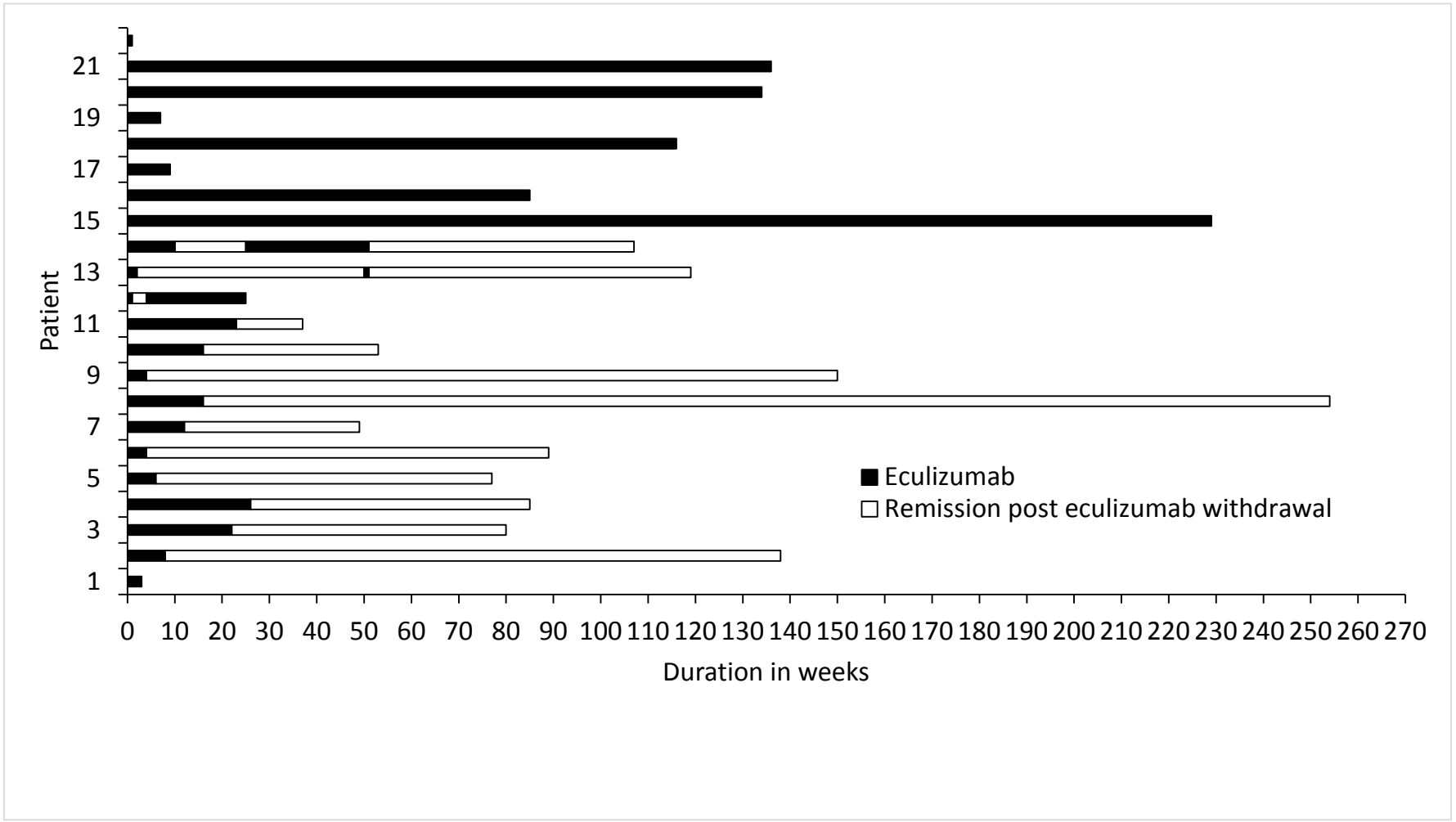
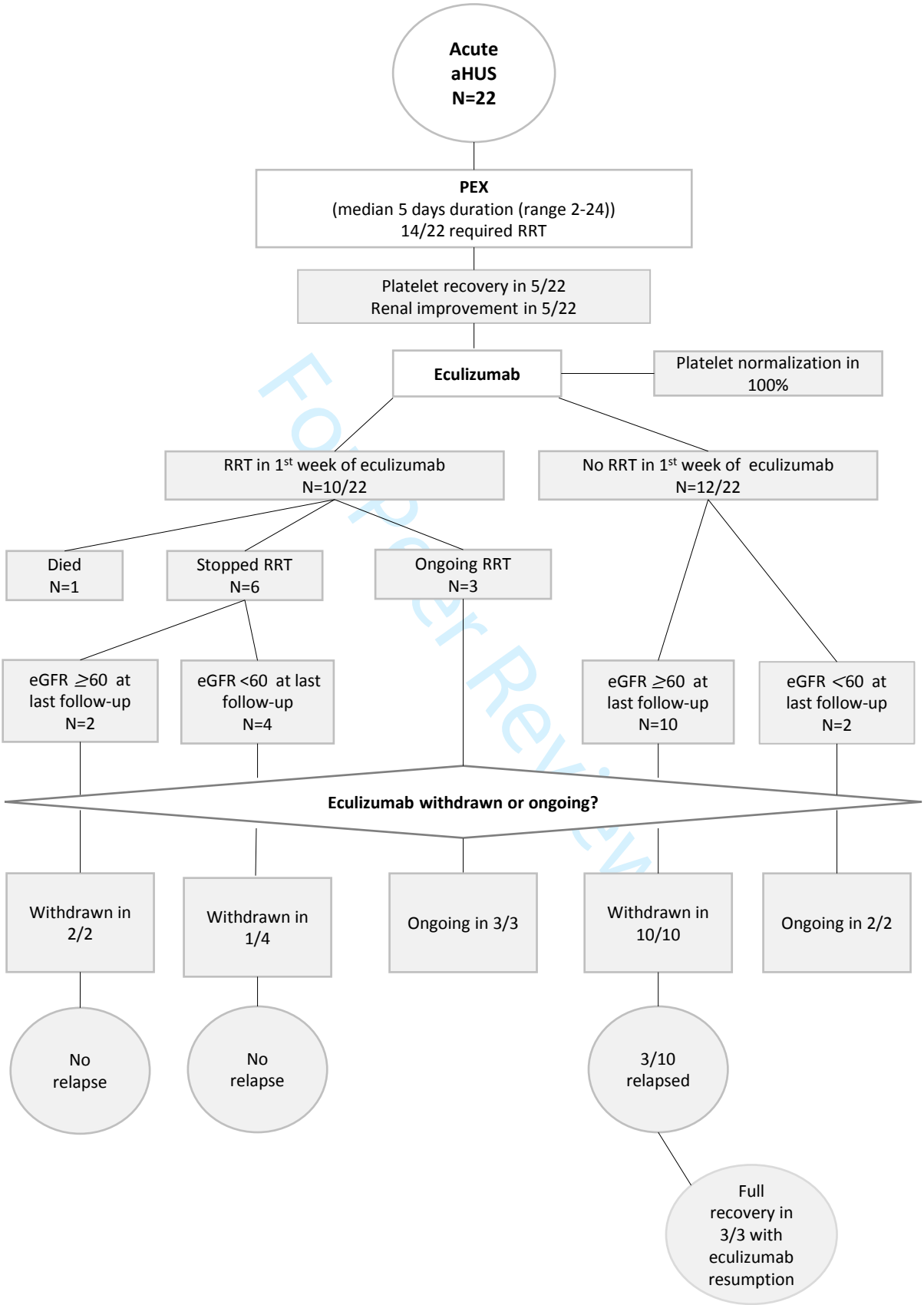
Atypical haemolytic uraemic syndrome in the eculizumab era: presentation, response to treatment and evaluation of an eculizumab withdrawal strategy.

Journal:	*British Journal of Haematology*
Manuscript ID	BJH-2018-01960.R1
Manuscript Type:	Ordinary Papers
Date Submitted by the Author:	24-Jan-2019

Complete List of Authors:	Neave, Lucy; University College London Hospitals NHS Foundation Trust, Department of Haematology Gale, Daniel; University College London, UCL Centre for Nephrology Cheesman, Simon; University College London Hospitals NHS Foundation Trust, Department of Pharmacy Shah, Raakhee; University College London Hospitals NHS Foundation Trust, Department of Haematology Scully, Marie; University College London Hospitals NHS Foundation Trust, Department of Haematology

Key Words:	aHUS, atypical haemolytic uraemic syndrome, eculizumab, withdrawal, TMA


Patient
21
19
17
15
13
11
9
7
5
3
1
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270
Duration in weeks
Eculizumab
Remission post eculizumab withdrawal



Atypical haemolytic uraemic syndrome in the eculizumab era: presentation, response to treatment and evaluation of an eculizumab withdrawal strategy.

Short Title: Eculizumab in aHUS

Authors: Lucy Neave[1], Daniel P Gale[2], Simon Cheesman[3], Raakhee Shah[3], Marie Scully[4]

[1] Department of Haematology, University College London Hospitals NHS Foundation Trust, London UK

[2] UCL Centre for Nephrology, University College London, London UK

[3] Department of Pharmacy, University College London Hospitals NHS Foundation Trust, London UK

[4] Department of Haematology, UCLH, Cardiometabolic programme- NIHR UCLH/UCL BRC, London, UK

Corresponding author:

Professor Marie Scully, Department of Haematology, University College London Hospital, 5th Floor Central, 250 Euston Road, London NW1 2PG, UK.
Telephone +44 203 447 9884 Fax +44 203 447 9145
Email m.scully@ucl.ac.uk

Key words:
aHUS, atypical haemolytic uraemic syndrome, eculizumab, withdrawal, TMA

Received 12 November 2018, accepted 5 February 2019

Summary

The complement inhibitor, eculizumab, has revolutionised the management of atypical haemolytic uraemic syndrome (aHUS), although the optimum treatment duration is debated. Twenty-two cases of acute aHUS managed with eculizumab were retrospectively reviewed, including outcomes after eculizumab withdrawal. Although 41% had an associated complement genetic abnormality, mutation status did not affect severity of clinical presentation. Sixty-four percent required renal replacement acutely, with a high incidence of nephrotic range proteinuria (47%). Eculizumab followed a median of 6 days of plasma exchange. After a median duration of therapy of 11 weeks (range 1-227), haematological recovery was seen in 100%, while 81% achieved at least partial renal recovery (median increase in estimated glomerular filtration rate (eGFR) 49 ml/min/1.73m^2). At median duration of follow-up of 85 weeks (range 4-255), 54.5% had eGFR ≥60 ml/min/1.73m^2, 27% had CKD, 14% were on dialysis, and 4.5% had died. Eculizumab was withdrawn in 59% (13/22) cases following complete haematological and renal recovery. Three of these 13 patients (23%) subsequently relapsed, with defined triggers in 2/3, but all made a full recovery with rapid resumption of eculizumab. There was a significant association between higher presenting creatinine and poorer renal outcomes. A strategy of eculizumab withdrawal in selected cases is both safe and cost effective.

Introduction

Atypical (or complement-mediated) haemolytic uraemic syndrome (aHUS) is a rare thrombotic microangiopathy (TMA), with an incidence of 1-2 per million (Noris and Remuzzi 2009). It is characterised by microangiopathic haemolytic anaemia (MAHA), consumptive thrombocytopenia, and multisystem end organ involvement with a predilection for the kidneys. Diagnosis is clinical, after exclusion of thrombotic thrombocytopenic purpura (TTP) [by ruling out severeADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) deficiency (activity <10%)], and other secondary causes of TMA, including infection-associated HUS due to shiga toxin-producing organisms (STEC) (Scully*, et al* 2017).

The last two decades have yielded significant developments in aHUS, both in elucidation of the pathophysiology and in management. Dysregulation of the alternative pathway of complement, as a result of an environmental trigger in a genetically susceptible individual is regarded as the key abnormality, leading to endothelial and platelet activation and, consequently, TMA (Jokiranta 2017). Pathogenic mutations in genes encoding complement factors H, B, I and 3 (*CFH*, *CFB*, *CFI*, *C3*), and

membrane cofactor protein (*CD46*), are demonstrated in 40-60% of affected individuals (Fremeaux-Bacchi*, et al* 2013, Noris and Remuzzi 2009, Schaefer*, et al* 2018). Factor H autoantibodies (Dragon-Durey*, et al* 2005, Hofer*, et al* 2014) and mutations in *DGKE* (encoding diacylglycerol kinase ε) (Lemaire*, et al* 2013) are rare causes usually seen in children.

Historically, the prognosis has been poor, with incomplete response rates to plasma exchange (PEX), and rates of end stage renal disease (ESRD) or death as high as 50-77% after 3-5 years (Fremeaux-Bacchi*, et al* 2013, Noris*, et al* 2010, Schaefer*, et al* 2018). However efficacy of the humanised monoclonal anti-C5 antibody eculizumab was demonstrated in open label phase II trials (Fakhouri*, et al* 2016, Greenbaum*, et al* 2016, Legendre *, et al* 2013, Licht*, et al* 2015a), inducing haematological remission, improving or stabilizing renal function, and preventing graft failure following renal transplant. US Food and Drug Administration and European Medicines Agency approvals were granted in 2011, and the National Health Service has funded the drug in England since 2013 under the coordination of the National aHUS Service.

