Full Title: Eculizumab in Shiga-Toxin producing E. Coli Haemolytic Uraemic Syndrome (ECUSTEC): A Randomised, Double-Blind, Placebo-Controlled Trial

Short Title: ECUSTEC trial

PROTOCOL: VERSION 4.0, 18th January 2018

Sponsor: The Newcastle upon Tyne Hospitals NHS Foundation Trust
Sponsor's Project Number: 7837

Chief Investigator: Dr Sally Johnson, The Newcastle Upon Tyne Hospitals NHS Foundation Trust

Coordinating Centre: Birmingham Clinical Trials Unit (BCTU)

Funder: National Institute for Health Research (NIHR) and the Medical Research Council (MRC) Efficacy and Mechanism Evaluation (EME) Programme (Ref. No.: 14/48/43)

EudraCT number: 2016-000997-39
ISRCTN: 89553116
MHRA CTA: 17136/0282/001-0001
REC Ref No:16/NE/0325

The Newcastle upon Tyne Hospitals NHS Foundation Trust

UNIVERSITY OF BIRMINGHAM

BCTU Birmingham Clinical Trials Unit

Amendments

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version:

Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment
1	12th December 2016	Version 2.0 12th December 2016	Substantial	Changes to the initial protocol requested by the MHRA including information about contraception, pregnancy testing, more frequent CNS examinations and SUSAR reporting.
2	1st April 2017	Version 3.0	Substantial	Changes to incorporate those requested by REC for Version 1.0 7th September 2016 and MHRA requested changes for Version 2.0 12th December 2016. Additional inclusion criteria and wording of an exclusion criteria. Further detail added regarding confirmation of vaccinations. Amendments to the assessments schedule, data collection, samples guidance and AE reporting sections. Other minor changes.
3.		Version 4.0	Substantial	The treatment window has been extended by 12 hours due to the operational difficulty of treating patients. Other minor changes.

Chief Investigator and Sponsor Signature Page

The Chief Investigator and the Sponsor have discussed this protocol which describes the ECUSTEC clinical trial. The Investigator agrees to perform the investigations and to abide by this protocol.

The Investigator agrees to conduct the trial in compliance with the approved protocol, Good Clinical Practice (GCP), the UK Regulations for CTIMPs (SI 2004/1031; as amended), the UK Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the Research Governance Framework (2005 2nd Edition, as amended) and other regulatory requirements as amended.

Chief Investigator:

Dr Sally Johnson

Consultant Paediatric Nephrologist

The Newcastle upon Tyne Hospitals NHS Foundation Trust

..... 07/02/2018 .....

Signature Date

Sponsor Representative:

~~Andrew Johnston~~ SEAN SCOTT

~~Research Management and Governance Manager~~ REGULATORY COMPLIANCE MANAGER.

The Newcastle upon Tyne Hospitals NHS Foundation Trust

Signature Date

..... 07/02/2018 .....

Principal Investigator Signature Page

Principal Investigator:

I have read and agree to the protocol, as detailed in this document. I agree to adhere to the protocol as outlined and agree that any suggested changes to the protocol must be approved by the Trial Steering Committee prior to seeking approval from the Main Research Ethics Committee (MREC) and/or Regulatory Authority.

I am aware of my responsibilities as an Investigator under the guidelines of Good Clinical Practice (GCP), the Declaration of Helsinki, local regulations (as applicable) and the trial protocol and I agree to conduct the trial according to these guidelines and to appropriately direct and assist the staff under my control, who will be involved in the trial.

Principal Investigator

Name: …….

Signature: ……...

Date: ……

Name of Institution

Institution: ……

The Principal Investigator should sign this page and return a copy to the ECUSTEC Trial Office.

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Safety Reporting

Fax SAE Forms to: 0121 415 9135/0121 415 9136 or email to ecustec@trials.bham.ac.uk

This protocol describes the ECUSTEC trial only. The protocol should not be used as a guide for the treatment of patients not taking part in the ECUSTEC trial. The trial will be conducted in accordance with the protocol and Good Clinical Practice (GCP). Every care has been taken in the drafting of this protocol but future amendments may be necessary, which will receive the required approvals prior to implementation.

Table of Abbreviations

A&E	Accident and Emergency
ABAS	Adaptive Behaviour Assessment System
ABPI	Association of the British Pharmaceutical Industry
AE	Adverse Event
AKI	Acute Kidney Injury
AR	Adverse Reaction
BCTU	Birmingham Clinical Trials Unit
BNF	British National Formulary
CHU-9D	Child Health Utility 9D
CI	Chief Investigator
CKD	Chronic Kidney Disease
CNS	Central Nervous System
CRF	Case Report Form
CRP	C- Reactive Protein
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of an Investigational Medicinal Product
DCF	Data Clarification Form
DMEC	Data Monitoring and Ethics Committee
DSUR	Development Safety and Update Report
ECG	Electrocardiogram
EEA	European Economic Area
eGFR	Estimated Glomerular Filtration Rate (Schwartz)
eGFRc	Estimated Glomerular Filtration Rate using cystatin C
EME	Efficacy and Mechanism Evaluation
FBC	Full Blood Count
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GP	General Practitioner
HR QoL	Health Related Quality of Life
HUS	Haemolytic Uraemic Syndrome
KO	Knock Out
ICER	Incremental Cost Effective Ratio
ICF	Informed Consent Form
ICH-GCP	International Conference on Harmonisation Guidelines for Good Clinical Practice
IMP	Investigational Medicinal Product
ISF	Investigator Site File
IV	Intravenous
LDH	Lactate Dehydrogenase
MHRA	Medicines and Healthcare Products Regulatory Authority
MRC	Medical Research Council
MREC	Multicentre Research Ethics Committee
NIHR	National Institute for Health Research

NIMP	Non-Investigational Medicinal Product
PCR	Polymerase Chain Reaction
PE	Plasma Exchange
PEDs QL	Paediatric Quality of Life InventoryTM
Penicillin	Phenoxymethylpenicillin
PI	Principal Investigator
PIC	Patient Identification Centre
PIS	Patient Information Sheet
PSS	Prescribed Specialist Service
QALY	Quality Adjusted Life Years
RCT	Randomised Controlled Trial
RRT	Renal Replacement Therapy
pRIFLE	Paediatric Risk, Injury, Failure, Loss, End-stage Renal Disease
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SD	Standard Deviation
SmPC	Summary of Product Characteristics
STEC	Shiga Toxin Escherichia Coli
Stx	Shiga toxin
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMA	Thrombotic Microangiopathy
TMG	Trial Management Group
TSC	Trial Steering Committee
UAR	Unexpected Adverse Reaction
VEG-F	Vascular Endothelial Growth Factor

Table of Contents

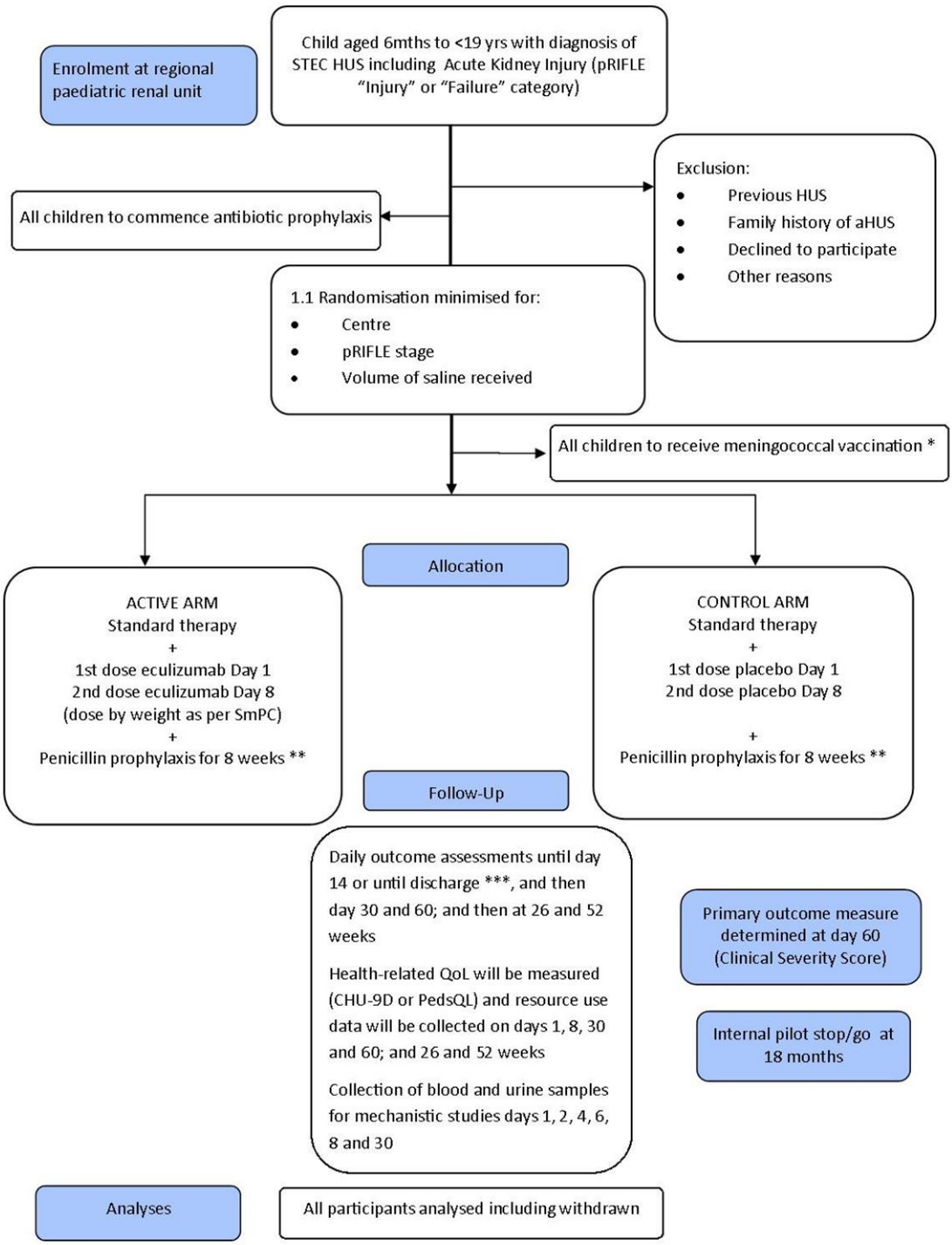
Summary and Trial Schema

Title	Eculizumab in Shiga-Toxin producing E. Coli Haemolytic Uraemic Syndrome (ECUSTEC): A Randomised, Double Blind, Placebo-Controlled Trial
Short Title/Acronym	The ECUSTEC Trial
Trial Design	Randomised, parallel group, double blind, placebo-controlled trial
Aim of Trial	To assess whether Eculizumab (Ecu) reduces the severity of Shiga-toxin producing Escherichia coli Haemolytic Uraemic Syndrome (STEC HUS) in children and young people
Sample Size	134
Inclusion Criteria	1] Age 6 months to <19 years 2] Weight ≥5kg 3] Diagnosis of HUS a. Micro-angiopathic haemolytic anaemia (indicated by fragmented red cells on blood film **OR** plasma lactate dehydrogenase (LDH) above local centre reference range) **AND** b. Thrombocytopenia (platelets <150x10^9/l) **AND** c. Acute Kidney Injury (AKI): "injury" or "failure" category of pRIFLE criteria despite correction of hypovolaemia 4] **EITHER** Reported diarrhoea within 14 days prior to diagnosis of HUS (defined according to World Health Organisation as "the passage of three or more loose or liquid stools per day - or more frequent passage than is normal for the individual") **OR** A stool culture or shiga toxin polymerase chain reaction or STEC serology result indicating STEC in the patient **OR** Household contact within 14 days prior to diagnosis of HUS 5] Patient intended to be able to receive trial drug within 48 hours of the on-call paediatric nephrologist formally taking over the care of the patient at the trial site providing inclusion criteria 3 is met, or within 48 hours of meeting inclusion criteria 3 if not met at the time the on-call paediatric nephrologist takes over the care of the patient. 6] Sexually active male or female patients must agree to practice an effective, reliable and medically approved contraceptive regimen for 6 months after enrolment. 7] Sexually active female patient has provided a negative

	pregnancy test ≤48 hours prior to randomisation 8] Patient/parent/guardian reported that vaccinations are up to date according to the routine UK (or equivalent) immunisation schedule 9] Written informed consent obtained from the patient's parents/guardians and written assent obtained from patients (where age appropriate). Patients aged 16 years and above will provide their own written informed consent.
Exclusion Criteria	1] Family history of atypical HUS 2] Previous episode of HUS 3] Known pre-existing eGFR <90ml/min/1.73m^2 4] Known or suspected pneumococcal infection 5] Known or suspected meningococcal infection 6] Prior to diagnosis, patient taking a drug known to be associated with HUS, e.g. calcineurin inhibitors, chemotherapy, quinine, oral contraceptive pill 7] Hypersensitivity to Ecu, murine proteins or any of the excipients listed in the Summary of Product Characteristics 8] Pregnancy or lactation 9] Malignancy 10] Known Disseminated Intravascular Coagulopathy 11] Refusal of consent, including consent for pregnancy testing, meningococcal vaccination or antibiotic prophylaxis 12] Currently participating in another clinical trial of an investigational medicinal product
Trial Description/Arms	Active arm: Standard therapy + 1st dose Ecu Day 1 and 2nd dose Ecu Day 8 Control arm: Standard therapy + 1st dose placebo Day 1 and 2nd dose placebo Day 8
Primary Outcome Measure	Clinical Severity Score assigned at day 60
Secondary Outcome Measures	• Overall Survival • Duration of renal replacement therapy (days) • Duration of thrombocytopenia (number of consecutive days until platelet count >150x10^9/l) • Duration of haemolysis (number of days until lactate dehydrogenase [LDH] within normal local centre reference range) • Number of packed red blood cell transfusions required and volume (ml/kg) • Duration markers of inflammation present (number of days until neutrophil cell count and C-reactive protein are in

	normal range for that centre) • CKD at 52 weeks (a composite endpoint of the presence of hypertension [average of 3 readings by manual method using centile charts for age/sex/height], albuminuria [urine albumin-creatinine ratio >2.5mg/mmol on early morning urine] or estimated glomerular filtration rate (eGFR) <90ml/min/1.73m2 at 52 weeks). Presence of any of these will constitute CKD at 52 weeks • eGFR measurement using a centralised cystatin C assay at 52 weeks • Persistent neurological defect at day 60 measured by structured expert assessment to include CNS examination, vision, hearing and neuropsychological assessment • Economic evaluation of cost per clinical severity score point, and cost per QALY gained, using PedsQL and CHU-9D assessments to measure health related quality of life
Follow-up Period	All participants will be followed up for 52 weeks

Trial Schema



*Meningococcal B vaccine (Bexsero™) and conjugate meningococcal ACYW vaccine (Nimenrix or Menveo) will be given to all ECUSTEC trial participants (unless Bexsero™ or ACWY already received as part of UK immunisation programme). Child <2yrs Nimenrix; ≥2yrs Menveo or Nimenrix. If platelet count $<50x10^9$/l defer vaccination until platelet $>50x10^9$/l

** erythromycin if penicillin allergic

*** assessment at day 21 and 30 if in-patient

Background and Rationale

Background

2.1. Shiga toxin producing Escherichia coli Haemolytic Uraemic Syndrome (STEC HUS) is the most common single cause of paediatric acute kidney injury (AKI), and affects approximately 100 UK children each year[1]. It has a 2-3% mortality rate and considerable morbidity with 50-60% of children requiring dialysis. A proportion (20-25%) develop severe disease with other organ involvement, including colonic necrosis and perforation (requiring laparotomy and bowel resection), central nervous system (CNS) disturbance including seizures, focal neurological defects and coma, pancreatitis (including temporary or permanent glucose intolerance) and myocardial dysfunction (including infarction). Long term complications such as chronic kidney disease (CKD) or more rarely permanent brain injury occur in up to 1/3 of survivors[2]. A meta-analysis demonstrated that 12% of patients with STEC HUS die or develop end-stage renal disease by 4.4 years of follow-up, with long term sequelae (hypertension, proteinuria, impaired glomerular filtration rate [GFR]) in approximately 25% of survivors[2]. Consequently, all cases require lifelong renal follow-up. Previous studies have failed to demonstrate improved short term or long term outcomes with interventions such as anticoagulation, plasma infusion, corticosteroids or oral therapy with a Shiga toxin (Stx) binding agent[3]. There is some evidence suggesting that early volume expansion with 0.9% saline may reduce the incidence of oligoanuria in STEC HUS[4].

Recently the field of HUS has been transformed through the delineation of causative genes for the closely related condition, atypical HUS (aHUS)[5]. aHUS describes patients with HUS without STEC infection, approximately 60% of whom have defects of the alternative complement pathway. Recent trials in aHUS show remarkable benefit from Ecu (Ecu, a monoclonal antibody that inhibits complement)[6,7]. There is clear evidence that Stx mediates glomerular endothelial thrombotic microangiopathy (TMA)[8], the pathological hallmark of STEC HUS, and there is increasingly compelling evidence that complement plays a role in pathogenesis. Patients exhibit transiently low plasma complement C3 levels during acute disease, indicating consumption, which correlates with disease severity. Serum complement activation products are elevated in the acute phase and correlate with disease severity[9]. Stx binds to and inhibits the protective function of complement factor H on cell surfaces which may make cells vulnerable to complement attack. Stx directly activates complement in fluid phase in vitro and reduces glomerular endothelial cell expression of another protective

complement regulator, CD59. Stx-treated endothelial cells show C3 deposition when treated with human serum (reviewed in [10]). Together, these findings suggest that Stx can activate complement and inhibit protective complement regulators. In a murine model of STEC HUS, complement blockade was protective against severe disease[11]. In contrast, no evidence of complement activation was detected in a nonhuman primate model of STEC HUS[12].

