ASEPTIC: Primary **A**ntibiotic prophylaxis using co-trimoxazole to prevent **S**pontan**E**ous bacterial **P**eritoni**TI**s in **C**irrhosis

Version	3.0
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Comprehensive Clinical Trials Unit Trial Adoption Group #	CTU/2017/308
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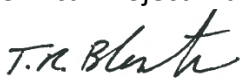
Signature	
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Table of Contents

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 participants 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 participants 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 [1]. The SPIRIT Statement Explanation and Elaboration document [2] 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	23rd January 2019
Secondary Identifying Numbers	CCTU Trial Adoption Group #: CTU/2017/308 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	Dr Alastair O'Brien, Reader & Consultant Hepatologist, University College London, University College Hospital & UCL Institute for Liver and Digestive Health **Address**: Upper 3rd Floor Division of Medicine Royal Free Campus Rowland Hill Street London NW3 2PF **Email**: a.o'brien@ucl.ac.uk
Public Title	A trial of using antibiotics to prevent infection in patients with advanced liver disease.
Scientific Title	Primary **A**ntibiotic prophylaxis using co-trimoxazole to prevent **S**pontan**E**ous bacterial **P**eritoni**TI**s in **C**irrhosis (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 24 months
Key Inclusion and Exclusion Criteria	**Inclusion criteria:** 1. Patients with liver cirrhosis and ascites with ascitic fluid protein count <2.0 g/dL (from sample taken within <12 weeks prior to randomisation) 2. Patients with ascitic polymorphonuclear count <250 cells/mm^3 and negative microbial culture at 5 days (on the last sample sent within <12 weeks prior to randomisation) 3. Patient at least 18 years of age 4. Documented informed consent to participate **Exclusion criteria:** 1. Patients with previous Spontaneous Bacterial Peritonitis (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 persistent hyperkalaemia (>6.5 mmol/L) related to pre-existing kidney disease with reduction not possible 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 (granulocytopenia, megaloblastic anaemia) 11. Severe thrombocytopenia with a platelet count <30 $x10^9$ /L 12. Patients with severe renal impairment, with eGFR <15 ml/min 13. Patients with skin conditions: exudative erythema multiform, Stevens-Johnson syndrome, toxic epidermal necrolysis and drug eruption with eosinophilia and systemic symptoms 14. Patients with congenital conditions: congenital glucose-6-Phosphate dehydrogenase deficiency of the erythrocytes, haemoglobin anomalies such as Hb Köln and Hb Zürich 15. Patients with acute porphyria 16. Any clinical condition which the investigator considers would make the patient unsuitable for the trial
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
Date of First Enrolment	Jul 2019
Target Sample Size	548
Primary Outcome(s)	Time to first incidence of spontaneous bacterial peritonitis up to 24 months following randomisation.
Key Secondary Outcomes	The following will be measured up to 24 months from randomisation: 1. All-cause mortality 2. Incidence of spontaneous bacterial peritonitis infection 3. Hospital admission rates 4. Incidence of *C. difficile*-associated diarrhoea

	5. Incidence of infections other than spontaneous bacterial peritonitis with hospital admission. 6. Incidence of other cirrhosis related events (e.g. variceal haemorrhage) 7. Incidence of renal dysfunction with creatinine >133 µmol/L (1.5mg/dL) at any point during hospital admission 8. Incidence of liver transplantation 9. Progression of liver disease assessed by increase in MELD score between baseline and end of trial follow up. 10. Safety and treatment-related adverse events 11. Treatment adherence (assessed by MARS questionnaire) 12. Health-related quality of life assessed using EQ-5D-5L questionnaire 13. Health and social care resource use assessed using Hospital Episode Statistics (HES) database 14. Mean incremental cost per quality adjusted life year gained (QALY) 15. Incidence of resolution of ascites with diuretic treatment not required for 6 months

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
Daizy Moualeu Kameni	UCL CCTU	Trial Manager
Kate Bennett	UCL CCTU	Trial Statistician
Rachael Hunter	UCL CCTU	Health Economist
Ekaterina Kuznetsova	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
Kate Bennett	UCL CCTU	Trial Statistician
James Blackstone	UCL CCTU	Clinical Project Manager
Daizy Moualeu Kameni	UCL CCTU	Trial Manager
TBA	UCL CCTU	Trial Manager (Monitor)
TBA	UCL CCTU	Data Manager

1.4.4. Trial Management Group

Name	Affiliation	Role and responsibilities
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
Stuart McPherson	Freeman Hospital	Principal Investigator
Jim Portal	Bristol Royal Infirmary	Principal Investigator
Paul Richardson	Royal Liverpool Hospital	Principal Investigator
Jennifer Ryan	Royal Free Hospital	Principal Investigator
Gavin Wright	Basildon Hospital	Principal Investigator
James Blackstone	UCL CCTU	Clinical Project Manager
Daizy Moualeu Kameni	UCL CCTU	Trial Manager
TBA	UCL CCTU	Trial Manager (Monitor)
Kate Bennett	UCL CCTU	Trial Statistician
Simon Skene	University of Surrey	Statistical Oversight
TBA	UCL CCTU	Data Manager

1.4.5. Trial Steering Committee

Name	Affiliation	Role and responsibilities
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

Name	Affiliation	Role and responsibilities
Professor Shahid Khan	Imperial College London	Chair
Mr Brennan Kahan	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
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
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 Rating 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
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.

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)[4] 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 procedure used to drain ascites.

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) in order to prevent the development of SBP; this is termed "primary prophylaxis".