At a cost of over £300 000 a year per patient, treatment carries significant financial burdens, as well as potential risks, including bacterial meningitis (Fakhouri*, et al* 2016), so the challenge now is to confirm that the drug is used in the most effective way possible, including determining the optimum treatment schedule. Whilst large-scale registries and prospective studies are needed to definitively address such questions [and are underway (Licht*, et al* 2015b)], in the short-term, retrospective cohort analyses are informative, though few have been published at present (Cataland*, et al* 2014, Cunningham*, et al* 2017, Fakhouri*, et al* 2014, Krishnappa*, et al* 2018, Sheerin*, et al* 2016).

We describe a cohort of 22 patients presenting to a UK TMA referral centre with acute aHUS and treated with eculizumab over a 6-year period. We highlight the presenting features and responses to eculizumab. We also assess the outcomes of a strategy of eculizumab withdrawal after achieving a complete or near-complete response.

Patients and Methods

All adult patients prescribed at least one dose of eculizumab for an acute presentation of aHUS in a single institution were retrospectively identified. Of 34 patients presenting with aHUS between 2012

and 2018, 9 responded to PEX only, 3 went on to receive an alternative C5 inhibitor on a clinical trial, while 22 patients received eculizumab. All 22 of these patients were included in the analysis, including those who were no longer followed up in our institution. Data regarding presenting clinical features, response to therapy and long-term outcomes of the aHUS episode was collected from the medical records as part of a service review, and analysed anonymously. All investigations had been performed as part of routine care.

In all cases, PEX with solvent detergent-treated plasma was initiated on admission. Renal replacement therapy (RRT) was commenced if indicated. The diagnosis of aHUS was made according to international consensus criteria (Scully*, et al* 2017) on the basis of: (i) presence of TMA (direct antiglobulin test-negative haemolytic anaemia with schistocytes on blood film and thrombocytopenia); (ii) exclusion of severe ADAMTS13 deficiency [(ADAMTS13 activity by fluorescence resonance energy transfer (Kokame*, et al* 2005) >10 IU/dL]; and (iii) exclusion of secondary TMAs (demonstration of normal coagulation screen, negative autoimmune serology, negative lupus anticoagulant and antiphospholipid antibody screening and negative reference laboratory STEC stool/serological investigations in all diarrhoeal cases, with imaging to exclude malignancy if indicated). End-organ damage was assessed via serum biochemistry, spot urine protein:creatinine ratio (UPCR), renal ultrasound, cardiac troponin I, electrocardiogram and, in selected cases, brain imaging. Renal biopsy was performed in cases of incomplete renal recovery where there was diagnostic uncertainty (N=2).

Following National aHUS Service approval to commence eculizumab, PEX was discontinued just prior to the first dose. Intravenous eculizumab was administered weekly for 4 weeks at a dose of 900 mg, followed by 1200 mg fortnightly starting on week 5. Meningococcal vaccination (against subtypes ACWY and B) was administered prior to initiation of eculizumab, followed by antibiotic prophylaxis (ciprofloxacin initially, followed by penicillin V) for the duration of therapy. If patients required ongoing RRT they were referred to their local renal centre. Following discharge, patients continued to receive eculizumab as an outpatient (and, in some cases, at home), with fortnightly blood and urine monitoring.

Withdrawal of eculizumab was considered in all patients who achieved complete haematological response [platelet count >150 x10^9/l and normal lactate dehydrogenase (LDH)] and complete or near-complete renal recovery [estimated glomerular filtration rate (eGFR) back to baseline without significant proteinuria]. The decision to stop eculizumab was made based on consensus clinician

opinion and patient preference, after a discussion of risks, benefits and available evidence. All patients who were offered the option to stop treatment elected to do so. Monitoring for relapse after withdrawal included symptoms review, full blood and reticulocyte counts, LDH, serum creatinine/eGFR and urinalysis/UPCR, initially fortnightly, but then at increasing time intervals and ultimately 6-monthly. All patients had access to a 24-h telephone helpline in the event of concerning symptoms in the interim.

Complement genotyping, performed via the National aHUS Service, included direct sequencing of coding exons of *CFH, CFI, CD46, C3* and *CFB,* and multiplex ligation-dependent probe amplification (MLPA) analysis for deletions and duplications of *CFH CFI, CD46*, *CFHR1* and *CFHR3*. Screening for factor H autoantibodies was also undertaken in the majority of cases.

Results

Summary of patients treated

Of the 22 patients identified, 68% were female. Median age at presentation was 32 years (range 16-67). All presented acutely with TMA: 21/22 were *de novo* presentations, whilst one patient had 4 previous episodes of 'relapsing TTP' but ADAMTS13 analysis performed for the first time on the index admission excluded TTP and led to a diagnosis of aHUS. All had native kidneys, and none were known to have chronic kidney disease (CKD) prior to presentation. Median duration of admission at our institution was 14 days (range 6-55), though 5 patients were discharged to local renal units for ongoing dialysis and 1 was repatriated to his local hospital. Median duration of follow-up from initiation of eculizumab was 85 weeks (range 4-255), excluding a patient who died on day 5. Five patients had ongoing outpatient management transferred to other centres (at 3, 7, 9, 37 and 124 weeks post-initiation of eculizumab, respectively) but data inclusive to those timepoints was included in the analysis. Key clinical information for all 22 patients is summarised in Table I.

Presenting features of aHUS

Gastroitestinal symptoms were common at presentation, affecting 64%, and were most commonly nausea and vomiting (36%), and abdominal pain (32%). STEC-negative diarrhoea was present in 23% of cases. Forty-one percent had neurological manifestations: seizure N=2; headache with or without visual disturbance N=4; transient diplopia N=1; transient facial and/or limb weakness N=2. Other presenting symptoms included: dark urine/ altered urine output (23%); bleeding/purpura (14%);

jaundice (9%); lethargy/malaise (5%). Three patients were diagnosed following detection of laboratory abnormalities in pregnancy. Thirteen patients were hypertensive at admission (though 3 had pre-existing hypertension).

Median nadir platelet count was 23 $x10^9$/l (range 7-85 $x10^9$/l) and median nadir haemoglobin (Hb) was 70g/l (range 62-103 g/l). Median presenting LDH was 1704 iu/l (range 582-4621 iu/l, normal range 135-214 iu/l). Reticulocytosis was notably absent in 45.5% of patients at presentation, though in all but one case this subsequently developed. Bilirubin remained normal in 22.7% of patients.

Median presenting creatinine was 323.5 µmol/l (range 80-1153, normal range 49-92). Proteinuria was demonstrated by urinalysis or UPCR in all cases. The median UPCR (N=17) was 199.5 mg/mmol (range 69->7000, normal range 0-13); it was >300 mg/mmol for 8/17 (47%). Median nadir eGFR was 11.5 ml/min/1.73m^2 (range 3-57), and 64% of patients (N=14) required RRT during the acute episode. Admission to the intensive care unit (ITU) was required by 14/22 (64%) patients, 4 of whom were intubated and ventilated. Whilst there were no overt cardiac manifestations at presentation, 77% (N=17) had elevated cardiac troponin T (median 64.5 ng/l, range 17-397, normal range 0-14). Despite neurological symptoms in 41%, brain magnetic resonance imaging was abnormal only in 3 cases (14%) (infarction/small vessel changes).

Clear triggers were identified in 50% of cases: pregnancy/postpartum in 4 (Patients 4, 5, 7 and 14 in Table I); influenza in 2; lower respiratory tract infections in 3; campylobacter diarrhoea in 1 (this was believed to be a trigger rather than the cause of the HUS, given that the TMA persisted despite resolution of the infection). Patient 10 initially presented with gallstone cholecystitis 5 months postpartum, but a frank TMA picture quickly evolved (along with post-ERCP pancreatitis) and the suspicion was that the gallstones resulted from a low grade postpartum HUS, as she had been noted to be hypertensive and proteinuric peripartum, with intermittent abdominal pain, malaise and nausea ever since.

ADAMTS13 activity on presentation was within the normal range for 86.3% (n=19) patients, and slightly low in 13.7% (n=3). Median activity was 72 iu/dl (range 56-91, normal range 60-146).

Response to plasma exchange

All 22 patients commenced PEX at admission, with a median PEX duration of 5 days (range 2-24). Platelet normalization (>150 x10^9/l) was achieved in 5/22 (range of PEX duration 5-18 days), with improvement in platelet count without normalization in 10/22 (range of PEX duration 2-24 days). Renal recovery to eGFR >90 ml/min/1.73m^2 was seen in 1 patient during PEX (Patient 9, PEX duration 24 days), but with incomplete platelet response. Four of 22 patients came off RRT during PEX but 7/22 remained RRT-dependent, while 3/22 required initiation of RRT.

Response to eculizumab

The median time from admission to initiation of eculizumab was 6 days [range 2-38 (with delays in the latter case due to funding issues due to non-UK nationality)]. Figure 1 illustrates the duration of therapy in all cases.

In terms of haematological response, all patients who were still thrombocytopenic at initiation (N=17) achieved sustained platelet counts ≥150 x10^9/l, after a median of 5 days (range 2-15). LDH normalised after median 22 days (range 3-74) for 14/16 patients for whom data was available, while 1 patient (patient 4) has persistently elevated LDH (but no other features of persistent TMA), and 1 patient already had a normal LDH at eculizumab initiation. Haemoglobin normalization occurred after median 43 days (range 11-211) in 17/18 patients for whom data was available, while 1 patient remains anaemic after 90 weeks of treatment, attributed to RRT-dependency and iron deficiency. Twenty of 22 (86%) patients maintained a normal platelet count for the duration of therapy. Patient 20 had two episodes of mild thrombocytopenia on therapy, with no other evidence of TMA, which resolved without any change to the eculizumab regime. Patient 22 developed a mild thrombocytopenia with elevated LDH (but stable renal function) 8 months into therapy in the context of a urinary tract infection with systemic features. Eculizumab was given 2 days early and all parameters normalised within 3 days.

In terms of renal response, renal function was maintained in the one patient who had normal renal function at eculizumab initiation (Patient 9). Of the 21 patients who had abnormal renal function at initiation of eculizumab, none showed renal deterioration on eculizumab and 17/21 (81%) showed improvement in eGFR (median increase in eGFR 49 ml/min/1.73m^2; range 22->80). The time for the

creatinine to reach a new baseline generally depended on the extent of renal impairment, ranging from 14 days to as long as 17 months.

Twelve of 21 patients made a complete or near-complete renal recovery (to eGFR ≥60 ml/min/1.73m^2 in 11/12 and eGFR 55 ml/min/1.73m^2 in Patient 8 who was 66 years old; resolution of proteinuria in 7/12), after a median of 23.5 days of eculizumab (range 14-51). 7 of those 12 patients (58%) had required RRT at presentation (duration 1-3 days in 6/7, but 68 days in Patient 11).

Four of 21 patients had residual CKD with eGFR 25-60 ml/min/1.73m^2 after eculizumab duration 119-232 weeks, two of whom (50%) had required RRT at initiation (duration 21 days and 6 months). Three of 21 patients remained on RRT and eculizumab at last follow-up, after 3, 7 and 85 weeks of eculizumab. One further patient (Patient 17) stopped RRT after 7 weeks but, due to transfer of care (while still on eculizumab), renal outcomes are unknown.

The final patient died during the acute admission (Patient 22). She required intubation and ventilation at presentation, for reduced consciousness and agitation, but had been extubated and was clinically improving, though still on RRT, when she suffered an unexpected cardiac arrest on day 5 of eculizumab. Whilst Patient 19 is also known to have died, following transfer to another institution after 7 weeks of eculizumab and RRT, the timing and circumstances of the death are unknown, and therefore cannot be reliably attributed to aHUS.

Predictors of renal response

Whilst recognizing the limitations of a retrospective cohort analysis, it is noticeable that patients with a final eGFR <60 ml/min/1.73m^2 after treatment, had significantly higher presenting creatinine levels than those who recovered eGFR to ≥60 ml/min/1.73m^2 [median 520 umol/l (range 236-1153) vs median 219.5 umol/l (range 80-402), p = 0.026 (Mann- Whitney test, U= 12.5)]. There was no significant correlation between renal outcome and peak UPCR, nadir platelet count, nadir Hb, peak LDH or time to eculizumab (Table II).

Complement abnormalities

Complement genetic abnormalities were identified in 40.9 % (9/22) of patients, involving *CD46* (N=4), *CFH* (N=3), *CFI* (N=2), *C3* (N=1) and *CFB* (N=1). Two patients (9%) had abnormalities in 2 genes (Table I).

Table III compares key clinical features of patients with normal and abnormal genetic screening. There was little obvious difference in severity of presentation or renal outcomes, though numbers are small and, for two cases in the abnormal genetic screening group and one in the normal group, care was transferred to other institutions before ultimate renal outcomes were known.

40.9% (N=9) had low C3 at presentation [median 0.77 g/l (range 0.38-0.88, normal range 0.9-1.8)], and this remained permanently or intermittently low despite clinical remission on eculizumab in 4/9 cases, suggesting poor correlation with disease activity. Low C3 was not a predictor of mutation status: 3/9 with low presenting C3 were subsequently found to have complement genetic abnormalities compared to 6/13 with normal C3.

Factor H autoantibody screening was performed in 59% of cases, and antibodies were not detected in any patients, though some samples were convalescent.

Withdrawal of eculizumab

Eculizumab was withdrawn in all 12 of the patients who made a complete or near complete renal response, after a median 11 weeks (range 1-26). The one patient who had normal renal function but persistent thrombocytopenia when eculizumab was initiated (Patient 9) stopped therapy after 4 weeks (following a complete haematological response), bringing the total number of patients in whom eculizumab was withdrawn to 13/22.