Ecu has been administered in STEC HUS. Lapeyraque described its use in three severely affected children with CNS involvement[13]. All three showed dramatic resolution of CNS symptoms. Ecu use was reported in three further children with STEC HUS and CNS involvement with prompt resolution of CNS features[14], though one later died from an intracranial haemorrhage, and in a series of seven children with acute CNS features[15], two of whom died and five had normal clinical and neuropsychological evaluations at 6 months. In 2011, 845 STEC HUS cases due to STEC O104 occurred in a well-publicised German outbreak, which was uncharacteristic of STEC HUS in UK children (most patients were adult females and there were higher rates of severe disease and CNS involvement). Ecu was given to 198 patients in a prospective trial with no control arm[16]. It was administered usually after plasma exchange, several days into hospital admission, and only to those most severely affected. Initial reports (abstracts) claim “rapid and sustained clinical improvements in TMA and systemic organ complications”[16]. However without a comparator group, this conclusion cannot be validated. One group compared best supportive care with plasma exchange with and without Ecu in adult patients[17]. Compared with those receiving plasma exchange (either with or without Ecu), here was a higher rate of death in the best supportive care group, but lower median hospital stays and creatinine at discharge. However, the best supportive care patients were older and some refused dialysis. Nine French patients (including one child) were given Ecu in the same outbreak, with no comparator group[18]. The authors concluded that early treatment with Ecu was associated with a rapid and efficient recovery. Loos reported paediatric data from this outbreak[19]. Of 90 children, 74% received supportive care, 19% received plasma exchange and 13 (14%) received Ecu, of whom 7 also had plasma exchange. Across all treatment groups, one patient died, 4.4% were left with CKD and 22% had ongoing CNS problems. The authors concluded that plasma exchange and Ecu showed no short-term benefit.

2.2. Rationale for current trial

The use of Ecu for the treatment of severe STEC HUS is increasing internationally, with no objective evidence of efficacy or safety in children or adults, and at a huge cost to the NHS

and other health services. There are no published prospective, controlled evaluations of Ecu in STEC HUS. There is a trial of Ecu currently being undertaken in France. ECULISHU (https://www.clinicaltrials.gov/ct2/show/NCT02205541) is a single-blind randomised controlled trial of Ecu in STEC HUS. ECULISHU examines whether giving 3 to 5 doses of Ecu reduces the severity of renal disease in children with STEC HUS, but without extra-renal involvement. Further, in the studies described above (in section 2.1), Ecu was administered late in the disease process and after other therapeutic strategies. However, there are data suggesting that complement activation occurs early in the disease process. It is therefore important that the efficacy and safety of Ecu in STEC HUS is properly evaluated in a prospective randomised controlled trial.

In our study, ECUSTEC, we are assessing giving Ecu early in the disease course, have a wider objective, to consider reduction in the overall disease severity, rather than just renal disease severity, have a double-blind design and are examining fewer doses of Ecu. ECUSTEC will also provide a health economic analysis to allow further assessment of the role of Ecu in managing STEC HUS in children.

2.3. Mechanistic Studies

Our current work, both in vivo and in vitro, supports the hypothesis that the podocyte is a central target of Shiga-toxin damage, which disrupts endothelial complement regulation via a reduction in podocyte Vascular Endothelial Growth Factor (VEGF) secretion, resulting in TMA[20]. We will seek evidence to support this hypothesis in patients with STEC HUS. Podocytes normally produce VEGF, which maintains the healthy glomerular endothelial phenotype[21]. A concept changing study by Eremina et al[22] demonstrated that reduced podocyte production of VEGF leads to glomerular endothelial TMA (the hallmark of HUS). Most work on STEC HUS views the glomerular endothelial cell as the target of Stx. We have generated a considerable body of preliminary work showing that Stx directly targets human podocytes to reduce podocyte VEGF secretion. Alongside this we have shown that VEGF upregulates protective complement factors on glomerular endothelial cells, and *in vivo* that podocyte specific VEGF-KO results in decreased glomerular endothelial Factor H expression (a protective factor) and increased complement deposition in the glomerular endothelium (C3 and C4). We propose to investigate whether systemic complement activation in acute STEC HUS reflects kidney complement activation. We will then relate this level of complement activation to markers of AKI and clinical severity. Furthermore, we will directly test reduced podocyte production of VEGF by measuring this in a systematic way in patient

urine during the disease course. Finally we will use the opportunity to collect patient neutrophils during active disease to directly test their role in delivering Stx to the podocyte, in co-culture experiments. These studies will together contribute work to develop a disease activity score to target Ecu treatment to those at high risk of severe disease.

Mutations in genes encoding complement proteins have been described in HUS triggered by Stx with affected patients reported to exhibit a more severe phenotype[23]. We will therefore interrogate the complement pathway genes (and other genes previously described) associated with aHUS[24] and assess the presence of certain allelic characteristics by clinical outcome. Common polymorphisms known to affect complement regulation will be compared to our previously established UK control population and to the ExAC database, to assess enrichment in the STEC-HUS group.

2.4. Risk and benefits

Ecu is licensed in paediatric patients ≥5kg with aHUS. The proposed dosage to be used in ECUSTEC is the same as that for aHUS in published clinical trials[7] and given in the Summary of Product Characteristics (SmPC) version updated on the 28th June 2016 (www.medicines.org.uk/emc/medicine/19966). Thus Ecu for STEC HUS is a re-purposed intervention, and the safety profile in the trial age group is well established. Two doses of Ecu are expected to provide complement blockade for at least 14 days, which corresponds with the timing of complement activation in STEC HUS[9]. Ecu is currently indicated for chronic administration in aHUS. Since STEC HUS is an acute disorder, and evidence shows that complement activation is transient[9], there is no rationale for chronic administration.

For trial participants, potential benefits may include a reduction in the acute severity of STEC HUS, and in long term complications such as CKD and CNS damage. The main risk for trial participants is a transient increase in the risk of meningococcal disease (approximately 0.3 cases of meningococcal disease per 100 patient years of Ecu use[25] – this equates to a risk of less than 1 in 4,000 for each trial participant), which will be minimised by the use of vaccination and prophylactic antibiotics. If effective, Ecu may prevent the need for acute dialysis in some patients and reduce its duration in others, thus reducing the risk of CKD (including need for chronic dialysis and renal transplantation). It may also reduce the incidence of chronic sequelae such as diabetes and neurodevelopmental disability.

Aims and Objectives

Research Objectives

3. In children aged 6 months to <19 years inclusive, we intend:

3.1.

1. To determine whether the severity of STEC HUS is less in those given Ecu compared with those given placebo
2. To assess the safety of Ecu in STEC HUS
3. To determine whether the incidence of CKD following STEC HUS is less in those receiving Ecu compared with those receiving placebo
4. To evaluate the cost-effectiveness of administration of Ecu in STEC HUS from the perspective of the NHS

Exploratory Objectives

3.2. Participants will be given the opportunity to participate in optional exploratory studies to increase understanding of the pathogenesis of STEC HUS. Exploratory studies will include:

1) To test the hypothesis that TMA in STEC HUS occurs via a Stx-mediated reduction in podocyte VEGF production leading to loss of key complement regulation we will:
    a. Investigate the time-course of systemic complement activation in STEC HUS
    b. Investigate whether complement activation correlates with markers of AKI and clinical severity
    c. Measure urinary VEGF systematically to determine whether podocyte VEGF production is reduced in STEC HUS
2) To test the role of patient neutrophils in delivering Stx toxin to the podocyte, in co-culture experiments (in selected patients)
3) To investigate genetic susceptibility to STEC-HUS by determining the frequency of genetic variants associated with altered complement regulation
4) To identify potential novel pathogenic mechanisms by undertaking whole exome sequencing of DNA of trial participants

4. Trial Design and Setting

4.1. Trial Design

Randomised, parallel-group, double-blind, placebo-controlled trial of Ecu in 134 children aged 6 months to less than 19 years, with STEC HUS.

4.1.1. Internal Pilot and "Stopping Rules''

The trial contains an internal pilot phase of 18 months (12 months recruitment, 6 months follow-up), the purpose of which is to determine whether the substantive trial will continue. Recruitment will continue during the 6 months follow-up period of the pilot phase. At the end of the pilot phase, the following progression rules will be used to guide the decision process as to whether the trial continues:

- That 26 participants are recruited in 12 months;
- That 20 of the 26 recruited participants (i.e. 10 of 13 participants in each arm) received the planned two doses of trial treatments as per the trial protocol
    - to assess logistics since some participants may be discharged prior to day 8, so will need to return to the renal unit to receive the second study dose
    - to assess tolerability as participants may be too unwell or may not have tolerated first dose, so do not receive the second dose;
- That at least 22 of the 26 recruited participants have completed 26 weeks follow up. This also includes assessing the completion of the primary outcome at 60 days. The clinical severity score is composed of multiple domains, all of which need to be completed in order to assign a score. Participants entered into the pilot phase of the trial will be monitored to check for any issues in the completion of the primary outcome so this can be addressed.
- That the independent Data Monitoring and Ethics Committee (DMEC) have reviewed the safety and efficacy data on the first 26 participants, and have not identified any tolerability (such that participants do not receive the two planned doses) or safety concerns (e.g. increase in meningococcal infection) with the use of Ecu in this patient population. The DMEC will also review the efficacy data using the trial primary outcome, the clinical severity score at 60 days post-randomisation, and a futility analysis will be performed. It will then be the responsibility of the DMEC to judge based on the interim data and futility analyses, whether there is sufficient evidence to support stopping the trial early as evidence of efficacy is proven or will not be proven.

The internal pilot phase will also enable us to:

- Identify any logistical issues or barriers to recruitment, which can be addressed, and the trial protocol revised if needed;
- Assess whether centres comply with their agreement to avoid giving plasma exchange in trial participants;
- Review the sample size assumptions, whilst accepting that the numbers in the internal pilot are small.

Outcome Measures

4.2 4.2.1. Primary Outcome Measure

The primary outcome measure is a purpose-developed, multi-domain clinical severity score (Appendix 1). A single score is assigned at day 60 to reflect cumulative morbidity up until that point.

The STEC HUS clinical severity score is a multi-domain score comprising severity of AKI and extra-renal events, developed by the protocol contributors for use as an outcome measure. The score has a range of 1 to 69; with higher scores indicating greater disease severity. Since severity of AKI is a significant prognostic factor, the score is weighted for severity of the AKI[2].

4.2.2. Secondary Outcome Measures

1. Overall survival
2. Duration of renal replacement therapy (days)
3. Duration of thrombocytopenia (number of consecutive days until platelet count >150x10^9/l)
4. Duration of haemolysis (number of days until lactate dehydrogenase (LDH) within local centre reference range)
5. Number of packed red blood cell transfusions required and volume (ml/kg)
6. Duration markers of inflammation present (number of days until neutrophil cell count and C-reactive protein are in normal range for that centre)
7. CKD at 52 weeks (a composite endpoint of the presence of hypertension [>95th centile for systolic blood pressure over an average of 3 readings by manual method using centile charts[26] for age/sex/height], albuminuria [urine albumin-creatinine ratio

>2.5mg/mmol on early morning urine] or estimated Glomerular Filtration Rate eGFR<90ml/min/1.73m^2 at 52 weeks). Presence of any of these will constitute CKD at 52 weeks.

8. eGFR measurement using a centralised cystatin C assay at 52 weeks.
9. Persistent neurological defect at day 60 measured by structured expert assessment to include CNS examination, vision, hearing and neuropsychological assessment
10. Economic evaluation of cost per clinical severity score point, and cost per QALY gained, using Paediatric Quality of Life Inventory (PedsQL) and Child Health Utility-9D (CHU-9D) assessments to measure health related quality of life (HR QoL).

Trial Setting and Recruitment

4.3 Patients with STEC HUS usually present to district hospitals (or the general paediatric department at regional centres) and are discussed with the regional paediatric renal unit. UK paediatric renal units have agreed to participate in the trial and promote it within their regional networks, encouraging early referral for consideration for participation in the trial. Referring district hospitals will be designated as Patient Identification Centres (PIC) where possible, and potentially eligible participants will be identified either at the PIC or upon referral to the renal unit.

The sequence and mechanism of referral will be as follows:

- The child is seen by a general paediatrician at the district hospital (which may or may not be a PIC) and STEC HUS is suspected (all inclusion criteria do not need to be met at this point)
- Clinical stabilisation takes place at the district general hospital/PIC
- The general paediatrician telephones the regional paediatric nephrologist on-call
- The paediatric nephrologist will accept the referral and arrange transfer which will be documented in the hospital notes
- If the child does not require transfer for clinical purposes, the paediatric nephrologist will explain that transfer will be accepted earlier than usual so that participation in the trial can be offered and explain that there will be no obligation to enter the trial
- Information regarding ECUSTEC will be offered pre-transfer if the referring hospital is a PIC – this will consist of an introductory letter (the ECUSTEC Letter for Participant Identification Centres) which gives a brief outline of the trial, so that participation in ECUSTEC can be explored further after arrival at the renal unit.

- If the parent/guardian/patient states that they do not wish to consider the ECUSTEC trial this information should be passed to the renal unit so as they are not approached further about the trial.

After arrival and stabilisation at the renal unit, and once a working clinical diagnosis of STEC HUS has been established, potential participants will undergo assessment for eligibility. Once eligibility is confirmed by a medically qualified doctor, the parents/guardians will be approached by an appropriately trained member of the clinical team who will discuss the trial with them, giving a comprehensive verbal explanation of the trial and time for them to ask questions. Ethically approved, written, Participant Information Sheets (PIS) will be provided. Age appropriate PIS for children and young people will also be available. If there is agreement to enter the trial, informed consent will be sought from the parent/guardian or patient if age ≥16 years, and assent from younger children if appropriate (according to age). **Although it is customary to provide information about a trial more than 24 hours before seeking informed consent, this will not be possible for ECUSTEC participants**. Our hypothesis is that Ecu is most effective when given early in the disease course. Thus participants/parents/guardians will be required to make a decision about the trial such that the trial drug is administered within 48 hours of arrival at the renal unit. In practice, the 48 hour clock starts at the time the on-call paediatric nephrology consultant formally takes over care of the patient at the trial site, providing they meet inclusion criteria 3 at the time of this formal commencement of paediatric nephrology care. If the patient does not meet inclusion criteria 3 at formal commencement of paediatric nephrology care, the 48 hour clock will start at the point when inclusion criteria 3 is met. Each participating centre will have specific logistic arrangements that will facilitate recruitment to the trial. However, since the window between arrival and recruitment is short, there may be some patients who cannot be offered participation for logistic reasons.

To conform to CONSORT guidelines, participating centres will be requested to complete an anonymised log (ECUSTEC Screening Log) for all patients screened including those who could not be randomised due to ineligibility, because they declined participation, because of local operational issues or any other reason.

Eligibility

Inclusion Criteria

5. The trial population will be children who fulfil the following inclusion criteria:

5.1.

1. Age 6 months to <19 years
2. Weight ≥5kg
3. Diagnosis of HUS
   a. Micro-angiopathic haemolytic anaemia (indicated by fragmented red cells on blood film **OR** plasma lactate dehydrogenase above local centre reference range)

   AND

   b. Thrombocytopenia (platelets <150x10^9/l)*

   AND

   c. Acute Kidney Injury (AKI): "injury" or "failure" category of pRIFLE criteria[27] (Table 1) despite correction of hypovolaemia (as per Figure 1)
4. **EITHER**

   Reported diarrhoea within 14 days prior to diagnosis of HUS (defined according to World Health Organisation as "the passage of three or more loose or liquid stools per day - or more frequent passage than is normal for the individual")

   OR

   Stool culture or shiga toxin polymerase chain reaction (PCR) or STEC serology result indicating STEC in the patient

   OR

   Household contact within 14 days prior to diagnosis of HUS
5. Patient intended to be able to receive trial drug within 48 hours of the on-call paediatric nephrologist formally taking over the care of the patient at the trial site providing inclusion criteria 3 is met, or within 48 hours of meeting inclusion criteria 3 if not met at the time the on-call paediatric nephrologist takes over the care of the patient.
6. Sexually active male or female patients must agree to be practicing an effective, reliable and medically approved contraceptive regimen for 6 months after enrolment. See section 5.3.2 for details on acceptable methods of effective contraception for this trial.
7. Sexually active female patient has provided a negative pregnancy test ≤48 hours prior to randomisation

8. Patient/parent/guardian reported that vaccinations are up to date according to the routine UK (or equivalent) immunisation schedule.*†
9. Written informed consent obtained from the patient's parents/guardians and written assent obtained from patients (where age appropriate). Patients aged 16 years and above will provide their own written consent.

* If the patient has received a platelet infusion prior to randomisation, the lowest documented platelet count prior to platelet infusion should be used.

† It is required that vaccination against Haemophilus influenza b and pneumococcus are complete. If vaccination against other organisms (e.g. MMR, HPV) is incomplete the patient remains eligible.

The RIFLE criteria are an accepted definition of AKI devised by the Acute Dialysis Quality Initiative. pRIFLE is a modified, validated paediatric version of the RIFLE criteria which is widely used in studies of paediatric AKI[27]. The ECUSTEC inclusion criteria require the "injury" or "failure" category to be reached – (Table 1). The eGFR can be calculated either from creatinine measured at the referring general paediatric unit or at the renal unit.eGFR will be determined by the Schwartz formula:

$$eGFR = height\ (cm) \times 36.5 / plasma\ creatinine\ (\mu mol/l)$$[28]

Height will be measured in order to determine eGFR, although if the clinical scenario precludes height measurement, this should be estimated from the corresponding centile from the child's weight.

	eGFR (Schwartz)		Urine output
Risk	≤ 75	OR	< 0.5 ml/kg/hr for 8 hrs
Injury	≤ 50		< 0.5 ml/kg/hr for 16 hours
Failure	≤ 35		< 0.3 ml/kg/hr for 24 hours or anuria for 12 hours
Loss	Need for Renal Replacement Therapy for > 4 weeks		
End-Stage	Need for Renal Replacement Therapy for > 3 months		

Table 1. pRIFLE criteria for Acute Kidney Injury[27]

Prior to confirmation of eligibility, the following protocol should be followed to ensure that "injury" or "failure" category of pRIFLE is met despite correction of hypovolaemia (Figure 1). To ensure recruitment remains possible within 48 hours, work towards potential enrolment should continue whilst awaiting the outcome of saline administration.