SBP is the most common serious infection in people with cirrhosis and carries significant morbidity and mortality[3]. 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[4,5], 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[6] and so all current guidelines focus on the minority of patients. NICE Guidance (NG50) recommends prophylaxis with norfloxacin or ciprofloxacin for ascitic fluid protein concentration <1.5 g/dL until the ascites has resolved, but the evidence for this, is dated[7]. The British Society of Gastroenterology (BSG) Guidelines on Management of Ascites in Cirrhosis (2006) states "For patients who have never had SBP in whom ascitic fluid 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 ascitic protein concentration <1.5 g/dL combined with impaired renal function and liver failure (Child's score >9 and bilirubin >3 mg/dL)[8]. 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 ascitic fluid 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. Therefore, these patients should be considered for long-term prophylaxis with norfloxacin. However, in those with ascitic fluid protein lower 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[9].

Although norfloxacin is recommended in all guidance[3], 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 ascitic fluid (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 hence an urgent need for high quality research.

Cirrhosis patients with an acute deterioration secondary complications of liver cirrhosis are termed as having acute decompensation, or if with extra hepatic organ failure then acute-on-chronic liver failure[10]. Decompensation includes: jaundice, ascites, hepatic encephalopathy, variceal bleeding, coagulopathy and hepatorenal syndrome. Cirrhosis patients are highly prone to bacterial infection secondary to immune dysfunction[11], with SBP the most common serious infection, and this frequently

triggers other organ dysfunction[3]. As a result, bacterial infection is a leading cause of death in these patients with a mortality of >40%[12].

Low AF protein has been shown to be the key clinical risk factor for development of SBP in many studies dating back 30 years[13-15]. Two prospective studies comprising 127 patients (13 with SBP)[13] and 110 patients (28 with SBP)[14] confirmed low AF protein concentration as an independent predictor of SBP. In addition, AF protein ≤1 g/dL was shown to predict the recurrence of SBP[16]. AF protein content mirrors host opsonisation activity with 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[13,17,18]. SBP risk only increases minimally (20-24%) between years two and three of follow up[13]. These data have been recently challenged by two post-hoc analyses of three large cohorts of hospitalised patients with decompensated cirrhosis that suggested ascitic protein count did not correlate with SBP risk[19,20]. However, neither study addressed the prospective risk, and low AF protein remains the most studied risk factor for SBP and an essential inclusion criterion for all international guidance and trials to date and measurement of AF protein is widely recommended to identify patients at high risk for SBP[8,9]. Several NHS trusts are only able to record AF protein counts of <2.0 g/dl and in order for our findings to have broad applicability if positive, we shall use this as the key inclusion criteria.

Therefore, an AF protein level of <2.0 g/dL will form the basis of the inclusion criteria and an estimated incidence of SBP in the placebo arm of 20-25% over 2 years is consistent with published literature[8,9,13,17,18]. This pragmatic approach is in line with established international practice including NICE guidance and, in the event of a positive outcome, would be applicable worldwide.

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[21]. 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[22]. 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[23]. SBP occurs in 25% of people who develop ascites, mostly in advanced liver disease, with 20-40% mortality[24]. Furthermore, those surviving hospital admission for infection have a mortality of 60% at 1 year[25]. The only effective treatment for advanced liver disease is transplantation, however only 800 adults transplants are performed per year and it is estimated that 60000 people have cirrhosis[21]; additional approaches are required to tackle this striking imbalance. With the effects of infection so profound, evidently a strategy of prevention is better than cure and could have substantial benefits. There are no immune restorative therapies in clinical practice 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[24]. However, this must be balanced against the risk of selecting drug resistant organisms and *Clostridium difficile* (*C. difficile)* associated diarrhoea (CDAD)[26,27]. 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[3]. Finally, current practice is guided by data from other countries which have very different rates of AMR[28] and may therefore not be applicable in the UK. There is therefore a huge necessity for this trial.

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[4,5]. Several studies contained mixed populations including those with previous SBP, secondary prophylaxis, for which an evidence base has been established[9]. 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±1.5, to 16.7% with no CP data and 30.3% with CP 10.4±1.5 and renal dysfunction. Quinolone primary prophylaxis reduced overall SBP incidence from 22/137 to 4/138 patients[3]. 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[8], however these are an extremely unwell group of patients and represent only a small minority of those with ascites[3]. It is unclear whether prophylaxis would benefit all patients with a low AF protein level.

Based on these studies we estimate a 60% reduction in development of SBP in the treatment group compared to placebo.

Once weekly ciprofloxacin has been studied but this approach lacks microbiological credibility; it is not recommended in US guidance due to the increased risk of the development of bacterial resistance[8]. Indeed, 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[29]. 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[30]. 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[26]. Excessive use of these antibiotics has been associated with increases in the prevalence of quinolone resistance amongst nosocomial Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumanii*, as well as *Staphylococcus aureus* and streptococci linked with community-acquired infections[28]. In addition, excess exposure to quinolones has been associated with colonisation and infection by healthcare-associated pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA)[31] and, in particular CDAD[32]. Moreover, quinolone usage may contribute significantly to the emergence of resistance to other classes of antibiotics, such as carbapenems[33] – 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[34,35]. 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[28]. 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[36]. 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[37]. Widespread use of quinolone prophylaxis could lead to a similar situation developing in the UK[38] and 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[39-41]. 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[42]. 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[43] and reduce the risk of hepatorenal syndrome and variceal bleeding[44]. 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[45]. 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 ≥9 with serum total bilirubin levels ≥51 µmol/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[40]. 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[46-48]. US guidance recommends either norfloxacin or co-trimoxazole for primary SBP prophylaxis in those with severe liver failure and/or renal dysfunction[8]. 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[49]. 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[50]. *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 drug used widely to treat community acquired urinary tract infection[51]. 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 prophylaxis actually protected against resistance to other antibiotics[52,53]. Although a recent study has reported significant associations between prophylaxis and non-susceptibility to penicillin and rifampin[54]. The studies of co-trimoxazole use to prevent SBP did not report AMR but these pre-date the current AMR era, were small and had only 6 months follow up[46-48].