The remaining 8/22 patients (excluding Patient 22 who died after 1 dose) remained on eculizumab therapy at last follow-up due to incomplete renal recovery. The median duration of therapy at last follow-up was 21.5 weeks (range 3-227).

Outcomes after eculizumab withdrawal

At last follow-up, 10/13 (76.9%) patients who stopped eculizumab (Patients 2-11) remained in remission, at a median duration of 66 weeks since stopping (range 14-238). This was despite reported potential triggers in 2 cases (viral infections, and a perianal abscess).

Of the 13 (23%) patients who stopped eculizumab, 3 relapsed (Patients 12, 13 and 14 in Table I), all within 1 year of stopping (at 3, 48 and 15 weeks respectively). Patient 12 was found incidentally to have an isolated mild thrombocytopenia (139 $x10^9$/l) on routine follow-up 23 days after stopping. Although there were otherwise no features of overt TMA, eculizumab was re-initiated in case this was a prelude to frank relapse, especially given that only 2 doses had initially been administered. A rapid recovery of platelet count ensued and the patient remains on treatment with a plan to stop again after 6 months. Patients 13 and 14 both presented with symptoms suggestive of relapse and platelet counts <30 $x10^9$/l, LDH >1000 iu/l and creatinine 200-300umol/l, after defined triggers (a viral infection 3 months postpartum, and flu A, respectively). Re-initiation of eculizumab in both cases on day 1 led to rapid full recovery, without need for PEX or RRT, and discharge home after 7 and 10 days, respectively. Eculizumab was subsequently stopped again in both cases (after 2 doses in Patient 13 and 6 months in Patient 14), and they remain in remission 17 and 14 months later, respectively.

In terms of predictors of relapse after stopping, there was a significantly higher risk of relapse in those with a complement genetic abnormality, than those without (3 of out 5 with mutations relapsed versus 0 out of 8 without, p=0.035 (Fisher exact test)). The genetic abnormalities in the 3 patients who relapsed are detailed in Table I, but included abnormalities in *C3* and *CFH* in Patient 12; in *CFB* in Patient 13; and in *CD46* in Patient 14. In addition, the duration of initial treatment in those who relapsed tended to be shorter than in those who did not (median 2 weeks (range 1-10) vs 14 weeks (4-26). C3 levels were not predictive of relapse as the three patients who did relapse had consistently normal C3 levels.

Adverse events

Eculizumab was well tolerated by all 22 patients, with no reported adverse reactions and no meningococcal infection. One patient (Patient 19) suffered several infections (recurrent pneumonia,

line infection, urinary candidiasis and *c. difficile* colitis) whilst receiving eculizumab but this was in the context of being intubated and ventilated in ITU, with a history of bronchiectasis.

Overall outcomes

The outcomes of all patients are summarised in Figure 2

Discussion

Whilst the retrospective nature of this cohort is a limitation, its size is comparable to the original prospective phase 2 trials, and 'real world' outcome data in this ultra-rare disease is scarce, so the findings are of value.

The presenting features of the cohort reiterate some important characteristics of aHUS: end organ involvement is not necessarily confined to the kidneys (Cataland and Wu 2014, Noris and Remuzzi 2009); neurological and gastrointestinal symptoms are common (Jamme*, et al* 2017, Schaefer*, et al* 2018); and whilst severe thrombocytopenia and mild renal impairment are more common in TTP (Cataland*, et al* 2012, Coppo*, et al* 2010), they do not exclude aHUS (Phillips*, et al* 2016) (nadir platelet count was <30 x10^9/l in 59% of patients, and peak creatinine was <200 umol/l in 14%). It is possible that our cohort is skewed to the less severe end of the renal spectrum renally, as cases presenting with severe renal impairment are often referred direct to nephrology, but in fact the proportion requiring RRT (64%) in the acute phase is similar to larger cohorts (Fakhouri*, et al* 2016, Sheerin*, et al* 2016). The 41% prevalence of complement genetic abnormalities is also in keeping with the existing literature, as is the finding of pregnancy as a common trigger (Fakhouri*, et al* 2010).

In terms of novel findings, nearly half of patients had nephrotic range proteinuria at presentation despite this not classically being associated with aHUS, and previous reports tending to be in children or cases with secondary causes (Noris*, et al* 2015). Whilst cardiovascular manifestations are reported (Noris and Remuzzi 2014), this is the first demonstration to our knowledge of a high prevalence (77%) of asymptomatic cardiac troponin elevation, suggesting frequent subclinical cardiac involvement (although renal impairment may have contributed to elevated troponin).

To summarise outcomes, at a median follow-up of 85 weeks (range 4-255), following a median duration of initial course of eculizumab of 11 weeks (range 1-227), 100% of patients showed resolution of thrombocytopenia and 81% showed improvement in eGFR (median increase in eGFR 49 ml/min/1.73m^2; range 22->90). Of the 14/22 patients who initially required RRT, 10 became dialysis independent. At last follow-up, 54.5 % had eGFR ≥60 ml/min/1.73m^2, 27% had CKD with eGFR <60 ml/min/1.73m^2 but not requiring RRT, 14% were on RRT and 4.5% had died. It should be noted that 2 of the 3 patients requiring RRT at last follow-up had their care transferred to another institution after only 3 and 7 weeks' of eculizumab, after which time there may have been some renal recovery.

The renal outcomes compare very favourably with data from the pre-eculizumab era [rates of ESRD or death 50-77% after 3-5 years (Fremeaux-Bacchi*, et al* 2013, Noris*, et al* 2010, Schaefer*, et al* 2018)], but also to previously published outcomes with eculizumab. Of the three Phase 2 trials in adults (Fakhouri*, et al* 2016, Legendre *, et al* 2013, Licht*, et al* 2015a), our cohort is most comparable to the 41 patients reported by Fakhouri *et al* (2016), who were not required to be plasma dependent or refractory and who generally received eculizumab early in the acute phase (although the proportion of relapses and renal transplants was higher in the Phase 2 cohort). Thirty-eight of 41 patients received the intended 26 weeks' of treatment, by which time point 98% achieved platelet normalisation. Fifty-four percent showed an increase in eGFR of >15 ml/min/1.73m^2 by 26 weeks, and 15% were dialysis-dependent at 26 weeks, from 58% at baseline and 46% at initiation of eculizumab.

The renal outcomes of our cohort are also comparable to published retrospective cohorts (Cunningham*, et al* 2017, Fakhouri*, et al* 2014, Gediz*, et al* 2016, Mallett*, et al* 2015, Sheerin*, et al* 2016). In a French series of 19 adult patients (Fakhouri*, et al* 2016), 63% required RRT at diagnosis, while at last follow-up (range 4-22 months, treatment ongoing in 74%), 16% required RRT, 37% had CKD and 47% had normal renal function. In a US cohort (N=52), 35% required dialysis prior to eculizumab, and 21% at 3 months (Cunningham*, et al* 2017). Of 23 incident patients in an analysis of the first year of the national specialised service in England (Sheerin*, et al* 2016), 15 (65%) required dialysis at eculizumab initiation, of whom 8 were able to stop dialysis after a duration of 1-30 weeks.

Whilst there are potential confounders, it is notable that a lower presenting creatinine was a significant predictor of a better renal response in this cohort. The same was not true in a post-hoc analysis of pooled data from the 4 prospective trials (Walle*, et al* 2017). The finding that mutation status did not affect the likelihood of renal recovery adds to existing data in this regard (Fakhouri*, et al* 2016, Sheerin*, et al* 2016, Walle*, et al* 2017).

If and when to withdraw eculizumab is an important and debated question, which has not yet been addressed prospectively as treatment was continued for the duration of the Phase 2 trials in the majority of cases. The 23% relapse rate post-withdrawal seen in this cohort is in keeping with 20-31% relapse rates reported in the largest four case series of patients who stopped in stable remission (a total of 86 cases) (Ardissino*, et al* 2014, Ardissino*, et al* 2015, Fakhouri*, et al* 2017, Merrill*, et al* 2017, Wijnsma*, et al* 2017). The time frame of relapse within 1 year is also comparable. There was a suggestion from two cohorts (Ardissino*, et al* 2015, Fakhouri*, et al* 2017), as in ours, that those with a mutation, especially *CFH* mutations, were more likely to relapse.

Whilst relapses did occur, all three patients who relapsed made rapid and complete recoveries with re-initiation of eculizumab, without needing PEX or RRT. The withdrawal strategy therefore led to no long-term adverse effects for these patients, or on outcomes of the cohort as a whole, given that the overall outcomes were comparable to those of the Phase 2 trial (Fakhouri*, et al* 2016), despite 59% of our cohort not receiving indefinite treatment.

Whilst the vast majority of published cases of relapse post-eculizumab withdrawal made a full recovery after early re-initiation of eculizumab (Ardissino*, et al* 2015, Ardissino*, et al* 2014, Fakhouri*, et al* 2017, Merrill*, et al* 2017, Wijnsma*, et al* 2017) a recent review (Macia*, et al* 2017) cites two cases in which re-initiation of eculizumab did not prevent deterioration to end stage renal failure (though timing to re-initiation is not given for one case, and the other case involves a patient who only received one dose initially). The potential benefits however are undeniable: just over 750 doses of eculizumab have been avoided in this cohort to date, with associated reduced risk of adverse effects (including meningococcal infection), reduced hospital attendances and service delivery burdens, and drug cost savings of over £11 million. Whilst a randomised controlled trial is needed to definitively assess the safety of eculizumab withdrawal, in the absence of such data this cohort adds to the growing body of evidence in support of such a strategy. Monitoring post-withdrawal, patient

education regarding potential symptoms of relapse, and pathways to ensure timely re-initiation of eculizumab in the event of relapse, are all vital however. In addition, our current approach is to give a minimum of 6 months' therapy before considering withdrawal, given that this data showed a trend towards a higher relapse risk following shorter durations of initial therapy.

Whilst the efficacy of eculizumab in aHUS is clear, many questions still remain to be answered with definitive prospective data, including the feasibility of dose tapering and stopping, how best to monitor disease activity, predictors of response, and whether therapy could be targeted to those who benefit most. Developing diagnostics to accurately differentiate aHUS from other TMAs is also key to ensure that eculizumab is used appropriately and in a timely fashion, to ensure maximum therapeutic benefit.

Author contributions:

LN collected and analysed the data and wrote the manuscript. DG reviewed and wrote the manuscript. SC and RS assisted with data collection and reviewed the manuscript. MS reviewed and wrote the manuscript.

Acknowledgments:

DG is supported by the St Peter's Trust

Disclosure and competing interests statement:

LN and RS have no completing interests to declare.

DPG has received honoraria from Alexion. SC has received speaker's fees from Alexion. MS has received honoraria and speaker's fees from Alexion.

References

Ardissino, G., Testa, S., Possenti, I., Tel, F., Paglialonga, F., Salardi, S., Tedeschi, S., Belingheri, M. & Cugno, M. (2014) Discontinuation of eculizumab maintenance treatment for atypical hemolytic uremic syndrome: a report of 10 cases. *Am J Kidney Dis,* **64,** 633-637.

Ardissino, G., Possenti, I., Tel, F., Testa, S., Salardi, S. & Ladisa, V. (2015) Discontinuation of eculizumab treatment in atypical hemolytic uremic syndrome: an update. *Am J Kidney Dis,* **66,** 172-173.

Cataland, S.R. & Wu, H.M. (2014) How I treat: the clinical differentiation and initial treatment of adult patients with atypical hemolytic uremic syndrome. *Blood,* **123,** 2478-2484.

Cataland, S.R., Yang, S. & Wu, H.M. (2012) The use of ADAMTS13 activity, platelet count, and serum creatinine to differentiate acquired thrombotic thrombocytopenic purpura from other thrombotic microangiopathies. *Br J Haematol,* **157,** 501-503.

Cataland, S.R., Holers, V.M., Geyer, S., Yang, S. & Wu, H.M. (2014) Biomarkers of terminal complement activation confirm the diagnosis of aHUS and differentiate aHUS from TTP. *Blood,* **123,** 3733-3738.

Coppo, P., Schwarzinger, M., Buffet, M., Wynckel, A., Clabault, K., Presne, C., Poullin, P., Malot, S., Vanhille, P., Azoulay, E., Galicier, L., Lemiale, V., Mira, J.P., Ridel, C., Rondeau, E., Pourrat, J., Girault, S., Bordessoule, D., Saheb, S., Ramakers, M., Hamidou, M., Vernant, J.P., Guidet, B., Wolf, M. & Veyradier, A. (2010) Predictive features of severe acquired ADAMTS13 deficiency in idiopathic thrombotic microangiopathies: the French TMA reference center experience. *PLoS One,* **5,** e10208.

Cunningham, J.M., Ahn, J. & Broome, C. (2017) Outcomes for Atypical Hemolytic Uremic Syndrome (aHUS) Treated with Eculizumab: A Single Center Analysis. *Blood,* **130,** 2331-2331.

Dragon-Durey, M.A., Loirat, C., Cloarec, S., Macher, M.A., Blouin, J., Nivet, H., Weiss, L., Fridman, W.H. & Fremeaux-Bacchi, V. (2005) Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol,* **16,** 555-563.

Fakhouri, F., Roumenina, L., Provot, F., Sallee, M., Caillard, S., Couzi, L., Essig, M., Ribes, D., Dragon-Durey, M.A., Bridoux, F., Rondeau, E. & Fremeaux-Bacchi, V. (2010) Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc Nephrol,* **21,** 859-867.

