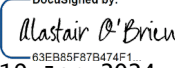




## ASEPTIC: Primary Antibiotic prophylaxis using co-trimoxazole to prevent Spontaneous bacterial Peritonitis in Cirrhosis

|   |                                 |
|---|---------------------------------|
| Version   | 8.0                             |
| Date  | 15 December 2023                |
| Sponsor   | University College London (UCL) |
| Comprehensive Clinical Trials Unit Trial Adoption Group # | CTU/2017/308                    |
| Trial registration  | 23 <sup>rd</sup> January 2019   |
| EudraCT #   | 2019-000581-38                  |
| REC #   | 19/SC/0311                      |

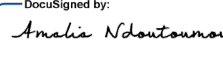
Authorisation: Chief Investigator

|           |  |
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| Role      | Professor of Hepatology  |
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| Date      | 19-Jan-2024  |

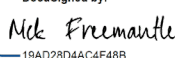
Authorisation: Sponsor/CCTU Director Representative

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| Role      | Director CCTU   |
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| Date      | 19-Jan-2024   |

Authorisation: Senior Operations Staff

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| Name      | Amalia Ndoutoumou   |
| Role      | Clinical Project Manager  |
| Signature | <small>DocuSigned by:</small><br><br><small>2B5833B861CC440...</small> |
| Date      | 19-Jan-2024   |

Authorisation: Senior Statistician

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| Name      | Prof Nick Freemantle  |
| Role      | Director CCTU   |
| Signature | <small>DocuSigned by:</small><br><br><small>19AD28D4AC4F48B...</small> |
| Date      | 19-Jan-2024   |



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## 1. Administrative information

This document was constructed using the Comprehensive Clinical Trials Unit (CCTU) at UCL Protocol template Version 5. It describes the ASEPTIC trial, sponsored by UCL and co-ordinated by CCTU.

It provides information about procedures for entering patients into the trial, and provides sufficient detail to enable: an understanding of the background, rationale, objectives, trial population, intervention, methods, statistical analyses, ethical considerations, dissemination plans and administration of the trial; replication of key aspects of trial methods and conduct; and appraisal of the trial's scientific and ethical rigour from the time of ethics approval through to dissemination of the results. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to registered investigators in the trial. Sites entering patients for the first time should confirm they have the correct version through a member of the trial team at CCTU.

CCTU supports the commitment that its trials adhere to the SPIRIT guidelines. As such, the protocol template is based on an adaptation of the Medical Research Council CTU protocol template (2012) and the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 Statement for protocols of clinical trials <sup>1</sup>. The SPIRIT Statement Explanation and Elaboration document <sup>2</sup> can be referred to, or a member of CCTU Protocol Review Committee can be contacted for further detail about specific items.

### 1.1. Compliance

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2008), the principles of Good Clinical Practice (GCP) as laid down by the Commission Directive 2005/28/EC with implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the Human Tissue (Quality and Safety for Human Application) Regulations 2007, the UK 2018 Data Protection Act, and the National Health Service (NHS) UK Policy Framework for Health and Social Care. International sites will comply with the principles of GCP as laid down by ICH topic E6 (Note for Guidance on GCP), Commission Directive 2005/28/EC, the European Directive 2001/20/EC (where applicable), the EU Tissue and Cells Directives 2004/23/EC, 2006/17/EC and 2006/86/EC, and other national and local applicable regulations. Agreements that include detailed roles and responsibilities will be in place between participating sites and CCTU.

Participating sites will inform CCTU as soon as they are aware of a possible serious breach of GCP or the Protocol, so that CCTU can fulfil its requirement to report the breach if necessary within the timelines specified in the UK Clinical Trials Regulations (currently 7 days). For the purposes of this regulation a 'serious breach' is one that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the subjects in the trial, or
- The scientific value of the trial.

## 1.2. Sponsor

UCL is the trial sponsor and has delegated responsibility for the overall management of the ASEPTIC trial to CCTU. Queries relating to UCL sponsorship of this trial should be addressed to the CCTU Director or via the Trial Team.

### 1.3. Structured trial summary

|   |   |
|---|---|
| Primary Registry and Trial Identifying Number | EudraCT #: 2019-000581-38   |
| Date of Registration in Primary Registry      | 23 <sup>rd</sup> January 2019   |
| Secondary Identifying Numbers                 | CCTU Trial Adoption Group #: CTU/2017/308<br>IRAS #: 262176   |
| Source of Monetary or Material Support        | National Institute of Health Research-Health Technology Assessment (NIHR-HTA)   |
| Sponsor                                       | University College London with sponsor responsibilities delegated to CCTU   |
| Contact for Public Queries                    | ctu.enquiries@ucl.ac.uk   |
| Contact for Scientific Queries                | <p>Prof Alastair O'Brien<br/>Professor &amp; Consultant Hepatologist<br/>University College London<br/>University College Hospital &amp; UCL Institute for Liver and Digestive Health</p> <p><b>Address:</b> Ground Floor<br/>Rayne Building<br/>5 University Street<br/>UCL Division of Medicine</p> <p>London WC1E 6JF</p> <p><b>Email:</b> a.o'brien@ucl.ac.uk</p>   |
| Public Title                                  | A trial of using antibiotics to prevent infection in patients with advanced liver disease   |
| Scientific Title                              | Primary Antibiotic prophylaxis using co-trimoxazole to prevent Spontaneous bacterial Peritonitis in Cirrhosis (ASEPTIC)   |
| Countries of Recruitment                      | United Kingdom  |
| Health Condition(s) or Problem(s) Studied     | Prevention of spontaneous bacterial peritonitis in patients with advanced liver disease   |
| Intervention(s)                               | Patients will receive either 960 mg co-trimoxazole or matching placebo medication taken orally once a day for 18 months   |
| Key Inclusion and Exclusion Criteria          | <p><b>Inclusion criteria:</b></p> <ol style="list-style-type: none"> <li>1. Patients with Child-Pugh Class B or C cirrhosis and presence of ascites requiring any diuretic treatment or <u>at least 1 or more</u> paracentesis within 3 months prior to enrolment.</li> <li>2. Patient at least 18 years of age</li> <li>3. Documented informed consent to participate.</li> </ol> <p><b>Exclusion criteria:</b></p> <ol style="list-style-type: none"> <li>1. Patients with current or previous Spontaneous Bacterial Peritonitis (defined as ascitic polymorphonuclear (PMN) cell count &gt;250/mm<sup>3</sup> with either positive or negative ascitic fluid culture without evident intra-abdominal surgically treatable</li> </ol> |



|            |   |
|------------|---|
|            | <p>source of infection. A white cell count <math>&gt;500</math> cell/mm<sup>2</sup> or positive microbial culture may be considered as evidence of previous SBP if the site PI considers this was in the context of a likely clinical diagnosis of SBP).</p> <ol style="list-style-type: none"> <li>2. Patients receiving palliative care with an expected life expectancy of <math>&lt;8</math> weeks</li> <li>3. Allergic to co-trimoxazole, trimethoprim or sulphonamides</li> <li>4. Pregnant or lactating mothers</li> <li>5. Patient enrolled in a clinical trial of investigational medicinal products (IMPs) that would impact on their participation in the study</li> <li>6. Patients with serum potassium (<math>&gt;5.5</math> mmol/L) related to pre-existing kidney disease which cannot be reduced*</li> <li>7. Patients receiving antibiotic prophylaxis (except for rifaximin)*</li> <li>8. Patients with long-term ascites drains*</li> <li>9. Women of child-bearing potential and males with a partner of child-bearing potential without effective contraception for the duration of trial treatment</li> <li>10. Patients with pathological blood count changes <ol style="list-style-type: none"> <li>a. Patients with haemoglobin (Hb) <math>&lt;70</math>g/L*</li> <li>b. Granulocytopenia defined as absolute neutrophil counts of less than 500 cells per microliter*</li> <li>c. Severe thrombocytopenia with a platelet count <math>&lt;30 \times 10^9</math> /L*</li> </ol> </li> <li>11. Patients with severe renal impairment, with eGFR <math>&lt;15</math> ml/min*</li> <li>12. Patients with skin conditions: exudative erythema multiform, Stevens-Johnson syndrome, toxic epidermal necrolysis and drug eruption with eosinophilia and systemic symptoms</li> <li>13. Patients with congenital conditions: congenital glucose-6-Phosphate dehydrogenase deficiency of the erythrocytes, haemoglobin anomalies such as Hb Köln and Hb Zürich</li> <li>14. Patients with acute porphyria</li> <li>15. Any clinical condition which the investigator considers would make the patient unsuitable for the trial</li> </ol> <p>*It is common for these investigations to change in patients with cirrhosis and long-term ascitic drains may be removed. Patients can be re-screened for eligibility if this occurs.</p> |
| Study Type | A multicentre, interventional, double-blind, placebo-controlled, parallel-arm, phase 3, randomised controlled trial to evaluate the use of co-trimoxazole as primary prophylaxis  |

|                         |   |
|-------------------------|---|
|                         | for spontaneous bacterial peritonitis to improve overall survival   |
| Date of First Enrolment | Sep 2019  |
| Target Sample Size      | 432   |
| Primary Outcome(s)      | Overall survival  |
| Key Secondary Outcomes  | <p>The following will be measured for a minimum period of 18 months from randomisation:</p> <ol style="list-style-type: none"> <li>1. Time to first incidence of spontaneous bacterial peritonitis (SBP) (SBP is defined as per the standard guidelines: ascitic fluid polymorphonuclear (PMN) cell count <math>&gt;250/\text{mm}^3</math> with either positive or negative ascitic fluid culture without evident intra-abdominal surgically treatable source of infection. A white cell count <math>&gt;500 \text{ cell}/\text{mm}^2</math> or positive microbial culture may be considered as evidence of previous SBP if the site PI considers this was in the context of a likely clinical diagnosis of SBP).</li> <li>2. Hospital admission rates</li> <li>3. Incidence of <i>C. difficile</i>-associated diarrhoea</li> <li>4. Incidence of infections other than SBP that require hospital admission.</li> <li>5. Incidence of other cirrhosis related events (e.g. variceal haemorrhage)</li> <li>6. Incidence of renal dysfunction with creatinine <math>&gt;133 \mu\text{mol}/\text{L}</math> (<math>1.5\text{mg}/\text{dL}</math>) at any point during hospital admission</li> <li>7. Effect of IMP on renal function for duration of the trial as measured by eGFR</li> <li>8. Effect of IMP on renal function for duration of the trial as measured by serum creatinine levels</li> <li>9. Incidence of anti-microbial resistance</li> <li>10. Incidence of liver transplantation</li> <li>11. Progression of liver disease assessed by increase in MELD score between baseline and end of trial follow up.</li> <li>12. Safety and treatment-related serious adverse events</li> <li>13. Treatment adherence (assessed by MARS questionnaire)</li> <li>14. Health-related quality of life assessed using EQ-5D-5L questionnaire.</li> <li>15. Health and social care resource use assessed using Hospital Episode Statistics (HES) database.</li> <li>16. Mean incremental cost per quality adjusted life year gained (QALY)</li> <li>17. Incidence of resolution of ascites with diuretic treatment not required for 6 months.</li> <li>18. Incidence of Transjugular intrahepatic portosystemic shunt (TIPS) insertion</li> </ol> |

### 1.4. Roles and responsibilities

These membership lists are correct at the time of writing; please see terms of reference documentation in the TMF for current lists.

#### 1.4.1. Protocol contributors

| Name                 | Affiliation          | Role                     |
|----------------------|----------------------|--------------------------|
| Alastair O'Brien     | UCL                  | Chief Investigator       |
| Louise China         | UCL                  | Clinical Research Fellow |
| Zainib Shabir        | UCL CCTU             | Clinical Project Manager |
| Marisa Chau          | UCL CCTU             | Clinical Project Manager |
| Daizy Moualeu Kameni | UCL CCTU             | Trial Manager            |
| David Gear           | UCL CCTU             | Trial Manager            |
| Kate Bennett         | UCL CCTU             | Trial Statistician       |
| Nick Freemantle      | UCL CCTU             | Oversight Statistician   |
| Hakim-Moulay Dehbi   | UCL CCTU             | Oversight Statistician   |
| Norin Ahmed          | UCL CCTU             | Trial Statistician       |
| Rachael Hunter       | UCL CCTU             | Health Economist         |
| Ekaterina Bordea     | UCL CCTU             | Health Economist Junior  |
| Liz Deane            | UCL CCTU             | Clinical Project Manager |
| Simon Skene          | University of Surrey | Statistical Oversight    |
| James Blackstone     | UCL CCTU             | Clinical Project Manager |

#### 1.4.2. Role of trial sponsor and funders

| Name     | Role  |
|----------|---|
| UCL      | Trial Sponsor   |
| UCL CCTU | Specific functions have been delegated to the UCL CCTU by the Sponsor. A Clinical Project Manager at the UCL CCTU will oversee the Trial Manager who will be responsible for the day-to-day management of the trial and providing support to the site staff. The CCTU will be involved in approaching sites, initiation visits, case report form development, database construction, and protocol and patient information development in collaboration with the Trial Management Group. |
| NIHR HTA | Trial Funder  |

#### 1.4.3. Trial Team

| Name                   | Affiliation | Role and responsibilities |
|------------------------|-------------|---------------------------|
| Alastair O'Brien       | UCL         | Chief Investigator        |
| Louise China           | UCL         | Clinical Research Fellow  |
| Norin Ahmed            | UCL CCTU    | Trial Statistician        |
| Amalia Ndoutoumou      | UCL CCTU    | Clinical Project Manager  |
| Iona Cullen-Stephenson | UCL CCTU    | Trial Manager             |
| Tasheeka Jeyapalan     | UCL CCTU    | Trial Manager (Monitor)   |
| Nishtha Kotriwala      | UCL CCTU    | Data Manager              |

**1.4.4. Trial Management Group**

| <b>Name</b>            | <b>Affiliation</b>             | <b>Role and responsibilities</b> |
|------------------------|--------------------------------|----------------------------------|
| Alastair O'Brien       | UCL/Royal Free Hospital        | Chief Investigator               |
| John Dillon            | University of Dundee           | Principal Investigator           |
| Michael Heneghan       | King's College Hospital        | Principal Investigator           |
| Steve Ryder            | Nottingham City Hospital       | Principal Investigator           |
| Louise China           | UCL                            | Clinical Research Fellow         |
| Indran Balakrishnan    | UCL                            | Microbiologist                   |
| Victoria Snowdon       | Addenbrooke's Hospital         | Principal Investigator           |
| Yiannis Kallis         | Royal London Hospital          | Principal Investigator           |
| Coral Hollywood        | Gloucestershire Royal Hospital | Principal Investigator           |
| Lynsey Corless         | Hull Royal infirmary           | Principal Investigator           |
| Ewan Forrest           | Glasgow Royal Infirmary        | Principal Investigator           |
| Stuart McPherson       | Freeman Hospital               | Principal Investigator           |
| Kush Abeysekera        | Bristol Royal Infirmary        | Principal Investigator           |
| Paul Richardson        | Royal Liverpool Hospital       | Principal Investigator           |
| Gavin Wright           | Basildon Hospital              | Principal Investigator           |
| Amalia Ndoutoumou      | UCL CCTU                       | Clinical Project Manager         |
| Iona Cullen-Stephenson | UCL CCTU                       | Trial Manager                    |
| Tasheeka Jeyapalan     | UCL CCTU                       | Trial Manager (Monitor)          |
| Norin Ahmed            | UCL CCTU                       | Trial Statistician               |
| Nick Freemantle        | UCL CCTU                       | Oversight Statistician           |
| Nishtha Kotriwala      | UCL CCTU                       | Data Manager                     |
| Ekaterina Bordea       | UCL CCTU                       | Health Economist Junior          |

**1.4.5. Trial Steering Committee**

| <b>Name</b>            | <b>Affiliation</b>                             | <b>Role and responsibilities</b> |
|------------------------|--|----------------------------------|
| Professor Phil Newsome | University of Birmingham                       | Chair                            |
| Dr Tim Clayton         | London School of Hygiene and Tropical Medicine | Member                           |
| Dr Ahmed Elsharkawy    | Queen Elizabeth Hospital Birmingham            | Member                           |
| Mr John Crookenden     | Patient Representative                         | PPI member                       |
| Mrs Martine Walmsley   | Patient Representative                         | PPI member                       |

**1.4.6. Independent Data Monitoring Committee**

| <b>Name</b>           | <b>Affiliation</b>              | <b>Role and responsibilities</b> |
|-----------------------|---------------------------------|----------------------------------|
| Professor Shahid Khan | Imperial College London         | Chair                            |
| Dr Zohra Zenasni      | Queen Mary University of London | Independent member               |
| Dr Nikhil Vergis      | Imperial College London         | Independent member               |

## 2. Abbreviations

|          |   |
|----------|---|
| AASLD    | American Association for the Study of Liver Disease |
| AE       | Adverse Event                                       |
| ACLF     | Acute-on-chronic liver failure                      |
| AF       | Ascitic Fluid                                       |
| AMR      | Anti-Microbial Resistance                           |
| AR       | Adverse Reaction                                    |
| BSG      | British Society of Gastroenterology                 |
| CA       | Competent Authority                                 |
| CI       | Chief Investigator                                  |
| CRF      | Case Report Form                                    |
| CTA      | Clinical Trial Authorisation                        |
| CCTU     | Comprehensive Clinical Trials Unit at UCL           |
| CDAD     | Clostridium Difficile Associated Diarrhoea          |
| CP       | Child's Pugh  |
| DSUR     | Development Safety Update Report                    |
| EASL     | European Association for the Study of Liver Disease |
| EC       | Ethics Committee                                    |
| EQ-5D-5L | The 5-level EQ-5D version                           |
| EU       | European Union                                      |
| GCP      | Good Clinical Practice                              |
| Hb       | Haemoglobin   |
| HEAP     | Health Economic Analysis Plan                       |
| HES      | Hospital Episode Statistics                         |
| HIV      | Human Immunodeficiency Viruses                      |
| ICH      | International Conference on Harmonisation           |
| IDMC     | Independent Data Monitoring Committee               |
| IMP      | Investigational Medicinal Product                   |

|       |   |
|-------|---|
| ITT   | Intention to Treat                                  |
| MARS  | Medication Adherence Report Scale                   |
| MELD  | Model for End-Stage Liver Disease                   |
| MHSDS | The Mental Health Services Data Set                 |
| MHRA  | Medicines and Healthcare products Regulatory Agency |
| NHS   | National Health Service                             |
| NICE  | National Institute for Health and Care Excellence   |
| PI    | Principal Investigator                              |
| PIS   | Participant Information Sheet                       |
| QA    | Quality Assurance                                   |
| QALY  | Quality Adjusted Life Year                          |
| QC    | Quality Control                                     |
| QMMP  | Quality Management and Monitoring Plan              |
| R&D   | Research and Development                            |
| REC   | Research Ethics Committee                           |
| SAE   | Serious Adverse Event                               |
| SAP   | Statistical Analysis Plan                           |
| SAR   | Serious Adverse Reaction                            |
| SBP   | Spontaneous Bacterial Peritonitis                   |
| SPC   | Summary of Product Characteristics                  |
| SUSAR | Suspected Unexpected Serious Adverse Reaction       |
| TIPS  | Transjugular intrahepatic portosystemic shunt       |
| TMF   | Trial Master File                                   |
| TMG   | Trial Management Group                              |
| TMT   | Trial Management Team                               |
| ToR   | Terms of Reference                                  |
| TSC   | Trial Steering Committee                            |
| UCL   | University College London                           |
| US    | United States                                       |

### 3. Glossary

**Acute decompensation (of cirrhosis)** - The acute development or worsening of the complications of cirrhosis and is the main cause of hospitalisation in cirrhotic patients.