Co-trimoxazole has been chosen for this trial as it is well tolerated, and effective with a substantial literature of prophylaxis in HIV; a >3000 patient study reported a 3% incidence of serious adverse events[55]. 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 and the very rare Stevens-Johnson skin rashes 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[56]. In HIV infected patients receiving high dose co-trimoxazole for *Pneumocystis jiroveci (P. carinii)* pneumonitis serum potassium increased by 1.1 mmol/L, an average 9.8±0.5 days after starting therapy[48]. 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[57]. It is therefore recommended that serum potassium is closely 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[58] (double our prophylactic dose) and caution is recommended in renal dysfunction. In clinical trials 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[27]. In a trial comparing co-trimoxazole with norfloxacin in which 25 participants were treated with co-trimoxazole, there was one drug discontinuation at 60 days secondary to worsening of renal function[26]. 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[48]. A UK study also reported no renal dysfunction[51], and AASLD guidance recommends co-trimoxazole for SBP prophylaxis in patients with ascites and renal impairment[8].

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[9]. The trial hypothesis is in agreement with this guidance that long-term primary prophylaxis should be prescribed unless liver function recovers and the ascites clears without the need for diuretic treatment or the patient receives a liver transplant; therefore, the trial period will be 2 years. This will test whether there is a waning of efficacy over time, whether there is a long term effect on mortality and whether our strategy impacts upon AMR. Previous studies have ranged from 1 month to 1 year and were too short to 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 due to this 2 year treatment period but since a positive outcome would lead to long-term prescription of co-trimoxazole then 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.

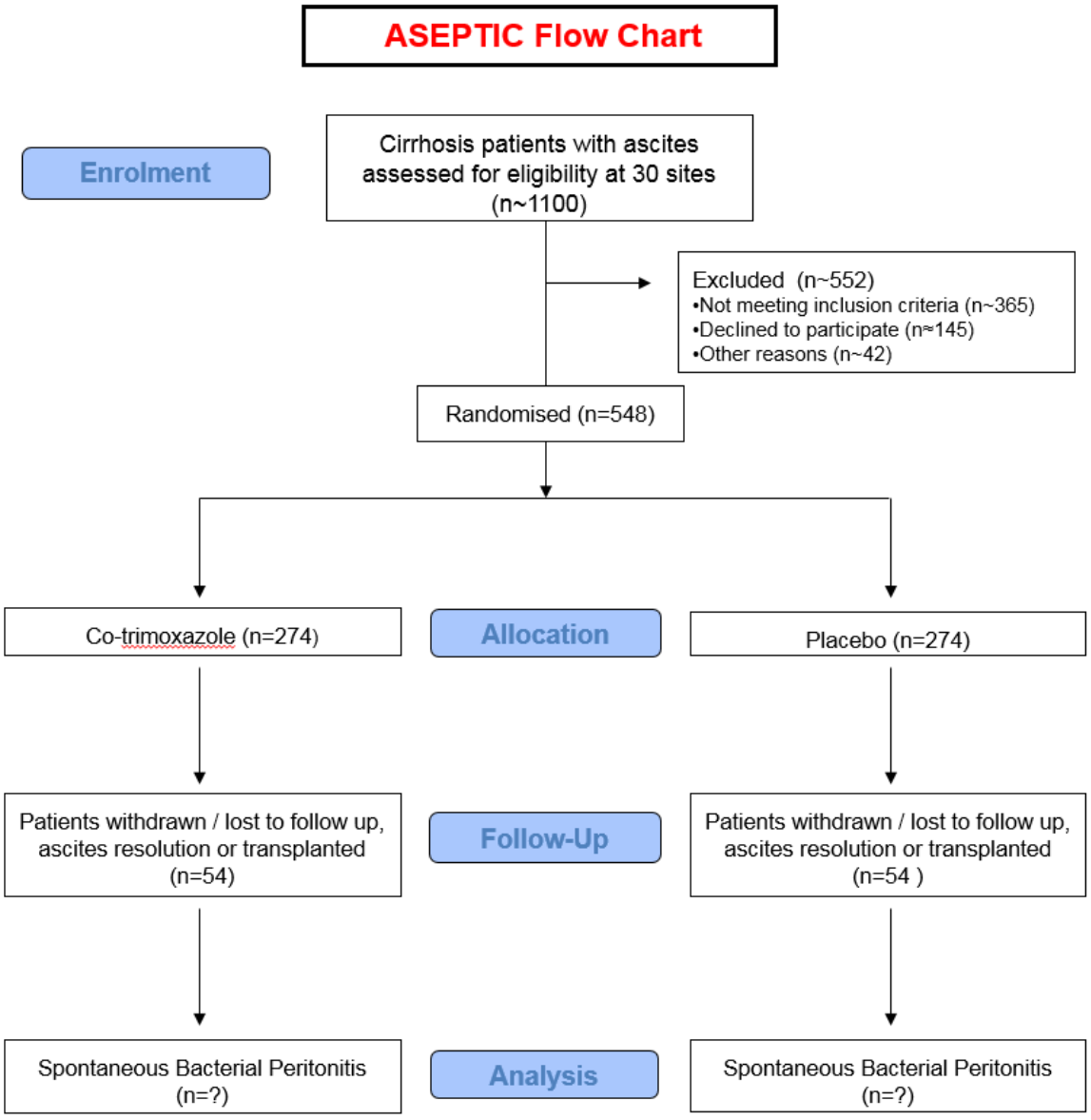
4.2. Objectives

The primary objective of ASEPTIC is to determine whether primary antibiotic prophylaxis with co-trimoxazole reduces the incidence of spontaneous bacterial peritonitis compared to placebo in adults with cirrhosis and ascites over a 2-year trial period.

