prolonged orocaecal transit times with the potential to functionally impact on drug-device interactions is included in the protocol as an additional safety measure. The thresholds for exclusion are defined in the exclusion criteria.

- **Orocaecal transit time**

Standardised radio-opaque markers such as the SITZMARKS capsule will be used to determine orocaecal transit time. Patients will ingest a single capsule at midnight prior to investigation. A single plain abdominal X-ray will be taken at 10 am the following day to assess the extent of elimination of the radiopaque markers. Patients with <20% retention of radio-opaque markers proximal to the ileocecal valve will be eligible for the study.

If the result from the above exam is indeterminate, a second assessment will be performed. This will either be Cine-MRI or Smart Pill method, depending on local availability. With both methods, the data will be transmitted remotely and analysed by a single operator in UCL within a maximum 5-day turnaround time.

- **Cine-MRI with gadolinium contrast – GIQUANT**

The dynamic series seen above can be acquired on any modern MRI scanner providing an excellent physiological view of the small bowel motility and orocaecal transit time. MRI is safe, well tolerated by patients and non-invasive making it an excellent modality for physiological investigations of the bowel. The patient will swallow a contrast pill and undergo a 20 minute MRI scan 10 hours later**.**

- **Smart Pill**

The SmartPill GI Monitoring System measures whole gut and regional gut (stomach, small bowel and colon) transit times and thus provide a measure of orocaecal transit. The smart pill is swallowed by the patient and data collected from a data receiver attached to the patient. This method will be used to assess orocaecal transit in instances in which MRI is unavailable or contraindicated.

In summary, oral administration of Yaq-001 to cirrhotic rats is associated with a significant reduction in organ injury and improvement in portal pressure, important clinical prognostic determinants. Carbon therapy attenuates the heightened endotoxin sensitivity observed in the BDL model and is associated with a normalisation of population and functional status in tissue resident and trafficking innate immune populations in the gut-liver axis with diminished inflammasomal activation. These changes are associated with a distinct shift in metabolomic phenotype associated with an improvement in body weight and expansion of autochthonous bacterial populations in the microbiome. Further studies are required to more fully ascertain the mechanisms of action of Yaq-001 in more detail but the observed changes in vivo demonstrate that the carbon is a good candidate for translation to clinical studies.

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International Standards

- ICH E6: Consolidated Guidance for Good Clinical Practice
  Section 5.16.2 Safety Information
  Section 7 Investigator's Brochure
  Appendix 2 Table of Contents for Investigator's Brochure

- ISO 14155: Clinical investigation of medical devices for human subjects – Good clinical practice
  Section 5.5
  Annex B Investigator's Brochure

ISO 13485: Medical Devices – Quality Management Systems – Requirements for Regulatory Purposes

# LIST OF PARTICIPANTING INVESTIGATORS / SITES

| Country | Site No. | Principal Investigator/ Site (Name and address) |
|---|---|---|
| United Kingdom | 01 | **Dr. Raj Mookerjee**<br>Institute of Liver and Digestive Disease<br>Royal Free Hospital; Rowland Street, London NW3 2PF, United Kingdom<br>Phone: +44 207 794 0500 |
| Spain | 02 | **Prof. Pere Gines**<br>Liver Unit, H. Clinic of Barcelona<br>Villaroel, 170, 08036 Barcelona, Spain<br>Phone: +34 93 227 17 13 |
| Spain | 03 | **Prof. Agustin Albillos**<br>Department of Gastroenterology and Hepatology, H. Ramón y Cajal<br>Carretera de Colmenar Viejo Km 9,100; 28034 Madrid, Spain<br>Phone: +34 91 336 85 92 |
| Spain | 04 | **Prof. Víctor Vargas**<br>Liver Unit – H. Vall d'Hebron<br>Passeig Vall d'Hebron, 119; 08021 Barcelona, Spain<br>Phone: +34 93 489 30 00 |

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|---|---|---|
| Italy | 05 | **Prof. Paolo Angeli**<br>Hepatic Emergencies Unit; Azienda Ospedaliera di Padova<br>Via Giustiniani, 2; 35128 Padova, Italy<br>Phone: +39 049 821 2 004 |
| Italy | 06 | **Prof. Paolo Caraceni**<br>Department of Medical and Surgical Sciences, Policlinico S.Orsola Malpighi<br>Via Albertoni, 15; 40138 Bologna, Italy<br>Phone: + 39 051 636 2 919 |
| Switzerland | 07 | **Prof. Reiner Wiest**<br>Department for Visceral Surgery and Medicine, Inselspital Universitaet Bern<br>Freiburgstrasse 4; 3010 Bern, Switzerland<br>Phone: +41 31 632 0291 |
| France | 08 | **Prof. François Durand**<br>Hepatology and Liver Intensive Care, Hospital Beaujon<br>100 Boulevard du General Leclerc; 82110 Clichy, France<br>Phone: + 33 01 40 87 50 00 |