**Albumin** - The most abundant protein in human blood plasma. Albumin is synthesised in the liver and therefore is commonly present at reduced circulating levels in advanced liver cirrhosis/chronic liver failure.

**Acute on Chronic liver failure** - A syndrome in patients with chronic liver disease with or without previously diagnosed cirrhosis which is characterised by acute hepatic decompensation resulting in liver failure (jaundice and prolongation of the International Normalised Ratio (INR)) and one or more extrahepatic organ failures that is associated with increased mortality within a period of 28 days and up to 3 months from onset.

**Ascites** – The build-up of fluid in the space surrounding the organs in the abdomen; the most common complication of cirrhosis.

**Child-Pugh score** - <https://www.mdcalc.com/child-pugh-score-cirrhosis-mortality> is used to assess the prognosis of liver cirrhosis.

| Parameter              | 1    | 2  | 3  |
|------------------------|------|--|--|
| Hepatic Encephalopathy | None | Grade 1-2<br>(or precipitant induced/suppressed with medication) | Grade 3-4<br>(or chronic/refractory)                     |
| Ascites                | None | Mild<br>(Grade I-II or diuretic responsive)                      | Moderate-Severe<br>(Grade III-IV or diuretic refractory) |
| Bilirubin (umol/L)     | <34  | 34-50  | >50  |
| Albumin (g/L)          | >35  | 28-35  | <28  |
| INR                    | <1.7 | 1.7-2.3  | >2.3   |

Childs A: 5-6

Childs B: 7-9

Childs C: 10-15

**Hepatic encephalopathy** - Confusion and coma as a result of liver failure.

**Jaundice** – The yellow discolouration of the skin and sclera of the eyes.

**Liver Cirrhosis** – A result of advanced liver disease, characterised by replacement of liver tissue by fibrosis (scar tissue) and regenerative nodules (lumps that occur due to attempted repair of damaged tissue). Cirrhosis is most commonly caused by alcohol, chronic viral hepatitis and fatty liver disease, but has many other causes. Liver cirrhosis is a pathological definition based on liver biopsy. However, this is an invasive procedure and uncommonly performed in patients admitted with complications of cirrhosis. Patients will be considered to have cirrhosis based on clinical judgment (including radiological imaging) as for standard UK practice.

**MELD** - The Model for End-Stage Liver Disease (MELD)<sup>4</sup> is a scoring system for assessing the severity of chronic liver disease.

$$\text{MELD} = 3.8 \cdot \log_e(\text{serum bilirubin [mg/dL]}) + 11.2 \cdot \log_e(\text{INR}) + 9.6 \cdot \log_e(\text{serum creatinine [mg/dL]}) + 6.4$$

**Paracentesis** – A medical procedure during which a drain is inserted to remove ascites from patients.

## 4. Introduction

### 4.1. Background and Rationale

We aim to determine the effectiveness of antibiotic prophylaxis for adults with cirrhosis and ascites but no previous episode of spontaneous bacterial peritonitis (SBP) to improve overall survival by reducing bacterial infections, especially SBP; this is termed “primary prophylaxis”.

In patients with liver cirrhosis, bacterial infection or sepsis carries a terrible mortality. SBP is the most common serious infection in people with cirrhosis and carries significant morbidity and mortality<sup>3</sup>. While antibiotic prophylaxis to prevent further infection has been established for those with a prior episode of SBP or presentation to hospital with upper gastrointestinal bleeding<sup>4,5</sup>, there remains considerable uncertainty over primary prophylaxis for SBP. This represents an important gap in our knowledge as 90% of SBP cases present in those with no previous episode<sup>6</sup> and so all current guidelines focus on the minority of patients. NICE Guidance (NG50) recommends prophylaxis with norfloxacin or ciprofloxacin for patients with ascitic fluid (AF) protein concentration <1.5 g/dL until the ascites has resolved, but the evidence for this, is dated<sup>7</sup>. The British Society of Gastroenterology (BSG) Guidelines on Management of Ascites in Cirrhosis (2006) states “For patients who have never had SBP in whom AF protein concentration is low (<1 g/dL), there is no consensus among experts regarding primary prophylaxis”. The American Association for the Study of Liver Disease (AASLD) recommends primary prophylaxis with norfloxacin or co-trimoxazole in patients with an AF protein concentration <1.5 g/dL combined with impaired renal function and liver failure (Child’s score >9 and bilirubin >3 mg/dL)<sup>8</sup>. The European Association for the Study of Liver Disease (EASL) states that one double-blind, placebo-controlled, randomised trial performed in patients with severe liver disease with AF protein lower than 1.5 g/dL and without prior SBP showed that norfloxacin (400 mg/day) reduced the risk of SBP and improved survival<sup>74</sup>. Therefore, these patients should be considered for long-term prophylaxis with norfloxacin. However, in those with AF protein greater than 1.5 g/dL, less severe liver disease and without prior history of SBP, the efficacy of quinolones in preventing SBP or improving survival is not clearly established and studies are needed. Finally, the duration of prophylaxis has not been established<sup>9</sup>.

Although norfloxacin is recommended in all guidance<sup>3</sup>, many UK NHS Trusts and pharmacies do not stock this drug. We conducted a national survey of primary prophylaxis for SBP through the British Society of Gastroenterology trial development group with responses from 23 centres that demonstrated a wide variation in clinical practice. Nine centres reported that they routinely used antibiotics as primary prophylaxis for SBP, seven did not routinely prescribe antibiotics as prophylaxis treatment and the remainder responded that they intermittently prescribe prophylaxis on a case-by-case or clinician dependent basis, often based on previous personal experiences. The antibiotics prescribed were ciprofloxacin (60%), norfloxacin (20%) or co-trimoxazole (20%). The majority of centres with trust guidelines for prescription would include patients with AF protein <1.5 g/dL or Child’s score B or C (advanced liver cirrhosis). There is evidently a lack of clarity over the optimum strategy and, given that infection represents a major cause of mortality in cirrhosis, an urgent need for high quality research.

Cirrhosis patients with an acute deterioration in their health secondary to complications of liver cirrhosis are termed as having acute decompensation (AD), or if extra hepatic organ failure co-exists, acute-on-chronic liver failure (ACLF)<sup>10</sup>. Decompensation includes: jaundice, ascites, hepatic



encephalopathy, variceal bleeding, coagulopathy and hepatorenal syndrome. Cirrhosis patients are highly prone to bacterial infection secondary to immune dysfunction<sup>11</sup>, with SBP the most common serious infection, and this frequently triggers other organ dysfunction<sup>3</sup>. As a result, bacterial infection is a leading cause of death in these patients with a mortality of >40%<sup>12</sup>.

In studies dating back 30 years, low AF protein has been shown to be a key risk factor for development of SBP<sup>13-15</sup>. Two prospective studies comprising 127 patients (13 with SBP)<sup>13</sup> and 110 patients (28 with SBP)<sup>14</sup> demonstrated low AF protein concentration as an independent predictor of SBP. In addition, AF protein  $\leq 1$  g/dL was shown to predict the recurrence of SBP<sup>16</sup>. AF protein content is considered to mirror host opsonisation activity and these studies demonstrated SBP incidence rates within 2 years of sampling of 20-25% at levels <1 g/dL and <1% in patients with protein levels >1.5 g/dL<sup>13,17,18</sup>. The authors also found that SBP risk increased minimally (20-24%) between years two and three of follow up<sup>13</sup>.

We originally used an AF protein of <2 g/dL in our inclusion criteria as many NHS trusts only record AF protein counts at this level rather than specific values. However, in our pilot ASEPTIC study we found that the incidence of low AF protein was extremely low, despite only screening patients with refractory ascites and very advanced liver disease. We screened 224 patients with ascites and only 38 had an AF protein <2g/dL. This includes one site that screened 30 patients with cirrhosis and ascites and did not identify a single patient with an AF protein concentration below this level. There are no large-scale UK data concerning AF protein values. Furthermore, the predictive value of the AF protein value has been recently challenged by two contemporary post-hoc analyses of three large cohorts of hospitalised patients with decompensated cirrhosis that suggested AF protein did not correlate with SBP risk<sup>19,20</sup>. Irrespective of the AF protein count, patients with persistent ascites are at very high risk of bacterial infection<sup>9</sup> and outcomes from infection have not improved in cirrhosis in the last 20 years. Based on our pilot study and more recent data we changed the inclusion criteria to persistent ascites without the need for an AF protein threshold to both substantially increase the number of patients eligible for recruitment and still include patients at great risk of SBP and subsequent mortality.

When we restarted recruitment in January 2021 following the first wave of COVID-19 in the UK, we received further feedback from research nurses and investigators that the eligibility requirement for ascites “despite 3 months of standard treatment” was significantly limiting recruitment.

Following the conclusion of the ASEPTIC pilot phase for recruitment and discussions with the UCL CCTU, Quality Management group and the ASEPTIC TMG group, IDMC and TSC, we have amended the inclusion criteria once more to improve recruitment to: ***‘Childs Pugh B or C cirrhosis and presence of ascites requiring any diuretic treatment or at least one or more paracentesis within 3 months of enrolment’***. This change will not affect our revised sample size/analysis and makes the study population more comparable to the ANSWER trial.

Most patients present for the first time with ascites to A&E and are subsequently hospitalised for ~1 week. This change would allow these patients to be recruited at discharge from hospital which sites believe will substantially increase recruitment. Furthermore, if the trial shows benefit of primary antibiotic prophylaxis, then this would be the time at which treatment would be commenced in the majority of patients eligible. Finally, sites have identified this as a good time to approach patients as they feel positive following response to treatment and have sufficient time to consider the information about the trial.

UK liver disease mortality rates have increased 400% since 1970, and it is now the third most common cause of premature death in the UK with incidence predicted to double over the next 20 years<sup>21</sup>. In 1999, liver disease surpassed lung cancer and breast cancer as a leading cause of years of working life lost and is set to overtake ischaemic heart disease within 2-3 years<sup>22</sup>. Finished admission episodes with a primary diagnosis of cirrhosis in English NHS hospitals rose 48.6% from 3783 in 2005/06 to 5621 in 2014/15, and the Chief Medical Officer for England has identified liver disease as a key population health problem<sup>23</sup>. SBP occurs in 25% of people who develop ascites, mostly in advanced liver disease, with 20-40% mortality<sup>24</sup>. Furthermore, those surviving hospital admission for infection have a mortality of 60% at 1 year<sup>25</sup>. The only effective treatment for advanced liver disease is transplantation, however only 800 adult transplants are performed per year and it is estimated that 60000 people have cirrhosis<sup>21</sup>; additional approaches are required to tackle this striking imbalance. With the effects of infection so profound, a strategy of prevention could have substantial benefits. However, there are no available immune restorative therapies and the only available strategy is prophylactic antibiotic treatment. Evidence exists that antibiotic prophylaxis halts, or at least delays, the development of infection, which may improve survival or bridge people to transplantation<sup>24</sup>. However, this must be balanced against the risk of selecting drug resistant organisms and *Clostridium difficile* (*C. difficile*) associated diarrhoea (CDAD)<sup>26</sup>. Yet this risk is unknown, as published data have been from relatively small (maximum 56 patients per group) single or dual centre studies with anti-microbial resistance (AMR) data only examined during the treatment period (6 months to 1 year), and most were performed in an era pre-dating the rise in AMR<sup>3</sup>. Finally, current practice is guided by data from other countries which have very different rates of AMR<sup>27</sup> and may not be applicable in the UK. There is therefore a huge necessity for this trial.

Our pilot study also indicates that the recording rate of SBP is likely to be a substantial underestimate. Unwell patients with advanced liver disease are admitted to hospital by the Acute Medical Admissions Units (AMU) teams and will commonly not see the specialist liver team until 24-72 hours after hospitalisation. Almost all diagnoses of SBP occur during emergency hospital admissions. An ascitic tap is required to diagnosis SBP, however, this is rarely performed by AMU teams as few of these doctors have been trained to do this. Therefore, an ascitic tap will be delayed, in the majority of cases, until performed by the liver teams at 24-72 hours. Although best practise is to wait until an ascitic tap has been taken before prescribing antibiotics, we have previously shown that half of these patients will be given empirical antibiotics at admission because of clinical concerns about their increased risk of sepsis<sup>9</sup>. The prescription of antibiotics before performing an ascitic tap substantially reduces the detection rate for SBP. This will cause lower SBP detection rates and we anticipate our original sample size (550 patients) would be insufficient to detect a meaningful difference between treatment arms.

Recent large-scale clinical trial data (Long-term albumin administration in decompensated cirrhosis (ANSWER) trial<sup>28</sup>) in patients with persistent ascites demonstrated an 18-month mortality of >20%. This study included an identical cohort of >400 patients to the cohort we anticipate recruiting in ASEPTIC and this has enabled us to power a study based on mortality as a primary endpoint. Unlike infection which can be difficult to record accurately, mortality is straightforward to record and evidently clinically meaningful. Indeed, the clinical trajectory that antibiotic prophylaxis aims to prevent is infection causing organ failure leading to death. Basing the survival probability in our control group on ANSWER provides a value of 0.66 at 18 months. All co-applicants, the Trial Management Group, trial team and all oversight groups agreed that a reduction of 38% in all-cause mortality, corresponding to a hazard ratio (HR) 0.62 with 77% of patients alive at 18 months in the treatment

group represented a clinically meaningful reduction in mortality. Our revised sample size calculation estimates 432 patients are required to demonstrate HR 0.62, with 90% power and two-sided alpha of 5% and a recruitment period of 2.5 years and 10% loss to follow up.

**Antibiotic Selection:** Two meta-analyses that included primary prophylaxis trials have shown that daily oral quinolones reduced both the risk of developing the first episode of SBP and mortality<sup>4,5</sup>. Several studies contained mixed populations including those with previous SBP, secondary prophylaxis, for which an evidence base has been established<sup>9</sup>. Of the three that focused on primary prophylaxis alone, the incidence of SBP in the placebo arms varied from 14% with Child's Pugh (CP) score  $8.5 \pm 1.5$ , to 16.7% with no CP data and 30.3% with CP  $10.4 \pm 1.5$  and renal dysfunction. Quinolone primary prophylaxis reduced overall SBP incidence from 22/137 to 4/138 patients<sup>3</sup>. The number needed to treat to prevent one episode of SBP at 6 months was calculated as 8.4. All three were single centre and included patients with AF protein counts of  $<1.5$  g/dL. Based on these studies, the AASLD gave a 1A recommendation for primary prophylaxis in patients with low AF protein, impaired renal function and advanced liver failure<sup>8</sup>, however these are an extremely unwell group of patients and represent only a small minority of those with ascites<sup>3</sup>. It is unclear whether prophylaxis would benefit all patients with persistent ascites.

Although British and European guidelines are based on treatment with quinolones, there is widespread microbiological concern over quinolone-associated AMR and CDAD rates. *Quinolones* are among the most frequently prescribed antibiotics worldwide<sup>29</sup>. They are used for the treatment of numerous infections owing to their excellent pharmacokinetics, good oral absorption, broad range of antimicrobial activity and relatively low incidence of side effects<sup>30</sup>. However, a clear relationship has been demonstrated between excessive quinolone use and the steady increase in the incidence of quinolone-resistant bacterial pathogens, both in hospital and the community<sup>26</sup>. Excessive use of these antibiotics has been associated with increases in the prevalence of quinolone resistance amongst nosocomial Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, as well as *Staphylococcus aureus* and streptococci linked with community-acquired infections<sup>28</sup>. In addition, excess exposure to quinolones has been associated with colonisation and infection by healthcare-associated pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA)<sup>31</sup> and, in particular CDAD<sup>32</sup>. Moreover, quinolone usage may contribute significantly to the emergence of resistance to other classes of antibiotics, such as carbapenems<sup>33</sup> – a phenomenon known as collateral resistance. As a result, quinolone usage carries significant risks both to the individual patient as well as the wider population. A recent trial of antibiotic prophylaxis in cirrhosis using norfloxacin in Egypt showed reduced efficacy compared to studies a decade previously, which may reflect increased AMR<sup>34,35</sup>. These studies suggest that quinolone prophylaxis may have serious adverse consequences. For example, Italy has high cephalosporin and carbapenem resistance rates amongst aerobic Gram-negative bacilli, exceeding 50% in some general hospitals – quinolone-based antibiotic prophylaxis may have exacerbated this<sup>28</sup>. One Italian centre has reported that the spread of multi-drug resistant infections has led to an increase in empirical antibiotic treatment failure, with antibiotic prophylaxis identified as a significant risk factor<sup>36</sup>. The high prevalence of resistance has led to another centre proposing the use of last-line antibiotics, such as meropenem and daptomycin, as first-line empirical treatment in SBP patients<sup>37</sup>. Widespread use of quinolone prophylaxis could lead to a similar situation developing in the UK<sup>38</sup> and we have therefore considered alternative strategies.