Key secondary objectives will include all-cause mortality, 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 2 years to prevent SBP in patients with cirrhosis and a low ascitic fluid protein count (<2.0 g/dL).



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; the dataset will include Hospital Episode Statistics (HES), the Mental Health Minimum Data Set (MHMDS) and mortality data.

The recruitment period will be 24 months, with a 9 month internal progression review to demonstrate deliverability of recruitment. It is expected to have approximately 15 open sites and >90 patients recruited during the 9 month review phase.

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 participants, enter data and collect samples
- The site should be able to store, prepare and dispense IMP 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 participants 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 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. Participants

5.3.1. Eligibility Criteria

All patients with cirrhosis, ascites and ascitic fluid protein concentration <2.0 g/dL, who have never had an episode of SBP and are not receiving prophylactic antibiotics (patients taking rifaximin will be eligible).

5.3.1.1. Participant 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 participant.

The eligibility criteria for this trial have been carefully considered and are the standards used to ensure that only medically appropriate participants are entered. Participants 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.

Participants 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. Participant Inclusion Criteria

1. Patients with liver cirrhosis and ascites with Ascitic fluid protein count <2.0 g/dL (from sample taken within 12 weeks prior to randomisation)
2. Patients with ascitic polymorphonuclear count <250 cells/mm^3 and negative microbial culture at 5 days (on the last sample sent within 12 weeks prior to randomisation)
3. Patient at least 18 years of age

4. Documented informed consent to participate

5.3.1.3. Participant Exclusion Criteria

1. Patients with previous Spontaneous Bacterial Peritonitis (SBP)
2. Patients receiving palliative care only with an expected life expectancy of <8 weeks
3. Allergic to co-trimoxazole, trimethoprim or sulphonamides
4. Pregnant or lactating mothers
5. Patient enrolled on a clinical trial of investigational medicinal products (IMPs) that would impact on their participation in the study
6. Patients with persistent hyperkalaemia (>6.5 mmol/L) related to pre-existing kidney disease that is not possible to reduce
7. 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 as mentioned in section 5.6 for the duration of trial treatment;
10. Patients with pathological blood count changes (granulocytopenia, megaloblastic anaemia)
11. Severe thrombocytopenia defined as platelets <30 $x10^9$/L
12. Patients with severe renal impairment, with eGFR <15 ml/min
13. Patients with skin conditions: exudative erythema multiform, Stevens-Johnson syndrome, toxic epidermal necrolysis and drug eruption with eosinophilia and systemic symptoms
14. Patients with congenital conditions: congenital glucose-6-Phosphate dehydrogenase deficiency of the erythrocytes, haemoglobin anomalies such as Hb Köln and Hb Zürich
15. Patients with acute porphyria
16. Any clinical condition which the investigator considers would make the patient unsuitable for the trial

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

Participants 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 participants, 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.

These include:

- Blood tests:
- Ascitic fluid sample for PMN count and microbial culture
- Pregnancy test: to be completed at screening and within 2 weeks prior to randomisation

5.4. Interventions

Participants will be randomised to receive either co-trimoxazole or placebo.

5.4.1. Products

- Co-trimoxazole, one capsules 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 24 months.

5.4.3. Dispensing

Co-trimoxazole and matching placebo will be manufactured and distributed by Sharp Clinical Services (UK) Ltd. All trial medication will be dispensed to the participants by the local pharmacy departments at each participating site to coincide with the participants' 3 monthly follow-up visits.

5.4.4. Dose modification, suspension, continuation, and withdrawal

5.4.4.1. Dose modification, suspension and continuation

- Renal dysfunction

If the patients eGFR reduces to <30 ml/min the IMP dosing will be reduced to alternate day dosing. If the eGFR rises to >30ml/min on 2 blood tests at least 3 months apart the IMP can be increased back to once daily dosing.

If the patients eGFR reduces to <15ml/min then the IMP will be stopped. If the eGFR rises to >15ml/min on 2 blood tests at least 3 months apart the IMP can be increased back to alternate day dosing. In these patients the IMP will not be increased any further to once daily dosing.

- 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. Blood samples will be taken 10 days (+/- 3 days) after commencement of IMP 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 which actually lowers serum potassium levels may be a more appropriate alternative that should be considered at an early stage. Participants and the site PI and physicians should be aware of the risks of hyperkalaemia in participants with renal dysfunction. If serum potassium rises to ≥6 mmol/L, co-

trimoxazole will be stopped and the patient will be re-challenged once the PI considers this to be safe. If hyperkalemia (potassium >6 mmol/l) occurs on more than one occasion, then when the IMP is restarted after cessation the dose will be reduced to alternate day dosing.

- Hospital admission non-SBP related

Treatment can be temporarily stopped if the participant experiences any severe adverse reactions or if in the opinion of the PI this is necessary. 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.

Participants that 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.

- Recovery from ascites

If ascites resolves off diuretic medication for greater than 6 months, participants must not continue to take the study 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 (24 months).

If during the trial the ascites returns, the patient can return to the treatment they were initially allocated. Further details will be available in the patient management plan.

5.4.4.2. Withdrawal

- 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 participant 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

Participants that develop SBP or upper gastrointestinal haemorrhage will be treated with standard antibiotics as per local guidelines and trial medication stopped. Those that develop SBP would have reached the primary endpoint and so would be withdrawn from trial treatment. Although the patient will 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. Those with upper gastrointestinal haemorrhage would be restarted on trial medication following cessation of antibiotics and recovery.