Fakhouri, F., Delmas, Y., Provot, F., Barbet, C., Karras, A., Makdassi, R., Courivaud, C., Rifard, K., Servais, A., Allard, C., Besson, V., Cousin, M., Chatelet, V., Goujon, J.M., Coindre, J.P., Laurent, G., Loirat, C. & Fremeaux-Bacchi, V. (2014) Insights from the use in clinical practice of eculizumab in adult patients with atypical hemolytic uremic syndrome affecting the native kidneys: an analysis of 19 cases. *Am J Kidney Dis,* **63,** 40-48.

Fakhouri, F., Hourmant, M., Campistol, J.M., Cataland, S.R., Espinosa, M., Gaber, A.O., Menne, J., Minetti, E.E., Provot, F., Rondeau, E., Ruggenenti, P., Weekers, L.E., Ogawa, M., Bedrosian, C.L. & Legendre, C.M. (2016) Terminal Complement Inhibitor Eculizumab in Adult Patients With Atypical Hemolytic Uremic Syndrome: A Single-Arm, Open-Label Trial. *Am J Kidney Dis,* **68,** 84-93.

Fakhouri, F., Fila, M., Provot, F., Delmas, Y., Barbet, C., Chatelet, V., Rafat, C., Cailliez, M., Hogan, J., Servais, A., Karras, A., Makdassi, R., Louillet, F., Coindre, J.P., Rondeau, E., Loirat, C. & Fremeaux-Bacchi, V. (2017) Pathogenic Variants in Complement Genes and Risk of Atypical Hemolytic Uremic Syndrome Relapse after Eculizumab Discontinuation. *Clin J Am Soc Nephrol,* **12,** 50-59.

Fremeaux-Bacchi, V., Fakhouri, F., Garnier, A., Bienaime, F., Dragon-Durey, M.A., Ngo, S., Moulin, B., Servais, A., Provot, F., Rostaing, L., Burtey, S., Niaudet, P., Deschenes, G., Lebranchu, Y., Zuber, J. & Loirat, C. (2013) Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. *Clin J Am Soc Nephrol,* **8,** 554-562.

Gediz, F., Payzin, B.K., Ecemis, S., Guler, N., Yilmaz, A.F., Topcugil, F. & Berdeli, A. (2016) Efficacy and safety of eculizumab in adult patients with atypical hemolytic uremic syndrome: A single center experience from Turkey. *Transfus Apher Sci,* **55,** 357-362.

Greenbaum, L.A., Fila, M., Ardissino, G., Al-Akash, S.I., Evans, J., Henning, P., Lieberman, K.V., Maringhini, S., Pape, L., Rees, L., van de Kar, N.C., Vande Walle, J., Ogawa, M., Bedrosian, C.L. & Licht, C. (2016) Eculizumab is a safe and effective treatment in pediatric patients with atypical hemolytic uremic syndrome. *Kidney Int,* **89,** 701-711.

Hofer, J., Giner, T. & Jozsi, M. (2014) Complement factor H-antibody-associated hemolytic uremic syndrome: pathogenesis, clinical presentation, and treatment. *Semin Thromb Hemost,* **40,** 431-443.

Jamme, M., Raimbourg, Q., Chauveau, D., Seguin, A., Presne, C., Perez, P., Gobert, P., Wynckel, A., Provot, F., Delmas, Y., Mousson, C., Servais, A., Vrigneaud, L., Veyradier, A., Rondeau, E. & Coppo, P. (2017) Predictive features of chronic kidney disease in atypical haemolytic uremic syndrome. *PLoS One,* **12,** e0177894.

Jokiranta, T.S. (2017) HUS and atypical HUS. *Blood,* **129,** 2847-2856.

Kokame, K., Nobe, Y., Kokubo, Y., Okayama, A. & Miyata, T. (2005) FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol,* **129,** 93-100.

Krishnappa, V., Gupta, M., Elrifai, M., Moftakhar, B., Ensley, M.J., Vachharajani, T.J., Sethi, S.K. & Raina, R. (2018) Atypical Hemolytic Uremic Syndrome: A Meta-Analysis of Case Reports Confirms the Prevalence of Genetic Mutations and the Shift of Treatment Regimens. *Ther Apher Dial,* **22,** 178-188.

Legendre , C.M., Licht , C., Muus , P., Greenbaum , L.A., Babu , S., Bedrosian , C., Bingham , C., Cohen , D.J., Delmas , Y., Douglas , K., Eitner , F., Feldkamp , T., Fouque , D., Furman , R.R., 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 , N.S., Trivelli , A., Zimmerhackl , L.B., Goodship , T. & Loirat , C. (2013) Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic–Uremic Syndrome. *New England Journal of Medicine,* **368,** 2169-2181.

Lemaire, M., Fremeaux-Bacchi, V., Schaefer, F., Choi, M., Tang, W.H., Le Quintrec, M., Fakhouri, F., Taque, S., Nobili, F., Martinez, F., Ji, W., Overton, J.D., Mane, S.M., Nurnberg, G., Altmuller, J., Thiele, H., Morin, D., Deschenes, G., Baudouin, V., Llanas, B., Collard, L., Majid, M.A., Simkova, E., Nurnberg, P., Rioux-Leclerc, N., Moeckel, G.W., Gubler, M.C., Hwa, J., Loirat, C. & Lifton, R.P. (2013) Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet,* **45,** 531-536.

Licht, C., Greenbaum, L.A., Muus, P., Babu, S., Bedrosian, C.L., Cohen, D.J., Delmas, Y., Douglas, K., Furman, R.R., Gaber, O.A., Goodship, T., Herthelius, M., Hourmant, M., Legendre, C.M., Remuzzi, G., Sheerin, N., Trivelli, A. & Loirat, C. (2015a) Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int,* **87,** 1061-1073.

Licht, C., Ardissino, G., Ariceta, G., Cohen, D., Cole, J.A., Gasteyger, C., Greenbaum, L.A., Johnson, S., Ogawa, M., Schaefer, F., Vande Walle, J. & Fremeaux-Bacchi, V. (2015b) The global aHUS registry: methodology and initial patient characteristics. *BMC Nephrol,* **16,** 207.

Macia, M., de Alvaro Moreno, F., Dutt, T., Fehrman, I., Hadaya, K., Gasteyger, C. & Heyne, N. (2017) Current evidence on the discontinuation of eculizumab in patients with atypical haemolytic uraemic syndrome. *Clin Kidney J,* **10,** 310-319.

Mallett, A., Hughes, P., Szer, J., Tuckfield, A., Van Eps, C., Cambell, S.B., Hawley, C., Burke, J., Kausman, J., Hewitt, I., Parnham, A., Ford, S. & Isbel, N. (2015) Atypical haemolytic uraemic syndrome treated with the complement inhibitor eculizumab: the experience of the Australian compassionate access cohort. *Intern Med J,* **45,** 1054-1065.

Merrill, S.A., Brittingham, Z.D., Yuan, X., Moliterno, A.R., Sperati, C.J. & Brodsky, R.A. (2017) Eculizumab cessation in atypical hemolytic uremic syndrome. *Blood,* **130,** 368-372.

Noris, M. & Remuzzi, G. (2009) Atypical hemolytic-uremic syndrome. *N Engl J Med,* **361,** 1676-1687.

Noris, M. & Remuzzi, G. (2014) Cardiovascular complications in atypical haemolytic uraemic syndrome. *Nature Reviews Nephrology,* **10,** 174.

Noris, M., Caprioli, J., Bresin, E., Mossali, C., Pianetti, G., Gamba, S., Daina, E., Fenili, C., Castelletti, F., Sorosina, A., Piras, R., Donadelli, R., Maranta, R., van der Meer, I., Conway, E.M., Zipfel, P.F., Goodship, T.H. & Remuzzi, G. (2010) Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol,* **5,** 1844-1859.

Noris, M., Mele, C. & Remuzzi, G. (2015) Podocyte dysfunction in atypical haemolytic uraemic syndrome. *Nature Reviews Nephrology,* **11,** 245.

Phillips, E.H., Westwood, J.P., Brocklebank, V., Wong, E.K., Tellez, J.O., Marchbank, K.J., McGuckin, S., Gale, D.P., Connolly, J., Goodship, T.H., Kavanagh, D. & Scully, M.A. (2016) The role of ADAMTS-13 activity and complement mutational analysis in differentiating acute thrombotic microangiopathies. *J Thromb Haemost,* **14,** 175-185.

Schaefer, F., Ardissino, G., Ariceta, G., Fakhouri, F., Scully, M., Isbel, N., Lommele, A., Kupelian, V., Gasteyger, C., Greenbaum, L.A., Johnson, S., Ogawa, M., Licht, C., Vande Walle, J. & Fremeaux-Bacchi, V. (2018) Clinical and genetic predictors of atypical hemolytic uremic syndrome phenotype and outcome. *Kidney Int*, **94**, 408-418.

Scully, M., Cataland, S., Coppo, P., de la Rubia, J., Friedman, K.D., Kremer Hovinga, J., Lammle, B., Matsumoto, M., Pavenski, K., Sadler, E., Sarode, R. & Wu, H. (2017) Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. *J Thromb Haemost,* **15,** 312-322.

Sheerin, N.S., Kavanagh, D., Goodship, T.H. & Johnson, S. (2016) A national specialized service in England for atypical haemolytic uraemic syndrome-the first year's experience. *Qjm,* **109,** 27-33.

Walle, J.V., Delmas, Y., Ardissino, G., Wang, J., Kincaid, J.F. & Haller, H. (2017) Improved renal recovery in patients with atypical hemolytic uremic syndrome following rapid initiation of eculizumab treatment. *J Nephrol,* **30,** 127-134.

Wijnsma, K.L., Duineveld, C., Volokhina, E.B., van den Heuvel, L.P., van de Kar, N. & Wetzels, J.F.M. (2017) Safety and effectiveness of restrictive eculizumab treatment in atypical haemolytic uremic syndrome. *Nephrol Dial Transplant*, **33**, 635-645.

Table I: Key clinical features of each of the 22 patients.

Patient	Age at presentation (years), sex	Previous or FH of aHUS	Genetic abnormality	Nadir platelet count ($x10^9$/l)	Nadir eGFR (ml/min /1.73m^2)	Duration of RRT	C3 low at presentation?	Eculizumab duration, weeks (doses, n)	Evidence of TMA activity while on eculizumab	Renal function at time of last dose (eGFR in ml/min /1.73m^2)	Eculizumab ongoing or withdrawn	Outcome after withdrawal (including duration off eculizumab)
*	51, M	No	VUCS *CFH* (c.3264A>C p.(Glu1088Asp) in exon 21)	31	6	Ongoing at 4.5 weeks*	No	3 (4)*	Remission not yet reached	Requiring RRT*	Ongoing*	n/a
	41, F	No	No	7	10	3 days	No	8 (7)	No	eGFR >60	Withdrawn	Ongoing remission (130 weeks)
	40, F	No	VUCS *CD46* (c.389+5G>A)	32	12	2 days	Yes	22 (14)	No	eGFR >60	Withdrawn	Ongoing remission (58 weeks)
	35, F	No	No	54	8	1 day	Yes	26 (16)	No	eGFR >60	Withdrawn	Ongoing remission (59 weeks)
	29, F	No	No	12	22	3 days	Yes	6 (6)	No	eGFR >60	Withdrawn	Remission for 71 weeks post-withdrawal, then restarted as prophylaxis during pregnancy
	42, M	Yes: previous 'relapsing TTP'	Pathogenic mutation in *CD46* (c.175 C>T p.(Arg59*))	13	49	No	No	4 (5)	No	eGFR >60	Withdrawn	Ongoing remission (85 weeks)
	23, F	No	No	62	14	2 days	No	12 (9)	No	eGFR >60	Withdrawn	Ongoing remission (37 weeks)
	66, F	No	No	14	13	3 days	Yes	16 (11)	No	eGFR 50-60	Withdrawn	Ongoing remission with stable eGFR (238 weeks)

16, M	No	No	14	24	No	Yes	4 (5)	No	eGFR >60	Withdrawn	Ongoing remission (146 weeks)
18, F	No	No	9	34	No	No	16 (11)	No	eGFR >60	Withdrawn	Ongoing remission (37 weeks)
17, F	No	No	61	10	10 weeks	Yes	23 (14)	No	eGFR >60	Withdrawn	Ongoing remission (14 weeks)*
26, M	No	Pathogenic mutation in *C3* (c.193A>C p.(Lys65Gln)); VUCS *CFH* (c.1548T>A p.(Asn516Lys))	17	57	No	No	1 (2)	No	eGFR >60	Withdrawn	Thrombocytopenia 3 weeks after withdrawal. Full recovery with eculizumab. Plan to stop after 6 months.
23, F	No	VUCS *CFB* (c.1112A>G(p.Asp371Gly))	20	12	No	No	2 (3)	No	eGFR >60	Withdrawn	Relapse 48 weeks after withdrawal. Full recovery with eculizumab (2 doses only). Ongoing remission 68 weeks later*.
20, F	No	Pathogenic mutation in *CD46* (c.286+2T>G in intron 2)	11	26	No	No	10 (8)	No	eGFR >60	Withdrawn	Relapse 15 weeks after withdrawal. Full recovery with eculizumab. Withdrawal again after 2 weeks. Ongoing remission 56 weeks later
30, F	No	No	55	3	6 months	No	229	No	Best eGFR 25 (22 months post-stopping) Now 10-15.	Ongoing	n/a
47, M	No	VUCS *CD46* (c.472G>C p.(Glu158Gln))	16	22	Ongoing at 19 months	Yes	85	No	Requiring RRT	Ongoing	n/a
25, F	Yes, mother known aHUS	Pathogenic *CFHR1-CFH* hybrid gene;	24	7	7 weeks	No	9 weeks (7 doses)*	n/a	eGFR 30*	Ongoing*	n/a

			CFI likely pathogenic variant (c.1246A>C p.(Ile416Leu))									
33, M	No	No	85	11	No	No	116 weeks	No	eGFR 30-40	Ongoing	n/a
50, M	No	No	22	8	Ongoing at nearly 8 weeks*	No	7 weeks (6 doses)*	No	Requiring RRT*	Ongoing*	n/a Subsequently died, circumstances unknown
31, F	No	No	41	8	No	Yes	134	2 possible subacute episodes (mild isolated thrombocytopenia)	eGFR 30-40	Ongoing	n/a
33, F	No	No	23	6	16 days	No	136	1 possible mild relapse	eGFR 50-60	Ongoing	n/a
67, F	No (but 'HELLP' in pregnancy)	Pathogenic mutation in *CFI* (c.561delG p.(Ala219fs) in exon 4)	42	7	Ongoing when died (day 11)	Yes	Died 4 days after 1st dose	n/a	On RRT when died	n/a	n/a

All mutations are heterozygous.

