Efficacy and safety of eculizumab in children with Shiga-toxin-producing *Escherichia coli* haemolytic uraemic syndrome: the ECUSTEC RCT

Natalie Ives,[1] Rebecca Woolley,[1] Moin A Saleem,[2] Catherine A Moakes,[1] Aoife Waters,[3] Rodney D Gilbert,[4] Hugh Jarrett,[1] Elizabeth Brettell,[1] Steve Nash,[5] Louise K Farmer,[2] Khadija Ourradi[4] and Sally A Johnson[6,7,8*]

[1]Birmingham Clinical Trials Unit, Institute of Applied Health Research, University of Birmingham, Birmingham, UK
[2]Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
[3]Department of Paediatric Nephrology, Great Ormond Street Hospital, London, UK
[4]Department of Paediatric Nephrology, Southampton Children's Hospital, Southampton, UK
[5]Campaigner on *Escherichia coli*, Hayes, Middlesex, UK
[6]Department of Paediatric Nephrology, Great North Children's Hospital, Newcastle, UK
[7]National Renal Complement Therapeutics Centre, Newcastle, UK
[8]Newcastle University Translational Clinical Research Institute, Newcastle, UK

[*]Corresponding author sally.johnson15@nhs.net

Published July 2024
DOI: 10.3310/RFTY4766

Scientific summary

Efficacy and safety of eculizumab in children with Shiga-toxin-producing *Escherichia coli* haemolytic uraemic syndrome: the ECUSTEC RCT

Efficacy and Mechanism Evaluation 2024; Vol. 11: No. 11
DOI: 10.3310/RFTY4766

NIHR Journals Library www.journalslibrary.nihr.ac.uk

Scientific summary

Background

Shiga-toxin-producing *Escherichia coli* haemolytic uraemic syndrome affects around 100 UK children each year, following gastrointestinal infection with Shiga-toxin-producing *E. coli*. Around half of affected children will need dialysis, about a quarter develop serious complications with long-term consequences and about 3% die. All patients require long-term follow-up because of the risk of developing chronic kidney disease (CKD).

No intervention has definitively been shown to reduce morbidity or mortality in this condition, and therefore treatment is supportive. Case reports and case series suggest that eculizumab (Soliris®, Alexion Pharmaceuticals, Boston, MA), an inhibitor of the complement system and an effective treatment for the related condition, atypical haemolytic uraemic syndrome, may be effective in this condition. Until very recently, there were no published data regarding the efficacy and safety of eculizumab in Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome, and yet despite this its use has risen globally. A recent randomised phase 3 clinical trial reported comparison of eculizumab with placebo in 100 children with Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome (Garnier A, Brochard K, Kwon T, Sellier-Leclerc A-L, Lahoche A, Allain Launay E, *et al*. Efficacy and safety of Eculizumab in pediatric patients affected by Shiga Toxin-Related Hemolytic and Uremic Syndrome: a randomized, placebo-controlled trial. *J Am Soc Nephrol* 2023;**34**:1561–73). Patients with severe multi-organ involvement were excluded. Four patients in the placebo group were withdrawn and subsequently received eculizumab. There was no difference between treatment groups in the proportion of children who required renal replacement therapy 48 hours after randomisation, In addition, no differences between groups were seen in the secondary outcome measures of extra-renal manifestations, duration of hospitalisation and mortality. During follow up, there was a slight difference in the proportion who exhibited renal sequelae at 12 months post randomisation (20 patients in the eculizumab group (43.48%) and 29 patients (64.44%) in the placebo group (P = 0.04). The authors concluded that eculizumab seemed to have no impact on the course of acute kidney injury and interpreted the 12-month follow-up data with caution. No data have been published which include the role of eculizumab in patients with severe manifestations of disease in a controlled setting.

Objectives of the main trial

The Eculizumab in Shiga-toxin-producing Escherichia coli Haemolytic Uraemic Syndrome (ECUSTEC) trial was designed to test the hypothesis that treatment with eculizumab reduces the severity of Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome in children aged 6 months–< 19 years. We also wanted to assess the safety of eculizumab and test the hypothesis that treatment with eculizumab reduces the incidence of CKD following Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome.

Objectives of the mechanistic substudies

The mechanistic component of the trial had the following objectives:

- to investigate the time course of systemic complement activation in Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome and its relation to the severity of disease
- to determine whether thrombotic microangiopathy (TMA) in Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome occurs via a Shiga-toxin-mediated reduction in podocyte vascular endothelial growth factor (VEGF) production, leading to loss of complement regulation

- to test whether neutrophils derived from patients with acute Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome deliver Shiga toxin to podocytes
- to assess whether any genetic variations in patients with Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome point to novel pathogenic mechanisms.