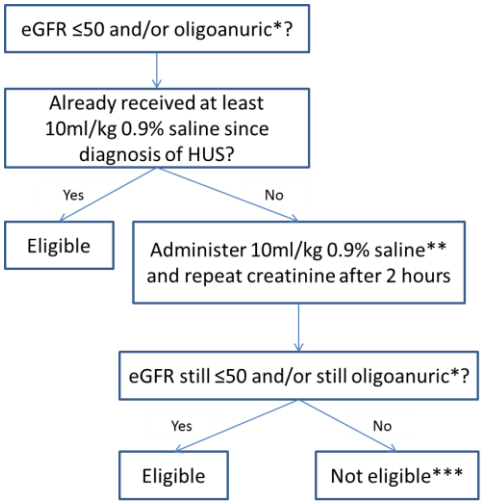


Figure 1. Protocol to ensure hypovolaemia is corrected. eGFR expressed as ml/min/1.73m^2

* oliguria defined as urine output <0.5ml/kg/hour for ≥16 hours

** unless clinical signs of hypervolaemia

*** may become eligible if renal function deteriorates further. An alternative crystalloid or colloid to 0.9% saline may be used if clinically indicated

5.2. Exclusion Criteria

Patients will not be entered if they fulfil any of the following exclusion criteria:

1. Family history of atypical HUS
2. Previous episode of HUS
3. Known pre-existing eGFR <90ml/min/1.73m^2
4. Known or suspected pneumococcal infection
5. Known or suspected meningococcal infection
6. Prior to diagnosis, patient taking a drug known to be associated with HUS, e.g. calcineurin inhibitors, chemotherapy, quinine, oral contraceptive pill
7. Hypersensitivity to Ecu, murine proteins or any of the excipients listed in the Summary of Product Characteristics
8. Pregnancy or lactation
9. Malignancy
10. Known Disseminated Intravascular Coagulopathy*

11. Refusal of consent, including consent for pregnancy testing, meningococcal vaccination or antibiotic prophylaxis
12. Currently participating in another clinical trial of an investigational medicinal product.

* testing of coagulation is not mandatory for inclusion in trial

5.3. Pregnancy and Birth Control

Sexually active female patients and male patient's partners should be advised to avoid becoming pregnant while receiving treatment with eculizumab.

The effect of eculizumab on the development of an embryo, foetus or unborn child is currently unknown. The outcome of pregnancies of participants will therefore be monitored in order to provide SAE data on congenital anomalies or birth defects. If a patient or the partner of a male trial patient becomes pregnant during the trial BCTU must be informed immediately (See section 10.6.4; for details on the reporting procedure).

5.3.1. Pregnancy Testing

All sexually active female patients must consent to and undergo a pregnancy test and provide a negative pregnancy test ≤48 hours prior to randomisation.

5.3.2. Contraceptive Advice

The effect of eculizumab on the development of an embryo, foetus or unborn child is currently unknown. Therefore sexually active male or female patients must agree to practice an effective, reliable and medically approved contraceptive regimen for 6 months after enrolment into the trial.

Acceptable methods of effective contraception for this trial are:

- Established* use of oral, injected or implanted hormonal methods of contraception.
- Placement of an intrauterine device (IUD) or intrauterine system (IUS).
- Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository). The use of barrier contraceptives should always be supplemented with the use of a spermicide.

The following should be noted:

- Failure rates indicate that, when used alone, the diaphragm or condom are not highly effective forms of contraception. Therefore the use of additional spermicides does confer additional theoretical contraceptive protection.
- However, spermicides alone are inefficient at preventing pregnancy. Therefore, spermicides are not a barrier method of contraception and must not be used alone.

- Absolute and continuous abstinence: when this is in line with the preferred and usual lifestyle of the patient. Please note that periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

*since use of combined oral contraceptive pill prior to randomisation is an exclusion criteria, this refers to established use after randomisation and alternative contraceptive measures should be used until combined oral contraceptive pill is established.

The suitability of the contraceptive method chosen for the individual participant needs to be approved by their responsible clinician.

6. Consent

It will be the responsibility of the Principal Investigator (PI) to ensure written informed consent is obtained for each participant prior to performing any trial related procedure. The responsibility for obtaining consent may be delegated by the PI to another clinician as captured on the ECUSTEC Site Signature and Delegation Log, but the PI must ensure that this has occurred prior to any trial related procedures being performed on the participant.

Parent/Guardian and Participant Information Sheet will be provided to facilitate this process. Investigators or delegate(s) will ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the potential participant and/or parent/guardian. They will also stress that participation is voluntary and that the potential participant and/or parent/guardian is free to refuse to take part and may withdraw from the trial at any time. The potential participant and/or parent/guardian will be given time to read the PIS and to discuss participation with others outside of the site research team.

The potential participant and/or parent/guardian will be given the opportunity to ask questions.

The Investigator or delegate(s) and potential participant or parent/guardian will then sign and date an Informed Consent Form (ICF). A copy of the ICF will be given to the participant and/or parent/guardian, a copy will be filed in the medical notes, and the original placed in the Investigator Site File (ISF). In addition, if the participant has given explicit consent a copy of the signed ICF will be sent to the Trials Office for review. Once the participant is randomised into the trial, the participant's unique trial identification number will be entered on the ICF maintained in the ISF.

Details of the informed consent discussions should be recorded in the participant's medical notes in accordance with Good Clinical Practice (GCP). This should include date of discussion, the name of the trial, outcome of the discussion, version number of the PIS given to participant, version number of ICF signed and date consent received, and that the person signing the consent form on behalf of the child has been determined to have the parental responsibility to do so. If a translator has been used this should be noted in the patient medical records. Where consent is obtained on the same day that the trial related assessments are due to start, a note should be made in the medical notes as to what time the consent was obtained and what time the procedures started. A copy of the PIS should be added to the medical notes.

If the participant reaches the age of 16 years once they are in the trial they can then give their own consent using the ECUSTEC Patient 16-18yrs consent form in association with the ECUSTEC Patient 16-18yrs PIS. This process should be recorded in the participant's medical notes. A copy of the ICF will be given to the participant, a copy will be filed in the medical notes, and the original placed in the Investigator Site File (ISF). In addition, if the participant has given explicit consent a copy of the signed ICF will be sent to the Trials Office.

At each visit the participant's and/or parent/guardian's willingness to continue in the trial should be ascertained and documented in the medical notes and on the study Case Report Form (CRF). Throughout the trial, the participant and/or parent/guardian will have the opportunity to ask questions about the trial. Any new information that may be relevant to the

participant's continued participation will be provided. Where new information becomes available which may affect the participants' and/or parent/guardian's decision to continue, participants and/or parent/guardians will be given time to consider and if happy to continue will be re-consented. Re-consent will be documented in the medical notes. The participant's and/or parent/guardian's right to withdraw from the trial will remain.

Electronic copies of the template PIS and ICF will be available from the Trials Office and the trial website (www.birmingham.ac.uk/ecustec) and will be printed or photocopied onto the headed paper of the local institution. Details of all participants approached about the trial will be recorded on the ECUSTEC Participant Screening/Enrolment Log and, with the participant's prior consent, their General Practitioner (GP) will also be informed that they are taking part in the trial using the EUSTEC Initial Letter to GP.

7. Enrolment and Randomisation

7.1. Vaccination and prophylactic antibiotics prior to randomisation

After all eligibility criteria have been confirmed and informed consent has been received, the **participants must start prophylactic antibiotics before they can be randomised into the ECUSTEC trial**. Prophylactic antibiotics will be continued orally for a period of 8 weeks. The patient should also be given meningococcal vaccination prior to randomisation unless platelet count is $<50x10^9$/l, in which case vaccination will be deferred until the platelet count is stable at $\geq 50x10^9$/l because of the risk of bleeding with intramuscular injection. Vaccination should also be deferred in those on systemic anticoagulation. Please refer to Section 8.2 for further details.

7.2. Randomisation

Randomisation will be provided by a secure online randomisation system at the Birmingham Clinical Trials Unit (BCTU) (available at https://www.trials.bham.ac.uk/ecustec). Unique log-in usernames and passwords will be provided to those who wish to use the online system and who have been delegated the role of randomising participants into the study as detailed on the ECUSTEC Trial Signature and Delegation Log. The online randomisation system will be available 24 hours a day, 7 days a week, apart from short periods of scheduled maintenance. A telephone toll-free randomisation service 0800 953 0274 is available

Monday to Friday, 09:00 to 17:00 UK time, except for bank holidays and University of Birmingham closed days.

After participant eligibility has been confirmed, informed consent has been received and prophylactic antibiotics have been started, the participant can be randomised into the trial. ECUSTEC Randomisation Notepads will be provided to investigators and should be completed and used to collate the necessary information prior to randomisation. The ECUSTEC Randomisation Notepad must be signed by the investigator to indicate that all of the eligibility criteria have been checked. It should also be noted in the medical records that the investigator has checked all of the eligibility criteria and that the patient meets all of the inclusion criteria and none of the exclusion criteria. The signed ECUSTEC Randomisation Notepad should be kept in the investigator site file and a copy sent to the Trials Office. All questions and data items on the Randomisation Notepad must be answered before a Trial Number can be given. If data items are missing, randomisation will be suspended, but can be resumed once the information is available. Only when all eligibility criteria and baseline data items have been provided will a Trial Number be allocated. Following randomisation a confirmatory email will be sent to the randomising clinician, local Principal Investigator, the named research nurse and the local pharmacy with a copy sent to the Chief Investigator.

Participants will be randomised at the level of the individual in a 1:1 ratio to either Ecu or placebo, which should be started as soon as possible.

A minimisation algorithm will be used to ensure balance in the allocation over the following variables:

- Centre
- pRIFLE category (Injury or Failure, see section 5)
- volume of 0.9% saline received in the 48h prior to randomisation (≤20ml/kg or >20ml/kg)

A ‘random element’ will be included in the minimisation algorithm, so that each patient has a probability (unspecified here), of being randomised to the opposite treatment that they would have otherwise received. Full details of the randomisation specification will be stored in a confidential document at BCTU.

Screening and Recruitment Logs

7.3. Investigators will keep their own study file log which links participants with their allocated trial number in the ECUSTEC Participant Recruitment and Identification Log. The Investigator must maintain this document which is **not** for submission to the Trials Office. The Investigator will also keep and maintain the ECUSTEC Screening Log which will be kept in the ISF, and should be available to be sent to the Trials Office upon request. The ECUSTEC Participant Recruitment and Identification Logs and ECUSTEC Screening Logs should be held in strict confidence. If the ECUSTEC Screening Log is requested by the Trials Office it must be fully anonymised before being sent.

The participant's GP should be notified that they are in the ECUSTEC trial, using the ECUSTEC Initial Letter to GP. The letter should include details of the vaccinations given to the participant.

Blinding

7.4. All site personnel will be blinded apart from those responsible for preparing the IMP (e.g. pharmacy), who will make sure that no other person, will have access to the study drugs and pharmacy documentation, and will remain independent to the treatment of all trial participants. After randomisation, the unblinded staff will receive the treatment allocation electronically from BCTU. The unblinded staff will prepare an intravenous (IV) infusion bag containing either sodium chloride 0.9% with Ecu or sodium chloride 0.9% (placebo) alone using aseptic technique. The prepared infusion bag will be labelled, using labels approved by the Sponsor's pharmacy and the Medicines and Health Regulatory Agency (MHRA), in an identical manner to maintain blinding. Trial drug labelling will comply with the applicable regulatory requirements and clinical trial specific labels will be attached to all treatment by local pharmacy.

7.5. Researchers at the co-ordinating centre and Sponsor will be similarly blinded, knowing only that an allocation has been made, but not the nature of the allocation, unless it becomes necessary to unblind (see below).

Emergency Unblinding

The blinded trial treatment allocation will only be broken for valid medical or safety reasons.

When to unblind:

During the time when the patient is taking trial treatment (the first 14 days), unblinding should only occur when there is a clinical need to know whether the patient is receiving Ecu, such that knowing which treatment the patient received will alter clinical management. For example:

i) the patient develops a condition where Ecu is contraindicated (e.g. untreated meningococcal sepsis)

ii) the patient develops a condition where Ecu treatment is required (e.g. atypical HUS is subsequently suspected). In this case it is recommended that the PI or delegate contacts the consultant on-call for the National aHUS Service (available at all times through switchboard at Newcastle Hospitals NHS Foundation Trust on 0191 233 6161) prior to unblinding to discuss the case.

iii) the research team at site become aware that the patient is, or may be, pregnant

Unblinding will also be required if the research team at site become aware that the patient (or their partner) is pregnant during the first 6 months from enrolment.

How to unblind?

The Principal Investigator, or co-investigator(s) listed on the delegation log in the Principal Investigator's absence, will have a secure login and password to access the ECUSTEC online system where the allocation will be revealed following entry of the necessary details. An email will be generated to alert the ECUSTEC Trials Office that the participant has been unblinded but the treatment allocation will not be revealed.

If it becomes necessary to unblind, only those who need to know the treatment allocation will be informed, thus where possible, other members of the research team at site will remain blinded, subject to clinical need. Unblinded participants will remain in the trial, and continue with trial follow-up assessments.

7.6 Unblinding in the event of a potential SUSAR

All potential SUSARs will be unblinded prior to reporting to the MHRA by the Sponsor in accordance with regulations. See Section 10.3 for further details.

8. Trial treatment / intervention

8.1. Name and description of Investigational Medicinal Products

IMPs	NIMPs
Eculizumab	Tetravalent Neisseria meningitis vaccine
Placebo (0.9% saline)	Bexsero™
	Phenoxymethylpenicillin
	Erythromycin

Table 2. Name of Investigational Medicinal Products

Arm	IMPs	Formulations
Eculizumab	Eculizumab	Brand: Soliris Formulation: 10mg/ml Concentrate for solution for infusion (30ml vial)
Placebo	Sodium chloride 0.9%	Brand: any brand with marketing authorisation within EEA Formulation: Intravenous Infusion bags

Table 3. Acceptable formulations of Investigational

8.2. Planned Interventions

Prevention of meningococcal disease

Ecu increases children's susceptibility to meningococcal disease, particularly due to uncommon serogroups (e.g. Y, W and X), although meningococcal disease due to any serogroup (including B or C) may occur.

To reduce the risk of meningococcal disease, all ECUSTEC trial participants should be given:

1. **Antibiotic prophylaxis**
2. **Vaccination against meningococcus**

3. Information on the early features of meningococcal disease

8.2.1. Antibiotic prophylaxis against meningococcus

All ECUSTEC trial participants should receive antibiotic prophylaxis against meningococcal disease for 8 weeks (see Table 4). Children who are allergic to penicillin should receive erythromycin. (If intolerant/allergic to erythromycin as well, discuss with a Microbiologist). Although there is evidence that some antibiotics may increase the severity of STEC HUS due to enhanced Stx release, penicillin and erythromycin do not increase Stx release[29]. **Participants should be supplied with two weeks of antibiotics upon discharge.**

It is essential that all participants receive their first dose of prophylactic antibiotics (see below) prior to receiving the first dose of trial drug. This is an eligibility criteria for the trial so participants cannot be randomised until the eligibility criteria is met.

Prophylactic antibiotics should be administered as follows:

Penicillin	
Age	Dose
6 months to <1 year	62.5 mg twice daily for 8 weeks
1–5 years	125 mg twice daily for 8 weeks
6–<19 years	250 mg twice daily for 8 weeks
Erythromycin if penicillin allergic	
Age	Dose
6 months – 2 years	125 mg twice daily for 8 weeks
>2–<9 years	250 mg twice daily for 8 weeks
9–<19 years	500 mg twice daily for 8 weeks

Table 4. Antibiotic prophylaxis

If oral antibiotic administration is not possible, intravenous penicillin should be administered at a dose of 25mg/kg twice daily until oral medication can be tolerated. In case of penicillin allergy and requirement for IV prophylaxis, discuss with Microbiologist.

8.2.2. Vaccination against meningococcus

Both Meningococcal B vaccine (Bexsero™) and conjugate meningococcal ACWY vaccine (Nimenrix or Menveo) will be given to all ECUSTEC trial participants (unless either have already received as part of an immunisation programme – see below). A flow chart is provided in Figure 2.

Meningococcal B vaccine (Bexsero™)

Bexsero™ is a newly licensed meningococcal vaccine which has been included in the routine immunisation schedule for the UK since 1st September 2015 for infants at 2, 4 and 12 months of age. Children born in the UK on or after 1st May 2015 will have been offered Bexsero™ within their routine immunisation schedule. If parents/guardians report that their child has received Bexsero™ as part of their routine immunisation schedule, further Bexsero™ vaccination is not required. However, vaccination status should be verified with their GP team at the earliest opportunity after receiving trial drug. If previous vaccination cannot subsequently be confirmed, Bexsero™ should be administered at the earliest opportunity (See Figure 2).

ACWY vaccine has been part of the UK immunisation protocol for children aged 14 since Autumn 2015. If parents/guardians report that their child has received ACWY vaccine as part of their routine immunisation schedule, further ACWY vaccination is not required. However, vaccination status should be verified with their GP team at the earliest opportunity after receiving trial drug. If previous vaccination cannot subsequently be confirmed, ACWY vaccine should be administered at the earliest opportunity (See Figure 2).

Prophylactic paracetamol at the time of vaccination and 2 further doses every 4-6 hours after vaccination, can reduce the incidence and intensity of post-vaccination febrile reactions and does not reduce the response to Bexsero™ (www.medicines.org.uk/emc/medicine/28407).