* denotes care was transferred to another institution: clinical details given correspond to the last follow-up visit at our institution.

aHUS: atypical haemolytic uraemic syndrome; eGFR: estimate glomerular filtration rate; F: female; FH: family history; HELLP: haemolysis, elevated liver enzyme levels, and low platelet levels; M: male; n/a: not available; RRT: renal replacement therapy; TMA: thrombotic microangiopathy; TTP: thrombotic thrombocytopenic purpura; VUCS = variant of unknown clinical significance.

Table II: Comparison of baseline characteristics and time to eculizumab initiation for patients attaining complete and incomplete renal recoveries.

	eGFR>60 ml/min/1.73m^2 at last follow-up	**eGFR <60 ml/min/1.73m^2 at last follow-up**	**Test statistic P value**
Presenting creatinine, µmol	219.5 (80-402)	520 (236-1153)	U = 12.5* P = 0.026
Peak UPCR$^{\#}$, mg/mmol	253 (69-1228)	145 (119->7000)	U= 18* P = 0.58
Nadir platelet count, x10^9/l	15.5 (9-62)	32 (14-85)	U= 21.5* P = 0.19
Nadir Hb, g/l	72 (41-103)	72 (59-78)	U = 30.5* P = 0.63
Peak LDH, iu/l	1408 (803-4621)	2184.5 (582-3704)	U = 31* P= 0.68
Time to eculizumab	≤ 7 days: N=10 > 7 days: N=2	≤ 7 days: N=3 > 7 days: N=3	P = 0.27**

All values are given as median (range) unless otherwise indicated.
Patients 1, 17, 19 and 22 are excluded from the analysis as care was transferred or death occurred before ultimate renal outcome was known.
All p values are two-tailed.
*Mann Whitney test
**Fisher exact test
$^{\#}$UPCR values were not available for 4 patients.

eGFR: estimate glomerular filtration rate; Hb: haemoglobin concentration; LDH: lactate dehydrogenase; UPCR: urine protein:creatinine ratio.

Table III: Comparison of clinical characteristics of patients with and without identified complement genetic abnormalities.

	Complement genetic abnormality detected (N=9)	**No complement genetic abnormality detected (N=13)**
C3 low at presentation	33% (N=3)	46% (N=6)
Nadir platelet count, x 10^9/l; median (range)	20 (11-42)	23 (7-85)
RRT during acute episode	55.6 % (N=5)	69.2% (N=9)
Peak UPCR, mg/mmol; median (range)**	660 (142-1406)	145 (69-1576)
Died during FU period	11.1% (N=1)	0[#]
Ongoing RRT at last FU	22.2% (N=2)*	7.7% (N=1)*
eGFR <60 ml/min/1.73m^2, not requiring RRT, at last FU	11.1% (N=1)*	38.4% (N=5)
eGFR ≥60 ml/min/1.73m^2, at last FU	55.5% (N=5)	53.4% (N=7)

* Final renal outcome unknown in 1 case (due to transfer of care to another institution)
** UPCR values were not available for 5 patients.
[#]Patient 19 (without a complement genetic abnormality) is known to have died after care was transferred to another institution, but the circumstances are unknown.

eGFR: estimate glomerular filtration rate; FU: follow-up; RRT: renal replacement therapy; UPCR: urine protein:creatinine ratio.

Figure legends

Figure 1: Duration of eculizumab (black bars) or remission following eculizumab withdrawal (white bars) in weeks for each patient (1-22).

First course of eculizumab was ongoing at last follow-up in Patients 1, 15-21. Patient 22 died 4 days following first eculizumab dose. Eculizumab was withdrawn in first remission in Patients 2-14. Patients 2-11 remained in remission at last follow-up. Patients 12-14 relapsed post withdrawal and restarted eculizumab. Durations of therapy including number of doses are also given in Table I.

Figure 2: Outcomes of all 22 patients

CM-HUS: complement-mediated haemolytic uraemic syndrome; eGFR: estimate glomerular filtration rate (ml/min/1.73m^2); PEX: plasma exchange; RRT: renal replacement therapy.

~~Title:~~

Atypical haemolytic uraemic syndrome in the eculizumab era: presentation, response to treatment and evaluation of an eculizumab withdrawal strategy.

Short Title: Eculizumab in aHUS

Authors: Lucy Neave[1], Daniel P Gale[2], Simon Cheesman[3], Raakhee Shah[3], Marie Scully[4]

~~Affiliations:~~

[1] Department of Haematology, University College London Hospitals NHS Foundation Trust, London UK

[2] UCL Centre for Nephrology, University College London, London UK

[3] Department of Pharmacy, University College London Hospitals NHS Foundation Trust, London UK

[4] Department of Haematology, UCLH, Cardiometabolic programme- NIHR UCLH/UCL BRC, London, UK

Corresponding author:

Professor Marie Scully, Department of Haematology, University College London Hospital, 5th Floor Central, 250 Euston Road, London NW1 2PG, UK.

Telephone +44 203 447 9884 Fax +44 203 447 9145

Email m.scully@ucl.ac.uk

~~Text word count: 4095 excluding Summary, Figure legends, Table text and legends and References~~

~~Summary word count: 194~~

~~Number of figures/tables: 2 figures, 3 tables~~

~~Number of references: 38~~

Key words:

aHUS, atypical haemolytic uraemic syndrome, eculizumab, withdrawal, TMA

Received 12 November 2018, accepted 5 February 2019

Summary

The complement inhibitor, eculizumab, has revolutionised the management of atypical haemolytic uraemic syndrome (aHUS), although the optimum treatment duration is debated. ~~22~~ Twenty-two cases of acute aHUS managed with eculizumab were retrospectively reviewed, including outcomes after eculizumab withdrawal. Although 41% had an associated complement genetic abnormality, ~~but~~ mutation status did not affect severity of clinical presentation. ~~64%~~Sixty-four percent required renal replacement acutely, with a high incidence of nephrotic range proteinuria (47%). Eculizumab followed a median of 6 days' ~~-~~ of plasma exchange. After a median duration of therapy of 11 weeks (range 1-227), haematological recovery was seen in 100%, while 81% achieved at least partial renal recovery (median increase in ~~eGFR -~~ estimated glomerular filtration rate (eGFR) 49 ml/min/1.73m^2). At median duration of ~~follow~~ follow-up of 85 weeks (range 4-255), 54.5% had eGFR ≥60 ml/min/1.73m^2, 27% had CKD, ~~-~~14% were on dialysis, and 4.5% had died. Eculizumab was withdrawn in 59% ~~-~~(13/22) cases following complete haematological and renal recovery. Three of these 13 patients (23%) ~~(3/13)~~ subsequently relapsed, with defined triggers in 2/3, but all made a full recovery with rapid resumption of eculizumab. There was a significant association between higher presenting creatinine and poorer renal outcomes. A strategy of eculizumab withdrawal in selected cases is both safe and cost effective.

Introduction

Atypical (or complement-mediated) haemolytic uraemic syndrome (aHUS) is a rare thrombotic microangiopathy (TMA), with an incidence of 1-2 per million (Noris and Remuzzi 2009). It is characterised by microangiopathic haemolytic anaemia (MAHA), consumptive thrombocytopenia, and multisystem end organ involvement with a predilection for the kidneys. Diagnosis is clinical, after exclusion of thrombotic thrombocytopenic purpura (TTP) ~~(~~[by ruling out severe ~~-~~ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) deficiency (activity <10~~%)), %)~~], and other secondary causes of TMA, including infection-associated HUS due to shiga toxin-producing organisms (STEC) (Scully, *et al* 2017).

The last two decades have yielded significant developments in aHUS, both in elucidation of the pathophysiology and in management. Dysregulation of the alternative pathway of complement, as a

result of an environmental trigger in a genetically susceptible individual is regarded as the key abnormality, leading to endothelial and platelet activation~~,~~ and, consequently, TMA (Jokiranta 2017). Pathogenic mutations in genes encoding complement factors H, B, I and 3 (*CFH*, *CFB*, *CFI*, *C3*), and membrane cofactor protein (*CD46*), are demonstrated in 40-60% of affected individuals (Fremeaux-Bacchi*, et al* 2013, Noris and Remuzzi 2009, Schaefer*, et al* 2018). Factor H autoantibodies (Dragon-Durey*, et al* 2005, Hofer*, et al* 2014) and mutations in *DGKE* (encoding diacylglycerol kinase ε) (Lemaire*, et al* 2013) are rare causes usually seen in children.

Historically, the prognosis has been poor, with incomplete response rates to plasma exchange (PEX), and rates of end stage renal disease (ESRD) or death as high as 50-77% ~~-~~after 3-5 years (Fremeaux-Bacchi*, et al* 2013, Noris*, et al* 2010, Schaefer*, et al* 2018). However efficacy of the humanised monoclonal anti-C5 antibody eculizumab was demonstrated in open label phase II trials (Fakhouri*, et al* 2016, Greenbaum*, et al* 2016, Legendre *, et al* 2013, Licht*, et al* 201~~5b~~5a), inducing haematological remission, improving or stabilizing renal function, and preventing graft failure following renal transplant. ~~FDA~~ US Food and Drug Administration and ~~EMA~~ European Medicines Agency approvals were granted in 2011, and the National Health Service ~~drug~~ has ~~been~~ funded ~~by~~ the drug ~~NHS~~ in England since 2013 under the coordination of the National aHUS Service.

At a cost of over £300 000 a year per patient, treatment carries significant financial burdens, as well as potential risks, including bacterial meningitis (Fakhouri*, et al* 2016), so the challenge now is to confirm that the drug is used in the most effective way possible, including determining the optimum treatment schedule. Whilst ~~large~~ large-scale registries and prospective studies are needed to definitively address such questions ~~([~~and are underway (Licht*, et al* 20~~15a~~15b)~~)]~~, in the ~~short~~ short-term, retrospective cohort analyses are informative, though ~~at present~~ few have been published at present (Cataland*, et al* 2014, Cunningham*, et al* 2017, Fakhouri*, et al* 2014, Krishnappa*, et al* 2018, Sheerin*, et al* 2016).

We describe a cohort of 22 patients presenting to a UK TMA referral centre with acute aHUS and treated with eculizumab over a ~~6~~ 6-year period. We highlight the presenting features and responses to eculizumab. We also assess the outcomes of a strategy of eculizumab withdrawal after achieving a complete or near-complete response.

Patients and Methods

All adult patients prescribed at least one dose of eculizumab for an acute presentation of aHUS in a single institution were retrospectively identified. Of 34 patients presenting with aHUS between 2012 and 2018, ~~-~~9 responded to ~~plasma exchange (~~PEX~~)~~ only, 3 went on to receive an alternative C5 inhibitor on a clinical trial, while 22 patients received eculizumab. All 22 of these patients were included in the analysis, including those who were no longer followed up in our institution. Data regarding presenting clinical features, response to therapy and ~~long~~ long~~-~~term outcomes of the aHUS episode was collected from the medical records as part of a service review, and analysed anonymously. All investigations had been performed as part of routine care.

In all cases, ~~plasma exchange (~~PEX~~)~~ with solvent ~~detergent~~ detergent~~-~~treated plasma was initiated on admission. Renal replacement therapy (RRT) was commenced if indicated. The diagnosis of aHUS was made according to international consensus criteria (Scully*, et al* 2017) on the basis of: (i) presence of ~~thrombotic microangiopathy~~TMA (direct antiglobulin test-negative haemolytic anaemia with schistocytes on blood film~~,~~ and thrombocytopenia); (ii) exclusion of severe ADAMTS13 ~~deficiency~~ deficiency [(ADAMTS13 activity by fluorescence resonance energy transfer (Kokame*, et al* 2005)~~)~~ >10 IU/dL~~);~~]; and (iii) exclusion of secondary TMAs (demonstration of normal coagulation screen, negative autoimmune serology, negative lupus anticoagulant and antiphospholipid antibody screening~~,~~ and negative reference laboratory STEC stool/serological investigations in all diarrhoeal cases, with imaging to exclude malignancy if indicated). End-organ damage was assessed via serum biochemistry, spot urine protein:creatinine ratio (UPCR), renal ultrasound, cardiac troponin I, electrocardiogram~~,~~ and, in selected cases, brain imaging. Renal biopsy was performed in cases of incomplete renal recovery where there was diagnostic uncertainty (N=2).