- Transplant

Patients who meet the inclusion criteria and none of the exclusion criteria but are on the transplantation list will still be included into the trial. If, during the trial the patient becomes eligible

to receive the transplant, the 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

Participants 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 5.11.3.4)

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 IMP received at site, storage of the IMP according to the SmPC, dispensing to patients, and destruction of expired or unused medication.

Participants should return all unused IMP at each follow-up visit. Unused, expired or returned IMP, stored at the local pharmacy until the sponsor confirms can be destroyed as per standard local procedures once approved by the sponsor.

5.4.6. Compliance and Adherence

Treatment adherence will be assessed by the research team with the Medication Adherence Rating 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. Participants will be educated about the possible dangers of non-compliance.

5.4.7. Concomitant Care

Participants must not be receiving long-term antibiotic prophylaxis. The participant is permitted to take rifaximin as this can be prescribed for hepatic encephalopathy. Participants 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 participants with renal dysfunction and those taking high dose spironolactone or amiloride. Patients with significant persistent renal dysfunction and hyperkalaemia (>6mmol/L) 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.

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 participants 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 (see section 4.4).

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 participants. 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.

5.4.10. Protocol Treatment Discontinuation

In consenting to the trial, participants are consenting to trial treatment, trial follow-up and data collection. However, an individual participant may stop trial treatment early or be stopped 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 participant's condition that in the clinician's opinion justifies the discontinuation of treatment
- Withdrawal of consent for treatment by the participant

As participation in the trial is entirely voluntary, the participant 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 participant's rights.

Participants 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 efficacy outcome will be the difference in the time to the first incidence of SBP between the randomised groups up to 24 months following randomisation, this will be termed time to first incidence.

SBP is defined as per the standard guidelines: ascitic fluid polymorphonuclear (PMN) cell count >250/mm^3 with either positive or negative ascitic fluid culture without evident intra-abdominal surgically treatable source of infection[3].

Previous studies have all shown a substantial reduction in SBP incidence with prophylaxis[3]. However, these trials were for a shorter duration, in an era of less AMR, at single/dual centres and rifaximin had not been approved for hepatic encephalopathy treatment prescriptions[3]. Therefore, the long term efficacy of prophylaxis in the modern era may be much less than the meta-analyses suggest. A reduction of >50% in incidence of SBP following antibiotic prophylaxis would be required to gain widespread clinical uptake in view of the concerns over possible antibiotic resistance or treatment related adverse events.

5.5.2. Secondary Outcomes

1. All-cause mortality
2. Incidence of spontaneous bacterial peritonitis infection
3. Hospital admission rates
4. Incidence of *C. difficile*-associated diarrhoea
5. Incidence of infections other than spontaneous bacterial peritonitis with hospital admission.
6. Incidence of other cirrhosis related events (e.g. variceal haemorrhage)
7. Incidence of renal dysfunction with creatinine >133 µmol/L (1.5mg/dL) at any point during hospital admission
8. Incidence of liver transplantation
9. Progression of liver disease assessed by increase in MELD score between baseline and end of trial follow up
10. Safety and treatment-related adverse events
11. Treatment adherence (assessed by MARS questionnaire)
12. Health-related quality of life assessed using EQ-5D-5L questionnaire
13. Health and social care resource use assessed using Hospital Episode Statistics (HES) database
14. Mean incremental cost per quality adjusted life year gained (QALY)
15. Incidence of resolution of ascites with diuretic treatment not required for 6 months

All the above outcomes are assessed up to 24 months following randomisation.

5.6. Participant Timeline

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.

The participant should be consented prior to any trial-specific procedures taking place.

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 child bearing 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 CTFG guidelines regarding the

recommendations related to contraception and pregnancy testing in clinical trials[73]; "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.

Female participants 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 participants' entry into the study, and this male is the sole partner for that participant.
- Total abstinence from intercourse with male partners (occasional abstinence is not a reliable form of contraception).

Baseline evaluation includes age, sex, date of birth, diagnosis, history and physical examination, liver and renal tests, ascitic fluid analysis and culture, and abdominal ultrasonography (within previous 6 months). The screening blood tests include full blood count with haemoglobin, white cell count, platelets, 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. Hospitalised participants should be randomised at discharge once medically stable.

Protocol treatment with trial medication (either co-trimoxazole or placebo) should start immediately following randomisation. Blood samples should be taken 7-14 days after commencement of treatment to check for hyperkalaemia. Participants should then attend for a follow-up visit every 3 months thereafter, up to 24 months, to collect medication and undergo routine blood testing as per standard care.

Treatment adherence should be assessed at the follow-up visits by the MARS questionnaire prior to a new medication being dispensed. This will be documented on a CRF. An adverse event CRF should also be completed at each visit and hyperkalaemia monitored by blood testing.

Hospital admissions since the previous visit to determine SBP and other infection rates should be documented on a CRF.

SBP is defined as per standard guidelines (see section 6.5.1). 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.

EQ-5D-5L should be completed by patients/site staff at baseline and every 6 months at patient visits thereafter (every other visit).

Figure 1: Participant timeline

Visit number	VISIT 1		VISIT 2	VISIT 3	VISIT 4	VISIT 5	VISIT 6	VISIT 7	VISIT 8	VISIT 9	VISIT 10	VISIT 11	Safety call
Month	(<12 wks from randomisation)	Month 0	Day 10[e] (+/- 3 days)	Month 1 (+/- 1 wk)	Month 3 (+/- 1wk)	Month 6 (+/- 1wk)	Month 9 (+/- 1wk)	Month 12 (+/- 1wk)	Month 15 (+/- 1wk)	Month 18 (+/- 1wk)	Month 21 (+/- 1wk)	Month 24 (+/- 1wk)	Month 25 (+/- 1wk)
	Screening	Randomisation	Treatment Phase										Follow-up
Eligibility screening	X												
Informed consent	X												
Medical history	X												
Pregnancy test *(if applicable)*	X[a]												
Ascitic fluid protein count	X[b]												
Blood tests[c], liver and renal function tests	X		X		X	X	X	X	X	X	X	X	
Randomisation		X											
Adverse reaction review				X	X	X	X	X	X	X	X	X	X
Hospital admission review			X	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	X	X	
EQ-5D-5L questionnaire		X				X		X		X		X	
Dispense trial medication[d]		X			X	X	X	X	X	X	X		