*Rifaximin* is a broad-spectrum antibiotic that eliminates gut microbes non-selectively, hence reducing the overall burden of intestinal bacteria and may also impair bacterial gut translocation<sup>39-41</sup>. Rifaximin is not absorbed into the systemic circulation, limiting systemic toxicity, side effects and the selective pressure for development of resistance. Rifaximin is NICE approved for maintaining remission from hepatic encephalopathy (HE) and widely prescribed for this indication<sup>42</sup>. Non-randomised studies to prevent bacterial infection in cirrhosis have shown mixed results. For example, rifaximin was shown to lower the infection rate in cirrhotic patients compared to no treatment, with as much as a 72% decrease in the risk of primary SBP<sup>43</sup> and reduce the risk of hepatorenal syndrome and variceal bleeding<sup>44</sup>. However, in a prospective cohort study comparing prophylaxis with rifaximin to prophylaxis with systemically absorbed antibiotic versus no prophylaxis, rifaximin did *not* reduce SBP occurrence in hospitalised cirrhotic patients compared with no treatment; only systemic antibiotics had an effect on reducing risk of SBP<sup>45</sup>. None of these studies were randomised, placebo-controlled trials. A French study is due to commence shortly comparing rifaximin with placebo in patients with ascites with an ascitic fluid protein (<1.5 g/dL) and impaired renal function or severe liver impairment (Child-Pugh score  $\geq 9$  with serum total bilirubin levels  $\geq 51$   $\mu\text{mol/L}$ ; <https://www.clinicaltrials.gov/ct2/show/NCT03069131?term=rifaximin+spontaneous+bacterial++peritonitis&rank=3>). However, in the UK this drug is widely prescribed for encephalopathy and therefore a substantial number of eligible patients in either arm would be prescribed this drug as an open-label agent, significantly confounding analyses<sup>40</sup>. Finally, it is expensive, with the current NHS cost of rifaximin (Targaxan, excluding VAT, from Drug Tariff, March 2014) being £259.23 for 56×550 mg capsules (1 month's supply).

*Co-trimoxazole* (*trimethoprim-sulfamethoxazole*) has been shown to have similar efficacy to norfloxacin in preventing SBP and is cost effective<sup>46-48</sup>. US guidance recommends either norfloxacin or co-trimoxazole for primary SBP prophylaxis in those with severe liver failure and/or renal dysfunction<sup>8</sup>. A US paper performed a cost-effectiveness analysis of norfloxacin and co-trimoxazole for SBP prophylaxis and found both to be cost-saving strategies with greater savings using co-trimoxazole<sup>49</sup>. Crucially, there are UK data comparing co-trimoxazole to norfloxacin for primary prophylaxis which showed similar efficacy but no admissions for CDAD in the co-trimoxazole group compared to 13/134 in the norfloxacin group<sup>50</sup>. *C. difficile* outbreaks can have devastating effects on morbidity and mortality in secondary care and nursing homes, and there seems to be some reluctance to use SBP prophylaxis citing concerns over CDAD. There is also potentially less concern over AMR when using co-trimoxazole compared to quinolones, with this narrow spectrum antibiotic used widely to treat community acquired urinary tract infection<sup>51</sup>. Indeed, studies in human immunodeficiency viruses (HIV) patients have shown that co-trimoxazole use has not coincided with a further increase in pneumococcal co-trimoxazole or multidrug resistance, and a meta-analysis in HIV suggested that there was some evidence that co-trimoxazole prophylaxis actually protected against resistance to other antibiotics<sup>52,53</sup>. Although a recent study has reported significant associations between prophylaxis and non-susceptibility to penicillin and rifampin<sup>54</sup>. The studies using co-trimoxazole to prevent SBP did not report AMR but these pre-date the current AMR era, were small and had only 6 months follow up<sup>46-48</sup>.

Co-trimoxazole has been chosen for this trial as it is well tolerated, cheap and effective with a substantial literature of prophylaxis in HIV; a >3000 patient study reported a 3% incidence of serious adverse events<sup>55</sup>. Although the majority of trials have used fluoroquinolone antibiotics, in 2019 the Medicines and Healthcare products Regulatory Agency (MHRA) recommended restricting use of these in prophylaxis because of very rare reports of disabling and potentially long-lasting or irreversible side

effects affecting the musculoskeletal and nervous systems. Co-trimoxazole offers an alternative without these adverse effects. Finally, co-trimoxazole will not compromise NICE guidance on the use of quinolones for secondary prophylaxis. Potential adverse events associated with co-trimoxazole are hyperkalaemia, the very rare but serious Stevens-Johnson skin rash and blood cell dyscrasias. Patients will be educated about possible adverse reactions, in particular skin rashes (especially Stevens-Johnson). Trimethoprim at doses of 10-20mg/kg/day has been shown to cause modest, clinically non-significant rises in serum creatinine<sup>56</sup>. In HIV infected patients receiving high dose co-trimoxazole for *Pneumocystis jirovecii* (*P. carinii*) pneumonitis serum potassium increased by 1.1 mmol/L, an average 9.8±0.5 days after starting therapy<sup>48</sup>. Changes in renal function did not account for the creatinine rise and levels returned to normal after cessation. High dose co-trimoxazole comprises 20 mg trimethoprim and 100 mg sulfamethoxazole per kg of body weight per day *i.e.* around 1500mg per day, ten times the dose we will use. It is believed that trimethoprim acts similarly to amiloride, blocking sodium channels at the distal tubules resulting in a reduction in transepithelial voltage and potassium secretion, with resultant hyperkalaemia (serum potassium ≥6 mmol/L)<sup>57</sup>. It is therefore recommended that serum potassium is monitored in these patients, particularly 7-10 days after starting the drug. Hyperkalaemia has also been noted in elderly non-HIV patients treated with lower doses<sup>58</sup> (that were *double* our prophylactic dose) and caution is recommended in renal dysfunction. In clinical trials in cirrhosis patients for SBP prophylaxis, hyperkalaemia has not been reported. In a study of 60 patients that included a serum creatinine of at least 177 µmol/L as one of the possible inclusion criteria, no adverse events were reported<sup>27</sup>. In a trial comparing co-trimoxazole with norfloxacin in which 25 patients were treated with co-trimoxazole, there was one drug discontinuation at 60 days secondary to worsening of renal function<sup>26</sup>. A retrospective study, again compared to norfloxacin, showed identical incidence of adverse events in each group with no worsening of renal failure secondary to co-trimoxazole<sup>48</sup>. A UK study also reported no renal dysfunction<sup>51</sup>, and AASLD guidance recommends co-trimoxazole for SBP prophylaxis in patients with ascites and renal impairment<sup>8</sup>.

Furthermore, it is off patent and low cost, with co-trimoxazole 80mg/400mg capsules (Actavis UK Ltd) priced at £2.37 for 28 capsules, making this fifty times less expensive than rifaximin. In view of the demonstrated equivalent efficacy, cheaper price and much lower concern over *C. difficile* diarrhoea and possibly AMR compared to quinolones, our consensus decision is that the treatment arm will be co-trimoxazole 960mg. However, in view of the possible beneficial effects of rifaximin, patients will be stratified according to its use at randomisation.

*Duration of therapy:* 2018 EASL Guidelines state that norfloxacin prophylaxis should be stopped in patients with long-lasting improvement of their clinical condition and disappearance of ascites<sup>9</sup>. Our trial treatment period of 18 months will test whether there is a waning of efficacy over time, whether there is a beneficial effect on mortality and whether our strategy impacts upon AMR. Previous studies have ranged from only 1 month to 1 year and our larger and longer study will test these crucial issues relevant to “real-world” prescribing of prophylaxis. It is possible that there may be more adverse events seen in the treatment group in this longer follow up but since a positive outcome would lead to long-term prescription of co-trimoxazole, this would also be very valuable information that will arise from this trial.

#### **4.1.1. Explanation for choice of comparators**

Our choice of comparator is placebo. There is no UK consensus regarding the necessity for prescribing primary prophylaxis in SBP with the majority of clinicians that took part in our national survey not prescribing antibiotics. Therefore, placebo is the best option for our trial.

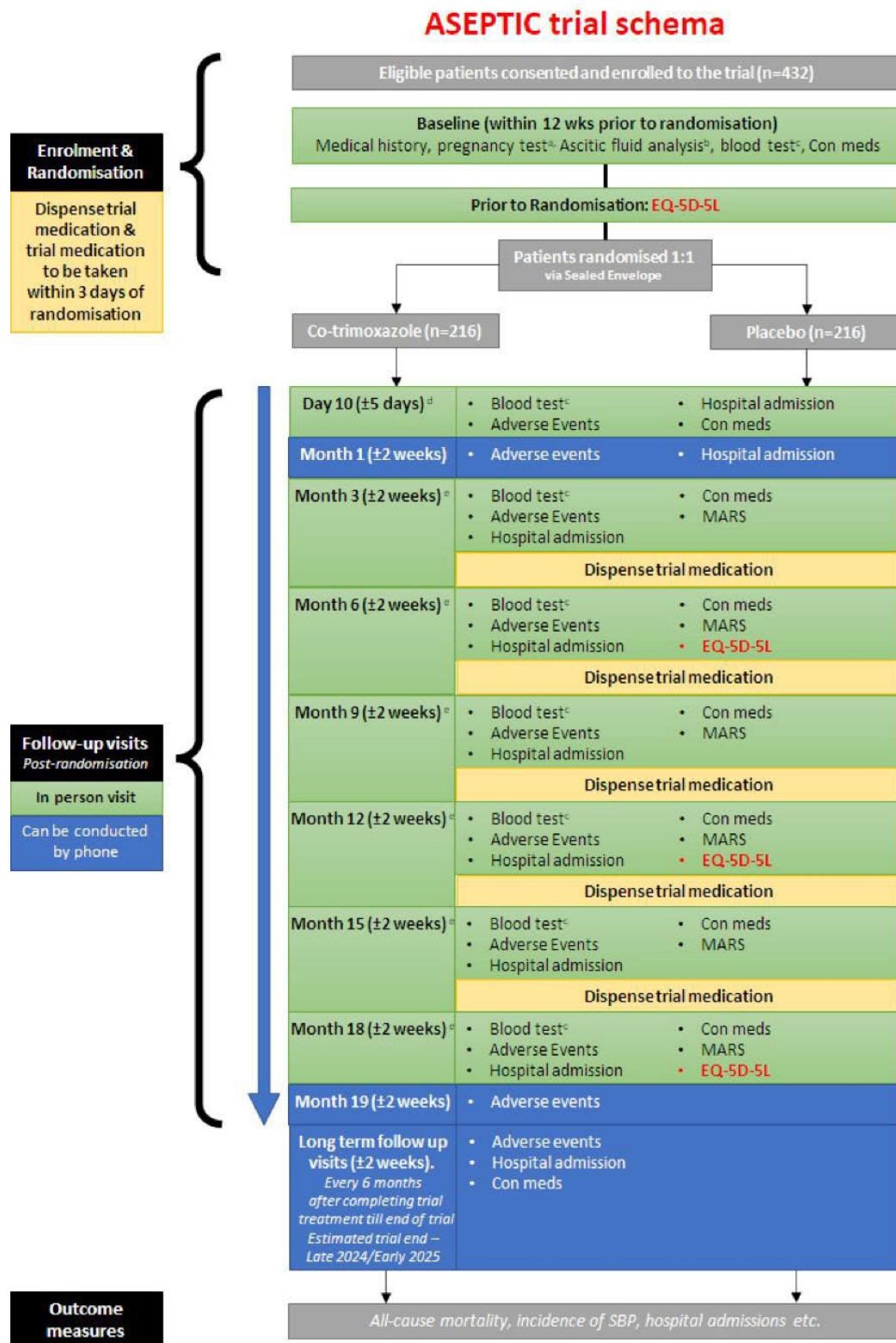
#### **4.2. Objectives**

The primary objective of ASEPTIC is to determine whether primary antibiotic prophylaxis with co-trimoxazole improves overall survival compared to placebo in adults with cirrhosis and ascites.

Key secondary objectives will include incidence of spontaneous bacterial peritonitis, Clostridium Difficile Associated Diarrhoea and Anti-Microbial Resistance incidence, cost-effectiveness, incidence of other complications of liver cirrhosis and patient hospitalisations during follow-up.

#### **4.3. Trial Design**

This is a multicentre placebo-controlled randomised double-blind trial that assesses efficacy, cost-effectiveness and safety of the use of co-trimoxazole for 18 months to improve overall survival in patients with cirrhosis and ascites.

<sup>a</sup> Pregnancy test must be completed within 2 weeks prior to randomisation<sup>b</sup> Ascitic fluid analysis can be done within 6 months prior to randomisation<sup>c</sup> Blood tests should include full blood count, with haemoglobin, white cell count, platelets, INR (or prothrombin time converted to INR), sodium, potassium, serum creatinine, and urea.

Liver function tests should include albumin, alanine aminotransferase (ALT), alkaline phosphatase, and bilirubin.

Renal function tests including eGFR.

<sup>d</sup> Blood test can be taken at GP or another clinic and the follow-up can be conducted over the phone only under exceptional circumstances.<sup>e</sup> If the patient permanently discontinues their trial medication, these follow-ups can be conducted over the phone and other data (e.g. bloods) can be taken from their routine hospital visits that fall within the trial follow-up windows.

## 5. Methods

### 5.1. Site Selection

The trial sponsor has overall responsibility for site and investigator selection and has delegated this role to CCTU.

#### 5.1.1. Study Setting

The trial will take place in secondary or tertiary care NHS hospitals that frequently manage patients with advanced liver disease.

An application will be made for access to data held by NHS Digital (or equivalent in other nations); the dataset will include Hospital Episode Statistics (HES), the Mental Health Minimum Data Set (MHMDs) and mortality data.

The recruitment period will be 30 months, with a 11-month internal progression review to demonstrate deliverability of recruitment. Please see section 5.8.1 for more details.

#### 5.1.2. Site/Investigator Eligibility Criteria

Once a site has been assessed as being suitable to participate in the trial, the trial team will provide them with a copy of this protocol and the relevant Summary of Product Characteristics (SPC).

To participate in the ASEPTIC trial, investigators and trial sites must fulfil a set of criteria that have been agreed by the ASEPTIC Sponsor and/or Trial Management Group (TMG) and that are defined below.

Eligibility criteria:

- A named clinician is willing and appropriate to take Principal Investigator responsibility
- Suitably trained and qualified staff are available to recruit patients, enter data and collect samples
- The site should be able to store, prepare and dispense trial medication appropriately
- The site should meet any other criteria agreed by the ASEPTIC Trial Management Group

##### 5.1.2.1. *Principal Investigator's (PI) Qualifications and Agreements*

The investigator(s) must be willing to sign an Investigator Agreement to comply with the trial protocol (confirming their specific roles and responsibilities relating to the trial, and that their site is willing and able to comply with the requirements of the trial). This includes confirmation of appropriate qualifications, by provision of a CV, familiarity with the appropriate use of any investigational products, agreement to comply with the principles of GCP, to permit monitoring and audit as necessary at the site, and to maintain documented evidence of all staff at the site who have been delegated significant trial related duties.

##### 5.1.2.2. *Resourcing at site*

The investigator(s) should be able to demonstrate a potential for recruiting the required number of suitable patients within the agreed recruitment period (i.e. the investigator(s) regularly treat(s) the target population). The Investigator should also have an adequate number of qualified staff and



facilities available for the foreseen duration of the trial to enable them to conduct the trial properly and safely.

Sites will be expected to complete a delegation of responsibilities log and provide staff contact details.

The site should have sufficient data management resources to allow prompt data completion and return to CCTU.

## 5.2. Site approval and activation

On receipt of the signed Clinical Trial Site Agreement, Investigator Agreement, approved delegation of responsibilities log and staff contact details, written confirmation of receipt will be sent to the site PI. The trial manager or delegate will notify the PI in writing of the plans for site activation. Sites will not be permitted to recruit any patients until a letter for activation has been issued. The Trial Manager or delegate will be responsible for issuing this after a green light to recruit process has been completed.

The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and, by the competent authority, and which was given favourable opinion by the Ethics Committee (EC). The PI or delegate must document and explain any deviation from the approved protocol and communicate this to the trial team at CCTU.

A list of activated sites may be obtained from the Trial Manager.

## 5.3. Patients

### 5.3.1. Eligibility Criteria

All patients with meeting all inclusion criteria and none of the exclusion criteria (see below) will be eligible.

#### 5.3.1.1. Patient selection

There will be **NO EXCEPTIONS** (waivers) to eligibility requirements at the time of randomisation. Questions about eligibility criteria should be addressed PRIOR to attempting to randomise the patient.

The eligibility criteria for this trial have been carefully considered and are the standards used to ensure that only medically appropriate patients are entered. Patients not meeting the criteria should not be entered into the trial for their safety and to ensure that the trial results can be appropriately used to make future treatment decisions for other people with similar diseases or conditions. It is therefore vital that exceptions are not made to these eligibility criteria.

Patients with cirrhosis and ascites that have been hospitalised or those that are attending hospital for ascitic drainage (paracentesis) at day-case units or outpatients visits, or those from liver transplant waiting list clinics will be identified and approached to participate.

Patients will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

#### 5.3.1.2. Patient Inclusion Criteria

1. Patients with Child-Pugh Class B or C cirrhosis and presence of ascites requiring any diuretic treatment or at least 1 or more paracentesis within 3 months prior to enrolment.
2. Patient at least 18 years of age
3. Documented informed consent to participate.