Meningococcal B vaccine (Bexsero™) should be administered **prior to first dose of trial drug** UNLESS

i. Platelet count <50x10^9/l (if so, vaccination should occur once platelet count is stable at 50x10^9/l or above, and certainly before discharge from the trial centre)

OR

ii. Patient is receiving systemic anticoagulation (not including intermittent use of anticoagulation during haemodialysis). If so, vaccination should occur once anticoagulation has been stopped for 24 hours and not anticipated to recommence, and certainly before discharge from the trial centre.

iii. Parent/guardian reports that Bexsero (or "Meningitis B vaccine") has been received as part of the UK (or equivalent) immunisation programme

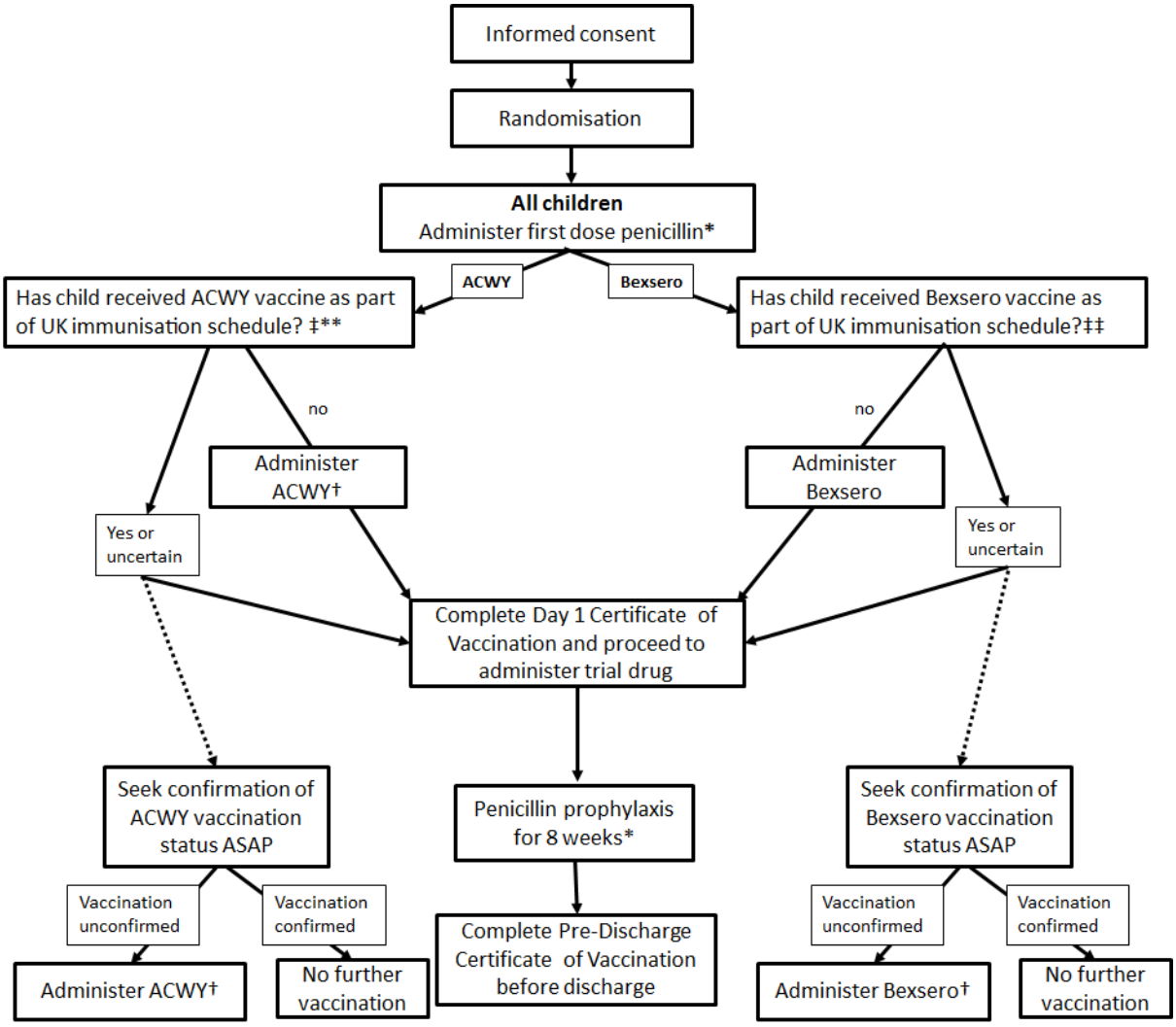
OR

iv. Parent/guardian is uncertain whether Bexsero has been received as part of the UK (or equivalent) immunisation programme

Previous Bexsero vaccination (as in ii) can be indicated verbally by parents/guardians but needs confirmation (e.g. red book documentation or written confirmation by GP practice team) prior to discharge from the trial site. Uncertain vaccination status (as in iii) should be clarified at the earliest opportunity with the GP practice team. If Bexsero vaccination is not confirmed, vaccination should take place prior to discharge from the trial site.

Feedback from parents about the trial protocol suggests that participants/parents/guardians are likely to welcome the option of completing the primary course of Bexsero™ vaccination even though the risk posed by Ecu would no longer be present. Funding for this is available within the ECUSTEC trial. Second doses can be offered and administered on day 60 (but this is optional for participants/ parents/guardians). Children under 2 would need a third dose, a booster, 12 months after completing the primary course. This would not be offered or administered by the ECUSTEC team, but a letter would be sent to the child's GP with details of how to claim remuneration if the participant/ parents/guardians wish this to be undertaken by their GP. Please use the ECUSTEC Meningococcal Day 30 Letter to inform participants/parents/guardians about their options at the day 30 trial visit (different versions of the letter are provided for children under or over 2yrs and for participants aged 16-18yrs). The GP should claim reimbursement from commissioners referencing the ECUSTEC trial and contact R&DFinancepreaward@nuth.nhs.uk for any queries.

Figure 2: Flow chart for prevention of meningococcal disease in ECUSTEC trial participants



* or erythromycin if penicillin allergic
**<2yrs Nimenrix; ≥2yrs Menveo or Nimenrix
† If platelet count <50x10⁹/l defer vaccination until platelet count >50x10⁹/l; if receiving systemic anti-coagulation defer vaccination until 24hrs after stopping anti-coagulation.
‡ ACWY is part of the UK immunisation programme for children aged 14yrs since Autumn 2015.
‡‡ Bexsero is part of the UK immunisation programme for children born on or after 30th April 2015.

Conjugate meningococcal ACWY vaccine (Nimenrix or Menveo)

In ECUSTEC participants we recommend Nimenrix for children under 2 years old. Children ≥2 years of age can be given either Nimenrix or Menveo.

This should be administered **prior to first dose of trial drug** UNLESS

i. Platelet count <50x10^9/l (if so, vaccination should occur once platelet count is 50x10^9/l or above, and certainly before discharge from the trial centre)

ii. Patient is receiving systemic anticoagulation (not including intermittent use of anticoagulation during haemodialysis). If so, vaccination should occur once anticoagulation has been stopped for 24 hours and not anticipated to recommence, and certainly before discharge from the trial centre.

iii. Parent/guardian reports ACWY vaccine has been received as part of the UK (or equivalent) immunisation programme

OR

Parent/guardian is uncertain whether ACWY vaccine has been received as part of the UK (or equivalent) immunisation programme

8.2.3. Information on the early features of meningococcal disease

All ECUSTEC trial participants should be informed of the features of meningococcal disease and how to access medical care immediately if they develop these features. Participants/parents/guardians will be given an ECUSTEC Meningitis Warning Card alerting them to signs and symptoms of meningococcal disease. An ECUSTEC Participant Card will be provided to participants/parents/guardians and should be carried at all times. It contains information about the participant's involvement in the trial and emergency contact details for local ECUSTEC staff. Website address links for further information regarding the early features of meningococcal disease will be provided on the ECUSTEC website (www.bham.ac.uk/ecustec).

Participants/parents/guardians should be reminded of the signs and symptoms of meningococcal disease at each of their assessments. It should be checked that the participants/parents/guardians are in possession of the ECUSTEC Meningitis Warning Card and the ECUSTEC Participant Card. This information should be documented in the patient medical records and the CRF. **If meningococcal disease is suspected urgent medical treatment in accordance with local clinical procedures should be started immediately.**

8.2.4. Documentation to be completed by site regarding meningococcal prevention and routine immunisation schedule

Sites to complete the ECUSTEC Day 1 Certificate of Vaccination for all participants in order for site pharmacist to dispense first dose of Ecu/placebo.

Confirmation that the participant has received Haemophilus influenza and pneumococcal infection vaccinations as part of the UK (or equivalent) immunisation programme must be received (e.g. red book documentation or written confirmation by GP practice team). This will be recorded on the ECUSTEC Day 8 Certificate of Vaccination and the ECUSTEC Pre-Discharge Certificate of Vaccination. Following this process, in the unlikely event that it becomes apparent that Haemophilus influenza and pneumococcal infection vaccinations have not been received, the GP should be notified so that these can be administered as soon as possible after discharge.

If meningococcal vaccination is deferred because of platelet count $<50x10^9$/l, vaccination should be administered as soon as the platelet count rises to $\geq 50x10^9$/l*. **The second dose of IMP will not be released unless the local investigator confirms that both vaccinations have been given (or Bexsero confirmed as not required), and antibiotic prophylaxis is continuing**, EXCEPT in the event that the platelet count remains $<50x10^9$/l when the investigator will agree to proceed to vaccination as soon as the platelet count is $\geq 50x10^9$/l. In this situation vaccination MUST be given prior to discharge from hospital and the Trial Office notified using the ECUSTEC Pre-discharge Certificate of Vaccination. If the platelet count has not risen to $\geq 50x10^9$/l by discharge please contact the CI for advice.

*If the participant has received a platelet transfusion, immunisation should be delayed until the patient has a stable platelet count $\geq 50 \times 10^9$/l.

Sites to send the ECUSTEC Day 1 Certificate of Vaccination for all participants to Alexion and the Trial Office no later than 48 hours after the 1st dose of Ecu (identified by unique trial identifier only).

Sites to complete the ECUSTEC Day 8 Certificate of Vaccination for all participants at day 8 and forward to the local trial site pharmacy in order for trial site pharmacist to dispense second dose of Ecu/placebo. Sites to forward a copy of the certificate to the ECUSTEC

Trials Office no later than 48 hours after the second dose of Ecu/placebo (identified by unique trial identifier only).

Sites to complete the ECUSTEC Pre-discharge Certificate of Vaccination for all participants prior to discharge from trial site and send to Alexion and the Trial Office no later than 48 hours after discharge (identified by unique trial identifier only).

The ECUSTEC Initial Letter to GP should be used to inform the participant's GP that the participant is participating in the trial, details of meningococcal vaccines received, that the participant has already commenced an 8 week course of antibiotics and that the GP will need to prescribe sufficient antibiotics to complete this course.

The ECUSTEC Day 60 Letter to GP should be used to inform the participant's GP that the day 60 assessment and the 8 week course of prophylactic antibiotics has been completed. It should also state whether the 2nd dose of Bexsero™ was administered with full details, if administered, and also whether the 3rd dose of Bexsero™ is indicated and whether participant/parent/guardian wishes to take up this option.

8.2.5. Investigational Medicinal Product

Two doses of Ecu or placebo will be administered in the dosing schedule shown in Table 5: Two doses are expected to provide complement blockade for at least 14 days, which corresponds with the timing of complement activation in STEC HUS. Ecu is currently indicated for chronic administration in aHUS. Since STEC HUS is an acute disorder, and evidence shows that complement activation is transient, there is no rationale for chronic administration.

Patient bodyweight	Day 1			Day 8 (+/- 1 day)		
	Placebo arm	Active arm		Placebo arm	Active arm	
	Volume of 0.9% Saline	Dose of eculizumab	Total infusion volume (made up with 0.9% Saline)	Volume of 0.9% Saline	Dose of eculizumab	Total infusion volume (made up with 0.9% Saline)
≥40 kg	180ml	900mg	180ml	180ml	900mg	180ml
20 to <40 kg	120ml	600 mg	120ml	120ml	600 mg	120ml
10 to <20 kg	120ml	600 mg	120ml	60ml	300 mg	60ml
5 to <10 kg	60ml	300 mg	60ml	60ml	300 mg	60ml

Table 5. Ecu and placebo dosing and infusion schedule

The administration of Ecu or placebo infusion should be initiated as early as practically possible following randomisation and ideally within 24 hours of arriving in the renal unit, but no later than 48 hours (or within 48 hours of meeting inclusion criteria 3 if not met on arrival). The day on which the first dose of trial drug is given is designated Day 1. Ecu or placebo will be administered by a suitably trained nurse (training will depend on the requirements of the individual NHS Trust) by IV infusion over 1 hour (up to 4 hours if necessary). A second dose will be administered 7 days (+/- 1 day) later (i.e. on day 8) by infusion over 1 hour (up to 4 hours if necessary. If participants have been discharged home before the second dose, they will return to site to have it administered as an out-patient. To facilitate this, reasonable travel expenses will be available on request.

If, due to unforeseen circumstances (e.g. loss of venous access, patient in theatre), the trial drug administration window for the first dose of IMP is missed, but randomisation has taken place and the trial drug has been prepared, the initial dose of trial drug should be administered and the time and date of the administration should be recorded in both the CRF and the medical notes. The participant should not be withdrawn from the trial and should receive a second dose as per protocol. The second dose should be given 7 (+/-1) days after the first dose.

8.2.6. Extravasation guidance

Please refer to your Trust guidelines for management of extravasation.

8.2.7. Supportive care

All participants will continue to receive full supportive care as follows:

1. Renal replacement therapy (RRT) will be initiated in the following circumstances:
    - Refractory electrolyte imbalance that poses a risk to the patient
    - Clinical signs of hypervolaemia (diuretics may be tried first if clinically appropriate)
    - Fluid restriction preventing sufficient nutrition
    - Oligoanuria (urine output <0.5ml/kg/hr) >6 hours in the absence of hypovolaemia (diuretics may be tried first if clinically appropriate)

In order to allow for differences in time taken to initiate RRT, the time at which the decision is taken to commence RRT will be recorded as the start of RRT. Stopping of RRT will be

guided by the judgement of the clinical team. The reasons for stopping RRT should be recorded.

2. Red cell transfusion will be performed if Hb <70g/l or if <75g/l with fall of greater than 20g/l evidenced in previous 24 hours. A 3 month course of oral folic acid therapy will be prescribed, to prevent folate deficiency following acute haemolysis.

3. Dialysis modality (haemodialysis vs. peritoneal dialysis vs. haemofiltration) will be guided by the child's clinical condition, preference of the attending team and availability of local expertise.

8.2.8. Plasma Exchange

Plasma exchange (PE) should NOT be used. PE counteracts the effect of Ecu by removing active drug and replacing exogenous plasma C5. PE has been used on an anecdotal basis in UK paediatric renal units. However, there is no published evidence to support the use of PE. Following extensive discussion between units in the lead-up to the ECUSTEC trial, all units have agreed not to undertake PE in ECUSTEC participants.

8.2.9. Plasma Infusion

Clinicians should be aware that plasma infusion counteracts the effect of eculizumab. In the unlikely event that plasma infusion (e.g. fresh frozen plasma, Octaplas™, cryoprecipitate) is clinically required (e.g. for correction of coagulopathy) a record of the product given, date and time of infusion and volume administered should be made on the CRF.

8.3. Treatment Supply and Storage

8.3.1. Treatment Supplies

The distribution of Ecu for routine clinical use is controlled and subject to a Risk Management Plan (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000791/WC500054208.pdf). In order to ensure trial centres have available stock of Ecu, an alternative Risk Management Plan for the use of Ecu within

the ECUSTEC trial has been proposed between the Trial Sponsor, drug manufacturer (Alexion Pharmaceuticals and the MHRA as detailed in Appendix 2).

Sodium chloride 0.9% (Placebo) - The provision of this is the responsibility of each individual participating site in accordance with standard hospital purchasing arrangement.

The provision of all vaccines and prophylactic antibiotics is the responsibility of each individual participating site in accordance with standard hospital purchasing arrangement. NIMPs used in this trial are not provided free of charge from the sponsor, as they are considered an NHS treatment cost.

8.3.2. Packaging and Labelling

Annex 13 compliant labels will be required and should be produced as per the MHRA approved template that will be provided to local pharmacies by the Trials Office. Local pharmacy will affix the labels at the point of preparation.

8.3.3. Drug Storage

Upon arrival at site pharmacy from Alexion, Ecu will be stored in a suitable fridge with temperature monitoring capabilities, segregated from routine clinical stock. Any temperature deviations should be reported to the Trials Office immediately upon becoming aware and stock should be quarantined until further guidance becomes available.

Sodium chloride 0.9% (Placebo) - Temperature monitoring should be in line with local requirements for the general medicine supplies held in pharmacy for routine care.

8.4. Drug Interaction or Contraindications

No drug interactions are reported for Ecu.

Ecu is contra-indicated in the following patients:

1) Patients with known hypersensitivity to Ecu, murine proteins or to any of the excipients listed in the SmPC.
2) Patients with unresolved Neisseria meningitidis infection
3) Patients who are not currently vaccinated against Neisseria meningitidis (unless they receive prophylactic treatment with appropriate antibiotics until 2 weeks after

vaccination). Note that the ECUSTEC protocol mandates 8 weeks antibiotic treatment.

Plasma exchange should not be undertaken in trial participants as this will remove the active product.

Accountability Procedures

8.5. Responsible site pharmacy personnel must maintain accurate accountability records of Ecu, including, but not limited to, the number of vials received, the number of vials dispensed to which participant, batch number, expiry date, and date of transaction. An accountability log of sodium chloride 0.9% (either as diluent for the Ecu or as placebo) must also be maintained including, but not limited to, batch number dispensed to which participant, expiry date and date of transaction. Used vials of Ecu should be disposed of according to normal local procedures.

ECUSTEC Accountability Logs will be provided by the Trials Office for the site pharmacy to log receipt and use of each Lot of Ecu and use of their own supply of sodium chloride 0.9%. “Use” includes disposal or destruction of Lots that have expired for any reason or have not been fully used (part use of Lots). Disposal and destruction should be achieved using local protocols.

8.6. Treatment Modification

The intervention requires two doses of Ecu or placebo.