Following National aHUS Service approval to commence eculizumab, PEX was discontinued just prior to the first dose. Intravenous eculizumab was administered weekly for 4 weeks at a dose of 900 mg, followed by 1200 mg fortnightly starting on week 5. Meningococcal vaccination (against subtypes ACWY and B) was administered prior to initiation of eculizumab, followed by~~-~~ antibiotic prophylaxis (ciprofloxacin initially, followed by penicillin V) for the duration of therapy. If patients required ongoing ~~renal replacement therapy (~~RRT~~)~~ they were referred to their local renal centre. Following discharge, patients continued to receive eculizumab as an outpatient (and, in some cases, at home), with fortnightly blood and urine monitoring.

Withdrawal of ~~-~~eculizumab was considered in all patients who achieved complete haematological response ~~({[platelets~~ platelet count >150 x10^9/l and normal lactate dehydrogenase (LDH)] and complete or near-complete renal recovery [estimated glomerular filtration rate (eGFR) back to baseline without significant proteinuria~~). -~~]. The decision to stop eculizumab was made based on consensus clinician opinion and patient preference, after a discussion of risks, benefits and available evidence. All patients who were offered the option to stop treatment elected to do so. Monitoring for relapse after withdrawal included symptoms review, ~~-~~full blood and reticulocyte counts, ~~lactate dehydrogenase~~LDH, serum creatinine/eGFR and urinalysis/U~~-~~PCR, initially fortnightly, but then at increasing time intervals and ultimately 6-monthly. All patients had access to a ~~24~~ 24~~-~~hour telephone helpline in the event of concerning symptoms in the interim.

Complement genotyping, performed via the National aHUS Service, included direct sequencing of coding exons of *CFH, CFI, CD46, C3* and *CFB,* and multiplex ligation-dependent probe amplification (MLPA) analysis for deletions and duplications of *CFH CFI, CD46*, ~~-~~*CFHR1* and *CFHR3*. Screening for factor H autoantibodies was also undertaken in the majority of cases.

Results

Summary of patients treated

Of the 22 patients identified, 68% were female. Median age at presentation was 32 years (range 16-67). All presented acutely with TMA~~-~~: 21/22 were *de novo* presentations, whilst one patient had 4 previous episodes of 'relapsing TTP' but ADAMTS13 analysis performed for the first time on the index admission excluded TTP and led to a diagnosis of aHUS. All had native kidneys, and none were known to have chronic kidney disease (CKD) prior to presentation. Median duration of admission at our institution was 14 days (range 6-55), though 5 patients were discharged to local renal units for ongoing dialysis and 1 was repatriated to his local hospital. Median duration of ~~follow~~ follow~~-~~up from initiation of eculizumab was 85 weeks (range 4-255), excluding a patient who died on day 5. ~~5~~ Five patients had ongoing outpatient management transferred to other centres (at 3, 7, 9, 37 and 124 weeks ~~post~~ post~~-~~initiation of eculizumab, respectively) but data inclusive to those timepoints was included in the analysis. Key clinical information for all 22 patients is summarised in Table I.

Presenting features of aHUS

~~GI~~ Gastroitestinal symptoms were common at presentation, affecting 64%, and were most commonly nausea and vomiting (36%), and abdominal pain (32%). STEC-negative diarrhoea was present in 23% of cases. ~~41%~~Forty-one percent had neurological manifestations: seizure N=2; headache with or without visual disturbance N=4; transient diplopia N=1; transient facial and/or limb weakness N=2. Other presenting symptoms included: dark urine/ altered urine output (23%); bleeding/purpura (14%); jaundice (9%); lethargy/malaise (5%). ~~3~~ Three patients were diagnosed following detection of laboratory abnormalities in pregnancy. ~~13~~ Thirteen patients were hypertensive at admission (though 3 had pre-existing hypertension).

Median nadir platelet count was 23 x10^9~~/L~~/l (range 7-85 x10^9~~/L~~/l) and median nadir haemoglobin (Hb) was 70g~~/L~~/l (range 62-103 g~~/L~~/l). Median presenting LDH was 1704 iu~~/L~~/l (range 582-4621 iu~~/l~~/l , normal range 135-214 iu~~/L~~/l). Reticulocytosis was notably absent in 45.5% of patients at presentation, though in all but one case this subsequently developed. Bilirubin remained normal in 22.7% of patients.

Median presenting creatinine was 323.5 ~~umol~~µmol~~/L~~/l (range 80-1153, normal range 49-92). Proteinuria was demonstrated by urinalysis or UPCR in all cases. The median UPCR (N=17) was 199.5 mg/mmol (range 69->7000, normal range 0-13~~),);~~ ~~and~~ it was >300 mg/mmol for 8/17 (47%). Median nadir eGFR was 11.5 ml/min/1~~,~~.73m^2 (range 3-57), and 64% of patients (N=14) required ~~renal replacement therapy~~RRT during the acute episode. ~~14/22 (64%) required~~ Admission ~~i~~to the intensive care unit (ITU) ~~admission~~was required by 14/22 (64%) patients, 4 of whom were intubated and ventilated. Whilst there were no overt cardiac manifestations at presentation, 77% (N=17) had elevated cardiac troponin T (median 64.5 ng~~/L~~/l, range 17-397, normal range 0-14). Despite neurological symptoms in 41%, ~~MRI~~ brain magnetic resonance imaging was abnormal only in 3 cases (14%) (infarction/small vessel changes).

Clear triggers were identified in 50% of cases: pregnancy/postpartum in 4 (Patients 4, 5, 7 and 14 in Table ~~1~~I); influenza in 2; lower respiratory tract infections in 3; campylobacter diarrhoea in 1 (~~the~~ this ~~latter~~ was believed to be a trigger rather than the cause of the HUS, given that the TMA

persisted despite resolution of the infection). Patient 10 initially presented with gallstone cholecystitis 5 months postpartum, but a frank TMA picture quickly evolved (along with post-ERCP pancreatitis) and the suspicion was that the gallstones resulted from a low grade postpartum ~~a~~HUS, as she had been noted to be hypertensive and proteinuric peripartum, with intermittent abdominal pain, malaise and nausea ever since.

ADAMTS13 activity on presentation was within the normal range for 86.3% (n=19) patients, and slightly low in 13.7% (n=3). Median activity was 72 iu/dl (range 56-91, normal range 60-146).

Response to plasma exchange

All 22 patients commenced ~~plasma exchange (~~PEX~~)~~ ~~from~~ at admission, with a median PEX duration of 5 days (range 2-24). Platelet normalization (>150 x10~~^~~^{9}~~/L~~/l) was achieved in 5/22 (range of PEX duration 5-18 days), with improvement in platelet count without normalization ~~-~~in 10/22 (range of PEX duration 2-24 days). Renal recovery to eGFR >90 ml/min/1.73m^2 was seen in 1 patient during PEX (Patient 9, PEX duration 24 days), but with incomplete platelet response. ~~4/~~Four of 22 patients came off RRT during PEX but 7/22 remained RRT-dependent, while 3/22 required initiation of RRT.

Response to eculizumab

The median time from admission to initiation of eculizumab was 6 days ~~{~~[range 2-38 (with delays in the latter case due to funding issues due to non-UK nationality~~)).)~~]. Figure 1 illustrates the duration of therapy in all cases.

In terms of haematological response, all patients who were still thrombocytopenic at initiation (N=17) achieved sustained platelet counts ≥150 x10^{9}~~/L~~/l, after a median of 5 days (range 2-15). LDH normalised after median 22 days (range 3-74) for 14/16 patients for whom data was available, while 1 patient (patient 4) has persistently elevated LDH (but no other features of persistent TMA), and 1 patient already had a normal LDH at eculizumab initiation. Haemoglobin normalization occurred after median 43 days (range 11-211) in 17/18 patients for whom data was available, while 1 patient

remains anaemic after 90 weeks of treatment, attributed to RRT-dependency and iron deficiency. ~~20/~~Twenty of 22 (86%) patients maintained a normal platelet count for the duration of therapy. Patient 20 had two episodes of mild thrombocytopenia on therapy, with no other evidence of TMA, which resolved without any change to the eculizumab regime. Patient 22 developed a mild thrombocytopenia with elevated LDH (but stable renal function) 8 months into therapy in the context of a urinary tract infection with systemic features. Eculizumab was given 2 days early and all parameters normalised within 3 days.

In terms of renal response, renal function was maintained in the one patient who had normal renal function at eculizumab initiation (~~Patient~~ Patient 9). Of the 21 patients who had abnormal renal function at initiation of eculizumab, none showed renal deterioration on eculizumab and 17/21 (81%) showed improvement in eGFR (median increase in eGFR 49 ml/min/1.73m^2; ~~(~~range 22->80~~)~~). The time for the creatinine to reach a new baseline generally depended on the extent of renal impairment, ranging from 14 days to as long as 17 months.

~~12/~~Twelve of 21 patients made a complete or near-complete renal recovery (to eGFR ≥60 ml/min/1.73m^2 in 11/12 and eGFR 55 ml/min/1.73m^2 in Patient 8 who was 66 years old; resolution of proteinuria in 7/12), after a median of 23.5 days of eculizumab (range 14-51). 7 of those 12 patients (58%) had required RRT at presentation (duration 1-3 days in 6/7, but 68 days in Patient 11).

~~4/~~Four of 21 patients had residual CKD with eGFR 25-60 ml/min/1.73m^2 after eculizumab duration 119-232 weeks~~., 2 of these 4 patients~~two of whom (50%) had required RRT at initiation (duration 21 d~~-~~ays and 6 months). Three of 21 patients ~~3/21~~ remained on RRT and eculizumab at last follow~~-~~up, after 3, 7 ~~-~~and 85 weeks of eculizumab. One further patient (Patient 17) stopped ~~-~~RRT after 7 weeks~~-~~ but, due to transfer of care (while still on eculizumab), renal outcomes are unknown.

The final patient died during the acute admission (Patient 22). She required intubation and ventilation at presentation, for reduced consciousness and agitation, but had been extubated and was clinically improving, though still on RRT, when she suffered an unexpected cardiac arrest on day 5 of eculizumab. Whilst Patient 19 is also known to have died, following transfer to another

institution after 7 weeks of eculizumab and RRT, the timing and circumstances of the death are unknown, and therefore cannot be reliably attributed to aHUS.

Predictors of renal response

Whilst recognizing the limitations of a retrospective cohort analysis, it is noticeable that patients with a final eGFR <60 ml/min/1.73m^2 after treatment, had significantly higher presenting creatinine levels than those who recovered eGFR to ≥60 ml/min/1.73m^2 ~~([~~median 520 umol~~/L~~/l (range 236-1153) vs median 219.5 umol~~/L~~/l (range 80-402), p = 0.026 (Mann- Whitney test, U= 12.5~~)).)].~~ There was no significant correlation between renal outcome and peak ~~urine protein:creatinine ratio~~UPCR, nadir platelet count, nadir Hb, peak LDH~~,~~ or time to eculizumab~~, as shown in~~ (Table II).

Complement abnormalities

Complement genetic abnormalities were identified in 40.9 % (9/22) of patients, involving *CD46* (N=4), *CFH* (N=3), *CFI* (N=2), *C3* (N=1) and *CFB* (N=1). ~~2~~ Two patients (9%) had abnormalities in 2 genes~~. Details are given in~~ (Table I).

Table III compares key clinical features of patients with normal and abnormal genetic screening. There was little obvious difference in severity of presentation or renal outcomes, though numbers are small and, for two cases in the abnormal genetic screening group~~,~~ and one in the normal group, care was transferred to other institutions before ultimate renal outcomes were known.

40.9% (N=9) had low C3 at presentation ~~([~~median 0.77 g~~/L~~/l (range 0.38-0.88, normal range 0.9-1.8~~)),)]~~, and this remained permanently or intermittently low despite clinical remission on eculizumab in 4/9 cases, suggesting poor correlation with disease activity. Low C3 was not a predictor of mutation status: 3/9 with low presenting C3 were subsequently found to have complement genetic abnormalities compared to 6/13 with normal C3.

Factor H autoantibody screening was performed in 59% of cases, and antibodies were not detected in any patients, though some samples were convalescent.

Withdrawal of eculizumab

Eculizumab was withdrawn in all 12 of the ~~-~~patients who made a complete or near complete renal response~~-~~, after a median 11 weeks (range 1-26). The one patient who had normal renal function but persistent thrombocytopenia when eculizumab was initiated (Patient 9) stopped therapy after 4 weeks (following a complete haematological response), bringing the total number of patients in whom eculizumab was withdrawn to 13/22.

The remaining 8/22 ~~-~~patients (excluding Patient 22 who died after 1 dose) ~~continued~~ remained on eculizumab therapy at last ~~follow~~ follow-up due to incomplete renal recovery. The median duration of therapy at last ~~follow~~ follow-up was 21.5 weeks (range 3-227).

Outcomes after eculizumab withdrawal

At last follo~~w u~~w-up, 10/13 (76.9%) ~~-~~patients who stopped eculizumab (Patients 2-11) remained in remission, at a median duration of 66 weeks since stopping (range 14-238). This was despite reported potential triggers in 2 cases (viral infections, and a perianal abscess).