#### 5.3.1.3. Patient Exclusion Criteria

1. Patients with current or previous Spontaneous Bacterial Peritonitis (defined as ascitic polymorphonuclear count  $>250$  cells/mm<sup>3</sup> with either positive or negative ascitic fluid culture without evident intra-abdominal surgically treatable source of infection. A white cell count  $>500$  cell/mm<sup>2</sup> or positive microbial culture may be considered as evidence of previous SBP if the site PI considers this was in the context of a likely clinical diagnosis of SBP ).
2. Patients receiving palliative care with an expected life expectancy of  $<8$  weeks
3. Allergic to co-trimoxazole, trimethoprim or sulphonamides
4. Pregnant or lactating mothers
5. Patient enrolled in a clinical trial of investigational medicinal products (IMPs) that would impact on their participation in the study
6. Patients with serum potassium ( $>5.5$  mmol/L) related to pre-existing kidney disease which cannot be reduced\*
7. Patients receiving antibiotic prophylaxis (except for rifaximin)\*
8. Patients with long-term ascites drains\*
9. Women of child-bearing potential and males with a partner of child-bearing potential without effective contraception for the duration of trial treatment
10. Patients with pathological blood count changes
  - a. Patients with haemoglobin (Hb)  $<70$ g/L\*
  - b. Granulocytopenia defined as absolute neutrophil counts of less than 500 cells per microliter\*
  - c. Severe thrombocytopenia with a platelet count  $<30 \times 10^9$  /L\*
11. Patients with severe renal impairment, with eGFR  $<15$  ml/min\*
12. Patients with skin conditions: exudative erythema multiform, Stevens-Johnson syndrome, toxic epidermal necrolysis and drug reaction with eosinophilia and systemic symptoms
13. Patients with congenital conditions: congenital glucose-6-Phosphate dehydrogenase deficiency of the erythrocytes, haemoglobin anomalies such as Hb Köln and Hb Zürich
14. Patients with acute porphyria
15. Any clinical condition which the investigator considers would make the patient unsuitable for the trial

*\*It is common for these investigations to change in patients with cirrhosis and long term ascitic drains may be removed. Patients can be re-screened for eligibility if this occurs.*

#### **5.3.1.4. Eligibility Criteria for Individuals Performing the Interventions**

Nursing and medical staff members of the clinical trial team at sites will have the appropriate qualifications to manage patients with complications of cirrhosis as for routine clinical care. Each member of the trial team at each site will have their roles within the trial, as delegated by the PI, documented on the ASEPTIC site delegation log. CVs of all staff working on the trial will be collected by UCL CCTU to document their qualifications and relevant experience. Protocol-specific training will be given to site staff; training must be completed before a site can be activated and prior to a member of staff starting work on the trial.

#### **5.3.1.5. Co-enrolment Guidance**

Patients may not be enrolled in any other clinical trial of an investigational medicinal product, without the permission of the Chief Investigator of the ASEPTIC trial and permission from the other trial. Co-enrolment on observational studies is allowed.

#### **5.3.1.6. Screening Procedures and Pre-randomisation Investigations**

Written informed consent to enter and be randomised into the trial must be obtained from patients, after explanation of the aims, methods, benefits and potential hazards of the trial and **BEFORE** any trial-specific procedures are performed or any blood is taken for the trial. The only procedures that may be performed in advance of written informed consent being obtained are those that would be performed on all patients in the same situation as usual standard of care.

Interventions and assessments prior to randomisation:

- Blood tests (for a list, please see section 5.6.1)
- Ascitic fluid sample for white cell count and/or protein count. It is standard practise at many sites to only process a sample for a PMN count if the white cell count is elevated. If the white cell count is not elevated, these patients will be considered not to have a diagnosis of SBP even if a PMN count is not processed. This is standard medical practise.
- Pregnancy test: to be completed at screening or following this so that the result is known within 2 weeks prior to randomisation, see below for more details.

A pregnancy test will be completed for all women of childbearing potential (women of childbearing potential excludes women who are postmenopausal or permanently sterilised, e.g. tubal ligation, hysterectomy, or bilateral salpingectomy). Women of childbearing potential and males with a partner of child bearing potential should be willing to use effective contraception with a “double barrier” method for the duration of trial treatment. According to the Clinical Trials Facilitation Group (CTFG) guidelines regarding the recommendations related to contraception and pregnancy testing in clinical trials<sup>73</sup>; “double barrier” method refers to simultaneous use of a physical barrier by each partner and it is defined as the combination of male condom AND either female cap, diaphragm or sponge with spermicide. If a pregnancy test has not been completed as part of standard of care, then it should be completed after consent and prior to randomisation. The screening pregnancy test must be completed within 2 weeks prior to randomisation. Partners of male patients will be informed of the risks of pregnancy and in the case of pregnancy during the trial, they will be asked to sign the consent form for the collection of their data at each follow up visit.

Female patients of childbearing potential must not become pregnant during the trial and so these women must have a negative serum or urine pregnancy test at screening, and agree to be consistent and use correctly two of the following acceptable methods of birth control during the study:

- Oral contraceptive, either combined or progestogen alone
- Injectable progestogen
- Implants of levonorgestrel or etonogestrel
- Estrogenic vaginal ring
- Percutaneous contraceptive patches
- Intrauterine device (IUD) or intrauterine system (IUS) with <1% failure rate as stated in the product label
- Double barrier (e.g. male condom plus spermicide, or female diaphragm or sponge plus spermicide)
- Bilateral tubal ligation
- Male partner sterilisation (vasectomy with documentation of azoospermia) prior to the female patients' entry into the study, and this male is the sole partner for that patient.
- Total abstinence from intercourse with male partners (occasional abstinence is not a reliable form of contraception).

#### 5.4. Interventions

After eligibility has been confirmed, patients satisfying the inclusion/exclusion criteria will be randomised using a secure online randomisation system (refer to section 5.9.1.1). Patients will be randomised in a 1:1 ratio to receive either co-trimoxazole or placebo.

##### 5.4.1. Products

- Co-trimoxazole, one capsule of 960 mg
- Placebo, one capsule

##### 5.4.2. Treatment Schedule

One capsule of 960mg co-trimoxazole or one capsule of placebo is taken daily with water for up to 18 months.

##### 5.4.3. Dispensing

Co-trimoxazole and matching placebo will be manufactured and distributed by Sharp Clinical Services (UK) Ltd. The trial medications are packaged in bottles, and one bottle contains 100 capsules. All trial medication will be dispensed to the patients by the local pharmacy departments at each participating site to coincide with randomisation and as close to the patients' 3 monthly follow-up visits. Due to the Covid-19 pandemic or exceptional circumstances, there may be situations where a patient may run out of IMP prior to the next follow-up visit (as a result of appointment scheduling and available capacity at site). If this is the case, as per MHRA and HRA guidelines, IMPs can be couriered to the patient's home address, as long as the patient has given verbal consent.

#### 5.4.4. Dose modification, suspension, continuation, and withdrawal

##### 5.4.4.1. Dose modification, suspension and continuation

Treatment may be temporarily stopped if the patient experiences any severe adverse reactions or if in the opinion of the PI is necessary. If that is the case, the patient will remain in the trial and continue to attend the follow up visit. The patient may be re-challenged following stopping of co-trimoxazole for at least a week if the PI considers that this would be safe.

- Renal dysfunction

If the patients eGFR reduces to <30 ml/min on trial treatment, the trial medication dosing will be reduced to alternate day dosing. If the eGFR rises to >30ml/min, the trial medication can be increased back to once daily dosing.

If the patients eGFR reduces to <15ml/min on trial treatment, then the trial medication will be stopped. If the eGFR rises to >15ml/min but remains <30 ml/min, the trial medication can be increased back to alternate day dosing. In these patients the trial medication will not be increased any further to once daily dosing.

The follow up blood test may be performed at any time from a week following any dose reduction/cessation.

- Hyperkalaemia

Hyperkalaemia usually occurs in patients following diuretic prescription which is often seen in patients and is monitored and controlled as part of standard care. Patients will be included only if serum potassium is  $\leq 5.5$ mmol/L. Blood samples will be taken 10 days (+/- 5 days) after commencement of trial medication and at each 3-monthly follow-up visit thereafter to monitor serum potassium levels. Physicians should be cautious, in line with standard medical practice, of prescribing diuretic medication or increasing the doses of diuretics which may also increase potassium (such as amiloride or spironolactone) during the course of the trial. Furosemide, a loop diuretic which actually lowers serum potassium levels may be an appropriate alternative that should be considered at an early stage. Patients and the site PI and physicians will be made aware of the risks of hyperkalaemia in patients with renal dysfunction. If serum potassium rises to  $\geq 6$  mmol/L, trial medication will be stopped and the patient will be re-challenged once the PI considers this to be safe. If hyperkalemia (potassium  $\geq 6$  mmol/L) related to trial medication occurs on more than one occasion, then when the trial medication is restarted, the dose will be reduced to alternate day dosing and remain at this dose for duration of study.

The follow up blood test may be performed at any time from a week following any dose reduction/cessation.

- Thrombocytopenia

The trial medication will be stopped if the platelet count falls below  $30 \times 10^9$  /L or if the platelet count has fallen to such an extent as to make the PI/clinical team concerned about an increased risk of bleeding. Having stopped the trial medication, if the platelet count rises to  $30 \times 10^9$  /L or a level at which the PI/clinical team is no longer concerned about an increased risk of bleeding, then the trial

medication can be restarted. If the patient has evidence of bleeding (e.g. gastrointestinal bleeding or recurrent nosebleeds requiring intervention) and platelet count  $<50 \times 10^9 /L$ , then trial medication should be stopped until the bleeding is controlled successfully.

The follow up blood test may be performed at any time from a week following any dose reduction/cessation.

- Hospital admission non-SBP related

The trial medication will be stopped on hospital admission. Patients who recover from a non-SBP related admission will be restarted on trial medication. There will be no unblinding of study medication unless considered important for patient care as assessed by the attending clinicians.

Those with upper gastrointestinal haemorrhage or any other reason to commence antibiotics during hospital admission will be restarted on trial medication following cessation of antibiotics and recovery. This will occur most commonly at discharge or shortly after, however it will be at the discretion of the treating clinician to decide if the patient is well enough to restart the trial medication.

- Recovery from ascites

If ascites resolves off diuretic medication for greater than 6 months, patients must not continue to take the trial medication as their health status has improved. Every effort will be made to keep these patients in the trial and they should continue to attend all follow-up visits until the end of the trial.

If during the trial the ascites returns, the patient can return to the treatment they were initially allocated.

#### **5.4.4.2. Withdrawal from trial treatment**

- Stevens-Johnsons syndrome

Co-trimoxazole is well-tolerated; however, it can cause significant side-effects in 1 of every 100 patients, usually skin rash.

Very rarely (1–7 cases per million people per year), Stevens-Johnson syndrome can develop (a severe skin rash) and treatment is immediate cessation of the drug. Under this circumstance the drug would not be restarted, and the patient would be withdrawn from trial treatment, every effort will be made to continue to follow-up the patient according to the protocol until the end of the trial.

- Hospital Admission SBP-related

Patients that develop SBP will be treated with standard antibiotics as per local guidelines and trial medication stopped. Once they have recovered from SBP these patients will then be commenced upon secondary antibiotic prophylaxis at discharge from hospital, as standard UK clinical practise. They will therefore be withdrawn from trial treatment but will continue their follow-up visits for the trial.

Transplant

Patients who meet the inclusion criteria and none of the exclusion criteria but are on the liver transplantation list can still be included in the trial. Patients put onto the transplantation waiting list during the trial would still receive trial medication. If a patient receives a liver transplant, that patient will be withdrawn from trial treatment, and every effort will be made to continue to follow-up the patient according to the protocol until the end of the trial.

- SUSAR Event

Patients that develop any SUSAR would be withdrawn from trial treatment and every effort will be made to continue to follow-up the patient according to the protocol for until the end of the trial.

The SUSAR should be reported to the CCTU within the designated timelines (see section 5.12.4)

#### 5.4.5. Accountability

The IMP accountability, storage and destruction will follow the IMP Management plan.

The trial pharmacist or delegate at each participating site will undertake accountability of trial medication supplies. Accountability must include tracking of all trial medication received at site, storage of the IMP according to the SmPC, dispensing to patients, and destruction of expired or unused medication.

Patients should return all unused trial medication at each follow-up visit. Unused, expired or returned trial medication, stored at the local pharmacy will need the sponsor to confirm that it can be destroyed as per standard local hospital procedures.

#### 5.4.6. Compliance and Adherence

Treatment adherence will be assessed by the research team with the Medication Adherence Report Scale (MARS) questionnaire at the 3-month follow-up visits prior to a new trial medication being dispensed. The MARS questionnaire is a registered questionnaire to evaluate patient adherence. This will be documented in the CRFs. Patients will be educated about the possible dangers of non-compliance.

#### 5.4.7. Concomitant Care

Patients must not be receiving long-term antibiotic prophylaxis. The patient is permitted to take rifaximin as this may be prescribed for hepatic encephalopathy. Patients will be stratified according to their use of rifaximin at randomisation and whether they are presently on the liver transplantation waiting list.

Site PIs and physicians will be aware of the potential risks of hyperkalaemia in patients with renal dysfunction and those taking high dose spironolactone or amiloride. Patients with significant persistent renal dysfunction and hyperkalaemia ( $\geq 6\text{mmol/L}$  serum potassium) that the PI believes cannot be managed with adjustment of diuretic medication will not be eligible. However renal dysfunction may improve in these patients and such patients may be reconsidered for eligibility at a later stage under such circumstances.

Caution should be exercised in patients taking any other drugs that can cause hyperkalaemia, for example ACE inhibitors, angiotensin receptor blockers and potassium-sparing diuretics such as

spironolactone. Concomitant use of trimethoprim-sulfamethoxazole (co-trimoxazole) may result in clinically relevant hyperkalaemia as mentioned in section 5.4.4 and addition of the loop diuretic furosemide that will reduce the potassium level should be considered.

#### 5.4.8. Special consideration

Special considerations should be taken for the following concomitant treatment and contraindications:

Zidovudine (a drug used for HIV treatment): in some situations, concomitant treatment with zidovudine may increase the risk of haematological adverse reactions to co-trimoxazole. Monitoring of haematological parameters will take place every 3 months in all patients.

Cyclosporin: reversible deterioration in renal function has been observed in patients treated with co-trimoxazole and cyclosporin following renal transplantation. However, it is exceptionally rare for a patient with ascites to have a renal transplant without a combined liver transplant. Patient that receive a liver transplant will be withdrawn from trial treatment but should continue to attend trial follow-up visits until the end of the trial.

Rifampicin: concurrent use of rifampicin and co-Trimoxazole results in a shortening of the plasma half-life of trimethoprim after a period of about one week. This is not thought to be of clinical significance.

When trimethoprim is administered simultaneously with drugs that form cations at physiological pH and are also partly excreted by active renal secretion (e.g. procainamide, amantadine), there is the possibility of competitive inhibition of this process which may lead to an increase in plasma concentration of one or both of the drugs. Appropriate monitoring will be undertaken under this circumstance.

Thiazides diuretics: in elderly patients concurrently receiving diuretics, mainly thiazides, there appears to be an increased risk of thrombocytopenia with or without purpura. However, thiazide diuretics are exceptionally rarely used in this group of patients as most will be treated with the aldosterone antagonist spironolactone or the loop diuretic furosemide.

Pyrimethamine: occasional reports suggest that patients receiving pyrimethamine at doses in excess of 25mg weekly may develop megaloblastic anaemia should co-trimoxazole be prescribed concurrently.

Warfarin: co-trimoxazole has been shown to potentiate the anticoagulant activity of warfarin via stereo-selective inhibition of its metabolism. Careful control of the anticoagulant therapy during treatment with co-trimoxazole is advisable and one of the newer class of anticoagulant may be advisable under these circumstances. PIs will be encouraged to discuss patients requiring anti-coagulation with haematology colleagues.

Phenytoin: co-trimoxazole prolongs the half-life of phenytoin and if co-administered could result in excessive phenytoin effect. Close monitoring of the patient's condition and serum phenytoin levels are advisable. However this anti-convulsant is uncommonly used.

Digoxin: concomitant use of trimethoprim with digoxin has been shown to increase plasma digoxin levels in a proportion of elderly patients. Patients will be appropriately monitored.



Methotrexate: co-trimoxazole may increase the free plasma levels of methotrexate. If co-trimoxazole is considered appropriate therapy in patients receiving other anti-folate drugs such as methotrexate, a folate supplement should be considered.

Lamivudine: administration of co-trimoxazole (160 mg/800 mg) causes a 40% increase in lamivudine exposure because of the trimethoprim component. Lamivudine has no effect on the pharmacokinetics of trimethoprim or sulfamethoxazole. However, this drug is almost never used for treatment of hepatitis B with other newer drugs such as tenofovir clinically preferred.

Interaction with sulphonylurea hypoglycaemic agents is uncommon but potentiation has been reported.

Repaglinide: trimethoprim may increase the exposure of repaglinide which may result in hypoglycaemia.

Azathioprine: There are conflicting clinical reports of interactions between azathioprine and trimethoprim-sulfamethoxazole, resulting in serious haematological abnormalities.

#### 5.4.9. Overdose of Trial Medication

Measures will be taken to minimise accidental overdose of trial medication by providing adequate education to trial patients. Accidental or deliberate overdose of trial medication will be treated accordingly. The re-introduction of trial medication dosing will be determined by the clinical investigator at the participating site. Any patient taking a deliberate overdose of trial medication should discontinue trial medication for the remaining duration of the trial and no further supply of trial medication given. Patients whose trial medication is discontinued will remain in follow-up for the duration of the trial where possible.