- The dosing schedule in Table 5 should be followed according to the weight of the child at randomisation. Since subsequent bodyweight changes are likely to reflect changes in fluid status rather than lean bodyweight, the dose should not be changed to an alternative weight bracket.
- With the exception of a serious infusion reaction (anaphylaxis) to the first dose there are no criteria for discontinuation of Ecu
- Penicillin V may need to be changed to erythromycin if an allergic reaction occurs

Trial procedures and assessments

Summary of assessments

	Trial Period																	
Timepoint	Month															6		12
	Week														5	9	26	52
	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	30	60	
	Window															+/- 7 days	-3/+7 days	+/-7 days	+/-7 days
ENROLMENT																			
Eligibility assessment		x																	
Informed consent		x																	
Randomisation		x																	
Allocation of study number		x																	
INTERVENTIONS																			
Ecu or placebo		x							x										
Prophylactic antibiotics‡		x																	
Meningococcal vaccines*		x															x		
ASSESSMENTS																			
Baseline assessment		x																	
Medical history		x																	
Height and weight		x														x	x	x	x
Cystatin C																			x
Targeted physical exam		x														x	x	x	x
FBC†		x	x	x	x	x	x	x	x	x	x	x	x	x	x				
Blood film		x																	
Plasma biochemistry †		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Plasma complement C3 and C4		x																	
STEC investigation - stools		x																	
STEC investigation - serum		x																	
Concomitant medication check		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Review signs and symtpoms for meningococcal disease, including occurrence of CNS signs and symptoms†		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Review signs and symptoms for septacaemia		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		
Blood pressure		x														x	x	x	x
Documentation of targeted events****†		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Optional anonymised feedback questionnaire																	x		
HR QoL		x							x							x	x	x	x
Blood sample to be sent to Bristol∞		x																	
Stool sample**																x			
Urine sample for exploratory studies***		x	x		x		x		x							x			
Plasma sample for exploratory studies***		x	x		x		x		x							x			
CNS Examination*****																	x		
Early morning urine sample******																x	x	x	x

Table 6. Schedule of Trial Assessment Visit

‡ Antibiotic prophylaxis to commence prior to randomisation and Ecu administration. To be administered daily until week 8 (day 56).

† Daily assessments until hospital discharge, if admission ≥14 days then weekly assessments from day 14 to discharge or day 60, which is ever is soonest.

* Meningococcal vaccine to be administered prior to trial drug as described in Section 8.2. If platelet count is $<50x10^9$/l, vaccination will be deferred until count is $\geq 50x10^9$/l. If the participant is receiving systemic anticoagulation, vaccination will be deferred until anticoagulation stopped for 24 hours and is not anticipated to recommence. Optional second dose of meningococcal vaccines at Day 60, see section 8.2.2.

** Participants should be provided with a stool sample collection pot along with collection instructions, on discharge. They should be asked to bring a sample of their/their child's stool to the day 30 assessment.

*** If the participant is discharged between day 1 and day 8 they are not required to return for urine and plasma samples for the mechanistic studies.

****Targeted events including renal replacement therapy, urine output, administration of blood products, concomitant medication, need for abdominal surgery, occurrence of CNS symptoms, occurrence of hyperglycaemia and insulin use, need for parenteral nutrition, myocardial infarction and additional infections.

***** CNS examination at Day 60 (-3/+7 days) if the participant had previous CNS features during acute disease.

****** Urine sample collection pots should be supplied at the previous assessment for the participant/parent to bring a sample of their first morning urine with them to the day 30, 60 and week 26 and 52 week assessment visits.

∞ Blood sample for genetic analysis to be sent to Bristol can be taken at day 1 or any day up to day 8.

9.2. Trial Procedures

Clinical information during hospital admission will be recorded in the hospital notes and on a separate trial (Case Report Form) CRF. Completed CRFs will be sent to BCTU for entry onto the trial database.

9.2.1. Baseline Assessment

At the baseline assessment (to correspond with the day that the 1st dose of trial drug is given), prior to drug administration, information will be captured on:

1. If the participant had diarrhoea in preceding 14 days, date of onset of diarrhoea
2. If the participant had diarrhoea, whether they had bloody diarrhoea
3. Patient history of positive STEC result in preceding 14 days
    a) Stool culture, stool PCR or serology
4. Family history of positive STEC result in preceding 14 days
    b) Stool culture, stool PCR or serology
5. Date and time of working diagnosis of HUS
6. Time of arrival

a. For those transferred from external site, time of arrival at trial site
b. For those referred internally, time care taken over by paediatric nephrologist

7. Volume of saline administered in the 48 hours prior to randomisation
8. Estimated urine output over the preceding 48 hours prior to randomisation
    a) Parental report
    b) Review of documentation sent by referring centre
9. Presence of any targeted symptoms in preceding 48 hours prior to randomisation
    a) Altered consciousness (excessive drowsiness, confusion, hallucination, irritability, agitation)
    b) Seizures
        i. Type and number occurred

10. Medication received in the 7 days prior to randomisation
    a) Antibiotics
    b) Paracetamol
    c) Ibuprofen
    d) Anti-motility agents (codeine, loperamide)

A targeted physical examination will be performed, including measurement of weight, height (if the clinical situation precludes this, an estimate of height should be made from the corresponding weight centile) and blood pressure (manual, average of 3 recordings).

Investigations, before the first dose of trial drug, will be performed as follows:

1. Full Blood Count (FBC) and blood film
2. Plasma biochemistry including electrolytes, urea, creatinine, lactate dehydrogenase (LDH), glucose, amylase, CRP, alanine transaminase,
3. STEC investigations – as part of routine clinical care all participants must have stool culture for E.coli O157, and stool PCR for evidence of STEC genes (if no stool is available during admission, a rectal swab may be sent). Serum will be sent for STEC serology. All STEC investigations for patients in England and Wales must be sent to the Public Health England Reference laboratory. STEC investigations for patients in Scotland should be sent to the Scottish E. coli O157/VTEC Reference Laboratory.
4. Plasma complement C3 and C4

Because of the multiple blood tests likely to be performed in the hours prior to IMP administration, the baseline results recorded should be those closest, but before, IMP administration.

9.2.2. Subsequent Assessments

All participants will be followed-up for 52 weeks from randomisation, with daily trial assessments until up until either hospital discharge or day 14 (whichever is soonest).). If hospital admission lasts >14 days, then assessments will continue to be taken weekly from day 14 to discharge or day 60 (whichever is soonest). The information from the in-patient assessments will be collated into a single CRF which can either be completed in real-time, or at the point of discharge.

All patients will be assessed at 30 and 60 days, and then at 26 and 52 weeks post randomisation for trial follow-up assessments. This is in keeping with the normal frequency of follow-up in clinical practice, although is more than some patients may require. Trial follow-up assessments may be made by any appropriately qualified health professional included on the site delegation log. The visits at day 30, and 26 and 52 weeks can take place +/- 7 days from the due date, and the day 60 visit can take place -3/+7 days from the due date. If the participant is still in the hospital at day 28, this fits into the time window for the day 30 assessment (day 30 +/-7 days), and so a second assessment at day 30 is not needed. Similarly for the day 56 (8 week assessment if still in hospital), and day 60 assessments.

At trial assessment visits, information will be captured regarding targeted events such as:

1. Use of (and type of) renal replacement therapy
2. Urine output
3. Administration of blood products (ml/kg)
    a. Red blood cells
    b. Platelets
    c. Plasma products
4. Targeted relevant concomitant medication
5. Need for abdominal surgery including outcome
6. Occurrence of CNS symptoms and signs
7. Occurrence of hyperglycaemia and use of insulin

8. Need for parenteral nutrition (and indication)
9. Myocardial infarction (on standard ECG +/- troponin +/- ECHO evidence)
10. Any targeted additional infections (e.g. central line infection, peritonitis).
11. Any signs and symptoms of meningitis and septicaemia

The following investigations will be performed daily until discharge:

1. FBC
2. Plasma biochemistry (including electrolytes, urea, creatinine, LDH, glucose, amylase, CRP, alanine transaminase)

The results of other investigations if performed (e.g. x-rays, magnetic resonance scans, echocardiogram etc.) will be recorded as follows:

- Echocardiogram
  - Evidence of cardiac failure or myocardial infarction
- Electrocardiograph (ECG)
  - Evidence of myocardial infarction
- Troponin T or Troponin I
  - Evidence of myocardial infarction
- Lipase
  - Raised value reported
- EEG
  - Normal or abnormal, if abnormal state abnormality

- MRI head
  - normal or abnormal, if abnormal state abnormality

The following information will also be collected following at discharge/day 14 (whichever is soonest):

- Family member known to have evidence of STEC infection subsequent to the participant's enrolment

At visits on day 30 and day 60 information will be captured regarding targeted events including:

1. Use of (and type of) renal replacement therapy

2. Administration of blood products (ml/kg)
3. Targeted relevant concomitant medication
4. Need for abdominal surgery including outcome
5. Occurrence of CNS symptoms and signs
6. Occurrence of hyperglycaemia and use of insulin
7. Need for parenteral nutrition (and indication)
8. Myocardial infarction (on standard ECG +/- troponin +/- ECHO evidence)
9. Any targeted additional infections (e.g. central line infection, peritonitis).
10. Any signs and symptoms of meningitis/septicaemia
11. Targeted adverse events

A targeted physical examination will be performed, including:

1. Blood pressure (manual, average of 3 recordings)
2. Weight and height
3. Signs of meningitis/septicaemia.

The following investigations will be performed:

1. Plasma biochemistry including creatinine, amylase (day 30 only), glucose

Early morning urine for albumin:creatinine ratio will be recorded. (Participants/parents should be provided with a urine sample collection pot at their previous assessment and asked to bring a sample of their/their child's first morning urine to the next assessment)
The results of other investigations if performed (e.g. x-rays, magnetic resonance scans, echocardiogram etc.) will be recorded as indicated above:

At the visits at 26 and 52 weeks information will be captured regarding targeted events including:

1. Use of (and type of) renal replacement therapy
2. Targeted relevant concomitant medication
3. Any targeted additional infections (e.g. central line infection, peritonitis).

A targeted physical examination will be performed, including:

1. Blood pressure (manual, average of 3 recordings)
2. Weight and height

The following investigations will be performed:

1. Plasma biochemistry including creatinine
2. Early morning urine for albumin:creatinine ratio will be recorded. (as above)

Feedback regarding the outcome of the trial assessments should be given to parents/participants as would occur in normal clinical practice.

Participants/parents/guardians should be reminded of the signs and symptoms of meningococcal disease at each of their assessments. It should be checked that the participant/parents/guardians are in possession of the ECUSTEC Meningitis Warning Card and the ECUSTEC Patient Study Card. This information should be documented in the patient medical records and the CRF. **If meningococcal disease is suspected urgent medical treatment in accordance with local clinical procedures should be started immediately.** Any clinical information regarding meningococcal disease will be recorded in the patient medical records and on the CRF.

9.2.3. Genetic blood sample

Between day 1 and day 8, a blood sample will be obtained and sent (to Bristol University) for storage prior to DNA analysis of genes previously associated with HUS, including: complement factor H, complement factor I, CD46, complement C3, complement factor B and diacylglycerol kinase (e). The sample should be collected **prior** to blood transfusion to avoid contamination of the DNA. If a blood transfusion has already taken place the sample should be collected at the latest date after transfusion which is prior to or on day 8.

9.2.4. Quality of life and cost data

HR QoL will be measured at baseline, day 8, day 30, day 60, 26 weeks and 52 weeks using parent rated CHU-9D and PedsQL questionnaires (completion dependent upon the participants age, please see table in Appendix 3).

Health service resource use information will be collected using standard CRF data collection forms at days 8 (after 2nd dose of Ecu), 30, 60 and weeks 26 and 52. The parent of each participant will be asked to recall visits to health professionals, medications and admissions at each follow-up assessment, and the information provided will be checked by searching their medical records. These data will be recorded on the CRF and in the medical records if not already noted.

9.2.5. Day 30 visit only

To determine whether Ecu leads to prolonged STEC excretion, a stool sample will be collected at day 30 and sent by post to the Public Health England Microbiological Reference Laboratory where they will be analysed for STEC (note that samples from Scotland and Wales should be sent to the Public Health England laboratory on this occasion, who will notify the Scottish E. coli O157/VTEC Reference Laboratory of any positive results). On discharge the parent/participant should be provided with a stool sample collection pot and stool sample collection instructions and asked to bring their/their child's stool sample to the day 30 assessment visit. Site staff should send the sample by post to the Public Health England Microbiological Reference Laboratory using the packaging provided by the Trials Office together with a Sample Form. The sample should only be identified by the participant's trial number and date of birth in the MMM/YYYY format. This sample should be noted on the ECUSTEC Samples Log. If the parent/participant fails to bring a stool sample with them to the day 30 assessment visit they should be given a stool sample collection pot and stool sample collection instructions together with the completed Sample Form and packaging materials and asked to collect a sample and send it themselves to the Public Health England Microbiological Reference Laboratory. The parent/participant should be asked to contact the clinic when they have sent the sample so as it can be tracked on the ECUSTEC Samples Log.

9.2.6. Day 60 visit only

1. On the day 60 visit a comprehensive CNS assessment will be performed if the participant had CNS features during acute disease. This will comprise: Examination by Consultant Paediatric Neurologist
2. Visual assessment by an optometrist and ophthalmologist
3. Hearing assessment by an audiologist
4. Neuropsychology assessment (supervised parental completion of the Adaptive Behaviour Assessment System Third Edition (ABAS-3) form by a neuropsychologist)

If impairment is detected within any of these assessments the assessor will be asked to make a judgement about whether this impairment has occurred since the onset of STEC HUS from the information available (e.g. parental history). The results of the 4 assessments will be collated on the ECUSTEC Neurological Results Worksheet by the paediatric neurologist and a CNS score will be assigned (see Appendix 1). These assessments are not required in those without CNS features during acute disease, as it is assumed that no long term CNS sequelae will have occurred. It is recognised that these assessments may need to take place on more than one visit which can take place at any time during the 60 (- 3 day and + 7 day) assessment window.

If full CNS assessment is undertaken at the day 60 visit, this feedback should take place after administering the quality of life questionnaires.

On the day 60 visit, participants/parents/guardians will be invited to fill in an optional anonymised ECUSTEC Trial Evaluation Questionnaire about their participation in the trial so far.

9.2.7. Week 52 visit only

At 52 weeks a blood sample will be sent to a central laboratory for estimation of GFR by creatinine and cystatin C. If patients are dialysis dependent at this time-point, a pre-haemodialysis sample should be taken for this assay, or a random sample on a patient receiving peritoneal dialysis. However the use of dialysis should be recorded on the CRF.

Participant and parent ECUSTEC Thank You Letters are provided by the Trials Office. Please ensure these are completed ready to give to the participant/parent/guardian at their week 52 visit. There are different versions available dependent on the age of the participant.

9.2.8. Exploratory studies

Optional blood and urine samples will be collected at days 1, 2, 4, 6, 8 and 30. If the participant is discharged between day 1 and day 8 they are not required to return for urine and blood sample collection for the exploratory studies. If the facilities for processing these samples are not in place outside routine working hours then samples due during these periods should not be collected.

9.2.9. Sample requirements, schedule, processing, storage and transport

Samples requirements and schedule are shown in Table 7. Specific Standard Operating Procedures will be provided for local laboratories.

Participants	Sample type	Day 1*	Day 2	Day 4	Day 6	Day 8	Day 30	Total blood volume over first 30 days	Max ml/kg over first 30 days	Week 52
Bodyweight <15kg	**Optional samples for mechanistic studies**									
	Blood (EDTA)	1ml	1ml	1ml	1ml	1ml	1ml	6 ml	4.0	
	Blood (Lithium Heparin)	2ml	2ml	2ml	-	2ml	2ml	10 ml		
	Non-optional samples									
	Blood (EDTA - genetics)	4ml						4ml		
	Serum									1ml
Bodyweight 15kg to <30kg	**Optional samples for mechanistic studies**									
	Blood (EDTA)	1ml	1ml	1ml	1ml	1ml	1ml	6 ml	2.0	
	Blood (Lithium Heparin)	4ml	4ml	4ml	-	4ml	4ml	20 ml		
	Non-optional samples									
	Blood (EDTA - genetics)	4ml						4ml		
	Serum									1ml
Bodyweight ≥30kg	**Optional samples for mechanistic studies**									
	Blood (EDTA)	1ml	1ml	1ml	1ml	1ml	1ml	6 ml	1.66	
	Blood (Lithium Heparin)	8ml	8 ml	8 ml	-	8 ml	8 ml	40 ml		
	Non-optional samples									
	Blood (EDTA - genetics)	4ml						4ml		
	Serum									1ml
	Optional samples for mechanistic studies									
Bodyweight ≥15kg, patients in Bristol**	Blood	-	10ml	-	-	-	-	36–46ml	3.33	
All participants	Urine***	20ml	20ml	20ml	20ml	20ml	20ml	-	-	

Table 7. Sample requirements and schedule. Tubes/containers will be provided.

* Day 1 samples can be taken at any point between obtaining informed consent and the time at which the first dose of trial drug is given. EDTA genetics sample can be taken at any day up to day 8.

** Up to 6 participants will be selected and an additional 10ml blood sample will be requested for neutrophil co-culture experiments. Because of the nature of these experiments, only participants treated at the site where the experiments are undertaken (Bristol) will be approached. Because of the additional blood volume, only patients ≥15kg will be approached.

*** if anuric then no urine sample will be obtained, if oliguric then maximum available sample up to 20ml volume should be obtained

The samples should be processed, stored and transported according to the instructions in the ECUSTEC Laboratory Manual.

An ECUSTEC Sample Log will be provided to sites by the Trials Office. Sites must log the details of all samples taken, processed, stored and transported as part of the ECUSTEC trial. All samples taken must only be identified by the participant's trial number and date of birth in the MMM/YYYY format.

9.3. Participants later found to not have evidence of STEC infection

Despite extensive testing, some participants enrolled in the trial will not have evidence of STEC isolated. These participants should remain under follow up in the trial. Further investigation may be required outside the remit of the trial. Participants suspected to have aHUS should be managed according to standard practice. Since the analysis of trial data will be performed on an intention to treat basis, these participants will not be withdrawn. Guidelines for unblinding in such cases are given in section 7.5 above.

9.4. Participant Withdrawals

Participants are free to withdraw from participation in the trial at any time upon request (by participant or parent/guardian) or be withdrawn from the trial by the investigator if considered in the participant's best interest.