Methods

Design

The trial was a randomised, double-blind, placebo-controlled, parallel-group trial of two doses of eculizumab in children (aged 6 months–< 19 years) with Shiga-toxin-producing *E. coli* Haemolytic Uraemic Syndrome. The trial had an internal pilot phase and included nested mechanistic laboratory studies and a cost-effectiveness evaluation, although the latter was not undertaken following the trial being stopped early.

The mechanistic substudies were optional; all participants in the main trial were offered the opportunity to participate in the substudies, which involved providing blood and urine samples over the first 30 days of the trial. Participant blood and urine samples were used to explore the evidence for, and time course of, complement activation in this condition. Using both patient samples and an in vitro cell co-culture model, evidence was sought to support the hypothesis that Shiga toxin causes a glomerular TMA as a consequence of its effect on podocyte VEGF production. This included measurement of patient urine and plasma complement factor H (CFH) and VEGF and plasma complement activation products [by both enzyme-linked immunosorbent assay (ELISA) and a novel degradomics technique]. In the co-culture experiments, glomerular endothelial cells were exposed to Shiga toxin in the presence and absence of podocytes.

Setting

The trial was conducted in 12 sites in NHS hospital settings across the UK with the support of 88 Patient Identification Centres.

Participants

Informed consent was sought from parents/guardians of eligible children (those aged 6 months–< 19 years weighing ≥ 5 kg, with a clinical diagnosis of Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome, including acute kidney injury (AKI) equivalent to the 'injury' or 'failure' category of the paediatric risk/injury/failure/loss/end criteria). Eligible young people aged 16–18 years provided their own consent for participation in the trial, with assent from younger children if appropriate (according to age).

Screening and randomisation

Screening began as soon as possible after a diagnosis of Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome was suspected. Once eligibility was confirmed and informed consent obtained, the participants were commenced on prophylactic antibiotics, and unless contra-indicated or already administered, participants were also vaccinated against meningococcal infection. Participants were then randomised into the ECUSTEC trial via a secure online central randomisation system. Participants were randomised at the level of the individual in a 1 : 1 ratio to either eculizumab or placebo, which was commenced as soon as possible after randomisation. A minimisation algorithm was used to ensure balance in the treatment allocation over the following variables: centre, severity of AKI and hydration status. To avoid predictability in the randomisation, a random element was included in the minimisation algorithm, so that each patient had a probability (unspecified here), of being randomised to the opposite treatment that they would have otherwise received.

Intervention

Each participant received two intravenous infusion doses of either eculizumab (dose according to first two doses of induction regimen for atypical haemolytic uraemic syndrome) or placebo. The first dose was given as soon as possible after randomisation (designated day 1), with the second dose given 7 days later (i.e. on day 8). They also received vaccination against meningococcal disease and an 8-week course of antibiotic prophylaxis. The participants, parents/guardians, clinical staff and research teams were blind to randomised treatment allocation throughout the study.

Outcome measures

Primary

The ECUSTEC clinical severity score (CSS), a purpose-developed, multidomain score comprising severity of AKI and extrarenal events. A single score is assigned at day 60 to reflect cumulative morbidity up until that point. The score ranges from 1 to 69 with higher scores indicating greater disease severity.

Secondary

- Overall survival.
- Duration of renal replacement therapy (days).
- Duration of thrombocytopenia (number of consecutive days until platelet count > 150 × 10^9/l).
- Duration of haemolysis (number of days until lactate dehydrogenase within 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).
- Persistent neurological defect at day 60 measured by structured expert assessment to include central nervous system examination, vision, hearing and neuropsychological assessment.
- CKD at 52 weeks (a composite end point of the presence of hypertension, albuminuria or estimated glomerular filtration rate (eGFR) < 90 ml/minute/1.73 m^2 at 52 weeks).
- eGFR measurement using a centralised cystatin C assay at 52 weeks.

Mechanistic studies

- Urine CFH levels.
- Urine VEGF levels.
- Presence of urine markers of podocyte damage (nephrin and Wilms tumour-1).
- Plasma VEGF and factor H levels.
- Plasma complement activation products (Bb, C3a, C4a and sC5b9 by ELISA and C3 and C4 activation markers by degradomics).
- Glomerular endothelial surface levels of factor H and C3d (a marker of complement activation) in a co-culture models of human conditionally immortalised podocytes and glomerular endothelial cells exposed to Shiga toxin.
- Whole exome sequencing and serum anti-factor H antibody levels.