#### 5.4.10. Protocol Treatment Discontinuation

In consenting to the trial, patients are consenting to trial treatment, trial follow-up and data collection. However, an individual patient may stop trial treatment early or cease involvement with the trial early for any of the following reasons:

- Pregnancy
- Unacceptable treatment toxicity or adverse event
- Inter-current illness that prevents further treatment
- Any change in the patient's condition that in the clinician's opinion justifies the discontinuation of treatment
- Withdrawal of consent for treatment by the patient

As participation in the trial is entirely voluntary, the patient may choose to discontinue trial treatment at any time without penalty or loss of benefits to which they would otherwise be entitled. Although not obliged to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason, whilst remaining fully respectful of the patient's rights.

Patients who discontinue protocol treatment, for any of the above reasons, should remain in the trial for the purpose of follow up and data analysis.

## 5.5. Outcomes

### 5.5.1. Primary Outcomes

The primary outcome will be overall survival. The maximum possible period of follow up, will be 48 months (assuming a recruitment period of 30 months, and 18 months treatment period for final patient recruited).

### 5.5.2. Secondary Outcomes

1. Time to first incidence of spontaneous bacterial peritonitis (SBP) (SBP is defined as per the standard guidelines: ascitic fluid polymorphonuclear (PMN) cell count  $>250/\text{mm}^3$  with either positive or negative ascitic fluid culture without evident intra-abdominal surgically treatable source of infection<sup>3</sup>). A white cell count  $>500 \text{ cell}/\text{mm}^2$  or positive microbial culture may be considered as evidence of previous SBP if the site PI considers this was in the context of a likely clinical diagnosis of SBP.
2. Hospital admission rates
3. Incidence of *C. difficile*-associated diarrhoea
4. Incidence of infections other than SBP with hospital admission.
5. Incidence of other cirrhosis related events (e.g. variceal haemorrhage)
6. Incidence of renal dysfunction with creatinine  $>133 \mu\text{mol}/\text{L}$  (1.5mg/dL) at any point during hospital admission
7. Effect of IMP on renal function for duration of the trial as measured by eGFR
8. Effect of IMP on renal function for duration of the trial as measured by serum creatinine levels
9. Incidence of anti-microbial resistance
10. Incidence of liver transplantation
11. Progression of liver disease assessed by increase in MELD score between baseline and end of trial follow up.
12. Safety and treatment-related serious adverse events
13. Treatment adherence (assessed by MARS questionnaire)
14. Health-related quality of life assessed using EQ-5D-5L questionnaire
15. Health and social care resource use assessed using Hospital Episode Statistics (HES) database
16. Mean incremental cost per quality adjusted life year gained (QALY)
17. Incidence of resolution of ascites with diuretic treatment not required for 6 months
18. Incidence of Transjugular intrahepatic portosystemic shunt (TIPS) insertion.

All the above outcomes are assessed for a minimum period of 18 months following randomisation. The maximum possible period of follow up will be 48 months (assuming a recruitment period of 30 months and 18 months treatment period for final patient recruited).

## 5.6. Patient Timeline

### 5.6.1. Enrolment and Randomisation

Table 1 specifies the assessments and interventions that are required before enrolment. The eligibility of the patient will be confirmed from screening data and clearly documented in patient medical notes and the study CRF. Baseline evaluations include age, sex, date of birth, diagnosis, medical history and physical examination, concomitant medications, liver and renal tests (within previous 12 weeks), ascitic fluid analysis and culture, and abdominal ultrasonography (within previous 6 months). The screening blood tests within 12 weeks prior to randomisation include full blood count with haemoglobin, white cell count, platelets, INR (or prothrombin time converted to INR), sodium, potassium (especially checking for hyperkalaemia), serum creatinine, urea, liver function tests including albumin, alanine aminotransferase (ALT), alkaline phosphatase and bilirubin. Renal function tests include eGFR. EQ-5D-5L will also need to be collected from patients at this time point.

After patient eligibility has been confirmed by the PI or delegated medically qualified doctor, enrolment can occur, and the patient will be randomly allocated to co-trimoxazole or placebo via the Sealed Envelope website. Hospitalised patients should be randomised at discharge once medically stable.

On the day of randomisation, delegated staff at site will enter the patient's initials, date of birth (month and year only), NHS number (which will be AES-256 encrypted), eligibility criteria fulfilment, and site details into the Sealed Envelope randomisation website, which will then allocate the randomised treatment. The treatment allocation will be concealed with only providing the kit codes to site. Usernames and passwords for Sealed Envelope will be provided to site staff during the site activation procedure.

Protocol treatment with trial medication (either co-trimoxazole or placebo) should start within 3 days from the date of randomisation. (Please note that if the PI/delegate thinks the patient is clinically well enough whilst the patient is an inpatient, then IMP can start whilst they are an inpatient and should be monitored by the clinical trial team).

### 5.6.2. Study follow-up

At Day 10 (+/- 5) days, blood samples should be taken after commencement of the trial medication to check for hyperkalaemia and to make any dose modifications to concomitant medications if needed. Under exceptional circumstances, if the patient is unable to attend this visit face to face, the site can send the patient to a GP or an equivalent clinic to do the blood test. The site must have a copy of the blood results sent to them and filed in the medical records. The site can conduct this visit over the telephone, and if needed, make any dose modifications based on the review of the blood results.

At Month 1 (+/- 2 weeks) post-randomisation, patients should then attend for a follow-up visit to check for adverse events (AEs) or any hospital readmissions. This visit can also be conducted over the phone.

At Month 3 post-randomisation (+/- 2 weeks) and every 3 months thereafter (+/- 2 weeks), up to 18 months; patients will need to attend the hospital to:

- review concomitant medications
- undergo routine blood testing as per standard care (this includes monitoring potassium levels for hyperkalaemia)
- assess for any AEs
- check if there are any hospital admissions since the previous visit to determine SBP\* and other infection rates
- assess for treatment adherence using the MARS questionnaire (needs to be done prior to receiving the next trial medication supply)
- dispense the next supply of trial medication. Unless the patient is on alternate dosing and has enough IMP to last until their next visit.
- 

*\* SBP is defined as per standard guidelines (see section 5.5.2). Ascitic fluid cultures should be performed using the conventional culture method and inoculating 10 mL of fluid in aerobic and anaerobic blood culture bottles at the bedside.*

At Month 6 post-randomisation (+/- 2 weeks) and every 6 months thereafter (+/- 2 weeks) **in addition** to the every 3-monthly assessments noted above, EQ-5D-5L should also be completed by the patient.

Hospital admissions, permanently stopping IMP and death will also need to be documented in the CRFs.

Sites will be provided with an appointment/safety card and can be given to patients at each visit from randomisation till the penultimate month 15 visit.

Where a patient misses a visit, but the data required is collected from their medical notes, this will not be classed as a protocol deviation. However, a note to file will be needed to record the missed visit. Missing data related to essential blood results or questionnaires will be classed as a protocol deviation.

For patients who permanently discontinue at any time their trial medication during Months 3-18, sites have the option to conduct these follow-ups over the telephone. Other data (e.g., bloods) can be obtained from the patient's medical records from their routine hospital visits that fall within the trial follow-up windows. Please note for patients where their ascites is resolved but later in the trial returns, and the patient would like to restart the trial medication, the patient will need to complete their follow-up visits face-to-face. For patients off IMP with no blood test results available, a missed visit will not be classed as protocol deviation. However, every effort should be made to include blood results if they have been taken as standard of care. If data from medical notes cannot be obtained from patients who are off IMP but are still being followed up, this will not constitute a deviation.

At Month 19 post-randomisation, there will be an end of study safety phone call to determine if the patient has experienced any AEs since randomisation. This can be conducted over the phone.

Patients will be followed up long term by telephone every 6 months (+/- 2 weeks) after the final visit (month 18) until the end of the trial.

Please see Table 1 for a list of Trial Schedule of Interventions and Assessments.

**Table 1: Trial schedule of interventions and assessments**

| Visit number   | VISIT 1                      |               | VISIT 2                             | VISIT 3                | VISIT 4               | VISIT 5                | VISIT 6                | VISIT 7                 | VISIT 8                 | VISIT 9                 | Safety call             | Long term follow-up  |
|--|------------------------------|---------------|-------------------------------------|------------------------|-----------------------|------------------------|------------------------|-------------------------|-------------------------|-------------------------|-------------------------|--|
| Month  | (<12 wks from randomisation) | Month 0       | Day 10 <sup>e</sup><br>(+/- 5 days) | Month 1<br>(+/- 2 wks) | Month 3<br>(+/- 2wks) | Month 6<br>(+/- 2 wks) | Month 9<br>(+/- 2 wks) | Month 12<br>(+/- 2 wks) | Month 15<br>(+/- 2 wks) | Month 18<br>(+/- 2 wks) | Month 19<br>(+/- 2 wks) | Every 6 months (+/- 2 wks) from Month 24 till end of trial |
|  | Screening                    | Randomisation | Follow-up                           |                        |                       |                        |                        |                         |                         |                         |                         |  |
| Eligibility screening                                    | X                            |               |                                     |                        |                       |                        |                        |                         |                         |                         |                         |  |
| Informed consent   | X                            |               |                                     |                        |                       |                        |                        |                         |                         |                         |                         |  |
| Medical history  | X                            |               |                                     |                        |                       |                        |                        |                         |                         |                         |                         |  |
| Pregnancy test<br>(if applicable)                        | X <sup>a</sup>               |               |                                     |                        |                       |                        |                        |                         |                         |                         |                         |  |
| Ascitic fluid analysis                                   | X <sup>b</sup>               |               |                                     |                        |                       |                        |                        |                         |                         |                         |                         |  |
| Blood tests, liver and renal function tests <sup>c</sup> | X                            |               | X                                   |                        | X                     | X                      | X                      | X                       | X                       | X                       |                         |  |
| Randomisation  |                              | X             |                                     |                        |                       |                        |                        |                         |                         |                         |                         |  |
| Adverse events review                                    |                              |               | X                                   | X                      | X                     | X                      | X                      | X                       | X                       | X                       | X                       | X  |
| Hospital admission review                                |                              |               | X                                   | X                      | X                     | X                      | X                      | X                       | X                       | X                       |                         | X  |
| Concomitant medication review                            | X                            |               | X                                   |                        | X                     | X                      | X                      | X                       | X                       | X                       |                         | X  |
| MARS questionnaire                                       |                              |               |                                     |                        | X                     | X                      | X                      | X                       | X                       | X                       |                         |  |
| EQ-5D-5L questionnaire                                   |                              | X             |                                     |                        |                       | X                      |                        | X                       |                         | X                       |                         |  |
| Dispense trial medication <sup>d</sup>                   |                              | X             |                                     |                        | X                     | X                      | X                      | X                       | X                       |                         |                         |  |

<sup>a</sup>: Pregnancy test must be completed within 2 weeks prior to randomisation

<sup>b</sup>: Ascitic fluid analysis can be done within 24 weeks prior to randomisation

<sup>c</sup>: Blood tests should include full blood count, with haemoglobin, white cell count, platelets, INR (or prothrombin time converted to INR), sodium, potassium, serum creatinine, and urea. Liver function tests should include albumin, alanine aminotransferase (ALT), alkaline phosphatase, and bilirubin. Renal function tests including eGFR.

<sup>d</sup>: At each indicated visit, one bottle of 100 capsules of trial medication is dispensed to the patient (either co-trimoxazole or placebo capsules). The patient should start taking their IMP within 3 days from the date of randomisation. Unless the patient is on alternate dosing and has enough IMP to last until their next visit.

<sup>e</sup>: Visit 2 should be completed at Day 10 from when the IMP started but can be completed between 5-15 days. The aim of this visit is to monitor the safety of the patient and to make any dose modifications to con-meds if needed. Under exceptional circumstances, if the patient is unable to attend a face-to-face visit, the site can send the patient to have their blood test at the GP or an equivalent clinic, provided that a copy of the blood results is sent back to the site (and is filed in the patient's medical records). The visit can be conducted over the telephone and make any dose modifications if required based on the blood results review.

### 5.6.3. Early Stopping of Trial Medication and Withdrawal

If a patient chooses to discontinue their trial treatment, they should continue to be followed-up as closely as possible to the follow-up schedule defined in the protocol, providing they are willing. They should be encouraged not to leave the whole trial, even though they no longer take the trial treatment.

If, however, the patient exercises the view that they no longer wish to be followed-up, the patient will be asked for consent for the research team to continue to collect data from their medical records. If the patient also declines for the research team to collect data from their medical records, then this view must be respected, and the patient should be withdrawn from the trial. CCTU should be informed of the withdrawal in writing using the appropriate ASEPTIC CRF. Data already collected will be kept and included in analyses according to the intention-to-treat principle for all patients who stop follow up early.

Patients who stop trial follow-up early will not be replaced.

### 5.6.4. Patient Transfers

If a patient moves from the area making continued follow-up at their consenting centre inappropriate, every effort should be made for them to be followed at another participating trial centre. Written consent should be taken at the new centre and then a copy of the patient's CRFs should be provided to the new centre. Responsibility for the patient remains with the original consenting centre until the new consent process is complete. The original consenting centre is responsible for responding to all data queries up until the point the patient transfers.

### 5.6.5. Loss to Follow-up

Patients will be followed-up for a minimum of 18 months following randomisation. Follow-up will be by contact at outpatient appointments. Every effort will be made to obtain follow up data on all patients. If a patient does not attend a clinic visit the site should attempt to contact the patient by telephone on at least 3 occasions. If it is not possible to make contact with the patient by phone, their General Practitioner (GP) should be contacted. If the patient cannot be contacted, this will be considered a missed visit in the first instance. The site should try and contact the patient again when the next visit is due.

A CRF will document hospital admissions since the previous visit, which can be acquired using routinely available hospital data to determine SBP and other infection rates. Obtaining follow-up data may also require tracing patients via their NHS number. We will apply for access to data held by NHS Digital (and its equivalent for other UK nations); the dataset will include Hospital Episode Statistics (HES), the Mental Health Minimum Data Set (MHMDs) and mortality data. The patient information sheet will include information that data will be collected using their NHS number and patients will be asked to give their consent freely. We will assess the feasibility of using HES to collect data on hospital admissions and other infection-related hospital use to assist with the economic evaluation.

Patients may only be deemed as "lost to follow up" if all avenues outlined here in section 5.6.5 have been exhausted and no data can be obtained from the patient's medical notes. Sites should speak to CCTU for advice on how to manage patients that cannot be contacted.

### 5.6.6. Trial Closure

The end of the trial for individual patients will be the date of their last visit. Trial closure is defined as the date when all data has been received, cleaned and all queries resolved at all sites. The REC and MHRA will be notified within 90 days of the trial closing.

## 5.7. Sample Size

For overall survival as the primary outcome, we anticipate a hazard ratio (HR) of 0.62 and calculated that 432 patients will be required to generate 187 events, incorporating a 10% cumulative probability of loss-to-follow-up by the end of the study. Guided by the Kaplan-Meier 18-month survival estimate amongst patients with cirrhosis and ascites in the control arm of the ANSWER trial<sup>7</sup>, and assuming an exponential survival distribution, we anticipate 66% survival in the control arm at 18 months. In the experimental arm, we expect 77% of patients surviving at 18 months. The recruitment will be uniform over a period of 2.5 years, and the follow up time will be 18 months minimum with a maximum potential follow-up of 48 months. This calculation was based on a two-sided 5% type-1 error rate and 90% power.

The primary event rate overall (blinded to allocation) will be monitored by the trial steering committee and follow up may be extended beyond the planned period to achieve the required number of events, with any extension agreed with the funder. Disruption to the trial schedule owing to the COVID-19 pandemic may necessitate an extension of the recruitment period.

## 5.8. Recruitment and Retention

### 5.8.1. Recruitment

Patients will be recruited from at least 30 secondary or tertiary care NHS hospitals that frequently manage patients with advanced liver disease. Patients with cirrhosis and ascites that may have been hospitalised will be identified and approached to participate, these patients will be randomised at discharge or once medically stable. Other patients will be screened when attending for ascitic drainage (paracentesis) at day care units, during outpatient visits and from liver transplant waiting list clinics.

The recruitment period will be 30 months, with a 11-month GO / NO GO internal progression review to demonstrate deliverability of recruitment. We will have 15 open centres who will recruit >80 patients during the 11-month phase (from protocol version 4.0 onwards). The 11-month pilot phase will commence when the first patient is randomised (post-Covid-19).

This would represent a GO/NO GO milestone for the trial based on recruitment numbers:

- (a) If our randomisation rate at 11 months is less than half that anticipated i.e. only 40 patients randomised, we will consider terminating the trial.
- (b) If our randomisation rate is between 50-75% of that predicted i.e. 40-60 patients randomised, we will review and improve our recruitment procedures and consider setting up more previously identified and assessed centres.



(c) If our randomisation rate is more than 75% of that predicted i.e. >60 patients randomised, then we will proceed with the trial as planned. *If our randomisation rate is 100% of that predicted (i.e. 80 patients randomised), then we will proceed with the trial as planned.*

### **Length of internal Pilot phase: 11 months**

|  | Red | Amber | Green |
|--|-----|-------|-------|
| Trial Recruitment  | 50% | 75%   | 100%  |
| Recruitment rate/site/month  | <1  | 1     | >1    |
| Number of new sites to be opened*  | 8   | 11    | 15    |
| Total number of patients recruited during this GO/NO GO internal progression phase | 40  | 60    | 80    |

\*15 sites have already been opened prior to version 4.0 protocol

### **5.8.2. Retention**

The importance of attending scheduled follow up appointments until trial completion will be explained to all patients at the start of the trial to ensure that only those able to commit to the trial protocol are recruited. Patients will be educated about the possible dangers of non-compliance. ASEPTIC also has a strong patient and public involvement (PPI) strategy to maximise patient benefit.

## **5.9. Assignment of Intervention**

### **5.9.1. Allocation**

#### **5.9.1.1. Sequence generation**

Patients will be randomised 1:1 to receive either co-trimoxazole or placebo. An independent online randomisation service ([www.sealedenvelope.com](http://www.sealedenvelope.com)) will be used to minimise allocation bias within the trial.