Participants who cease trial drug will be followed up for the entire duration of the trial provided that consent for their ongoing participation in the trial is not withdrawn.

At each assessment visit, parents/guardians and participants will be asked to confirm whether they wish to continue in the trial (and this should be documented in the CRF and the patient medical records) and may choose any of the following;

- The participant may wish to withdraw from the investigational treatment, but is willing to be followed-up according to the trial protocol (i.e. has agreed that follow-up data can be collected and used in the final analysis)
- The participant may not want to attend trial specific follow-up visits but is willing to be followed-up according to standard practice (i.e. has agreed that follow-up data can be collected at standard clinic visits and used in the trial final analysis)
- The participant is not willing to be followed up for trial purposes at any further visits (but has agreed that any data collected prior to the withdrawal of consent can be used in the trial final analysis).
- The Participant wishes to withdraw and that none of their trial data, including that already collected, be used for any trial purposes (complete withdrawal)

Full details of the reason(s) for withdrawal should be recorded on the CRFs and patient medical records including date, reason and type of withdrawal. Participants who withdraw from trial treatment but continue with on-going follow-up and data collection should be followed-up in accordance with the trial protocol. Participants who withdraw will continue to be managed according to standard best practice.

10. Adverse Event Reporting

10.1. Reporting Requirements

Safety will be assessed continuously throughout the trial. The collection and reporting of adverse events (AEs) and serious adverse events (SAEs) will be in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and its subsequent amendments. The Investigator will assess the seriousness and causality (relatedness) of all AEs experienced by the participant with reference to the Reference Safety Information. The Reference Safety Information (RSI) for the ECUSTEC trial will be the Soliris® (Ecu) Summary of Product Characteristics (SmPC) Section 4.8 (28th June 2016). The Trials Office will review the SmPC annually and, under the guidance of the Chief Investigator update the RSI when required.

Annual Development Safety Update Reports (DSURs) will be submitted to the main ethics committee and MHRA. The DSUR will be compiled by the Trials Office, reviewed and signed by the CI and submitted by the Trials Office.

Adverse Events (AE) Definition and Reporting

10.2.

AE: Any untoward medical occurrence in a trial participant to whom a research treatment or procedure has been administered, including occurrences which are not necessarily caused by or related to that treatment or procedure.

Although all AEs should be recorded in the source data (patient's medical notes), it is known that STEC HUS causes anaemia and renal failure, as well as significant alterations to laboratory parameters and, for trial purposes, we only require the following to be reported via the ECUSTEC CRFs:

1. The development of any significant infections (grade 3 and above) will be documented at each trial assessment.
2. Infusion reactions to trial interventions.
3. The presence of STEC in a stool sample which will be collected at day 30 (see section 9.2.4).

10.3. Serious Adverse Events (SAE) Definition and Reporting

If an AE meets the criteria of a SAE for this trial and occurs within 90 days of the first dose of meningococcal vaccination or prophylactic antibiotic (whichever occurs first) it will be reported to the trial office. To ensure consistent reporting of SAEs, the ECUSTEC trial will code events in accordance with the CTCAE v4.03 document, provided to sites and available on request from the ECUSTEC trial office. For each diagnosis/event symptom described a code should be given by the site in accordance with the SOC table, provided by the ECUSTEC office.

SAE: Any adverse event which:

- results in death;
- is life-threatening*;
- requires hospitalisation** or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- results in a congenital anomaly or a birth defect;
- or, is otherwise considered medically significant by the Investigator

**The term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.*

*** Patients must be formally admitted – waiting in outpatients or A&E does not constitute an SAE (even though this can sometimes be overnight). Similarly, planned hospitalisations that clearly are not related to the condition under investigation or hospitalisations/prolongation of hospitalisation due to social reasons should not be considered as SAEs.*

- Hospitalisations for routine treatment or monitoring of the studied indication, not associated with any deterioration in condition are **not** considered SAEs

Events identified as SAEs require completion of an **SAE form**.

A trial-specific SAE form will be forwarded to BCTU within 24h of the research staff becoming aware of the event. The Trials Office will report all SAEs to the CI and Sponsor. The CI must review the causality assessment made by the PI (or delegate) and evaluate whether the event is expected or unexpected against the reference safety information. The approved reference safety information for the ECUSTEC trial will be the Soliris® (Ecu) Summary of Product Characteristics Section 4.8 (version 28th June 2016). Events categorised as Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the Main REC and MHRA within the required timeframes. All SUSARs will be unblinded prior to reporting to the MHRA (by the Sponsor accessing the unblinding service as detailed in Section 7.5). The Trials Office will record and report all SUSARs to the Sponsor. The Trials Office will inform the Sponsor and CI upon receipt of a SUSAR. The Sponsor will ensure that all SUSARs are entered in the European database.

10.3.1. Events that do not require expedited (immediate) reporting

Participants receiving treatment for STEC HUS may require one or more general anaesthetic +/- surgical procedures to provide dialysis access (such as peritoneal dialysis catheter or central venous catheter) to undergo renal replacement therapy. This information will be captured within the trial assessments and does not need expedited reporting. For this reason, the following SAEs do not require expedited (immediate) reporting by site and are not regarded as unexpected for the purpose of this trial:

- General anaesthetic +/- surgical procedures undertaken solely to provide dialysis access

An SAE Form should still be completed for these events but can be faxed to the Trials Office in line with the normal reporting timelines for clinical data (2 weeks).

STEC HUS and the treatment of STEC HUS is commonly associated with infections, which should be both recorded in the clinical notes, and on an SAE Form for those infections which meet the SAE criteria. However, infections do not require **expedited** reporting unless the clinical team believe that the nature of the infection differs from the usual course of STEC HUS.

That is, when reporting infections that meet the criteria of an SAE, ONLY meningococcal infections and those infections which differ from the usual pattern seen in STEC HUS must be reported as SAEs within 24hrs of becoming aware of the event.

10.4. Reporting period and procedure

The reporting period should start immediately after the administration of prophylactic antibiotics and should last until 30 days after the subsequent vaccination at 60 days. That is, a total of 90 days from first vaccination/antibiotic for routine reporting.

SAEs that are judged to be at least possibly related to the IMP must still be reported in an expedited manner irrespective of how long after IMP administration the reaction occurred.

Note: Death from any cause should be reported on an SAE Form and returned to the Trial Office.

10.4.1. Adverse Events

Where required by the trial, AEs should be collected on a CRF (and where applicable on an SAE Form). All CRFs should be completed and returned by post within 2 weeks of the trial visit/event.

10.4.2. Serious Adverse Events

AEs defined as serious and which require reporting as an SAE should be reported on an SAE Form. When completing the form, the Investigator will be asked to define the causality and the severity of the AE. On becoming aware that a participant has experienced an SAE, the Investigator (or delegate) must complete, date and sign an SAE Form.

To fax the SAE Form: 0121 415 9135 or 0121 415 9136

To email the SAE Form: ecustec@trials.bham.ac.uk

On receipt the Trials Office will allocate each SAE a unique reference number which will be forwarded to the site as proof of receipt. If confirmation of receipt is not received within 1 working day please contact the Trials Office. The SAE reference number should be quoted on all correspondence and follow-up reports regarding the SAE and filed with the actual SAE in the ISF.

For SAE Forms completed by someone other than the Investigator the Investigator will be required to countersign the original SAE Form to confirm agreement with the causality and severity assessments. The form should then be returned to the Trials Office and a copy kept in the ISF.

Investigators should also report SAEs to their own Trust in accordance with local practice.

10.4.3. Provision of follow-up information

Participants should be followed up until resolution or stabilisation of the event. Follow-up information should ideally be provided on a new SAE Form, quoting the SAE reference number provided by the trials office and indicating that the form is a follow-up to previous information. The PI is also required to indicate if the new information provided changes their assessment of causality and sign accordingly.

10.5. Reporting Procedure – Trials Office

On receipt the Trials Office will allocate each SAE a unique reference number which will be forwarded to the site as proof of receipt within 3 working days. The SAE reference number will be quoted on all correspondence and follow-up reports regarding the SAE and filed with the actual SAE in the Trial Master File (TMF).

On receipt of an SAE Form causality will be reviewed independently by the CI or Deputy and the expectedness assessment will be made. An SAE judged by the investigator or co-investigator to have a reasonable causal relationship with the trial medication will be regarded as a Serious Adverse Reaction (SAR). The CI or Deputy will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected (i.e. is not defined in the reference safety information) it will be classified as a Suspected Unexpected Serious Adverse Reaction (SUSAR).

Reporting to the Competent Authority and Research Ethics Committee

10.6.1. Suspected Unexpected Serious Adverse Reactions

10.6 The Trials Office will record and report any SUSARs to the Sponsor and will inform the Sponsor and CI on receipt of a SUSAR. The Sponsor will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to the MHRA and Research Ethics Committee (REC) within 7 days after the Trials Office has been notified. Detailed follow-up information will be provided within an additional 8 days.

All other events categorised as SUSARs will be reported by the Sponsor within 15 days after the Trials Office has been notified.

10.6.2. Serious Adverse Reactions

The Trials Office will report details of all SAEs and SARs (including SUSARs) to the MHRA, REC and Sponsor annually from the date of the Clinical Trial Authorisation, in the form of a Development Safety Update Report (DSUR).

The CI will review and sign the DSUR. A copy is also sent to the Sponsor at the time of sending out the DSUR.

10.6.3. Adverse Events

Details of all AEs collated by the ECUSTEC trials office will be reported to the MHRA on request.

10.6.4. Monitoring pregnancies for potential Serious Adverse Events

There is an identified risk of congenital anomalies or birth defects in the offspring of participants as a result of their participation in the trial. The outcome of pregnancies of participants will therefore be monitored in order to provide SAE data on congenital anomalies or birth defects. In the event that a participant or their partner becomes pregnant during the SAE reporting period a pregnancy notification form will be completed (providing the participant's details) and returned to the Trials Office.

- If it is the participant who is pregnant, outcome data will be provided on a follow-up pregnancy notification form.

- Where the participant's partner is pregnant consent must first be obtained and the participant should be given a pregnancy release of information form or appropriate form to give to their partner. If the partner is happy to provide information on the outcome of their pregnancy they should sign the pregnancy release of information form.

Once consent has been obtained details of the outcome of the pregnancy will be provided on a follow-up pregnancy notification form and an SAE Form will be completed if there are birth defects or congenital abnormalities to report.

10.6.5. Other safety issues identified during the course of the trial

The MHRA and REC will be notified immediately if a significant safety issue is identified during the course of the trial.

The Sponsor will also be informed before or at the time that the REC and MHRA are informed.

10.7. Investigators

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to Principal Investigators. A copy of any such correspondence should be filed in the Investigator Site File.

10.8. Data Monitoring Committee

The independent DMEC will review all SAEs.

10.9. Reporting to third parties

SUSARs will be reported to Alexion Pharmaceuticals, the manufacturer of Ecu. The Trials Office will report a minimal data set of all individual events categorised as a fatal or life threatening SUSAR to Alexion within 7 days after the Trials Office has been notified. Detailed follow-up information will be provided within an additional 8 days. All other events categorised as SUSARs will be reported within 15 days after the Trials Office has been notified. SUSARs sent to Alexion will only be identified by the participant's trial number.

10.10. Urgent Safety Measures

If any urgent safety measures are taken, the BCTU shall immediately, and in any event no later than 3 days from the date the measures are taken, give written notice to the REC and MHRA of the measures taken and the circumstances giving rise to those measures.

11. Data Handling and Record Keeping

11.1. Source Data

Source data is defined as: all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. In order to allow for the accurate reconstruction of the trial and clinical management of the participant, source data will be accessible and maintained.

The CRFs are not the source data for clinical information, however the HR QoL questionnaires will be considered source data. This data is entered directly onto the CRF and these are clearly identified and detailed below:

- PedsQL questionnaire
- CHU-9D questionnaire

Source data is kept as part of the participants' medical notes generated and maintained at site.

11.2. CRF Completion

Data reported on each CRF will be consistent with the source data and any discrepancies will be explained. In the event of data being obtained by telephone contact with the participant/parent this must be recorded directly into the medical notes and transposed onto CRFs appropriately. Staff delegated to complete CRFs will be trained to adhere to GCP.

The following guidance applies to data and partial data:

- Time format and unknown times – all times should be in accordance with the 24hr clock
- Rounding conventions – rounding should be in the normal way
- Trial-specific interpretation of data fields – where guidance is needed additional information will be supplied
- Entry requirements for concomitant medications.(generic or brand names) – generic names should be used where possible
- Missing/incomplete data – should be clearly indicated – all blank fields will be queried by the trial office

- Repeat laboratory tests – the data used to inform clinical decisions should always be supplied. If a test is repeated it is either to confirm or clarify a previous reading. Confirmatory tests should use the original test values. Protocol and GCP non-compliances should be reported to the Trials Office on discovery.

In all cases it remains the responsibility of the site's Principal Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

Data Management

11.3. The ECUSTEC Trial will not use double data entry. Data is validated by pop-ups on the database when out of range and by random checks. All missing and ambiguous data will be queried via Data Clarification Forms (DCFs). Responses should be made on the DCF. The original DCF should be copied and the copy attached to the CRF to which it relates. The original DCF should be returned to ECUSTEC Trial Office. A data management plan will be devised by the Trials Office.

11.4. Archiving

It is the responsibility of the Principal Investigator to ensure all essential trial documentation and source documents (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, participants' hospital notes, copies of CRFs etc.) at their site are securely retained for at least 25 years.

No documents will be destroyed without prior approval from the Trials Office.

12. Quality control and quality assurance

12.1. Site Set-up and Initiation

All participating Principal Investigators will be asked to sign the necessary agreements including a Clinical Trial Agreement, and supply a current signed copy of their CV and a recent GCP training certificate to the Trials Office. GCP training should be should be updated and maintained in accordance with local Trust policy. All members of the site research team will also be required to sign a site signature and delegation log. Prior to commencing recruitment all sites will undergo a process of initiation. Key members of the site research team will be required to attend either a meeting or a teleconference covering aspects of the trial design, protocol procedures, Adverse Event reporting, collection and

reporting of data and record keeping. Sites will be provided with an ISF and a Pharmacy File containing essential documentation, instructions, and other documentation required for the conduct of the trial. The Trials Office must be informed immediately of any change in the site research team.

Monitoring

12.2 Monitoring will be carried out as required following a risk assessment and as documented in the monitoring plan. Any monitoring activities will be reported to the trials team and any issues noted will be followed up to resolution.

On-site Monitoring

12.3 On-site monitoring visits may be triggered, for example by poor CRF return, poor data quality, low SAE reporting rates, excessive number of participant withdrawals or deviations. If a monitoring visit is required the Trials Office will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the ECUSTEC trial staff access to source documents as requested. On-site monitoring may also be ad hoc in accordance with BCTU quality management system processes.

12.3.1. Central Monitoring

The Trials Office will be in regular contact with the site research team to check on progress and address any queries that they may have. The Trials Office will check incoming CRF for compliance with the protocol, data consistency, missing data and timing. Sites will be asked for missing data or clarification of inconsistencies or discrepancies.

12.4.

Audit and Inspection

The Principal Investigator will permit trial-related monitoring, quality checks, audits, ethical reviews, and regulatory inspection(s) at their site, providing direct access to source data/documents. The Principal Investigator will comply with these visits and any required follow up. Sites are also requested to notify the Trials Office of any MHRA inspections.

12.5

Notification of Serious Breaches

In accordance with Regulation 29A of the Medicines for Human Use (Clinical Trials) Regulations 2004 and its amendments, the Sponsor of the trial is responsible for notifying the licensing authority in writing of any serious breach of the conditions and principles of

GCP in connection with that trial or the protocol relating to that trial, within 7 days of becoming aware of that breach.

For the purposes of this regulation, a “serious breach” is a breach which is likely to effect to a significant degree the safety or physical or mental integrity of the participants of the trial; or the scientific value of the trial. Sites are therefore requested to notify the Trials Office of any suspected trial-related serious breach of GCP and/or the trial protocol. Where the Trials Office is investigating whether or not a serious breach has occurred, sites are also requested to cooperate with the Trials Office in providing sufficient information to report the breach to the MHRA where required and in undertaking any corrective and/or preventive action. Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or GCP, and/or poor recruitment. Any major problems identified during monitoring may be reported to relevant stakeholders e.g. Trial Management Group, Trial Steering Committee, the REC and the relevant regulatory bodies. This includes reporting serious breaches of GCP and/or the trial protocol to the REC and MHRA.

The Sponsor will provide decisions on serious breaches and shall notify the MREC and MHRA in writing of any serious breach of:

1. the conditions and principles of GCP in connection with the trial; or
2. the protocol relating to the trial, as amended from time to time, within 7 days of becoming aware of that breach.

The Sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase.

13. End of Trial Definition

The end of trial will be 6 months after the last data capture. This will allow sufficient time for the completion of protocol procedures, data collection and data input. The Trials Office will notify the MHRA and REC that the trial has ended within 90 days of the end of trial. Where the trial has terminated early, the Trials Office will inform the MHRA and REC within 15 days of the end of trial. The Trials Office will provide them with a summary of the clinical trial report within 12 months of the end of trial.

The CI supported by the Trials Office has taken on sponsor responsibility for end of trial notification and summary report submission and a copy of the end of trial notification as well

as the summary report is also sent to Newcastle upon Tyne NHS Foundation Trust at the time of sending these are sent to the MHRA and REC.

The Trials Office will be responsible for posting clinical trial summary results in the European Clinical Trials Database (EudraCT) within 6 months after the trial has ended. The Trials Office will disseminate the final report to trial sites.

Once the results are published following the end of the trial participants can be informed which treatment arm of the trial they were recruited to.

Statistical Considerations

14. Sample Size

14.1 The justification for the sample size is based on retrospective pilot data collected on 94 patients with STEC HUS presenting to five of the potential trial centres over several years who met the trial inclusion criteria. These data gave a mean clinical severity score of 13.16, standard deviation (SD=9.66; range: 2 to 45). A difference in clinical severity score of 5 points is a moderate effect size (0.52) and equates to a meaningful clinical benefit. For example, a reduction of 5 points would equate to the difference between 9 days on dialysis and 4 days on dialysis. This would be a 5 day reduction in hospital stay and concomitant reduction in painful procedures and distress for children and families. Alternatively, this could equate to avoiding a surgical laparotomy to investigate an acutely distended abdomen, or avoiding development of cardiac failure.