Results

The target sample size was 134 participants, but recruitment was stopped early due to low recruitment and the impact of the COVID-19 pandemic. At the point the trial was stopped, 108 children had been screened for participation, of whom 87 were deemed eligible to participate. Thirty-six children were consented and randomised; 17 were randomised to eculizumab and 19 were randomised to placebo. One participant withdrew from the trial and one participant died. The majority of baseline data of the

participants were comparable across the two groups; however, the participants in the placebo group were slightly older and consequently heavier than those in the eculizumab group.

Reasons for slow recruitment included a fall in the incidence of Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome during the trial period (up to a 37% reduction) and a lack of out-of-hours infrastructure for undertaking acute interventional clinical trials in children.

The mean CSS at day 60 for participants randomised to eculizumab was 11.5 [standard deviation (SD) 8.4] compared to 14.6 (SD 7.7) for participants randomised to placebo [adjusted mean difference: −2.5, 95% confidence interval (CI) −7.8 to 2.8, $p = 0.3$]. Five participants (three eculizumab, two placebo) experienced an adverse event, and there were seven serious adverse events (SAEs) in six participants (five eculizumab, one placebo). None of the SAEs were considered related to the trial treatment.

Mechanistic substudies

Of the 36 participants recruited to the main trial, 32 consented to take part in the mechanistic studies and provided blood and/or urine samples. In anuric patients, only blood samples were collected.

Urine factor H and vascular endothelial growth factor levels in serial samples

The highest urine factor H levels were at day 1 (150 ng/ml), diminishing by day 4 (30 ng/ml), and completely normalising by day 30 (undetectable).

The highest urine VEGF levels were at day 1 (average 1300 ng/ml) and by day 30 the levels were below 20 ng/ml.

Markers of podocyte damage

Western blots of urine cell pellets showed acute podocyte loss during active disease, which recovered by day 8.

Plasma factor H and vascular endothelial growth factor levels

No difference was seen for plasma levels of either factor H or VEGF at day 1 or day 30.

Plasma degradomics analysis

In a sample of five patients, N-termini consistent with complement C3 and C4 activation were much more abundant at day 1 compared with day 3.

In vitro cell co-culture

In response to Shiga toxin, there was a reduction in glomerular endothelial factor H levels, accompanied by evidence of complement activation (increased C3d levels) and this was critically dependent on the presence of podocytes. Shiga toxin had no effect when added to endothelial cells alone.

Plasma complement activation products

Mean plasma levels of Bb were elevated in both groups at baseline (4.38 mcg/ml in the eculizumab group and 10.38 mcg/ml in the placebo group, normal range 0.48–1.62 mcg/ml). They were also elevated at day 2 (5.91 mcg/ml in the eculizumab group and 4.09 mcg/ml in the placebo group) and day 4 (4.90 mcg/ml in the eculizumab group and 3.16 mcg/ml in the placebo group). At day 6 and day 8, Bb levels remained elevated in the placebo group (6.21 and 3.47 mcg/ml respectively) but were normal in the eculizumab group. In both groups, mean Bb levels were in the normal range at day 30.

Mean plasma levels of C3a were elevated in both groups at baseline and at days 2 and 4. At days 6 and 8, mean levels remained elevated in the placebo group while mean levels in the eculizumab group were in the normal range. Levels were in the normal range for both groups by day 30.

Mean plasma levels of C4a were elevated at all time points in both groups but fell significantly at day 30. Mean levels were 3852, 3026, 3423, 3067, 3425 and 1623 ng/ml at days 1, 2, 4, 6, 8 and 30 in the eculizumab group (normal range 110–699 ng/ml) and 3970, 3573, 2673, 4348, 2844 and 1992 ng/ml at the same respective time points in the placebo group.

Mean plasma levels of sC5b9 were normal in both groups at all time points with the exception of day 4 and day 6 in the placebo group, which were elevated (487 and 514 ng/ml respectively, normal range 95–467 ng/ml).

In the placebo group, a linear relationship was not established between CSS and baseline Bb (r = 0.43, p = 0.2); C3a (r = −0.16, p = 0.7); C4a (r = 0.15, p = 0.7) or sC5b9 (r = −0.17, p = 0.7). Similarly, a linear relationship was not established between CSS and the maximum value of Bb (r = 0.45, p = 0.1); C3a (r = 0.15, p = 0.6); C4a (r = 0.23, p = 0.4) or sC5b9 (r = −0.22, p = 0.5).

Delivery of Shiga toxin to podocytes from patient-derived neutrophils

Insufficient patient samples were obtained to complete this part of the work.

Genetic variations in patients with Shiga-toxin-producing Escherichia coli *haemolytic uraemic syndrome*

Data from whole exome sequencing have been obtained and analysis is ongoing.