Randomisation will use a minimisation algorithm incorporating a random element, stratifying by active participation on liver transplant waiting list, rifaximin prescription at enrolment and centre. Following discussions with the Independent Data and Monitoring Committee (IDMC) and Trial Steering Committee (TSC) in early 2022, two new stratification variables will be added to the randomisation process. These are if the alcohol was considered a significant cause of cirrhosis, and if the patient is actively consuming alcohol at randomisation. To ensure maximum balance is achieved across the stratification factors, minimisation will be carried out on these factors separately.

#### **5.9.1.2. Allocation concealment mechanism**

A single labelled bottle of trial medication will be dispensed following randomisation, and at each subsequent 3 monthly follow-up visits, unless the patient is on alternate dosing and has enough IMP to last until their next visit. Due to the Covid-19 pandemic or in exceptional circumstances, there may be situations where a patient may run out of IMP prior to the next follow-up visit (as a result of appointment scheduling and available capacity at site). If this is the case, as per MHRA and HRA guidelines, IMPs can be couriered to the patient's home address, as long as the patient has given verbal consent.

The unique kit code allocated to a patient at each clinic visit will be revealed to the investigator or research nurse through Sealed Envelope (a password protected, secure web-based system) on entry of the patient's identification number and date of birth.

The investigator or prescribing research nurse will provide details of the allocated kit code assigned to each patient to enable dispensing of trial medication by the pharmacy department. Trial medication will only be dispensed upon receipt of the prescription form and copy of the confirmation from Sealed Envelope showing the allocated kit code.

A full accountability trail will be maintained from receipt of trial medication in the pharmacy, to the point of dispensing and destruction of undispensed trial medication. The site pharmacist will remain blinded to trial arm and trial medication (co-trimoxazole/placebo) allocation.

Please see Section 5.9.2 'Blinding' for more information on how to manage the trial if the study encounters IMP supply issues.

#### **5.9.1.3. Allocation Implementation**

The responsibility for enrolling patients and prescribing trial treatment lies with the PI. Eligibility decisions will be made in line with the approved protocol. Other physicians employed at the same clinical site may enrol and prescribe trial treatments to patients only if they have received appropriate training on the trial and appear on the ASEPTIC Trial Delegation Log, approved by the PI. Nurses or pharmacists delegated to the ASEPTIC study at site who are registered independent prescribers will be deemed suitable to prescribe ASEPTIC IMP also, where also signed off by the PI on the delegation log to do so. Registered Independent Prescribers will include nurses and nurses who are registered and accredited. Randomisation will be carried out at each recruiting centre by the research nurse or delegated individual using the online randomisation service provided by Sealed Envelope.

#### **5.9.2. Blinding**

The trial patients and clinicians will be blinded to treatment allocation. The co-trimoxazole and placebo capsules will appear identical.

A detailed statistical analysis plan will be approved by the Trial Steering Committee before any analysis of unblinded data, including health economics, quality of life and serious adverse events.

#### **Co-trimoxazole supply issue**

There may be a possibility during the trial that there is a supply issue with co-trimoxazole. If this arises, to ensure we reduce the impact this will have on the trials' scientific value and avoid interruptions in patient treatment, the trial team will implement the following contingency plans in the order below:

- A. Identify an alternative brand that will fit within the capsule (for the over-encapsulation). This will maintain the double blinding and the scientific integrity of the trial. If this is not possible, then:
- B. Alter the study design to be single-blinded. Due to the supply issue, we will need to change manufacturers and so the appearance of the IMP **may** look different.

Furthermore, we will only implement Plan B for as long as needed. Plan B will only be implemented until the trial team manages to secure stock supply of co-trimoxazole and therefore will revert back to the original plan or Plan A. This will be dependent on the manufacturer's timeline for co-trimoxazole production and availability for use.

If the trial medication stock that are stored at the Supplier is running low, and the new Drug Campaign is not ready for use, the trial team will move stock that is stored at a participating site that has sufficient supplies to a site with little or no stock. This contingency plan will only be implemented under exceptional circumstances. The process for this IMP transfer will be detailed in the ASEPTIC IMP Transfer Management Plan.

### 5.9.3. Emergency Unblinding

There will be no unblinding of study medication unless considered important for the patient's care as assessed by the attending clinicians. In the event emergency unblinding becomes necessary, this can occur at any time through the 24-hour online randomisation system Sealed Envelope. Each patient will have an appointment/safety card with contact details to be used in an emergency.

## 5.10. Data Collection, Management and Analysis

### 5.10.1. Data Collection Methods

Each patient will be given a unique trial Patient Identification Number (PIN). Data will be collected at the time-points indicated in the Trial Schedule (Table 1).

Data will be collected from the trial sites using paper Case Record Forms (CRFs) or entered into the database by a delegated site staff member for remote data entry. Data will be stored on secure servers based at UCL. Training on CRF completion for site staff listed on the delegation of responsibilities log will be provided.

Data collection, data entry and queries raised by a member of the ASEPTIC trial team will be conducted in line with the CCTU and trial specific Data Management Standard Operating Procedure and CRF completion guidelines.

Identification logs, screening logs and enrolment logs will be kept at the trial site in a locked cabinet within a secured room.

Clinical trial team members will receive trial protocol training. All data will be handled in accordance with the Data Protection Act 2018 (and subsequent updates and amendments).

### 5.10.2. Data Management

Data will be entered in the approved ASEPTIC database, which is protected in accordance to established CCTU procedures.

Patients will be given a unique trial Patient Identification Number (PIN). Data will be entered under this identification number onto the central database (InferMed's MACRO) stored on the servers based at UCL. The database will be password-protected and only accessible to members of the ASEPTIC trial team at CCTU, delegated site staff members, and external regulators if requested. The servers are protected by firewalls and are patched and maintained according to best practice. The physical location of the servers is protected by CCTV and security door access. The database and coding frames have been developed by the Clinical Trial Manager in conjunction with CCTU. The database software provides a number of features to help maintain data quality, including maintaining an audit trail, allowing custom validations on all data, allowing users to raise data query requests, and search facilities to identify validation failure/ missing data.

The database and coding frames have been developed by the Clinical Trial Manager in conjunction with CCTU. The database will only be available to specified users who will require a username and password for access. MACRO supports a role-based security model, granting different users different database privileges. MACRO implements data validations to assist data quality, including range checks on individual items and consistency checks between multiple items. This will be compliant with all necessary regulatory requirements including audit trail to allow for date/time stamped corrections accompanied by justification/explanation for any data amendments.

After completion of the trial, the database will be retained on the servers of UCL for on-going analysis of secondary outcomes.

The identification, screening and enrolment logs, linking patients' identifiable data to the pseudo-anonymised PIN, will be held locally by the trial site. This will either be held in written form in a locked filing cabinet or electronically in password-protected form on hospital computers. Logs containing identifiable data should not be transferred to the CCTU. After completion of the trial the identification, screening and enrolment logs will be stored securely by the sites for 5 years unless otherwise advised by CCTU.

An application will be made for access to data held by NHS Digital (or equivalent for all other UK nations); the dataset will include Hospital Episode Statistics (HES), the Mental Health Minimum Data Set (MHMDS) and mortality data. The patient information sheet will include information that data will be collected using their NHS number and patients will be asked to give their consent freely. We will assess the feasibility of using HES to collect data on hospital admissions and other infection-related hospital use to assist with the economic evaluation.

### 5.10.3. Non-Adherence and Non-Retention

If the patient chooses to discontinue taking the trial medication, this will need to be documented in our CRF. Reasons for treatment discontinuation, if possible, will be recorded by the trial team. Every effort will be made to continue to follow-up the patient according to the protocol until the end of the trial. Patients will be educated about the possible dangers of non-compliance.

## 5.11. Statistics

### 5.11.1. Statistical Analysis Plan

A detailed statistical analysis plan, including a full specification of the analysis principals and details will be written prior to the first unblinded analysis and approved in advance by the Trial Steering Committee. All statistical tests will use a 2-sided p-value of 0.05, unless otherwise specified, and all confidence intervals presented will be 95% and 2-sided.

### 5.11.2. Statistical Methods

#### Primary outcome

The primary endpoint is overall survival. The Kaplan-Meier survival curves of the two treatment groups will be compared using the log-rank test. An unadjusted Cox proportional hazards model will be fitted, and the unadjusted hazard ratio and 95% confidence interval will be presented. An adjusted Cox model will then be fitted, adjusted for the stratification factors (active participation on liver transplant waiting list, rifaximin prescription at enrolment, liver cirrhosis due to alcohol, current alcohol use at randomisation). Centre may be included as a covariate depending on number of centres and the number of events per centre) by including them as covariates in the model. Should there be problems with fitting the adjusted model, we will exclude covariates as necessary. Evidence of non-proportional hazards would mean that the Cox proportional hazards model is not appropriate, and that the effects estimated from the model could be misleading.

In the event that non-proportional hazards a series of analysis will be performed:

- Stratified log rank test;
- LER LED – the life expectancy difference (LED) and life expectancy ratio (LER) will be presented<sup>72</sup>. LED is the difference between mean survival times in the intervention and control arms. LER is the ratio of these two times.
- Odds of death at 18 months will be calculated via logistic regression model.

All outcomes are assessed for a minimum period of 18 months following randomisation. The maximum possible period of follow up will be 48 months (assuming a recruitment period of 30 months and 18 months treatment period for final patient recruited).

#### Secondary outcomes:

Other binary secondary outcomes will be analysed using logistic regression, continuous outcomes using linear regression, and time to event outcomes using Kaplan-Meier methods. All secondary outcomes will be unadjusted.

The proportion of patients experiencing any serious adverse event rates in the two randomised groups will be compared using Fisher's exact test.

Quality of life during the trial in the two treatment arms will be compared by fitting a hierarchical linear regression model containing treatment time, baseline quality of life and treatment as fixed effects, with random patient effects to account for multiple values from each patient.

Regular reports concerning patient safety, death and SBP events will be prepared for the Independent Data Monitoring Committee (IDMC), who will monitor the event rate with regard to the sample size assumptions to ensure the trial is adequately powered, maintaining trial team blinding. The IDMC may request a formal interim analysis if a report raises concerns.

#### **5.11.2.1. Additional Analyses - Subgroup**

Results on the primary efficacy outcome will be presented by stratum, i.e. according to the levels of the stratifying variables used in the randomisation process. Interactions between each of these variables and treatment will be added in turn to the primary analysis model to investigate whether the treatment effect differs according to the levels of these factors.

The factors for subgroup analysis will be the following:

- Rifaximin prescription at randomisation
- Active participation on liver transplant list at randomisation
- Liver cirrhosis due to alcohol
- Current alcohol use at randomisation
- Gender
- Ascitic fluid protein count (cut-off 2g/dL)
- Long-term ascites (ascites persistent for more than 3 months at enrolment)

#### **5.11.2.2. Analysis Population and Missing Data**

The main analysis will be conducted following the intention-to-treat principle in accordance with the randomised intervention. All efforts will be made to minimise the amount of missing data, particularly for the primary outcome. Should there be substantial amounts of missing data, we will consider further analyses to examine the effect that missing data may have on our findings.

#### **5.11.3. Health Economics**

The aim of the economic evaluation is to calculate the mean incremental cost per quality adjusted life year (QALY) gained of using co-trimoxazole to prevent spontaneous bacterial peritonitis and improve overall survival in cirrhosis patients. The analysis will be done from health and social care perspective using 18-month trial data.

QALYs will be calculated based on EQ-5D-5L completed at baseline and every 6 months during the 18-month follow-up. If a patient does not attend a site for a planned visit, the EQ-5D-5L questionnaire will be posted to the patient or completed by telephone. Mortality data will also be used to calculate QALYs.

We will apply to the NHS Digital (or equivalent for other UK nations) for access to Hospital Episode Statistics (HES) database. It will allow us to collect high quality data on patients' resource use. This will include data on hospital admissions including length of in-hospital stay and type of ward and number of day-case visits. We will also collect information regarding care package assigned at discharge from patient records. Unit costs will be obtained from publicly available data sources where possible. To calculate the cost of co-trimoxazole we will collect data on prescriptions and if they were filled. The unit cost will be obtained from the British National Formulary (BNF).

The HES dataset will also contain the data on mortality, hospital admissions and other resource use related to managing cirrhosis and infections to assist with the key primary and secondary outcomes.

#### **5.11.3.1. Health Economic Analysis Plan (HEAP)**

A full HEAP will be developed for the within-trial analysis and will be subject to approval by the Trial Steering Committee. The primary analysis will be a within-trial intention-to-treat analysis. There will be no economic modelling conducted as a part of trial. 18-month follow-up is considered long enough to capture all important drivers of cost-effectiveness of co-trimoxazole used in patients with advanced liver disease.

Health-related quality of life (HRQoL) will be measured using the EQ-5D-5L, which will be collected at baseline, and every 6 months for each individual patient. Utility scores will be calculated using UK-specific tariffs. QALYs will be calculated as the area under the curve adjusting for baseline differences and other variables as specified in the HEAP.

Resource use will be valued from the perspective of the health and social services. The cost saving is expected to result from preventing hospital re-admissions. Therefore, data on hospital admissions, length of stay and type of ward is of particular importance. Other cost components included in the analysis will consist of (but not necessarily limited to) the cost of co-trimoxazole, day-case visits, any tests undertaken, outpatient attendances, primary care contacts, A&E attendances, and prescribed medications. Resource use unit costs will be taken from publicly available data sources where possible. Drugs will be costed using the BNF.

We will report descriptive statistics, including percentages, means and standard deviations for all health and social care resource use at 18 months for co-trimoxazole versus placebo. 95% confidence intervals for difference in costs between the two groups will be based on the bootstrapped results adjusting for variables specified in the analysis plan. Bootstrapped results will be also used to report the incremental cost per QALY gained.

Cost-effectiveness acceptability curves will be constructed using the bootstrapped adjusted results to report the probability that co-trimoxazole is cost-effective compared to placebo for prevention of spontaneous bacterial peritonitis in patients with advanced liver disease for a range of values of willingness to pay for a QALY gain.

### **5.12. Data Monitoring**

#### **5.12.1. Data Monitoring Committee**

An Independent Data Monitoring Committee (IDMC) will meet at least annually. This will consist of a clinician with expertise in liver disease, a clinical trialist and a statistician. No member of the IDMC will be an investigator linked to the trial. The IDMC will receive safety and efficacy reports and advise the Trial Steering Committee (TSC) on whether the trial should continue unchanged.

Further details of the roles and responsibilities of the IDMC, including membership, relationships with other committees, decision making processes, and the timing and frequency of interim analyses (and description of stopping rules and/or guidelines where applicable) are described in detail in the ASEPTIC IDMC Terms of Reference (ToR).



### 5.12.2. Interim Analyses

No formal interim analysis is planned within the study, but periodic reports concerning patient safety and key efficacy outcomes will be prepared for the IDMC as agreed in the ToR. The IDMC may request an interim analysis if a report raises concerns.

### 5.12.3. Data Monitoring for Harm

All adverse events (AEs) or serious adverse events (SAEs) occurring during the trial observed by the investigator or reported by the patient will be recorded in the patient's medical records as per standard practise and if applicable, on the appropriate ASEPTIC CRFs.

### 5.12.4. Safety reporting

Definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial.

**Table 2: Adverse Event Definitions**

|  |  |
|--|--|
| <b>Adverse Event (AE)</b>  | Any untoward medical occurrence in a patient or clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this product.   |
| <b>Adverse Reaction (AR)</b>   | Any untoward and unintended response to an investigational medicinal product related to any dose administered  |
| <b>Unexpected Adverse Reaction (UAR)</b>   | An adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unauthorised product or summary of product characteristics (SPC) for an authorised product.  |
| <b>Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)</b>   | Any AE or AR that at any dose: <ul style="list-style-type: none"> <li>• results in death</li> <li>• is life threatening*</li> <li>• requires hospitalisation or prolongs existing hospitalisation**</li> <li>• results in persistent or significant disability or incapacity</li> <li>• is a congenital anomaly or birth defect</li> <li>• or is another important medical condition***</li> </ul> |
| <p>* The term life threatening here refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that might hypothetically cause death if it was more severe (e.g. a silent myocardial infarction)</p> <p>** Hospitalisation is defined as an in-patient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisation for pre-existing conditions (including elective procedures that have not worsened) do not constitute an SAE</p> <p>*** Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. Important AEs or ARs that may not be immediately life threatening or result in death or hospitalisation, but may seriously jeopardise the patient by requiring intervention to prevent one of the other outcomes listed in the table (e.g. a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not require hospitalisation, or development of drug dependency).</p> |  |



Adverse events include:

- An exacerbation (i.e. increase in the frequency or intensity) of a pre-existing illness episodic event or symptom (initially recorded at the screening/baseline visit), that is detected after trial drug administration/intervention an increase in the frequency or intensity of a pre-existing episodic event or condition
- a condition (regardless of whether PRESENT prior to the start of the trial) that is DETECTED after trial drug administration. (This does not include pre-existing conditions recorded as such at baseline – as they are not detected after trial drug administration.)
- continuous persistent disease or a symptom present at baseline that worsens following administration of the trial treatment
- Occurrence of a new illness, episodic event or symptom, that is detected after trial drug administration/intervention

Adverse events do NOT include:

- Medical or surgical procedures: the condition that leads to the procedure is the adverse event
- Pre-existing disease or a condition present before treatment that does not worsen
- Hospitalisation where no untoward or unintended response has occurred e.g. elective cosmetic surgery
- Overdose of medication without signs or symptoms

#### **5.12.4.1. Other Notifiable Adverse Events**

##### **Pregnancy**

In pregnancy, co-trimoxazole has a risk of teratogenicity in the first trimester and neonatal haemolysis and methaemoglobinaemia in the third trimester (<https://bnf.nice.org.uk/drug/co-trimoxazole.html>). Although pregnancy during the trial is highly unlikely (incidence of 3 in >2000 patients<sup>10</sup>), patients will be told of the importance of avoiding pregnancy during trial treatment. If a patient becomes pregnant whilst on treatment they will be withdrawn from further treatment as part of the trial. If a patient does become pregnant while on treatment, this needs to be reported as a Notifiable Adverse Event (NAE) in the same way as an SAE, and immediately on awareness at site. At the end of the trial, patients will continue to have specialised follow-up at an appropriate clinic. Should it become apparent that a patient has conceived whilst on trial treatment then this may be brought to the attention of the general practitioner and local obstetric services.