14.2 To detect a difference of 5 points in the clinical severity score between groups using a 2-sided t-test and assuming a SD of 9.66, with 80% power and a type I error rate of 5% (α=0.05), a total of 60 participants per group will need to be randomised. Assuming and adjusting for a 10% loss to follow-up drop-out rate, 134 participants (67 per group) will need to be recruited.

Analysis of Outcome Measures

A separate Statistical Analysis Plan will be produced and will provide a more comprehensive description of the planned statistical analyses. A brief outline of these analyses is given below. The primary comparison groups will be composed of those randomised to Ecu versus those randomised toplacebo. In the first instance, all analyses will be based on the intention to treat principle, i.e. all participants will be analysed in the groups to which they were

allocated irrespective of compliance or other protocol violation. For all major outcome measures, summary statistics and differences between groups, (e.g. mean differences, relative risks), will be presented, with 95% confidence intervals and p-values from two-sided tests also given. Analyses will be adjusted for the minimisation variables listed in section 7.2 where possible. No adjustment for multiple comparisons will be made.

14.2.1. Primary Outcome Measure

The primary outcome measure is the multi-domain clinical severity score at day 60. The score has a range of 1 to 69; with higher scores indicating greater disease severity. When the clinical severity score was calculated using data collected on 94 patients, from the same population planned for ECUSTEC, it followed a near normal distribution. Therefore, the primary analysis will use a linear regression model, adjusting for the minimisation variables, to compare the mean clinical severity score at day 60 between the ecu and placebo arms. An unadjusted analysis will be performed as a secondary analysis using a two-sample t-test. The mean difference in severity score between arms and 95% confidence interval will be reported. If normality does not hold, then medians and interquartile ranges will be reported, and non-parametric methods will be used.

14.2.2. Analysis of the Secondary Outcomes

The secondary outcomes include both continuous and categorical data. Continuous outcomes (e.g. number of days patient on RRT, eGFR at 52 weeks) will be analysed as per the primary outcome. The number of days a participant has thrombocytopenia, haemolysis and presence of markers of inflammation during the acute phase (defined as up to 14 days post-randomisation) will be categorised using pre-specified categories defined in the Statistical Analysis Plan, and logistic regression models fitted. Binary outcome measures (e.g. overall survival, persistent neurological defect at day 60, CKD at 52 weeks) will be reported as proportions and a log-binomial regression model fitted. For count data (e.g. number of packed red blood cell transfusions required), a Poisson model or other appropriate count model will be fitted. Mean log count differences will be reported alongside the respective 95% confidence interval.

14.2.3. Planned Subgroup Analyses

Subgroup analyses will be limited to the same variables (except centre which was included as a minimisation variable for logistic reasons) used in the minimisation algorithm (see section 7.2). Tests for statistical heterogeneity (e.g. by including the treatment group by

subgroup interaction parameter in the regression model) will be performed prior to any examination of effect estimate within subgroups. The results of subgroup analyses will be treated with caution and will be used for the purposes of hypothesis generation only.

14.2.4. Missing Data and Sensitivity Analyses

Every attempt will be made to collect full follow up data on all study participants, it is thus anticipated that missing data will be minimal. Participants with missing primary outcome data will not be included in the primary analysis in the first instance. This presents a risk of bias, and sensitivity analyses will be undertaken to assess the possible impact of the risk. Full details will be included in the Statistical Analysis Plan. Further sensitivity analysis may include an analysis in the per-protocol population.

14.3. Planned Interim Analyses

Interim analyses will be conducted for presentation to the independent DMEC. This will include an analysis of the primary and major secondary outcomes and full assessment of safety (serious adverse events) Further details of DMEC arrangements are given in section 15.5 and will be included in the Statistical Analysis Plan.

14.4. Planned Final Analyses

The primary analysis for the study will occur once all participants have completed the 60 day assessment and corresponding outcome data has been entered onto the study database and validated as being ready for analysis. This analysis will include data items up to and including the 60 day assessment and no further. Longer term data out to 52 weeks will be analysed separately once participants have completed the corresponding assessments.

14.5. Assessment of cost effectiveness

We will include a trial based economic evaluation from a NHS/PSS perspective to assess the cost-effectiveness of Ecu versus placebo. The economic evaluation will be based on cost per clinical outcome using the primary outcome for the trial, the clinical severity score, as well as cost per Quality-Adjusted Life Year (QALY) gained. To facilitate this analysis, health service resource use information will be collected using standard data collection forms to estimate the incremental cost of using Ecu compared to placebo. Unit cost data will be derived from nationally represented sources such as the British National Formulary (BNF), the National Schedule for Reference Costs and the Unit Costs of Health and Social Care (PSSRU). Utility data will be estimated in two ways according to the age group of the

sample. This is because methods for estimating utility values for children aged less than 5 years are not well established. For children aged 5 years and above the age-specific proxy version of the CHU-9D instrument will be completed and the resulting descriptive profiles will be converted into utility values using conventional methods. For all children and young people aged 6 months and above the age specific proxy version of the PedsQL instrument will be completed. A mapping algorithm will be applied to generate utility scores for children aged between 2 and 4 years. The mapping algorithm will be determined using regression methods to model the relationship between the CHU-9D and PedsQL scores for children for whom both will have been collected (i.e. those aged 5 years and above). In addition, a version of the CHU-9D for children aged less than 5 years that is under development will be piloted as a basis for estimating utility values and exploring the potential to mapping utility values for patients younger than 2 years. Outcome data will be collected at day 8 (after 2nd dose of Ecu), 30 and 60 days and 26 and 52 weeks post-randomisation, in line with the clinical outcome data. Resource use data will be collected at day 8, 30 and 60 days and 26 and 52 weeks post-randomisation. Appropriate sensitivity analysis will be applied to explore how sensitive the results are to data parameters and assumptions within the analyses. Results will be expressed as incremental cost-effectiveness ratios (ICERs) using ICER plots and cost-effectiveness acceptability curves (CEACs) to represent the probability of being cost-effective at different willingness to pay thresholds.

A model-based economic evaluation will estimate the long-term cost-effectiveness of using Ecu for patients with HUS. It is anticipated a mathematical model will be constructed to determine the long-term (beyond 12 months) outcomes and costs associated with either Ecu or placebo. The model structure will be informed by expert opinion within the team and by reviewing modelling studies that have been undertaken which consider long-term outcomes associated with HUS. Outcomes will be in the form of survival and estimated quality of life and will use data collected from the trial and literature on long-term outcomes associated with HUS. Costs in the model will include those for the Ecu and placebo pathways derived from the trial-based analysis and literature-based estimates for long-term outcomes associated with HUS. The model will be run over the patients' lifetime, with costs and benefits discounted at a rate of 3.5%. As for the trial-based analysis, the analysis will be conducted from an NHS and PSS perspective, extensive sensitivity analysis will be undertaken, and the results will be presented using ICERs and CEACs.

Trial Organisational Structure

Sponsor

15. Sponsorship and indemnity arrangements for the ECUSTEC trial will be undertaken by the Newcastle Upon Tyne Hospitals NHS Foundation Trust.

15.1.

Trials Office

The ECUSTEC Trial Office will be based at the BCTU at the University of Birmingham.

15.2.

Trial Management Group (TMG)

15.3. The TMG will be comprise of the CI, other lead investigators and members of BCTU. The TMG will be responsible for the day-to-day running and management of the ECUSTEC trial, and will convene at regular intervals.

Trial Steering Committee (TSC)

15.4.

The role of the TSC is to provide the overall supervision of the trial. The TSC will monitor trial progress, and conduct and advise on scientific credibility. The TSC will consider and act, as appropriate, upon the recommendations of the DMEC. Further details of the remit and role of the TSC are available in the TSC charter.

15.5.

Data Monitoring and Ethics Committee (DMEC)

An independent DMEC will oversee the safety of the participants in the trial.

Data analyses will be supplied in confidence to the independent DMEC, who will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further participants. The DMEC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group. The DMEC will meet prior to the trial opening and then again at the end of the pilot phase (at 18 months or after 26 patients have been recruited and completed 6 month follow-up, whichever occurs first) to review the data and advise on the continuation of the study using the stopping rules described in section 4.1.1. Since this is an internal pilot, and this data will be included in the main analysis of the ECUSTEC trial, the data will remain confidential, except to members of the DMEC and the trial statistician(s) performing the analyses. If the trial continues, the DMEC will meet at least annually, or as per a timetable agreed by the DMEC prior to the trial commencement.

Additional meetings may be called if recruitment is much faster than anticipated and the DMEC may, at their discretion, request to meet more frequently or continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified. The DMEC will report directly to the Trial Steering Committee who will convey the findings of the DMEC to MHRA, funders, and/or sponsors as applicable.

The DMEC may consider recommending the discontinuation of the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise participant safety. The trial would also stop early if the interim analyses showed differences between treatments that were deemed to be convincing to the clinical community. In this case, the DMEC will advise the chair of the TSC if, in their view, any of the randomised comparisons in the trial have provided both (a) "proof beyond reasonable doubt"† that for all, or for some, types of patient one particular treatment is definitely indicated or definitely contraindicated in terms of a net difference in the major endpoints, and (b) evidence that might reasonably be expected to influence the patient management of many clinicians who are already aware of the other main trial results. The TSC can then decide whether to close or modify any part of the trial. Unless this happens, however, the TMG, TSC, the investigators and all of the central administrative staff (except the statisticians who supply the confidential analyses) will remain unaware of the interim results.

† Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but a difference of at least $p<0.001$ (similar to Haybittle-Peto stopping boundary) in an interim analysis of a major endpoint may be needed to justify halting, or modifying, the study prematurely. If this criterion were to be adopted, it would have the practical advantage that the exact number of interim analyses would be of little importance, so no fixed schedule is proposed.

15.6.

Finance

This is an investigator-initiated and investigator-led trial funded by the National Institute for Health Research (NIHR) and the Medical Research Council (MRC) Efficacy and Mechanism Evaluation (EME) Programme (Ref. No.: 14/48/43).

16. Ethical Considerations and Research Governance

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996 (website: http://www.wma.net/en/30publications/10policies/b3/index.html).

The trial will be conducted in accordance with the Research Governance Framework for Health and Social Care, the applicable UK Statutory Instruments, (which include the Medicines for Human Use Clinical Trials 2004 and subsequent amendments and the Data Protection Act 1998 and Human Tissue Act 2008" and Human Tissue (Scotland) Act 2006 and Guidelines for Good Clinical Practice (GCP). This trial will be carried out under a Clinical Trial Authorisation in accordance with the Medicines for Human Use Clinical Trials regulations. The protocol will be submitted to and approved by the REC prior to circulation.

Before any participants are enrolled into the trial, the Principal Investigator at each site is required to obtain local approvals. Sites will not be permitted to enrol participants until written confirmation of approval is received by the Principal Investigator.

It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary local approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual participants.

16.1. Quality Assurance

Monitoring of this trial will be to ensure compliance with GCP. A risk proportionate approach to the initiation, management and monitoring of the trial will be adopted (as per the MRC/DH/MHRA Joint Project: Risk-adapted Approaches to the Management of Clinical Trials of Investigational Medicinal Products) and outlined in the trial-specific risk assessment.

17. Confidentiality and Data Protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998.

Participants will always be identified using only their unique trial identification number, on the CRF and in correspondence between the Trials Office and the participating site and between

Alexion and the participating site. Participant date of birth will need to be collected at randomisation in the DD/MMM/YYYY format. The HR QOL questionnaires have specific age ranges, so the full date of birth is needed to ensure the correct questionnaires are completed for the participant's age. However the participant will only be identified once in the trial by their date of birth in the MMM/YYYY format and their full date of birth will be hidden within the database system.

The Investigator must maintain documents not for submission to the Trials Office (e.g. Participant Identification Logs) in strict confidence. In the case of specific issues and/or queries from the regulatory authorities, it will be necessary to have access to the complete trial records, provided that participant confidentiality is protected.

The Trials Office will maintain the confidentiality of all participant's data and will not disclose information by which participants may be identified to any third party. Representatives of the Trials Office and sponsor may be required to have access to participant's notes for quality assurance purposes but participants should be reassured that their confidentiality will be respected at all times.

Personal data about the participants will be collected during the trial and will be held securely and treated as strictly confidential, in line with the Data Protection Act 1998. Data will be transferred from the participating trial centres to the trial office at the BCTU and held on a secure database server. Participants will be made aware of this prior to entry into the trial, and will be asked to consent to this. Any data processed outside the BCTU will be anonymised. In line with GCP, once data collection is complete on all participants, all data will be stored for at least 25 years.

18. Insurance and Indemnity

Indemnity arrangements for the ECUSTEC trial will be undertaken by the Newcastle Upon Tyne Hospitals NHS Foundation Trust.

As this is not an industry-sponsored trial, ABPI guidelines on indemnity will not apply. There will be no special arrangements for compensation for any non-negligent harm as a result of participating in the trial. In terms of liability, NHS Trust and Non-Trust Hospitals have a duty

of care to patients treated, whether or not the patient is taking part in a clinical trial. Compensation is only available via NHS indemnity in the event of clinical negligence being proven.

Publication Policy

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The Chief Investigator will coordinate dissemination of data from this trial. All publications and presentations, including abstracts, relating to the main trial will be authorised by the ECUSTEC TMG. Results of this trial will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the relevant members of the TMG and authorship will be determined by mutual agreement.

All publications using data from this trial to undertake original analyses will be submitted to the TMG for review before release. To safeguard the scientific integrity of the trial, data from this trial will not be presented in public before the main results are published without the prior consent of the TMG.

Any secondary publications and presentations prepared by Investigators must be reviewed by the TMG. Manuscripts must be submitted to the TMG in a timely fashion and in advance of being submitted for publication, to allow time for review and resolution of any outstanding issues. Authors must acknowledge that the trial was performed with the support of the Newcastle upon Tyne Hospitals NHS Foundation Trust and National Institute for Health Research (NIHR) and the Medical Research Council (MRC) Efficacy and Mechanism Evaluation (EME) Programme. A list of contributors will be included where PICs and an individual at each PIC will be listed.

Reference List

20.

1. Lynn RM, O'Brien SJ, Taylor CM, Adak GK, Chart H, Cheasty T, Coia JE, Gillespie IA, Locking ME, Reilly WJ, Smith HR, Waters A, Willshaw GA. Childhood hemolytic uremic syndrome, United Kingdom and Ireland. Emerg Infect Dis. 2005 Apr;11(4):590-6.
2. Garg AX, Suri RS, Barrowman N, Rehman F, Matsell D, Rosas-Arellano MP, Salvadori M, Haynes RB, Clark WF. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. JAMA. 2003 Sep 10;290(10):1360-70
3. Michael M, Elliott EJ, Ridley GF, Hodson EM, Craig JC. Interventions for haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. Cochrane Database Syst Rev. 2009 Jan 21;(1):CD003595
4. Hickey CA, Beattie TJ, Cowieson J, Miyashita Y, Strife CF, Frem JC, Peterson JM, Butani L, Jones DP, Havens PL, Patel HP, Wong CS, Andreoli SP, Rothbaum RJ, Beck AM, Tarr PI. Early volume expansion during diarrhea and relative nephroprotection during subsequent hemolytic uremic syndrome. Arch Pediatr Adolesc Med. 2011 Oct;165(10):884-9.
5. Kavanagh D1, Goodship TH, Richards A. Atypical hemolytic uremic syndrome. Semin Nephrol. 2013 Nov;33(6):508-30
6. Legendre CM, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, Bingham C, Cohen DJ, Delmas Y, Douglas K, Eitner F, Feldkamp T, Fouque D, Furman RR, Gaber O, Herthelius M, Hourmant M, Karpman D, Lebranchu Y, Mariat C, Menne J, Moulin B, Nürnberger J, Ogawa M, Remuzzi G, Richard T, Sberro-Soussan R, Severino B, Sheerin NS, Trivelli A, Zimmerhackl LB, Goodship T, Loirat C. Terminal complement inhibitor Ecu in atypical hemolytic-uremic syndrome. N Engl J Med. 2013 Jun 6;368(23):2169-81
7. Greenbaum LA, Fila M, Ardissino G, Al-Akash SI, Evans J, Henning P, Lieberman KV, Maringhini S, Pape L, Rees L, van de Kar NC, Vande Walle J, Ogawa M, Bedrosian CL, Licht C. Ecu is a safe and effective treatment in pediatric patients with atypical hemolytic uremic syndrome. Kidney Int. 2016 Mar;89(3):701-11.
8. Cheung V, Trachtman H. Hemolytic uremic syndrome: toxins, vessels, and inflammation. Front Med (Lausanne). 2014 Nov 4;1:42.
9. Thurman JM, Marians R, Emlen W, Wood S, Smith C, Akana H, Holers VM, Lesser M, Kline M, Hoffman C, Christen E, Trachtman H. Alternative pathway of complement in children with diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2009 Dec;4(12):1920-4
10. Keir LS, Saleem MA. Current evidence for the role of complement in the pathogenesis of Shiga toxin haemolytic uraemic syndrome. Pediatr Nephrol. 2014 Oct;29(10):1895-902.
11. Morigi M, Galbusera M, Gastoldi S, Locatelli M, Buelli S, Pezzotta A, Pagani C, Noris M, Gobbi M, Stravalaci M, Rottoli D, Tedesco F, Remuzzi G, Zoja C. Alternative pathway activation of complement by Shiga toxin promotes exuberant C3a formation that triggers microvascular thrombosis. J Immunol. 2011 Jul 1;187(1):172-80
12. Lee BC, Mayer CL, Leibowitz CS, Stearns-Kurosawa DJ, Kurosawa S. Quiescent complement in nonhuman primates during E coli Shiga toxin-induced hemolytic uremic syndrome and thrombotic microangiopathy. Blood. 2013 Aug 1;122(5):803-6.

13. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, Boppel T, Kirschfink M, Oualha M, Proulx F, Clermont MJ, Le Deist F, Niaudet P, Schaefer F. Ecu in severe Shiga-toxin-associated HUS. N Engl J Med. 2011 Jun 30;364(26):2561-3
14. Ekinci Z1, Candan C, Alpay H, Canpolat N, Akyüz SG, Gündüz Z, Dursun I, Bek K, Dursun H, Işıyel E, Öktem F, Tabel Y, Akil İ, Delibaş A, Gülleroğlu K, Akıncı N, Dinçel N, Özkaya O, Söylemezoğlu O. Hemolytic uremic syndrome outbreak in Turkey in 2011. Turk J Pediatr. 2013 May-Jun;55(3):246-52.
15. Gitiaux C, Krug P, Grevent D, Kossorotoff M, Poncet S, Eisermann M, Oualha M, Boddaert N, Salomon R, Desguerre I. Brain magnetic resonance imaging pattern and outcome in children with haemolytic-uraemic syndrome and neurological impairment treated with Ecu. Dev Med Child Neurol. 2013 Aug;55(8):758-65.
16. Rolf A. Stahl, The STEC HUS Study Group. Open-Label, Multi-Center Trial of Ecu in Patients with Shiga-Toxin-Producing E. coli Hemolytic Uremic Syndrome (STEC HUS). Oral Presentation to American Society of Nephrology, San Diego, USA, November 3rd 2012.
17. Kielstein JT, Beutel G, Fleig S, Steinhoff J, Meyer TN, Hafer C, Kuhlmann U, Bramstedt J, Panzer U, Vischedyk M, Busch V, Ries W, Mitzner S, Mees S, Stracke S, Nürnberger J, Gerke P, Wiesner M, Sucke B, Abu-Tair M, Kribben A, Klause N, Schindler R, Merkel F, Schnatter S, Dorresteijn EM, Samuelsson O, Brunkhorst R; Collaborators of the DGfN STEC HUS registry.Best supportive care and therapeutic plasma exchange with or without Ecu in Shiga-toxin-producing E. coli O104:H4 induced haemolytic-uraemic syndrome: an analysis of the German STEC HUS registry. Nephrol Dial Transplant. 2012 Oct;27(10):3807-15.
18. Delmas Y, Vendrely B, Clouzeau B, Bachir H, Bui HN, Lacraz A, Hélou S, Bordes C, Reffet A, Llanas B, Skopinski S, Rolland P, Gruson D, Combe C. Outbreak of Escherichia coli O104:H4 haemolytic uraemic syndrome in France: outcome with Ecu. Nephrol Dial Transplant. 2014 Mar;29(3):565-72.
19. Loos S, Ahlenstiel T, Kranz B, Staude H, Pape L, Härtel C, Vester U, Buchtala L, Benz K, Hoppe B, Beringer O, Krause M, Müller D, Pohl M, Lemke J, Hillebrand G, Kreuzer M, König J, Wigger M, Konrad M, Haffner D, Oh J, Kemper MJ. An outbreak of Shiga toxin-producing Escherichia coli O104:H4 hemolytic uremic syndrome in Germany: presentation and short-term outcome in children. Clin Infect Dis. 2012 Sep;55(6):753-9.
20. Keir LS, Spooner RA, Welsh GI, Coward RJ, Satchell S, Richards A , Saleem MA. The role of the podocyte in the renal pathogenesis of Diarrhoea associated Haemolytic Uraemic Syndrome (D+HUS). Oral Presentation at European Society of Paediatric Nephrology, Krakow, Poland. September 2012
21. Eremina V, Sood M, Haigh J, Nagy A, Lajoie G, Ferrara N, Gerber HP, Kikkawa Y, Miner JH, Quaggin SE. Glomerular-specific alterations of VEGF-A expression lead to distinct congenital and acquired renal diseases. J Clin Invest. 2003 Mar;111(5):707-16.
22. Eremina V, Jefferson JA, Kowalewska J, Hochster H, Haas M, Weisstuch J, Richardson C, Kopp JB, Kabir MG, Backx PH, Gerber HP, Ferrara N, Barisoni L, Alpers CE, Quaggin SE. VEGF inhibition and renal thrombotic microangiopathy. N Engl J Med. 2008 Mar 13;358(11):1129-36
23. Alberti, M, Valoti, E, Piras, R, Bresin, E, Galbusera, M, Tripodo, C, Thaiss, F, Remuzzi, G, Noris, M: Two patients with history of STEC-HUS, posttransplant recurrence and complement gene mutations. Am J Transplant, 13: 2201-2206, 2013.
24. Bu F, Maga T, Meyer NC, Wang K, Thomas CP, Nester CM, Smith RJ. Comprehensive genetic analysis of complement and coagulation genes in atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2014 Jan;25(1):55-64.

25. Drug Safety and Risk Management Advisory Committee: Briefing Document for Soliris (Ecu). Oct 2014
26. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. The fourth report on the diagnosis, evaluation, and treatment of high blood pressure in children and adolescents. Pediatrics. 2004;114(2 Suppl 4th Report):555
27. Akcan-Arikan A, Zappitelli M, Loftis LL, Washburn KK, Jefferson LS, Goldstein SL. Modified RIFLE criteria in critically ill children with acute kidney injury. Kidney Int. 2007 May;71(10):1028-35.
28. Schwartz GJ, Work DF. Measurement and estimation of GFR in children and adolescents. Clin J Am Soc Nephrol. 2009 Nov;4(11):1832-43.
29. Yoh M, Frimpong EK, Voravuthikunchai SP, Honda T. Effect of subinhibitory concentrations of antimicrobial agents (quinolones and macrolide) on the production of verotoxin by enterohemorrhagic Escherichica Coli O157:H7. Can J Microbiol. 1999 Sep;45(9):732-9.
30. Lang RM, Bierig M, Devereux RB et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr 2005; 18(12): 1140-63.
31. Zoghbi WA, Enriquez-Sarano M, Foster E et al. Recommendations for evaluation for the severity of native valvular regurgitation with two-dimensional and Doppler echocardiography. J Am Soc Echocardiogr 2003; 16(7): 777-802.
32. Thygesen K, Alpert JS, Jaffe AS et al. Third universal definition of myocardial infarction. Circulation 2012; 126(16): 2020-35.

Definitions

Term	Description
21 Adverse Event (AE)	Any untoward medical occurrence in a participant or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. Comment: An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational medicinal product, whether or not related to the investigational medicinal product.
Adverse Reaction (AR)	All untoward and unintended responses to an IMP related to any dose administered. Comment: An AE judged by either the reporting Investigator or Sponsor as having causal relationship to the IMP qualifies as an AR. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.
Serious Adverse Event (SAE)	Any untoward medical occurrence or effect that: • Results in death <for phase III trials with long term follow-up where survival is an endpoint consider adding text "(unrelated to original disease)"> • Is life-threatening* • Requires hospitalisation or prolongation of existing inparticipants' hospitalisation • Results in persistent or significant disability or incapacity • Is a congenital anomaly/birth defect • Or is otherwise considered medically significant by the Investigator** Comments: The term severe is often used to describe the intensity (severity) of a specific event. This is not the same as serious, which is based on participants/event outcome or action criteria. * Life threatening in the definition of an SAE refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe. ** Medical judgment should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life threatening or do not result in death or hospitalisation but may

	jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should be considered serious.
Serious Adverse Reaction (SAR)	An Adverse Reaction which also meets the definition of a Serious Adverse Event
Unexpected Adverse Reaction (UAR)	An AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator Brochure for an unapproved IMP or (compendium of) Summary of Product Characteristics (SPC) for a licensed product). When the outcome of an AR is not consistent with the applicable product information the AR should be considered unexpected.
Source data	All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A SAR that is unexpected i.e. the nature, or severity of the event is not consistent with the applicable product information. A SUSAR should meet the definition of an AR, UAR and SAR.
Trials Office	The team of people, including the Chief Investigator, responsible for the overall management and coordination of the trial.
Unexpected Event	The type of event that is not listed in the protocol as an expected occurrence.
Source data	All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial
Trials Office	The team of people, including the Chief Investigator, responsible for the overall management and coordination of the trial.

Table 9.

Appendix 1: Clinical Severity Score

Renal	Lowest eGFR >50	1
	Lowest eGFR 26-50, no oligoanuria*	2
	Lowest eGFR ≤ 25, no oligoanuria*	3
	Oligoanuria* but no dialysis (or renal replacement therapy, RRT) required	4
	Dialysis/RRT <48 hours	5
	Dialysis/RRT 2 days	6
	Dialysis/RRT 3 days	7
	Dialysis/RRT 4 days	8
	Dialysis/RRT 5 days	9
	Dialysis/RRT 6 days	10
	Dialysis/RRT 7 days	11
	Dialysis/RRT 8 days	12
	Dialysis/RRT 9 days	13
	Dialysis/RRT 10 days	14
	Dialysis/RRT 11 days	15
	Dialysis/RRT 12 to 13 days	16
	Dialysis/RRT 14 to 17 days	17
	Dialysis/RRT 18 to 20 days	18
	Dialysis/RRT 21 to 27 days	19
	Dialysis/RRT 28 to 34 days	20
	Dialysis/RRT 35 to 41 days	21
	Dialysis/RRT 42 to 48 days	22
	Dialysis/RRT 49 to 55 days	23
	Dialysis/RRT >55 days	24
CNS	No obvious CNS involvement	0
	Altered consciousness (Agitation, irritability, hallucinations, confusion, excessive drowsiness)	2
	Single seizure	4
	Two or more seizures 24 hrs apart**	6
	Transient focal neurological defect (>24 hrs*** but <1 week)	7
	Persistent focal neurological defect (present at day 60 and persistent for more than 1 week)	10
	Persistent global (≥ 2 brain functions - vision/hearing/cognitive/motor/sensory/memory) neurological defect at day 60	15
Pancreas	No clinical or biochemical evidence pancreatitis	0
	Raised amylase and/or lipase† without clinical symptoms/signs	2
	Hyperglycaemia without insulin requirement	6
	Pancreatitis with sequelae (laparotomy, parenteral nutrition††, insulin required)	8
	Chronic sequelae of pancreatitis at day 60 (parenteral nutrition††, insulin, other)	10
Gastro-intestinal	No abdominal surgery reguired (except related to peritoneal dialysis catheter)	0

	Laparoscopy/laparotomy required for abdominal symptoms	5
	Intestinal perforation AND/OR bowel resection required	8
	Stoma formation	10
Cardiac	No cardiac involvement (normal CVS examination - except hypertension/volume overload)	0
	Cardiac failure confirmed by ECHO††† (impaired systolic ventricular# function or chamber enlargement## or valve regurgitation###)	4
	Cardiac failure confirmed by ECHO with dilated cardiomyopathy	6
	Myocardial infarction (on standard ECG +/- troponin +/- ECHO evidence)####	10

*****oligoanuria defined as urine output <0.5ml/kg/hr for 12 hours

** Multiple seizures occurring within a 24 hr period considered part of the same event

***Todd's paresis following a seizure should resolve within 24 hrs

† lipase measurement not mandatory, however if measured and found to be elevated this would count

†† only if parenteral nutrition is required because of pancreatitis, not for other indications

††† ECHO only mandatory if clinical signs of cardiac failure or myocardial infarction

Impaired systolic ventricular function: left ventricular ejection fraction <55% (measured by volume estimation method such as modified Simpson's rule) OR fractional shortening <25% (using 2-dimensional or M-mode)[30].

Chamber enlargement: Left ventricular end-diastolic diameter (LVEDD) ≥2 z-scores (SD, using M-mode in parasternal long axis)[30].

Valve regurgitation: new mitral valve regurgitation ≥ moderate (vena contracta width ≥0.3cm, regurgitant volume ≥30ml/beat, regurgitant fraction ≥30%, effective regurgitant orifice area ≥0.2cm^2)[31].

Diagnosis requires troponin evidence of myocardial infarction AND at least one of symptoms of ischaemia OR ECG evidence OR Echo evidence[32].

Troponin evidence: any cardiac troponin measurement greater than the 99th centile upper reference limit.

ECG evidence: new significant localised ST-segment-T wave changes OR pathological Q waves OR left bundle branch block.

Echo evidence: new regional wall motion abnormality OR new mitral valve regurgitation due to papillary muscle rupture.

Within each domain, highest score at any point in first 60 days is recorded and score for each domain is added together to give total clinical severity score.

Abbreviations: eGFR estimated glomerular filtration rate; RRT renal replacement therapy; CNS central nervous system; CVS cardiovascular system; ECHO echocardiogram.

Appendix 2: Agreed Alternative Risk Minimisation Measures

Background: ECUSTEC is a randomised, double-blind, placebo-controlled trial investigating the use of Ecu in Shiga-Toxin producing E. Coli Haemolytic Uraemic Syndrome. The hypothesis is that Ecu is most effective when given early in disease course. The first dose of Ecu/placebo will be given within 48 hours of the patient arriving at the trial site.

Challenges: The controlled distribution system imposed on Ecu requires an individual certificate of meningococcal vaccination (or antibiotic prophylaxis administration if meningococcal vaccination is contra-indicated) to be completed and provided to Alexion before any shipment can be made. However, the time critical nature of ECUSTEC would require stock to be available at each participating trial site to ensure timely administration of the drug for trial participants.

We propose alternative risk minimisation measures for the prevention of meningococcal disease for ECUSTEC Trial participants, with the understanding that ECUSTEC will be conducted in accordance with Clinical Trials Regulation and Good Clinical Practice.

Agreed risk minimisation measures:

- The trial to be conducted in accordance to a clinical trial protocol approved by the MHRA.
- The trial protocol to stipulate the requirement for meningococcal vaccination as well as antibiotic prophylaxis for all consented trial participants irrespective of the treatment allocation (i.e. Ecu vs. Placebo).
- Recruitment of participants to occur only when all inclusion criteria and none of the exclusion criteria have been met.
- All trial participants to be systematically followed up in accordance to the approved ECUSTEC clinical trial protocol.
- Alexion to distribute Ecu of an agreed quantity to each trial site along with educational risk minimisation materials (for all participants whether receiving Ecu or placebo) only upon receipt of confirmation from the trial sponsor that all trial approvals (MHRA approval, REC approval, HRA approval) are in place for that site.

- All orders relating to the trial are to be requested by sites using a trial specific order form.
- Trial sites to segregate the trial Ecu stock from general pharmacy stock.
- Trial sites to fully document the receipt and dispensing of the product to facilitate traceability of the product.
- Sites to complete the ECUSTEC Day 1 Certificate of Vaccination for all participants in order for site pharmacist to dispense first dose of Ecu/placebo
- Sites to send the ECUSTEC Day 1 Certificate of Vaccination for all participants to Alexion no later than 48 hours after the 1st dose of Ecu (identified by unique trial identifier only)
- Sites to complete the Pre-discharge ECUSTEC Certificate of Vaccination for all participants prior to discharge from trial site and send to Birmingham Clinical Trials unit
- Sites to send the Pre-discharge ECUSTEC Certificate of Vaccination for all participants to Alexion no later than 48 hours after discharge from (identified by unique trial identifier only)
- To minimise wastage, trial stock approaching its expiry/ unused trial stock at the end of the trial to be re-distributed to general pharmacy stock, and this will be clearly documented by trial sites.
- Adverse event reporting will take place according to Good Clinical Practice
- Alexion will be notified in the event of a Serious Unexpected Serious Adverse Reaction

Appendix 3. ECUSTEC Questionnaires Table of Completion by Age

ECUSTEC Questionnaires Table of Completion by Age		
Age of Child (years)	**Parent Completed PedsQL**	**Parent Completed Child Health Utility 9D (CHU-9D)**
≤1	Use version 1-12 months	No form available
>1	Use version 13-24 months	No form available
2	Use version 2-4 years	No form available
3	Use version 2-4 years	Use CHU-9D for under 5 years
4	Use version 2-4 years	Use CHU-9D for under 5 years
5	Use version 5-7 years	Use CHU-9D
6	Use version 5-7 years	Use CHU-9D
7	Use version 5-7 years	Use CHU-9D
8	Use version 8-12 years	Use CHU-9D
9	Use version 8-12 years	Use CHU-9D
10	Use version 8-12 years	Use CHU-9D
11	Use version 8-12 years	Use CHU-9D
12	Use version 8-12 years	Use CHU-9D
13	Use version 13-18 years	Use CHU-9D
14	Use version 13-18 years	Use CHU-9D
15	Use version 13-18 years	Use CHU-9D
16	Use version 13-18 years	Use CHU-9D
17	Use version 13-18 years	Use CHU-9D
18	Use version 13-18 years	Use CHU-9D

Appendix 4: Clinical Severity Score Cardiac Component

No cardiac involvement (normal CVS examination – except hypertension/volume overload) = 0

Cardiac failure confirmed by Echo (impaired systolic ventricular function or chamber enlargement or valve regurgitation) = 4

Impaired systolic ventricular function: left ventricular ejection fraction <55% (measured by volume estimation method such as modified Simpson's rule) OR fractional shortening <25% (using 2-dimensional or M-mode) [1].

Chamber enlargement: Left ventricular end-diastolic diameter (LVEDD) ≥2 z-scores (SD, using M-mode in parasternal long axis) [1].

Valve regurgitation: new mitral valve regurgitation ≥ moderate (vena contracta width ≥0.3cm, regurgitant volume ≥30ml/beat, regurgitant fraction ≥30%, effective regurgitant orifice area ≥0.2cm^2) [2].

Cardiac failure confirmed by Echo with dilated cardiomyopathy = 6

Impaired systolic ventricular function AND chamber enlargement (both as above).

Myocardial infarction (on standard ECG +/- troponin +/- Echo evidence) = 10

Diagnosis requires troponin evidence of myocardial infarction AND at least one of symptoms of ischaemia OR ECG evidence OR Echo evidence [3].

Troponin evidence: any cardiac troponin measurement greater than the 99th centile upper reference limit.

ECG evidence: new significant localised ST-segment-T wave changes OR pathological Q waves OR left bundle branch block.

Echo evidence: new regional wall motion abnormality OR new mitral valve regurgitation due to papillary muscle rupture.