[a]: Pregnancy test must be completed within 2 weeks prior to randomisation
[b]: Ascitic cell and protein count can be done within 6-12 weeks prior to randomisation
[c]: Blood tests should include full blood count, with haemoglobin, white cell count, platelets, INR, sodium, potassium, urea, serum creatinine, and urea. Liver function tests should include albumin, alanine aminotransferase (ALT), alkaline phosphatase, and bilirubin. Renal function tests including eGFR.
[d]: At each indicated visit, one bottle of 100 capsules of trial medication is dispensed to the patient (either co-trimoxazole or placebo capsules)
[e]: Visit 2 should be completed at Day 10 but can be completed between 7-13 days. The aim of this visit is to monitor the safety of the patients and make any dose modifications to con-meds. All other visits must be completed within +/- 1 week from the due date

5.6.1. Early Stopping of Follow-up

If a participant 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 and facilitated not to leave the whole trial, even though they no longer take the trial treatment. If, however, the participant exercises the view that they no longer wish to be followed-up either, this view must be respected and the participant withdrawn entirely from the trial. CCTU should be informed of the withdrawal in writing using the appropriate ASEPTIC trial documentation. Data already collected will be kept and included in analyses according to the intention-to-treat principle for all participants who stop follow up early.

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

5.6.2. Participant Transfers

If a participant 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 participant's CRFs should be provided to the new centre. Responsibility for the participant 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 participant transfers.

5.6.3. Loss to Follow-up

Patients will be followed-up for 24 months following randomisation. Follow-up will be by contact at outpatient appointments. Every effort will be made to maintain contact with all patients. 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 participants via their NHS number. We will apply for access to data held by NHS Digital; 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 participants 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.

5.6.4. Trial Closure

The end of the trial for individual participants 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

Previous studies have all shown a substantial reduction in SBP incidence with prophylaxis[3]. However, these trials were for a shorter duration in an era of less AMR, at single/dual centres and rifaximin had not been approved for hepatic encephalopathy treatment prescriptions[3]. Therefore, the long-term efficacy of prophylaxis in the modern era may be much less than the meta-analyses suggest.

The primary endpoint is event-free survival up to 24 months from randomisation, measured time to incidence of SBP. In order to demonstrate a reduction of >50% in incidence of SBP following antibiotic prophylaxis, to gain widespread clinical uptake in view of the concerns over possible antibiotic resistance or treatment related adverse events, the sample size calculations have been based on a

55% reduction. Assuming a relative reduction in the cumulative event probabilities of SBP to be approximately 55% (hazard ratio 0.45) with expected event probabilities after 2 years of 22.5% in the control group and 14.6% in the co-trimoxazole group, the estimated sample sizes for a two-sample comparison of survivor functions by log-rank test is 548 (274 per treatment arm), to achieve 90% power and a two-sided 5% significance level. This allows for 20% loss-to-follow-up (withdrawal/loss-to-follow-up/resolution of ascites/transplant).

5.8. Recruitment and Retention

5.8.1. Recruitment

Patients will be recruited from up to 30 secondary or tertiary care NHS hospitals that frequently manage patients with advanced liver disease. Patients with cirrhosis and ascites that have been hospitalised will be identified and approached to participate, these patients will be randomised at discharge 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 24 months, with a 9-month internal progression review to demonstrate deliverability of recruitment. It is expected to have approximately 15 open centres and >90 patients recruited during the 9-month phase.

5.8.2. Retention

The importance of attending scheduled follow up appointments until trial completion will be explained to all participants at the start of the trial to ensure that only those able to commit to the trial protocol are recruited. Participants 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

Participants will be randomised 1:1 to receive either co-trimoxazole or placebo. An independent online randomisation service (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. 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 at baseline, and at each subsequent 3 monthly follow-up visit.

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

The investigator will provide details of the allocated kit code assigned to each participant 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.

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

A blinded review of the evidence of infection will be performed to validate the infection diagnoses used for primary and secondary outcomes. The endpoint review panel will assess whether there is evidence from the CRFs to support such a diagnosis.

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.

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 a 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 participant will be given a unique trial Participant Identification Number (PIN). Data will be collected at the time-points indicated in the Trial Schedule (Figure 1).

Data will be collected from the trial sites using paper Case Record Forms (CRFs) and transferred to CCTU. The data will be entered into the database by a member of the ASEPTIC trial team and stored on secure servers based at UCL. Training on paper CRF completion and storage 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 by a member of the ASEPTIC trial team at CCTU and protected using established CCTU procedures.

Participants will be given a unique trial Participant Identification Number (PIN). Data will be entered under this identification number onto the central database (InferMed's MACRO) stored on the servers based at CCTU. The database will be password protected and only accessible to members of the ASEPTIC trial team at CCTU, 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 participant 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; 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 participants 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

Withdrawal of consent will be documented on a Withdrawal CRF. Once consent has been withdrawn trial treatment will cease, but trial follow-up should continue if possible. Reasons for treatment discontinuation, if possible, will be recorded by the trial team. Participants will be educated about the possible dangers of non-compliance.

5.10.4. Statistics

5.10.4.1. 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. All statistical analysis will be performed using Stata (StatCorp, College Station, TX, USA).