Conclusions

In children with Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome, the mean CSSs at day 60 were similar between those randomised to eculizumab and those randomised to placebo. However, since the trial was stopped early and did not recruit to the planned sample size, this cannot be interpreted as evidence of no effect. In order to deliver successful clinical trials of investigational medicinal products in acutely unwell children, a review of out-of-hours paediatric research infrastructure may be required.

In the mechanistic substudies, we have established that urine factor H and VEGF levels are sensitive measures of early disease activity, and have demonstrated complement activation in patient serum using both ELISA and sophisticated proteomics technology. Urine factor H and VEGF levels and plasma degradomics for C3 and C4 proteins could all be further explored as new biomarkers of acute Shiga-toxin-producing *E. coli* haemolytic uraemic syndrome. Our co-culture cell work has demonstrated that podocyte cross-talk is responsible for factor H and complement activation levels on endothelial cells. Collectively this strongly supports the mechanistic hypothesis of a complement-mediated disease driven via the podocyte as the target cell.

Trial registration

This trial is registered as EudraCT2016-000997-39 and ISRCTN89553116.

Funding

This award was funded by the National Institute for Health and Care Research (NIHR) Efficacy and Mechanism Evaluation (EME) programme (NIHR award ref: 14/48/43) and is published in full in *Efficacy and Mechanism Evaluation*; Vol. 11, No. 11. See the NIHR Funding and Awards website for further award information.

Efficacy and Mechanism Evaluation

ISSN 2050-4373 (Online)

A list of Journals Library editors can be found on the NIHR Journals Library website

Efficacy and Mechanism Evaluation (EME) was launched in 2014 and is indexed by Europe PMC, DOAJ, Ulrichsweb™ (ProQuest LLC, Ann Arbor, MI, USA) and NCBI Bookshelf.

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

Editorial contact: journals.library@nihr.ac.uk

The full EME archive is freely available to view online at www.journalslibrary.nihr.ac.uk/eme.

Criteria for inclusion in the *Efficacy and Mechanism Evaluation* journal
Manuscripts are published in *Efficacy and Mechanism Evaluation* (EME) if (1) they have resulted from work for the EME programme, and (2) they are of a sufficiently high scientific quality as assessed by the reviewers and editors.

EME programme

The Efficacy and Mechanism Evaluation (EME) programme funds ambitious studies evaluating interventions that have the potential to make a step-change in the promotion of health, treatment of disease and improvement of rehabilitation or long-term care. Within these studies, EME supports research to improve the understanding of the mechanisms of both diseases and treatments.

The programme supports translational research into a wide range of new or repurposed interventions. These may include diagnostic or prognostic tests and decision-making tools, therapeutics or psychological treatments, medical devices, and public health initiatives delivered in the NHS.

The EME programme supports clinical trials and studies with other robust designs, which test the efficacy of interventions, and which may use clinical or well-validated surrogate outcomes. It only supports studies in humans and where there is adequate proof of concept. The programme encourages hypothesis-driven mechanistic studies, integrated within the efficacy study, that explore the mechanisms of action of the intervention or the disease, the cause of differing responses, or improve the understanding of adverse effects. It funds similar mechanistic studies linked to studies funded by any NIHR programme.

The EME programme is funded by the Medical Research Council (MRC) and the National Institute for Health and Care Research (NIHR), with contributions from the Chief Scientist Office (CSO) in Scotland and National Institute for Social Care and Health Research (NISCHR) in Wales and the Health and Social Care Research and Development (HSC R&D), Public Health Agency in Northern Ireland.

This article

The research reported in this issue of the journal was funded by the EME programme as award number 14/48/43. The contractual start date was in April 2016. The draft manuscript began editorial review in November 2021 and was accepted for publication in December 2022. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The EME editors and production house have tried to ensure the accuracy of the authors' manuscript and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this article.

This article presents independent research. The views and opinions expressed by authors in this publication are those of the authors and do not necessarily reflect those of the NHS, the NIHR, the MRC, the EME programme or the Department of Health and Social Care. If there are verbatim quotations included in this publication the views and opinions expressed by the interviewees are those of the interviewees and do not necessarily reflect those of the authors, those of the NHS, the NIHR, the EME programme or the Department of Health and Social Care.

This article was published based on current knowledge at the time and date of publication. NIHR is committed to being inclusive and will continually monitor best practice and guidance in relation to terminology and language to ensure that we remain relevant to our stakeholders.

Published by the NIHR Journals Library (www.journalslibrary.nihr.ac.uk), produced by Newgen Digitalworks Pvt Ltd, Chennai, India (www.newgen.co).