~~3/~~Of the 13 (23%) patients ~~in whom~~who stopped eculizumab~~, was stopped~~3 relapsed (Patients 12, 13 and 14 in Table I), all within 1 year of stopping (at 3, 48 and 15 weeks respectively). Patient 12 was found incidentally to have an isolated mild thrombocytopenia (139 x10^9~~/L~~/l) on routine follo~~w u~~w-up 23 days after stopping. Although there were otherwise no features of overt TMA, eculizumab was re-initiated in case this was a prelude to frank relapse, especially ~~since~~ given that only 2 doses had initially been administered. A rapid recovery of platelet count ensued and the patient remains on treatment with a plan to stop again after 6 months. Patients 13 and 14 both presented with symptoms suggestive of relapse and ~~platelets~~ platelet counts <30 x10^9~~/L~~/l, LDH >1000 ~~-~~iu~~/L~~/l and creatinine 200-300umol~~/L~~/l, ~~-~~after defined triggers (a viral infection 3 months postpartum, and flu A,

respectively). Re-initiation of eculizumab in both cases on day 1 led to rapid full recovery, without need for PEX or RRT, and discharge home after 7 and 10 days, respectively. Eculizumab was subsequently stopped again in both cases (after 2 doses in Patient 13 and 6 months in Patient 14), and they remain in remission 17 and 14 months later, respectively.

In terms of predictors of relapse after stopping, there was a significantly higher risk of relapse in those with a complement genetic abnormality, than those without (3 of out 5 with mutations relapsed versus 0 out of 8 without, p=0.035 (Fisher exact test)). The genetic abnormalities in the 3 patients who relapsed are detailed in Table I, but included abnormalities in *C3* and *CFH* in Patient 12; in *CFB* in Patient 13; and in *CD46* in Patient 14. In addition, the duration of initial treatment in those who relapsed tended to be shorter than in those who did not (median 2 weeks (range 1-10) vs 14 weeks (4-26). C3 levels were not predictive of relapse as the three patients who did relapse had consistently normal C3 levels.

Adverse events

Eculizumab was well tolerated by all 22 patients, with no reported adverse reactions and no meningococcal infection. One patient (Patient 19) suffered several infections (recurrent pneumonia, line infection, urinary candidiasis and *c. difficile* colitis) whilst receiving eculizumab but this was in the context of being intubated and ventilated in ITU, with a history of bronchiectasis.

Overall outcomes

The outcomes of all patients are summarised in Figure 2

Discussion

Whilst the retrospective nature of this cohort is a limitation, its size is comparable to the original prospective phase 2 trials, and 'real world' outcome data in this ultra-rare disease is scarce, so the findings are of value.

The presenting features of the cohort reiterate some important characteristics of aHUS: end organ involvement is not necessarily confined to the kidneys (Cataland and Wu 2014, Noris and Remuzzi 2009); neurological and gastrointestinal symptoms are common (Jamme*, et al* 2017, Schaefer*, et al* 2018); and whilst severe thrombocytopenia and mild renal impairment are more common in TTP (Cataland*, et al* 2012, Coppo*, et al* 2010), they do not exclude aHUS (Phillips*, et al* 2016) (nadir platelet count was <30 x10^9/L/l in 59% of patients, and peak creatinine was <200 umol/L/l in 14%). It is possible that our cohort is skewed to the less severe end of the renal spectrum renally, as cases presenting with severe renal impairment are often referred direct to nephrology, but in fact the proportion requiring RRT (64%) in the acute phase is similar to larger cohorts (Fakhouri*, et al* 2016, Sheerin*, et al* 2016). The 41% prevalence of complement genetic abnormalities is also in keeping with the existing literature, as is the finding of pregnancy as a common trigger (Fakhouri*, et al* 2010).

In terms of novel findings, nearly half of patients had nephrotic range proteinuria at presentation despite this not classically being associated with aHUS, and previous reports tending to be in children or cases with secondary causes (Noris*, et al* 2015). Whilst cardiovascular manifestations are reported (Noris and Remuzzi 2014), this is the first demonstration to our knowledge of a high prevalence (77%) of asymptomatic cardiac troponin elevation, suggesting frequent subclinical cardiac involvement (although renal impairment may have contributed to elevated troponin).

To summarise outcomes, at a median follow-up of 85 weeks (range 4-255), following a median duration of initial course of eculizumab of 11 weeks (range 1-227), 100% of patients showed resolution of thrombocytopenia and 81% showed improvement in eGFR (median increase in eGFR 49 ml/min/1.73m^2; (range 22->90)). Of the 14/22 patients who initially required RRT initially, 10 became dialysis independent. At last follow-up, 54.5 % had eGFR ≥60 ml/min/1.73m^2, 27% had CKD with eGFR <60 ml/min/1.73m^2 but not requiring RRT, 14% were on RRT, and 4.5% had died. It should be noted that 2 of the 3 patients requiring RRT at last follow-up had their care transferred to another institution after only 3 and 7 weeks' of eculizumab, after which time there may have been some renal recovery.

The renal outcomes compare very favourably with data from the pre-eculizumab era ~~(~~[rates of ESRD or death 50-77%~~ ~~after 3-5 years ~~(Fremeaux-Bacchi, *et al* 2013, Noris, *et al* 2010, Schaefer, *et al* 2018)),~~ (Fremeaux-Bacchi, *et al* 2013, Noris, *et al* 2010, Schaefer, *et al* 2018)], but also to previously published outcomes with eculizumab. Of the three Phase 2 trials in adults (Fakhouri, *et al* 2016, Legendre , *et al* 2013, Licht, *et al* 201~~5b~~5a), our cohort is most comparable to the 41 patients ~~in~~ reported by ~~the third (~~Fakhouri~~,~~ *et al* (2016), who were not required to be plasma dependent or refractory and who generally received eculizumab early in the acute phase (although the proportion of relapses and renal transplants was higher in the Phase 2 cohort). ~~38/~~Thirty-eight of 41 patients received the intended 26 weeks' of treatment, by which time point 98% achieved platelet normalisation. ~~54%~~Fifty-four percent showed an increase in eGFR of >15 ml/min/1.73m^2 by 26 weeks, and 15% were ~~dialysis~~ dialysis-dependent at 26 weeks, from 58% at baseline and 46% at initiation of eculizumab.

The renal outcomes of our cohort are also comparable to published retrospective cohorts (Cunningham, *et al* 2017, Fakhouri, *et al* 2014, Gediz, *et al* 2016, Mallett, *et al* 2015, Sheerin, *et al* 2016). In a French series of 19 adult patients (Fakhouri, *et al* 2016), 63% required RRT at diagnosis, while at last follo~~w u~~w-up (range 4-22 months, treatment ongoing in 74%), 16% required RRT, 37% had CKD~~,~~ and 47% had normal renal function. In a US cohort (N=52), 35% required dialysis prior to eculizumab, and 21% at 3 months (Cunningham, *et al* 2017). Of 23 incident patients in an analysis of the first year of the national specialised service in England (Sheerin, *et al* 2016), 15 (65%) required dialysis at eculizumab initiation, of whom 8 were able to stop dialysis after a duration of 1-30 weeks.

Whilst there are potential confounders, it is notable that a lower presenting creatinine was a significant predictor of a better renal response in this cohort. The same was not true in a post-hoc analysis of pooled data from the 4 prospective trials (Walle, *et al* 2017). The finding that mutation status did not affect the likelihood of renal recovery adds to existing data in this regard (Fakhouri, *et al* 2016, Sheerin, *et al* 2016, Walle, *et al* 2017).

If and when to withdraw eculizumab is an important and debated question, which has not yet been addressed prospectively as treatment was continued for the duration of the Phase 2 trials in the majority of cases. The 23% relapse rate ~~post~~ post-withdrawal seen in this cohort is in keeping with

20-31% relapse rates reported in the largest four case series of patients who stopped in stable remission (a total of 86 cases) (Ardissino, *et al* ~~2015~~2014, Ardissino, *et al* 2015~~4~~, Fakhouri, *et al* 2017, Merrill, *et al* 2017, Wijnsma, *et al* 2017). The time frame of relapse within 1 year is also comparable. There was a suggestion from two cohorts (Ardissino, *et al* 2015, Fakhouri, *et al* 2017), as in ours, that those with a mutation, especially *CFH* mutations, were more likely to relapse~~, especially *CFH* mutations~~.

Whilst relapses did occur, all three patients who relapsed made rapid and complete recoveries with re-initiation of eculizumab, without needing PEX or RRT. The withdrawal strategy therefore led to no ~~long~~ long-term adverse effects for these patients, ~~n~~or on outcomes of the cohort as a whole, given that the overall outcomes were comparable to those of the Phase 2 trial (Fakhouri, *et al* 2016), despite 59% of our cohort not receiving indefinite treatment.

Whilst the vast majority of published cases of relapse ~~post~~ post-eculizumab withdrawal made a full recovery after early re-initiation of eculizumab (Ardissino, *et al* 2015, Ardissino, *et al* 2014, Fakhouri, *et al* 2017, Merrill, *et al* 2017, Wijnsma, *et al* 2017) a recent review (Macia, *et al* 2017) cites two cases in which re-initiation of eculizumab did not prevent deterioration to ~~ESRF~~ end stage renal failure (though timing to re-initiation is not given for one case, and the other case involves a patient who only received one dose initially). The potential benefits however are undeniable: just over 750 doses of eculizumab have been avoided in this cohort to date, with associated reduced risk of adverse effects (including meningococcal infection), reduced hospital attendances and service delivery burdens, and drug cost savings of over £11 million. Whilst a randomised controlled trial is needed to definitively assess the safety of eculizumab withdrawal, in the absence of such data this cohort adds to the growing body of evidence in support of such a strategy. Monitoring ~~post~~ post-withdrawal, patient education regarding potential symptoms of relapse, and pathways to ensure timely re-initiation of eculizumab in the event of relapse, are all vital however. In addition, our current approach is to give a minimum of 6 months' therapy before considering withdrawal, given that this data showed a trend towards a higher relapse risk following shorter durations of initial therapy.

Whilst the efficacy of eculizumab in aHUS is clear, many questions still remain to be answered with definitive prospective data, including the feasibility of dose tapering and stopping, how best to

monitor disease activity, predictors of response, and whether therapy could be targeted to those who benefit most. Developing diagnostics to accurately differentiate aHUS from other TMAs is also key to ensure that eculizumab is used appropriately and in a timely fashion, to ensure maximum therapeutic benefit.

Author contributions:

LN collected and analysed the data and wrote the manuscript. DG reviewed and wrote the manuscript. SC and RS assisted with data collection and reviewed the manuscript. MS reviewed and wrote the manuscript.

Acknowledgments:

DG is supported by the St Peter's Trust

Disclosure and competing interests statement:

LN and RS have no completing interests to declare.

DPG has received honoraria from Alexion. SC has received speaker's fees from Alexion. MS has received honoraria and speaker's fees from Alexion.

References

Ardissino, G., Testa, S., Possenti, I., Tel, F., Paglialonga, F., Salardi, S., Tedeschi, S., Belingheri, M. & Cugno, M. (2014) Discontinuation of eculizumab maintenance treatment for atypical hemolytic uremic syndrome: a report of 10 cases. *Am J Kidney Dis,* **64,** 633-637.

Ardissino, G., Possenti, I., Tel, F., Testa, S., Salardi, S. & Ladisa, V. (2015) Discontinuation of eculizumab treatment in atypical hemolytic uremic syndrome: an update. *Am J Kidney Dis,* **66,** 172-173.

~~Ardissino, G., Testa, S., Possenti, I., Tel, F., Paglialonga, F., Salardi, S., Tedeschi, S., Belingheri, M. & Cugno, M. (2014) Discontinuation of eculizumab maintenance treatment for atypical hemolytic uremic syndrome: a report of 10 cases. *Am J Kidney Dis,* **64,** 633-637.~~

Cataland, S.R. & Wu, H.M. (2014) How I treat: the clinical differentiation and initial treatment of adult patients with atypical hemolytic uremic syndrome. *Blood,* **123,** 2478-2484.

Cataland, S.R., Yang, S. & Wu, H.M. (2012) The use of ADAMTS13 activity, platelet count, and serum creatinine to differentiate acquired thrombotic thrombocytopenic purpura from other thrombotic microangiopathies. *Br J Haematol,* **157,** 501-503.

Cataland, S.R., Holers, V.M., Geyer, S., Yang, S. & Wu, H.M. (2014) Biomarkers of terminal complement activation confirm the diagnosis of aHUS and differentiate aHUS from TTP. *Blood,* **123,** 3733-3738.

~~Cataland, S.R. & Wu, H.M. (2014) How I treat: the clinical differentiation and initial treatment of adult patients with atypical hemolytic uremic syndrome. *Blood,* **123,** 2478-2484.~~

~~Cataland, S.R., Yang, S. & Wu, H.M. (2012) The use of ADAMTS13 activity, platelet count, and serum creatinine to differentiate acquired thrombotic thrombocytopenic purpura from other thrombotic microangiopathies. *Br J Haematol,* **157,** 501-503.~~

Coppo, P., Schwarzinger, M., Buffet, M., Wynckel, A., Clabault, K., Presne, C., Poullin, P., Malot, S., Vanhille, P., Azoulay, E., Galicier, L., Lemiale, V., Mira, J.P., Ridel, C., Rondeau, E., Pourrat, J., Girault, S., Bordessoule, D., Saheb, S., Ramakers, M., Hamidou, M., Vernant, J.P., Guidet, B., Wolf, M. & Veyradier, A. (2010) Predictive features of severe acquired ADAMTS13 deficiency in idiopathic thrombotic microangiopathies: the French TMA reference center experience. *PLoS One,* **5,** e10208.

Cunningham, J.M., Ahn, J. & Broome, C. (2017) Outcomes for Atypical Hemolytic Uremic Syndrome (aHUS) Treated with Eculizumab: A Single Center Analysis. *Blood,* **130,** 2331-2331.