5.10.4.2. Statistical Methods

Primary outcome:

The primary analysis of the primary outcome of time to SBP will be a log-rank test to compare the survival distributions of the two treatment groups. A p-value of <0.05 would be interpreted as a statistically significant treatment effect. As secondary analyses, the following strategy will be used. 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, and 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 are observed, the life expectancy difference (LED) and life expectancy ratio (LER) will be presented[72]. [Ref]. LED is the difference between mean survival times in the intervention and control arms. LER is the ratio of these two times. A further secondary analysis will compare the incidence of SBP between arms using a logistic regression model, adjusted similarly.

Secondary outcomes:

Other binary secondary 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 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.

5.10.4.3. 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. In addition, a subgroup analysis will examine whether the treatment effect differs in men and women. As the trial has not been powered to detect subgroup effects, this should be considered exploratory.

The groups will be defined as follows:

- Rifaximin prescription at randomisation - Yes vs No
- Active participation on liver transplant list at randomisation – Yes vs No
- Gender – Male vs Female

5.10.4.4. 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.

5.10.5. 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 in cirrhosis patients. The analysis will be done from health and social care perspective using 24-month trial data.

QALYs will be calculated based on EQ-5D-5L completed at baseline and every 6 months during the 24-month follow-up. If a participant does not attend a site for a planned visit, the EQ-5D-5L questionnaire will be posted to the participant or completed by telephone. Mortality data will also be used to calculate QALYs.

We will apply to the NHS Digital 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 other key secondary outcomes.

5.10.5.1. Health Economic Analysis Plan

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. 24-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 (HRQL) will be measured using to 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 24 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.

5.11. Data Monitoring

5.11.1. Data Monitoring Committee

An Independent Data Monitoring Committee (IDMC) will meet every 6 months. 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 TSC on whether the trial should continue unchanged.

Further details of the roles and responsibilities of the Independent Data Monitoring Committee (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).

5.11.2. 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.

5.11.3. 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 and on the appropriate ASEPTIC CRFs.

5.11.4. 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 1: Adverse Event Definitions

Adverse Event (AE)	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.
Adverse Reaction (AR)	Any untoward and unintended response to an investigational medicinal product related to any dose administered
Unexpected Adverse Reaction (UAR)	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.
Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)	Any AE or AR that at any dose: • results in death • is life threatening* • requires hospitalisation or prolongs existing hospitalisation** • results in persistent or significant disability or incapacity • is a congenital anomaly or birth defect • or is another important medical condition***

* 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)

** 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

*** 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 participant 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).

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

5.11.4.1. 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[10]), 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 birth, 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. Participants 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.

5.11.4.2. 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 that are not considered to be related to trial medication and no CRF needs to be completed.

All non-serious adverse reactions, whether expected or not, where the adverse event is considered to be related to trial medication must be reported. Adverse reactions must be recorded in the patient's medical notes and recorded on the Adverse Reaction Log CRF at each follow-up visit.

Adverse Events:

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

Adverse Reactions:

All ARs should be recorded in the participant's medical notes as per usual clinical practice and recorded on the adverse reaction log.

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 (Table 2) as protocol defined exceptions to SAE reporting.

Protocol-defined exceptions to SAE reporting:

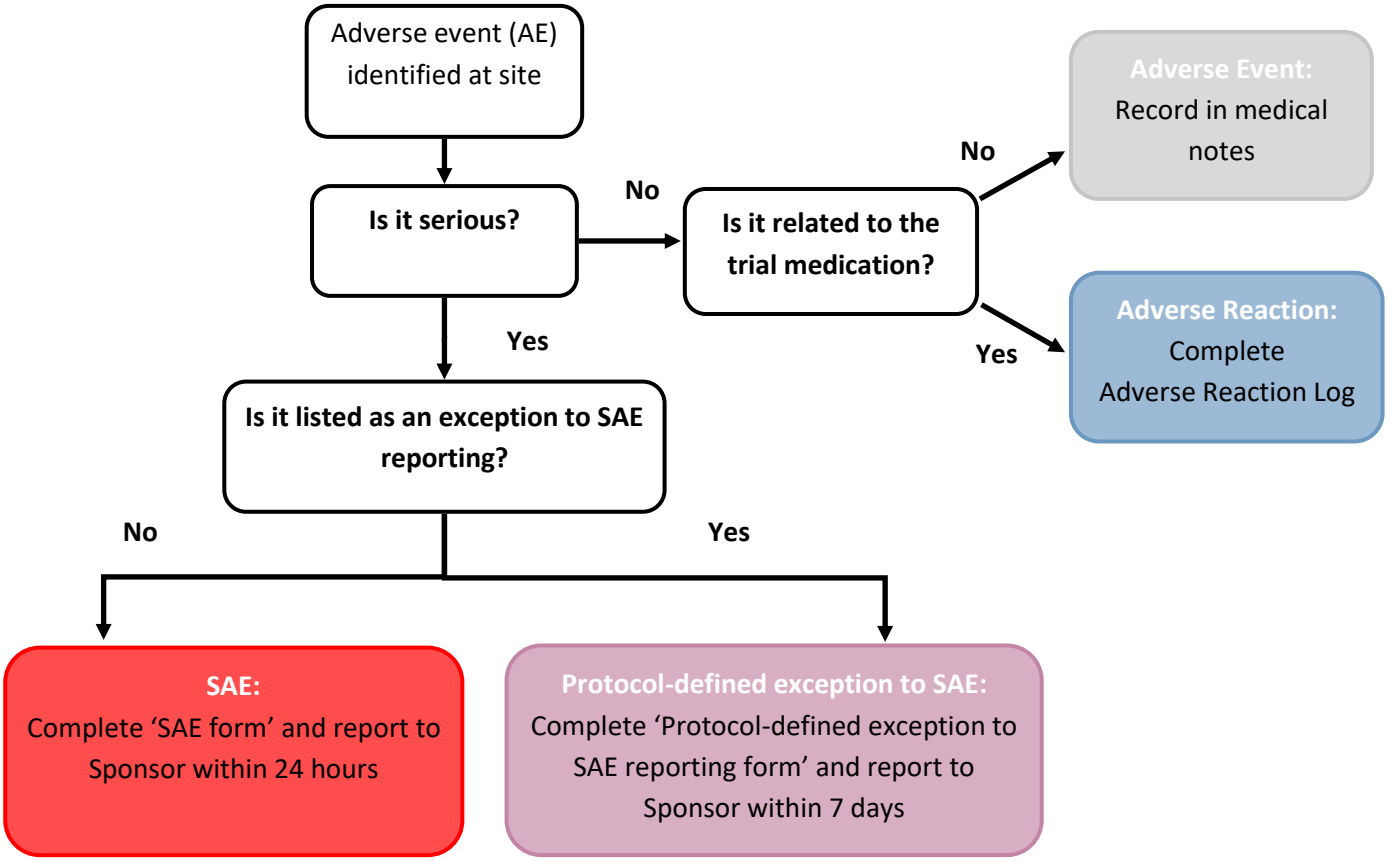
The events listed in Table 2 are expected serious adverse events associated with advanced liver disease. If an event listed occurs and meets one or more of the criteria for a '**serious** adverse event', expedited reporting as an SAE is not necessary. Instead, report the event to CCTU within 7 days using the dedicated 'protocol-defined exception to SAE reporting form' (see Table 2 for details).