##### **Follow-up of pregnancy**

Pregnancies will be followed up until one month after birth for both the mother and newborn child/children, and the pregnancy outcome reported on a pregnancy outcome form. Patients should be asked to contact the site trial team if they become pregnant at any time whilst on the treatment or for 30 days after the end of trial treatment. Pregnancy follow-up will be closely monitored using the trial database. The trial team at UCL CCTU will regularly check the database for pregnancy outcome forms that have not been received within 10 months of notification of pregnancy. In the event of an outstanding pregnancy outcome form a request for the form will be sent, as a matter of urgency, to the site. If there is no response to the query within the timelines given, UCL CCTU may perform a triggered on-site monitoring visit. Patients should be aware that they should monitor for safety events and pregnancies for 30 days after the end of their participation in the trial. Any pregnancies or safety

events should be reported to the site trial team immediately. The site trial team may contact the patient to check for any safety events or pregnancies.

In the event of miscarriage, birth defect or congenital abnormality this should be reported as a separate SAE and causality and expectedness assessed by the PI or medical delegate. This can also be reported as a resolution to the original pregnancy report, but it must also be reported separately as a new event.

In the event of elective abortion, this does not need to be reported as an SAE. The site staff should document all details of a patient's pregnancy, reporting and follow-up and details of any forms sent to UCL CCTU including the pregnancy outcome form, in the source notes.

#### **5.12.4.2. Investigator responsibilities relating to safety reporting**

All non-serious adverse events, whether expected or not, should be recorded in the patient's medical notes. It is not necessary to report non-serious adverse events and no CRF needs to be completed.

##### **Adverse Events:**

All AEs should be recorded in the patient's medical notes as per usual clinical practice.

##### **Serious Adverse Events:**

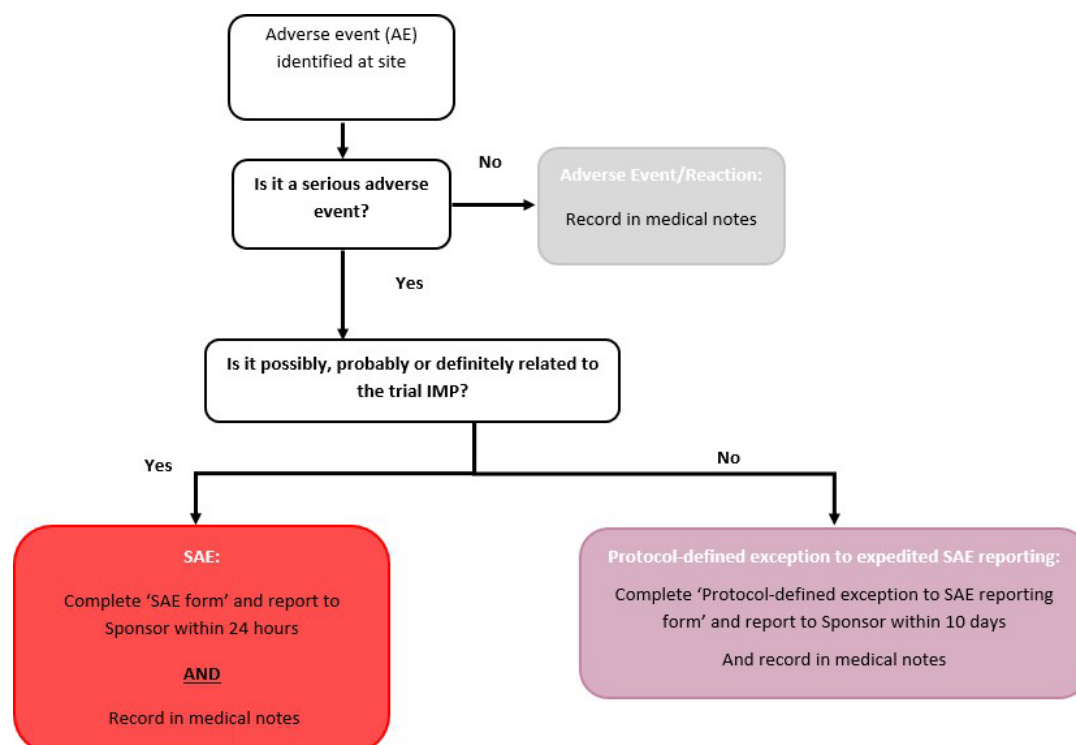
SAEs should be notified to CCTU immediately and no longer than within 24 hours of the investigator becoming aware of the event, with the exception of the events listed below as protocol defined exceptions to SAE expedited reporting.

##### **Protocol-defined exceptions to SAE expedited reporting:**

If an event occurs, and meets one or more of the criteria for a '**serious** adverse event' AND is not considered (possibly, probably or definitely) related to the trial medication, **expedited reporting as an SAE is NOT needed**. Instead, report the event to CCTU within 10 days using the dedicated 'protocol-defined exception to SAE reporting form'.

As 'Death' is the primary outcome for the trial and this patient group has a high mortality rate due to the disease progression, sites do not require to expedite reporting for this. The only exception is if the PI/delegate considers the death as possibly, probably or definitely related to the trial medication, and therefore expedited reporting is required.

Any other safety adverse events meeting the criteria of a '**serious** adverse event', should be reported to CCTU immediately (within 24 hours) using an SAE form if the investigator believes an event is (possibly, probably, or definitely) **related** to the trial drug.

**Figure 1: Safety reporting**

#### 5.12.4.2.1. Seriousness assessment

When an AE or AR occurs, the investigator responsible for the care of the patient must first assess whether or not the event is serious using the definition given in Table 2. If the event is classified as 'serious' then an SAE form must be completed and CCTU (or delegated body) notified immediately (no longer than 24 hours) (except those specified in 'protocol-defined exemption to SAE expedited reporting' (see Section 5.12.4.2) as not requiring expedited reporting to the Sponsor).

#### 5.12.4.2.2. Severity or grading of Adverse Events

The severity of all ARs and SAEs in this trial should be graded using the toxicity gradings in the Common Terminology Criteria for Adverse Events (CTCAE version 5.0):

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 activities of daily living (ADL).\*

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL.\*\*

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death-related to AE.

\*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

\*\*Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

#### 5.12.4.2.3. Causality

The investigator must assess the causality of all serious adverse events or reactions in relation to the trial therapy using the definitions in Table 3.

**Table 3: Causality definitions**

| Relationship           | Description   | Event type    |
|------------------------|---|---------------|
| Unrelated              | There is no evidence of any causal relationship   | Unrelated SAE |
| Unlikely to be related | There is little evidence to suggest that there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition or other concomitant treatment)      | Unrelated SAE |
| Possibly related       | There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition or other concomitant treatment) | SAR           |
| Probably related       | There is evidence to suggest a causal relationship and the influence of other factors is unlikely   | SAR           |
| Definitely related     | There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.  | SAR           |

If an SAE is considered to be related to trial treatment, and treatment is discontinued, interrupted or the dose modified, refer to the relevant Interventions sections of the protocol (see section 5.4.4).

#### 5.12.4.2.4. Expectedness

If there is at least a possible involvement of the trial medications (including any comparators), the sponsor will assess the expectedness of the event. If information on expectedness is provided by the investigator this should also be taken into consideration by the sponsor. An unexpected adverse reaction is one that is not reported in the current approved version of the IB or SPCs for the trial, or one that is more frequently reported or more severe than previously reported. See Section 4.8 of the relevant SPC for a list of expected side effects associated with the drugs being used in this trial. If a SAR is assessed as being unexpected it becomes a SUSAR (suspected, unexpected, serious adverse reaction) and MHRA and REC reporting guidelines apply (see Notifications sections of the protocol).

#### 5.12.4.3. Notifications

##### 5.12.4.3.1. Notifications by the Investigator to CCTU

CCTU must be notified of all SAEs immediately (i.e. within 24 hours of the investigator becoming aware of the event). If the SAE is exempt from expedited reporting the CCTU should be notified within 10 working days.

Investigators should notify CCTU of any SAEs and other Notifiable Adverse Events (NAEs) occurring from the time of randomisation until 30 days after the last protocol treatment administration, including SARs and SUSARs.

Any subsequent events that may be attributed to treatment should be reported to the MHRA using the yellow card system (<https://yellowcard.mhra.gov.uk/the-yellow-card-scheme/>).

The SAE form must be completed by the investigator (or the consultant named on the delegation of responsibilities log who is responsible for the patient's care) who will provide the grading and causality for the event. In the absence of the responsible investigator, the SAE form should be completed and signed by a member of the site trial team and emailed as appropriate within the timeline. The responsible investigator should check the SAE form at the earliest opportunity, make any changes necessary, sign and then email to CCTU. Detailed written reports should be completed as appropriate. Systems will be in place at the site to enable the investigator to check the form for clinical accuracy as soon as possible.

The minimum criteria required for reporting an SAE are the patient identification number and partial date of birth, name of reporting investigator and sufficient information on the event to confirm seriousness. Any further information regarding the event that is unavailable at the time of the first report should be sent as soon as it becomes available.

The SAE form must be scanned and sent by email to the trial team at CCTU in an **encrypted** format to:

**[ctu.aseptic@ucl.ac.uk](mailto:ctu.aseptic@ucl.ac.uk)**

Patients must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline values, or until the event has stabilised. Follow-up should continue after completion of protocol treatment and/or trial follow-up if necessary. Every effort will be made to resolve SAEs by End of Study. Follow-up SAE forms (clearly marked as follow-up) should be completed and emailed to CCTU as further information becomes available. Additional information and/or copies of test results etc. may be provided separately. The patient must be identified by trial number, date of birth and initials only. The patient's name should not be used on any correspondence and should be blacked out and replaced with trial identifiers on any test results.

#### 5.12.4.3.2. CCTU responsibilities

A medically qualified member of staff will be appointed as the sponsor clinical reviewer (usually the Chief Investigator (CI) or a medically qualified delegate) and will perform a clinical review of all SAE reports received. The sponsor clinical reviewer will complete the assessment of expectedness in light of the Reference Safety Information (RSI).

CCTU is undertaking the duties of trial sponsor and is responsible for the reporting of SUSARs and other SARs to the regulatory authorities (MHRA) and the ECs as appropriate. Fatal and life threatening SUSARs must be reported to the competent authorities within 7 days of CCTU becoming aware of the event; other SUSARs must be reported within 15 days.

CCTU will keep investigators informed of any safety issues that arise during the course of the trial.

The trial manager or delegate at CCTU will submit Development Safety Update Reports (DSURs) to competent authorities.

### 5.12.5. Quality Assurance and Control

#### 5.12.5.1. Risk Assessment

The Quality Assurance (QA) and Quality Control (QC) considerations for the ASEPTIC trial are based on the standard CCTU Quality Management Policy that includes a formal Risk Assessment, and that acknowledges the risks associated with the conduct of the trial and proposals of how to mitigate them through appropriate QA and QC processes. As defined by the MHRA Risk Adapted Approach, the IMP is type B. Indeed, the IMP has a marketing authorisation, nevertheless, the drug is used outside its marketing authorisation for a new therapeutics indication.

Risks are defined in terms of their impact on: the rights and safety of patients; project concept including trial design, reliability of results and institutional risk; project management; and other considerations.

QA is defined as all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP and applicable regulatory requirements. QC is defined as the operational techniques and activities performed within the QA system to verify that the requirements for quality of the trial related activities are fulfilled.

#### 5.12.5.2. Central Monitoring at CCTU

CCTU staff will review Case Report Form (CRF) data for errors and missing key data points. The trial database will also be programmed to generate reports on errors and error rates. Essential trial issues, events and outputs, including defined key data points, will be detailed in the ASEPTIC trial Data Management Plan.

#### 5.12.5.3. On-site (or remote) Monitoring

The frequency, type and intensity of routine and triggered on-site monitoring (subject to Covid-19, remote monitoring may be required if Covid-19 restrictions are still in place) will be detailed in the ASEPTIC Quality Management and Monitoring Plan (QMMP). The QMMP will also detail the procedures for review and sign-off of monitoring reports. In the event of a request for a trial site inspection by any competent authority UCL CCTU must be notified as soon as possible.

##### 5.12.5.3.1. Direct access to participant records

Participating investigators must agree to allow trial related monitoring, including audits, REC review and regulatory inspections, by providing access to source data and other trial related documentation as required. Patient consent for this must be obtained as part of the informed consent process for the trial.

#### 5.12.5.4. Trial Oversight

Trial oversight is intended to preserve the integrity of the trial by independently verifying a variety of processes and prompting corrective action where necessary. The processes reviewed relate to patient enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect patients, including reporting of harms; completeness, accuracy and timeliness of data collection; and will verify adherence to applicable policies detailed in the Compliance section of the protocol. Independent trial oversight complies with the CCTU trial oversight policy.

In multi-centre trials this oversight is considered and described both overall and for each recruiting centre by exploring the trial dataset or performing site visits as described in the ASEPTIC QMMP.

#### 5.12.5.4.1. Trial Team

The Trial Team (TT) will be set up to assist with developing the design, co-ordination and day to day operational issues in the management of the trial, including budget management. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TT terms of reference.

#### 5.12.5.4.2. Trial Management Group

A Trial Management Group (TMG) will be set up to assist with developing the design, co-ordination and strategic management of the trial. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TMG terms of reference.

#### 5.12.5.4.3. Independent Trial Steering Committee

The Independent Trial Steering Committee (TSC) is the independent group responsible for oversight of the trial in order to safeguard the interests of trial patients. The TSC provides advice to the CI, CCTU, the funder and sponsor on all aspects of the trial through its independent Chair. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TSC terms of reference.

#### 5.12.5.4.4. Independent Data Monitoring Committee

The Independent Data Monitoring Committee (IDMC) is the only oversight body that has access to unblinded accumulating comparative data. The IDMC is responsible for safeguarding the interests of trial patients, monitoring the accumulating data and making recommendations to the TSC on whether the trial should continue as planned. The membership, frequency of meetings, activity (including review of trial conduct and data) and authority will be covered in the IDMC terms of reference. The IDMC will consider data in accordance with the statistical analysis plan and will advise the TSC through its Chair.

#### 5.12.5.4.5. Trial Sponsor

The role of the sponsor is to take on responsibility for securing the arrangements to initiate, manage and finance the trial. UCL is the trial sponsor and has delegated the duties as sponsor to CCTU via a signed letter of delegation.

## 6. Ethics and Dissemination

### 6.1. Ethics Committee Approval

Before initiation of the trial at any clinical site, the protocol, all informed consent forms and any material to be given to the prospective patient will be submitted to the relevant EC for approval. Any subsequent amendments to these documents will be submitted for further approval. Before initiation of the trial at each additional clinical site, the same/amended documents will be submitted for local permissions.

The rights of the patient to refuse to participate in the trial without giving a reason must be respected. After the patient has entered the trial, the clinician remains free to give alternative treatment to that specified in the protocol, at any stage, if s/he feels it to be in the best interest of the patient. The



reasons for doing so must be recorded. After randomisation the patient must remain within the trial for the purpose of follow up and data analysis according to the treatment option to which they have been allocated. However, the patient remains free to change their mind at any time about the protocol treatment and follow-up without giving a reason and without prejudicing their further treatment.

### **6.2. Competent Authority Approvals**

This protocol will be submitted to the national Competent Authority (MHRA).

This is a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC. Therefore, a CTA is required in the UK.

The progress of the trial, safety issues and reports, including expedited reporting of SUSARs, will be reported to the Competent Authority, regulatory agency or equivalent in accordance with relevant national and local requirements and practices.

### **6.3. Other Approvals**

The protocol will be submitted by those delegated to do so to the relevant R&D department of each participating site or to other local departments for approval as required in each country. A copy of the local permissions (or other relevant approval as above) and of the Patient Information Sheet (PIS) and consent form on local headed paper must be forwarded to the co-ordinating centre before patients are randomised to the trial.

The protocol has received formal review and methodological, statistical, clinical and operational input from the CCTU Protocol Review Committee.

### **6.4. Protocol Amendments**

Approval for substantial amendments to the protocol will be sought by the Trial Team at UCL CCTU from the REC and the appropriate regulatory bodies. Approved protocol amendments will be communicated by the Trial Team at UCL CCTU to all investigators.

### **6.5. Consent**

Patients will be provided with a Patient Information Sheet (PIS) and given time to read it fully.

Following a discussion with a medical qualified investigator or suitable trained and authorised delegate, any questions will be satisfactorily answered and if the patient is willing to participate, written informed consent will be obtained. During the consent process it will be made completely and unambiguously clear that the patient is free to refuse to participate in all or any aspect of the trial, at any time and for any reason, without incurring any penalty or affecting their treatment.

Consent will be re-sought if new information becomes available that affects the patient's consent in any way. This will be documented in a revision to the PIS and the patient will be asked to sign an updated consent form. These will be approved by the ethics committee prior to their use.

A copy of the approved consent form is available from the CCTU trial team.



### **6.5.1. Consent in Ancillary Studies**

No ancillary studies have been finalised. Collection and archiving of samples to allow ancillary studies is included in the main protocol. Ancillary study proposals will be subject to separate review and approval by an Independent Research Ethics Committee.

## **6.6. Confidentiality**

Any paper copies of personal trial data will be kept at the participating site in a secure location with restricted access. Only non-identifiable data will be kept at the UCL CCTU office with only authorised UCL CCTU staff members having access. Only staff working on the trial will have password access to this information.