# CLINICAL INVESTIGATION PLAN SYNOPSIS

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| **CIP Number:** | Yaq001-S-001 |
| **Title:** | Safety and tolerability of Yaq-001 in patients with cirrhosis |
| **Short name:** | CARBALIVE-SAFETY study |
| **Clinical phase:** | First-in-human clinical investigation |
| **Purpose:** | To ascertain the safety and tolerability of oral administration of Yaq-001 in patients with cirrhosis |
| **Study design:** | Multicentre, randomized, double blinded, placebo controlled trial to evaluate safety and tolerability of oral administration of Yaq-001 therapy in two dosing cohorts.<br><br>56 cirrhotic patients with diuretic-responsive ascites will be enrolled. Patients will be randomized to two dosing cohorts:<br><br>• Cohort 1 (1:1 randomization)<br>  ○ Standard medical treatment + Yaq-001 (4 g/ day) – n= 14.<br>  ○ Standard medical treatment + placebo-control (placebo for 4 g of Yaq-001/ day) – n= 14.<br>• Cohort 2 (1:1 randomization)<br>  ○ Standard medical treatment + Yaq-001 (8 g/ day) – n= 14.<br>  ○ Standard medical treatment + placebo-control (placebo for 8 g of Yaq-001/ day) – n= 14.<br><br>Study patients will be dosed daily with Yaq-001 for 12 weeks. Assessments of DSMB will take place after 4 and 12 weeks. Investigational centres specialized in the management of patients with liver cirrhosis will participate in the study.<br><br>For each patient, the study duration will be up to 15 weeks, including the screening (up to 2 weeks), treatment (12 weeks) and 7-day follow up period.<br><br>The total study duration is estimated to be approximately 12 months from screening of first patient until study completion of the last patient. |



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| **Investigational product:** | Yaq-001: highly adsorbent activated carbon with tailored pore size distribution<br>Placebo control: methylcellulose dyed to match Yaq-001 |
| **Objectives:** | **Primary objective**:<br>Assessment of the safety and tolerability of Yaq-001<br>**Secondary objectives**:<br>• Assessment of changes in blood endotoxin activity<br>• Assessment of changes in organ function<br>• Assessment of changes in nutritional status |
| **Inclusion criteria:** | 1. Male and female patients<br>2. Age ≥ 18 years at screening<br>3. Clinical diagnosis of cirrhosis for any cause. Liver biopsy is not required<br>4. Cirrhotic patients with diuretic-responsive ascites and Child-Pugh score = 7-11 inclusive<br>5. Abstinence from alcohol for at least 4 weeks prior to screening |
| **Exclusion criteria:** | 1. Refusal or inability (lack of capacity) to give informed consent<br>2. Prohibited medication within 4 weeks before the start of the study treatment: all oral antibiotics, immunosuppressants, long acting benzodiazepines or barbiturates and antiviral medication<br>3. Change in dose of proton pump inhibitor therapy within 4 weeks before the start of the study treatment<br>4. Patients with once daily medications in which orocaecal transit time is greater than 10 hours<br>5. Patients requiring medication in which the dosing schedule is three times per day or greater<br>6. Antiviral therapy for hepatitis C within 3 months prior to screening<br>7. Hospital admission for liver-related indication for at least 4 weeks (except paracentesis)<br>8. BMI > 35 or BMI < 18<br>9. *Clostridium Difficile* diarrhoea within 4 weeks before the start of the study treatment<br>10. Uncontrolled infection (chronic viral hepatitis is not an exclusion criterion)<br>11. Human immunodeficiency virus<br>12. Presence of a transjugular intrahepatic portosystemic shunt (TIPSS)<br>13. Participation in any clinical study of an investigational medicinal product within 30 days of five half-lives of the investigational product, whichever is longer<br>14. Presence of clinically relevant cardiovascular, pulmonary, gastro-intestinal, renal, hepatic, metabolic, haematological, neurological, psychiatric, systemic, ocular, gynaecologic or any acute infection disease or signs of acute illness that, in the opinion of the investigator, might compromise the patient's safe participation in the trial.<br>15. Presence of the history of cancer within the past 5 years with exception of hepatocellular carcinoma within Milan criteria, adequately treated localised basal cell carcinoma of the skin, in situ cervical carcinoma or solid malignancy surgical excised in total without recurrence for five years.<br>16. Women of child bearing potential. Only postmenopausal women or with surgical sterilization will be included. |

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| **Primary endpoints:** | Safety assessments will be performed at Day 1, Weeks 1, 4, 8 and 12<br>Safety assessment will include:<br>• Physical examination<br>• Vital signs: blood pressure, pulse rate, respiratory rate and body temperature<br>• Clinical laboratory tests: haematology, coagulation, clinical chemistry and urinalysis<br>• 12-lead ECG<br>• Reported and observed adverse events. |
| **Prohibited concomitant medication:** | • Once daily medications in patients with orocaecal transit time greater than 10 hours<br>• Medications in which the dosing schedule is three times per day or greater<br>• Antiviral therapy for hepatitis C |
| **Timing of concomitant medications:** | Morning doses should be taken at least 10 hours after carbon therapy<br>Evening doses should be taken at least 4 hours before carbon therapy |
| **Statistical considerations:** | This study is not powered for statistical significance. The clinical safety and all other data generated will be described using descriptive statistics |



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