The events listed below should be reported to CCTU immediately (within 24 hours) using an SAE form if the investigator believes an event is **related** to the trial drug.

Table 2: Protocol-defined exceptions to SAE reporting

Infection (all cause)
Ascites
Jaundice
Hepatic encephalopathy
Variceal or Gastrointestinal bleed
Peripheral oedema

Figure 2: Safety reporting



5.11.4.2.1. Seriousness assessment

When an AE or AR occurs, the investigator responsible for the care of the participant must first assess whether or not the event is serious using the definition given in Table 1. 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).

5.11.4.2.2. 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; hospitalization or prolongation of hospitalization 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.

5.11.4.2.3. 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 (eg the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (eg the participant's clinical condition or other concomitant treatment)	Unrelated SAE
Possibly related	There is some evidence to suggest a causal relationship (eg 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 (eg the participant'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.

5.11.4.2.4. 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 4.3 of the relevant SPC for a list of expected toxicities 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).

5.11.4.3. Notifications

5.11.4.3.1. 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 (the consultant named on the delegation of responsibilities list who is responsible for the participant'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 trial 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**

Participants 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 participant must be identified by trial number, date of birth and initials only. The participant's name should not be used on any correspondence and should be blacked out and replaced with trial identifiers on any test results.

5.11.4.3.2. 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.

5.11.5. Quality Assurance and Control

5.11.5.1. 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 participants; 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.

5.11.5.2. 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.

5.11.5.3. On-site Monitoring

The frequency, type and intensity of routine and triggered on-site monitoring 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.

5.11.5.3.1. 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. Participant consent for this must be obtained as part of the informed consent process for the trial.

5.11.5.4. 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 participant enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect participants, 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.

5.11.5.4.1. 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.

5.11.5.4.2. 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.

5.11.5.4.3. 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 participants. 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.

5.11.5.4.4. 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 participants, 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.

5.11.5.4.5. 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 participant 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 participant to refuse to participate in the trial without giving a reason must be respected. After the participant 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

participant. The reasons for doing so must be recorded. After randomisation the participant 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 participant 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 Participant Information Sheet (PIS) and consent form on local headed paper must be forwarded to the co-ordinating centre before participants 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 participant is willing to participate, written informed consent will be obtained. During the consent process it will be made completely and unambiguously clear that the participant 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 participant's consent in any way. This will be documented in a revision to the PIS and the participant 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 participant's personal data is ensured by not collecting participant 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 participant will be issued a patient identification code, and this will be the primary identifier for the participant, with secondary identifiers of month and year of birth and initials.

The participant's consent form will carry their name and signature, but these will be kept at the trial site (participant's hospital) and not with the participant'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 participants for injury caused by their participation in the clinical trial. Participants 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 participant 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 participant's right to seek compensation via the non-negligence route.

Participants 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. Participants 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	14th May 2019	N/A
V2.0	10th July 2019	Changes made following receipt of MHRA grounds for non-acceptance: 1) Exclusion criteria (section 1.3 and 5.3.1.3) updated in accordance with contraindication in the SmPC to include: • Severe renal impairment with eGFR <15 ml/min • Pathological blood count changes (granulocytopenia, megaloblastic, anaemia) • Skin conditions: exudative erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis and drug eruption with eosinophilia and systemic symptoms • Congenital conditions: congenital glucose-6-Phosphate dehydrogenase deficiency of the erythrocytes, haemoglobin anomalies such as Hb Köln and Hb Zürich • Acute porphyria 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 3) Information on dose modifications for renal dysfunction and hyperkalemia added (section 5.4.4.1) 4) Definitions of double barrier methods of contraception updated (section 5.6) 5) Details of all test required as part of blood tests added (section 5.6)
V3.0	13th July 2019	1) Secondary outcomes clarified 2) Participant timeline updated (section 5.6) The demography has been removed, the term 'SBP diagnosis review' has been updated to 'hospital admission review', and the term 'hospital admission review' have been included at the Day 10 and Month 1 follow-up visits.

		3) Pharmacovigilance processes updated (section 5.11.4). 4) 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 using the dedicated 'protocol-defined exception to SAE reporting form'. 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. 5) Participant timeline updated (section 5.6) 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.

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