Confidentiality of patient's personal data is ensured by not collecting patient names on CRFs that will be sent to UCL CCTU and storing the data in a pseudonymised fashion at UCL CCTU. At trial enrolment the patient will be issued a patient identification code, and this will be the primary identifier for the patient, with secondary identifiers of month and year of birth and initials.

The patient's consent form will carry their name and signature, but these will be kept at the trial site (patient's hospital) and not with the patient's data at the UCL CCTU. The patient consent forms will only be accessed by UCL CCTU staff for purposes of monitoring the consent procedure at the site.

## **6.7. Declaration of Interests**

The investigators named on the protocol have no financial or other competing interests that impact on their responsibilities towards the scientific value or potential publishing activities associated with the trial.

## **6.8. Indemnity**

UCL holds insurance against claims from patients for injury caused by their participation in the clinical trial. Patients may be able to claim compensation if they can prove that UCL has been negligent. However, as this clinical trial is being carried out in a hospital, the hospital continues to have a duty of care to the patient in the clinical trial. UCL does not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. This applies whether the hospital is an NHS Trust or not. This does not affect the patient's right to seek compensation via the non-negligence route.

Patients may also be able to claim compensation for injury caused by participation in this clinical trial without the need to prove negligence on the part of UCL or another party. Patients who sustain injury and wish to make a claim for compensation should do so in writing in the first instance to the Chief Investigator, who will pass the claim to UCL's insurers, via the Sponsor's office.

Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to UCL, upon request.

## **6.9. Finance**

ASEPTIC is fully funded by an NIHR HTA grant number 17/67/01. It is not expected that any further external funding will be sought.

### **6.10. Archiving**

The investigators agree to archive and/or arrange for secure storage of ASEPTIC trial materials and records for a minimum of 5 years after the close of the trial unless otherwise advised by the CCTU.

### **6.11. Access to Data**

Requests for access to trial data will be considered, and approved in writing where appropriate, after formal application to the TSC. Considerations for approving access are documented in the TSC Terms of Reference. After the end of the trial Data requests will be considered by the CI and UCL CCTU Senior Management Team.

### **6.12. Ancillary and Post-trial Care**

Ancillary and post-trial care will be standard medical care.

### **6.13. Publication Policy**

#### **6.13.1. Trial Results**

The publication of results will comply with the UCL and UCL CCTU Publication Policies and will include submission to open access journals.

To maximise the chances of early adoption we would need to actively promote the results from the trial. Our team incorporates a number of key opinion leaders and chief figures within UK Hepatology. The results will be actively disseminated through peer-reviewed publications, presentations and posters at policy and academic meetings, media and social media. Many of our project applicants and collaborators are chief figures of such bodies as the British Society of Gastroenterology (BSG) and the British & European Association for the Study of the Liver (BASL & EASL). Indeed, the BSG supports our study. Our consortium includes key members of writing committees for guidelines on management of many aspects of liver disease both nationally and internationally. If successful, these bodies would be crucial in drafting and promoting new guidelines to incorporate co-trimoxazole as primary prophylaxis for SBP. Further publicity and engagement with the public and health care users would be generated by the British Liver Trust and the UCL Public Engagement Unit which would provide expertise, support and training.

The results of the trial will be disseminated regardless of the direction of effect.

#### **6.13.2. Authorship**

The TMG will nominate a writing group, which will consist of members of the TMG and will be responsible for drafting the manuscript for publication. These individuals will be named on the final publication.

## **7. Ancillary Studies**

No ancillary studies have been finalised. Collection and archiving of samples to allow ancillary studies is included in the main protocol. Ancillary study proposals will be subject to separate review and approval.

## 8. Protocol Amendments

| Protocol Version Number | Protocol Date              | Summary of Changes   |
|-------------------------|----------------------------|--|
| V1.0                    | 14 <sup>th</sup> May 2019  | N/A  |
| V2.0                    | 10 <sup>th</sup> July 2019 | <p>Changes made following receipt of MHRA grounds for non-acceptance:</p> <ol style="list-style-type: none"> <li>1) Exclusion criteria (section 1.3 and 5.3.1.3) updated in accordance with contraindication in the SmPC to include: <ul style="list-style-type: none"> <li>• Severe renal impairment with eGFR &lt;15 ml/min</li> <li>• Pathological blood count changes (granulocytopenia, megaloblastic, anaemia)</li> <li>• Skin conditions: exudative erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis and drug eruption with eosinophilia and systemic symptoms</li> <li>• Congenital conditions: congenital glucose-6-Phosphate dehydrogenase deficiency of the erythrocytes, haemoglobin anomalies such as Hb Köln and Hb Zürich</li> <li>• Acute porphyria</li> </ul> </li> <li>2) Screening procedures (section 5.3.1.1.6 and 5.6) updated to specify that pregnancy test must be completed at screening and within 2 weeks prior to randomisation</li> <li>3) Information on dose modifications for renal dysfunction and hyperkalemia added (section 5.4.4.1)</li> <li>4) Definitions of double barrier methods of contraception updated (section 5.6)</li> <li>5) Details of all test required as part of blood tests added (section 5.6)</li> </ol> |
| V3.0                    | 13 <sup>th</sup> July 2019 | <ol style="list-style-type: none"> <li>1) Secondary outcomes clarified</li> <li>2) Patient timeline updated (section 5.6)</li> <li>3) The demography has been removed, the term 'SBP diagnosis review' has been updated to</li> <li>4) 'hospital admission review', and the term 'hospital admission review' have been included at the Day 10 and Month 1 follow-up visits.</li> <li>5) Pharmacovigilance processes updated (section 5.11.4).</li> <li>6) The protocol now includes protocol-defined exceptions to SAE reporting of adverse events associated with advanced liver disease. If an event listed in the protocol occurs and meets one or more of the criteria for a 'serious adverse event', expedited reporting as an SAE is not necessary. Instead, the event should be reported to CCTU within 7 days</li> </ol>   |

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|  |  | <p>using the dedicated ‘protocol-defined exception to SAE reporting form’.</p> <p>Co-trimoxazole is an established marketed drug. It is anticipated that this cohort of immunosuppressed patients will experience a substantial number of adverse events during the course of the study due to their underlying liver condition. Therefore, the trial team has taken the decision to only collect adverse reactions as part of the trial data set, and not to collect information all adverse events unrelated to the trial treatment. All adverse events will be recorded in the patient’s medical notes.</p> <p>7) Patient timeline updated (section 5.6)</p> <p>It has been clarified following MHRA requirement. Precision about the definition of “double barrier” method of contraception and reference of the CFTG has been added.</p> |
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| V4.0 | 25 <sup>th</sup><br>August<br>2020 | <ol style="list-style-type: none"> <li>1. Inclusion criteria amended to remove ascitic fluid protein count threshold (&lt;2g/dL) and wording of 'ascites persisting for <math>\geq 3</math> months despite standard treatment added.</li> <li>2. Inclusion criteria; Polymorphonuclear count &lt;250 cells/mm<sup>3</sup> or white cell count moved to an exclusion criteria as part of the definition of spontaneous bacterial peritonitis (SBP) (Polymorphonuclear count &gt;250 cells/mm<sup>3</sup> and/or white cell count &gt;500 cell/mm<sup>3</sup> and/or positive microbial culture)</li> <li>3. Exclusion criteria - persistent hyperkalaemia related to pre-existing kidney disease that is not possible to reduce changed from &gt;6.5 mmol/L to &gt;5.5 mmol/L threshold (section 5.3.1.3)</li> <li>4. Expanded exclusion criteria: Patients with pathological blood count changes <ol style="list-style-type: none"> <li>i. Patients with haemoglobin (Hb) &lt;70g/L*</li> <li>ii. Granulocytopenia defined as absolute neutrophil counts of less than 500 cells per microliter*</li> <li>iii. Severe thrombocytopenia with a platelet count &lt;30 x10<sup>9</sup> /L*</li> </ol> </li> <li>5. Note added regarding rescreening patients for eligibility if the blood investigations may change.<br/><i>'*It is common for these investigations to change in patients with cirrhosis. Patients can be re-screened for eligibility if this occurs.'</i></li> <li>6. Primary outcome changed from first incidence of SBP to overall survival (section 5.5.1).</li> <li>7. Secondary outcomes added: <ol style="list-style-type: none"> <li>a. First incidence of SBP following randomisation</li> <li>b. Incidence of anti-microbial resistance</li> <li>c. Incidence of Transjugular intrahepatic portosystemic shunt (TIPS) insertion</li> <li>d. Safety and treatment-related serious adverse event</li> </ol> </li> <li>8. Patient treatment period amended from 24 months to 18 months post-randomisation. Follow up changed to minimum 18 months with a maximum possible follow up period of 48 months (assuming a recruitment period of 30 months and 18 months treatment period for final patient recruited).</li> <li>9. Concomitant medication review added at day 10</li> <li>10. Expanded follow-up windows to:<br/>Day 10 (+/- 5 days) – <i>previously +/- 3 days</i><br/>Month 1 onwards (+/- 2 weeks) – <i>previously (+/- 1 week)</i></li> </ol> |
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|  |  | <ol style="list-style-type: none"> <li>11. Update to section 5.4.4.2 and the title “withdrawal” has been changed by “withdrawal from trial treatment”.</li> <li>12. The wording of the figure 1 safety report has been updated (section 5.12.4.2)</li> <li>13. More precision are given to the potential risk of decrease of platelet count (section 5.4.4.1). There is a minor risk of platelet count which can fall below <math>&lt;30 \times 10^9 /L</math>, in that case the risk of bleeding increases and the patient must be withdrawn from trial treatment until the platelet count rises <math>&gt;30 \times 10^9 /L</math></li> <li>14. Renal dysfunction: the timeframe of 3 months is removed as a minimum reasonable timeframe for confirming that the renal function is back to a normal range (section 5.4.4.1)</li> <li>15. ‘Medical Adherence Rating Scale’ changed to ‘Medical Adherence Reporting Scale’.</li> <li>16. Sample size changed from 548 to 432 needed in order to show difference in overall survival between trial arms (section 5.7)</li> <li>17. Monitoring updated to allow remote monitoring as mitigation for COVID-19</li> <li>18. AF protein count to be recorded as a subgroup analysis.</li> <li>19. Clarification of criteria for patient to be considered lost to follow up (section 5.6.5)</li> <li>20. Addition of internal 9-month pilot phase (suggested by HTA)</li> <li>21. <b>Covid-19 mitigation:</b> Added a possibility in couriering the IMP to the patient if the trial medical runs out prior to the next visit (section 5.4.3 &amp; 5.9.1.2)</li> <li>22. Update to background and rationale (section 4.1) with details of removal of AF protein count inclusion criteria, changing primary outcome from incidence of SBP to Overall Survival, revised sample size calculation &amp; update to selection of co-trimoxazole as antibiotic for investigation.</li> <li>23. Update to trial schema to reflect updated trial timeline for patients (section 4.3)</li> <li>24. Pregnancy test and contraception requirements moved to this section 5.3.1.6</li> <li>25. Update to section 5.4.4.1 – Dose modification/suspension/continuation <ul style="list-style-type: none"> <li>• Thrombocytopenia section added</li> <li>• Suspension of IMP if patient experiences SAR or in PI opinion.</li> <li>• Hyperkalemia dose modification amended to specify ‘If hyperkalemia (potassium <math>\geq 6</math> mmol/L) related to trial medication’</li> </ul> </li> </ol> |
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|  |  | <ul style="list-style-type: none"> <li>• Section added about dose modifications/suspensions/continuations if patient experiences thrombocytopenia</li> <li>• Addition of 'trial medication will be stopped on hospital admission.'</li> <li>• Addition of upper gastrointestinal haemorrhage criteria or other reason for patients to resume antibiotics whilst in hospital.</li> </ul> <p>26. Section 5.4.9 – overdose of trial medication. Clarification of patients remaining in follow up even if trial medication discontinued.</p> <p>27. Section 5.6 – updated patient timeline (table 1). Conmed and adverse event review added to day 10 visit. Month 25 visit moved to month 19 and visits 10 and 11 (M21 and M24) removed.</p> <p>28. Section 5.11.2 – Update to statistical methods for primary outcome of overall survival</p> <p>29. Update to safety reporting:</p> <ul style="list-style-type: none"> <li>• ARs no longer need to be reported to sponsor</li> <li>• Only SAEs considered possibly, probably or definitely related to IMP need expedited reporting. All other SAEs can be reported within 10 days to sponsor</li> <li>• List of protocol-defined exceptions to SAE reporting removed</li> <li>• Death does not need expedited reporting unless related to IMP</li> </ul> |
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| V5.0 | 20 <sup>th</sup> August 2021 | <ol style="list-style-type: none"> <li>1. Change inclusion criteria #1 to remove definition of persistent ascites. Inclusion criteria #1 now reads; 'Patients with Child-Pugh Class B or C cirrhosis and presence of ascites requiring any diuretic treatment or at least 1 or more paracentesis within 3 months prior to enrolment.'</li> <li>2. Addition of Child-Pugh Score to glossary in line with the change to inclusion criteria.</li> <li>3. Additional input to background and rationale to explain reasons for changing inclusion criteria #1.</li> <li>4. References to pilot phase for recruitment changed from 9 months to 11 months as agreed with funder in June 2021.</li> <li>5. Addition of Registered Independent Prescribers, including research nurses who are registered with the Nursing and Midwifery Council (NMC) to section 5.9.1.2 and 5.9.1.3. This is to allow registered research nurses to prescribe IMP for patients at randomization and follow-up visits, where delegated to do so.</li> <li>6. Addition of cut-off for protein count of 2g/dL for subgroup analysis of protein count.</li> <li>7. Addition of subgroup analysis to compare patients with long term ascites (persisting for 3 months or more at time of enrolment) versus patients with ascites that has persisted less than 3 months.</li> <li>8. Updating of Prof Nick Freemantle's (UCL CCTU) role on the trial in section 1.4.</li> </ol> |
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| V6.0 | 06 Oct 2022 | <ol style="list-style-type: none"> <li>1. Widen the window of when IMP should start following randomisation.</li> <li>2. Extended the pre-screening ascitic fluid analysis, culture and abdominal ultrasonography window from 12 weeks to 6 months.</li> <li>3. If the patient is admitted to hospital or is an inpatient, it will be at the discretion of the treating clinician if the patient is well enough to start/restart the trial medication.</li> <li>4. Addition of two new stratification variables. These are if the alcohol was considered a significant cause of cirrhosis, and if the patient is actively consuming alcohol at randomisation.</li> <li>5. For Day 10 visit, under exceptional circumstances, if the patient is unable to attend this visit face to face, the site can send the patient to a GP or an equivalent clinic to do the blood test. The site must have a copy of the blood results sent to them and filed in the medical records. The site can conduct this visit over the telephone, and if needed, make any dose modifications based on the review of the blood results.</li> <li>6. For patients who permanently discontinue trial medication at any time during Months 3-18, these follow-ups can be completed over the telephone. The only exception is if the reason to permanently discontinue trial medication was because the ascites is resolved, but later in the trial the ascites return, and they restart their trial medication, then the visits will need to be face-to-face.</li> <li>7. Removal of ascitic fluid analysis and blood tests, liver and renal function tests in the long-term follow-up phase of the study.</li> <li>8. If the patient no longer wishes to be followed up in the trial, the patient will be asked for consent for the research team to continue to collect data from their medical records.</li> <li>9. Pharmacists who are independent prescribers can prescribe trial medication provided that they are on the delegation log and signed off by the PI.</li> <li>10. Updated staff names on the trial.</li> </ol> |
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|------|-------------|---|
| V7.0 | 07 Feb 2023 | <ol style="list-style-type: none"> <li>1. Addition of some contingency plans if there is an IMP supply issue. These are:               <ol style="list-style-type: none"> <li>a. IMP transfer of existing stock from one participating site to another</li> <li>b. To ensure continuation of IMP supply to sites:                   <ol style="list-style-type: none"> <li>i. Identify an alternative brand that will fit the capsule for the over-encapsulation. If not feasible, then we will implement the next contingency plan:</li> <li>ii. Alter the study design to be single-blinded.</li> </ol> </li> </ol> </li> <li>2. Removed Participant Management Plan – this was incorrectly referenced in the protocol and is not needed for the trial.</li> <li>3. Clarified that only delegated medically qualified doctors can only confirm patient eligibility into the trial.</li> <li>4. Addition of two more secondary outcomes.</li> <li>5. Clarified the primary outcome statistical analyses.</li> <li>6. Addition of two more subgroup analyses.</li> </ol>  |
| V8.0 | 15 Dec 2023 | <ol style="list-style-type: none"> <li>1. Corrections, formatting, and minor amendments to the overall text. Protocol version history updated.</li> <li>2. Front page: Updated signatories' names:<br/>Sponsor/CCTU Director Representative: Prof Nick Freemantle replaced Laura Farrelly.<br/>Senior Operations Staff: Amalia Ndoutoumou replaced Marisa Chau</li> <li>3. Section 1.4.1 Protocol contributors: Updated name of Health Economist to Ekaterina Bordea (previously Ekaterina Kuznetsova)</li> <li>4. Section 1.4.3 Trial Team: Updated trial team staff:<br/>Clinical Project Manager: Amalia Ndoutoumou replaced Marisa Chau<br/>Trial Manager: Iona Cullen-Stephenson replaced Amandeep Virk</li> <li>5. Section 1.4.4 Trial Management Group: Updated TMG Membership:<br/>Clinical Project Manager: Amalia Ndoutoumou replaced Marisa Chau<br/>Trial Manager: Iona Cullen-Stephenson replaced Amandeep Virk<br/>Updated name of Health Economist to Ekaterina Bordea (previously Ekaterina Kuznetsova)</li> <li>6. Clarification on what constitutes a protocol deviation (Section 5.6.2).</li> <li>7. Clarification over the classification of a patient being lost to follow-up (Section 5.6.5).</li> <li>8. Clarification that IMP does not need to be dispensed at a visit if the patient is on alternate dosing and therefore has enough IMP to last until their next visit.</li> </ol> |

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