Dragon-Durey, M.A., Loirat, C., Cloarec, S., Macher, M.A., Blouin, J., Nivet, H., Weiss, L., Fridman, W.H. & Fremeaux-Bacchi, V. (2005) Anti-Factor H autoantibodies associated with atypical hemolytic uremic syndrome. *J Am Soc Nephrol,* **16,** 555-563.

Fakhouri, F., Roumenina, L., Provot, F., Sallee, M., Caillard, S., Couzi, L., Essig, M., Ribes, D., Dragon-Durey, M.A., Bridoux, F., Rondeau, E. & Fremeaux-Bacchi, V. (2010) Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc Nephrol,* **21,** 859-867.

Fakhouri, F., Delmas, Y., Provot, F., Barbet, C., Karras, A., Makdassi, R., Courivaud, C., Rifard, K., Servais, A., Allard, C., Besson, V., Cousin, M., Chatelet, V., Goujon, J.M., Coindre, J.P., Laurent, G., Loirat, C. & Fremeaux-Bacchi, V. (2014) Insights from the use in clinical practice of eculizumab in adult patients with atypical hemolytic uremic syndrome affecting the native kidneys: an analysis of 19 cases. *Am J Kidney Dis,* **63,** 40-48.

Fakhouri, F., Hourmant, M., Campistol, J.M., Cataland, S.R., Espinosa, M., Gaber, A.O., Menne, J., Minetti, E.E., Provot, F., Rondeau, E., Ruggenenti, P., Weekers, L.E., Ogawa, M., Bedrosian, C.L. & Legendre, C.M. (2016) Terminal Complement Inhibitor Eculizumab in Adult Patients With Atypical Hemolytic Uremic Syndrome: A Single-Arm, Open-Label Trial. *Am J Kidney Dis,* **68,** 84-93.

Fakhouri, F., Fila, M., Provot, F., Delmas, Y., Barbet, C., Chatelet, V., Rafat, C., Cailliez, M., Hogan, J., Servais, A., Karras, A., Makdassi, R., Louillet, F., Coindre, J.P., Rondeau, E., Loirat, C. & Fremeaux-Bacchi, V. (2017) Pathogenic Variants in Complement Genes and Risk of Atypical Hemolytic Uremic Syndrome Relapse after Eculizumab Discontinuation. *Clin J Am Soc Nephrol,* **12,** 50-59.

~~Fakhouri, F., Hourmant, M., Campistol, J.M., Cataland, S.R., Espinosa, M., Gaber, A.O., Menne, J., Minetti, E.E., Provot, F., Rondeau, E., Ruggenenti, P., Weekers, L.E., Ogawa, M., Bedrosian, C.L. & Legendre, C.M. (2016) Terminal Complement Inhibitor Eculizumab in Adult Patients With Atypical Hemolytic Uremic Syndrome: A Single-Arm, Open-Label Trial. *Am J Kidney Dis,* **68,** 84-93.~~

~~Fakhouri, F., Roumenina, L., Provot, F., Sallee, M., Caillard, S., Couzi, L., Essig, M., Ribes, D., Dragon-Durey, M.A., Bridoux, F., Rondeau, E. & Fremeaux-Bacchi, V. (2010) Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc Nephrol,* **21,** 859-867.~~

Fremeaux-Bacchi, V., Fakhouri, F., Garnier, A., Bienaime, F., Dragon-Durey, M.A., Ngo, S., Moulin, B., Servais, A., Provot, F., Rostaing, L., Burtey, S., Niaudet, P., Deschenes, G., Lebranchu, Y., Zuber, J. & Loirat, C. (2013) Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. *Clin J Am Soc Nephrol,* **8,** 554-562.

Gediz, F., Payzin, B.K., Ecemis, S., Guler, N., Yilmaz, A.F., Topcugil, F. & Berdeli, A. (2016) Efficacy and safety of eculizumab in adult patients with atypical hemolytic uremic syndrome: A single center experience from Turkey. *Transfus Apher Sci,* **55,** 357-362.

Greenbaum, L.A., Fila, M., Ardissino, G., Al-Akash, S.I., Evans, J., Henning, P., Lieberman, K.V., Maringhini, S., Pape, L., Rees, L., van de Kar, N.C., Vande Walle, J., Ogawa, M., Bedrosian, C.L. & Licht, C. (2016) Eculizumab is a safe and effective treatment in pediatric patients with atypical hemolytic uremic syndrome. *Kidney Int,* **89,** 701-711.

Hofer, J., Giner, T. & Jozsi, M. (2014) Complement factor H-antibody-associated hemolytic uremic syndrome: pathogenesis, clinical presentation, and treatment. *Semin Thromb Hemost,* **40,** 431-443.

Jamme, M., Raimbourg, Q., Chauveau, D., Seguin, A., Presne, C., Perez, P., Gobert, P., Wynckel, A., Provot, F., Delmas, Y., Mousson, C., Servais, A., Vrigneaud, L., Veyradier, A., Rondeau, E. & Coppo, P. (2017) Predictive features of chronic kidney disease in atypical haemolytic uremic syndrome. *PLoS One,* **12,** e0177894.

Jokiranta, T.S. (2017) HUS and atypical HUS. *Blood,* **129,** 2847-2856.

Kokame, K., Nobe, Y., Kokubo, Y., Okayama, A. & Miyata, T. (2005) FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *Br J Haematol,* **129,** 93-100.

Krishnappa, V., Gupta, M., Elrifai, M., Moftakhar, B., Ensley, M.J., Vachharajani, T.J., Sethi, S.K. & Raina, R. (2018) Atypical Hemolytic Uremic Syndrome: A Meta-Analysis of Case Reports Confirms the Prevalence of Genetic Mutations and the Shift of Treatment Regimens. *Ther Apher Dial,* **22,** 178-188.

Legendre , C.M., Licht , C., Muus , P., Greenbaum , L.A., Babu , S., Bedrosian , C., Bingham , C., Cohen , D.J., Delmas , Y., Douglas , K., Eitner , F., Feldkamp , T., Fouque , D., Furman , R.R., 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 , N.S., Trivelli , A., Zimmerhackl , L.B., Goodship , T. & Loirat , C. (2013) Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic–Uremic Syndrome. *New England Journal of Medicine,* **368,** 2169-2181.

Lemaire, M., Fremeaux-Bacchi, V., Schaefer, F., Choi, M., Tang, W.H., Le Quintrec, M., Fakhouri, F., Taque, S., Nobili, F., Martinez, F., Ji, W., Overton, J.D., Mane, S.M., Nurnberg, G., Altmuller, J., Thiele, H., Morin, D., Deschenes, G., Baudouin, V., Llanas, B., Collard, L., Majid, M.A., Simkova, E., Nurnberg, P., Rioux-Leclerc, N., Moeckel, G.W., Gubler, M.C., Hwa, J., Loirat, C.

& Lifton, R.P. (2013) Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. *Nat Genet,* **45,** 531-536.

Licht, C., Greenbaum, L.A., Muus, P., Babu, S., Bedrosian, C.L., Cohen, D.J., Delmas, Y., Douglas, K., Furman, R.R., Gaber, O.A., Goodship, T., Herthelius, M., Hourmant, M., Legendre, C.M., Remuzzi, G., Sheerin, N., Trivelli, A. & Loirat, C. (2015a) Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int,* **87,** 1061-1073.

Licht, C., Ardissino, G., Ariceta, G., Cohen, D., Cole, J.A., Gasteyger, C., Greenbaum, L.A., Johnson, S., Ogawa, M., Schaefer, F., Vande Walle, J. & Fremeaux-Bacchi, V. (20~~15a~~15b) The global aHUS registry: methodology and initial patient characteristics. *BMC Nephrol,* **16,** 207.

~~Licht, C., Greenbaum, L.A., Muus, P., Babu, S., Bedrosian, C.L., Cohen, D.J., Delmas, Y., Douglas, K., Furman, R.R., Gaber, O.A., Goodship, T., Herthelius, M., Hourmant, M., Legendre, C.M., Remuzzi, G., Sheerin, N., Trivelli, A. & Loirat, C. (2015b) Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int,* **87,** 1061-1073.~~

Macia, M., de Alvaro Moreno, F., Dutt, T., Fehrman, I., Hadaya, K., Gasteyger, C. & Heyne, N. (2017) Current evidence on the discontinuation of eculizumab in patients with atypical haemolytic uraemic syndrome. *Clin Kidney J,* **10,** 310-319.

Mallett, A., Hughes, P., Szer, J., Tuckfield, A., Van Eps, C., Cambell, S.B., Hawley, C., Burke, J., Kausman, J., Hewitt, I., Parnham, A., Ford, S. & Isbel, N. (2015) Atypical haemolytic uraemic syndrome treated with the complement inhibitor eculizumab: the experience of the Australian compassionate access cohort. *Intern Med J,* **45,** 1054-1065.

Merrill, S.A., Brittingham, Z.D., Yuan, X., Moliterno, A.R., Sperati, C.J. & Brodsky, R.A. (2017) Eculizumab cessation in atypical hemolytic uremic syndrome. *Blood,* **130,** 368-372.

Noris, M. & Remuzzi, G. (2009) Atypical hemolytic-uremic syndrome. *N Engl J Med,* **361,** 1676-1687.

Noris, M. & Remuzzi, G. (2014) Cardiovascular complications in atypical haemolytic uraemic syndrome. *Nature Reviews Nephrology,* **10,** 174.

Noris, M., Caprioli, J., Bresin, E., Mossali, C., Pianetti, G., Gamba, S., Daina, E., Fenili, C., Castelletti, F., Sorosina, A., Piras, R., Donadelli, R., Maranta, R., van der Meer, I., Conway, E.M., Zipfel, P.F., Goodship, T.H. & Remuzzi, G. (2010) Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol,* **5,** 1844-1859.

Noris, M., Mele, C. & Remuzzi, G. (2015) Podocyte dysfunction in atypical haemolytic uraemic syndrome. *Nature Reviews Nephrology,* **11,** 245.

~~Noris, M. & Remuzzi, G. (2009) Atypical hemolytic-uremic syndrome. *N Engl J Med,* **361,** 1676-1687.~~

~~Noris, M. & Remuzzi, G. (2014) Cardiovascular complications in atypical haemolytic uraemic syndrome. *Nature Reviews Nephrology,* **10,** 174.~~

Phillips, E.H., Westwood, J.P., Brocklebank, V., Wong, E.K., Tellez, J.O., Marchbank, K.J., McGuckin, S., Gale, D.P., Connolly, J., Goodship, T.H., Kavanagh, D. & Scully, M.A. (2016) The role of ADAMTS-13 activity and complement mutational analysis in differentiating acute thrombotic microangiopathies. *J Thromb Haemost,* **14,** 175-185.

Schaefer, F., Ardissino, G., Ariceta, G., Fakhouri, F., Scully, M., Isbel, N., Lommele, A., Kupelian, V., Gasteyger, C., Greenbaum, L.A., Johnson, S., Ogawa, M., Licht, C., Vande Walle, J. & Fremeaux-Bacchi, V. (2018) Clinical and genetic predictors of atypical hemolytic uremic syndrome phenotype and outcome. *Kidney Int~~.~~,* **94**, 408-418.

Scully, M., Cataland, S., Coppo, P., de la Rubia, J., Friedman, K.D., Kremer Hovinga, J., Lammle, B., Matsumoto, M., Pavenski, K., Sadler, E., Sarode, R. & Wu, H. (2017) Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. *J Thromb Haemost,* **15,** 312-322.

Sheerin, N.S., Kavanagh, D., Goodship, T.H. & Johnson, S. (2016) A national specialized service in England for atypical haemolytic uraemic syndrome-the first year's experience. *Qjm,* **109,** 27-33.

Walle, J.V., Delmas, Y., Ardissino, G., Wang, J., Kincaid, J.F. & Haller, H. (2017) Improved renal recovery in patients with atypical hemolytic uremic syndrome following rapid initiation of eculizumab treatment. *J Nephrol,* **30,** 127-134.

Wijnsma, K.L., Duineveld, C., Volokhina, E.B., van den Heuvel, L.P., van de Kar, N. & Wetzels, J.F.M. (2017) Safety and effectiveness of restrictive eculizumab treatment in atypical haemolytic uremic syndrome. *Nephrol Dial Transplant*~~.~~, **33**, 635-645.

Table I: Key clinical features of each of the 22 patients.

Patient	Age at presentation (years), sex	Previous or FH of aHUS	Genetic abnormality	Nadir platelet count ($x10^9$/l)	Nadir eGFR (ml/min /1.73m^2)	Duration of RRT	C3 low at presentation?	Eculizumab duration, weeks (doses, n)	Evidence of TMA activity while on eculizumab	Renal function at time of last dose (eGFR in ml/min /1.73m^2)	Eculizumab ongoing or withdrawn	Outcome after withdrawal (including duration off eculizumab)
1*	51, M	No	VUCS *CFH* (c.3264A>C p.(Glu1088Asp) in exon 21)	31	6	Ongoing at 4.5 weeks*	No	3 (4)*	Remission not yet reached	Requiring RRT*	Ongoing*	n/a
2	41, F	No	No	7	10	3 days	No	8 (7)	No	eGFR >60	Withdrawn	Ongoing remission (130 weeks)
3	40, F	No	VUCS *CD46* (c.389+5G>A)	32	12	2 days	Yes	22 (14)	No	eGFR >60	Withdrawn	Ongoing remission (58 weeks)
4	35, F	No	No	54	8	1 day	Yes	26 (16)	No	eGFR >60	Withdrawn	Ongoing remission (59 weeks)
5	29, F	No	No	12	22	3 days	Yes	6 (6)	No	eGFR >60	Withdrawn	Remission for 71 weeks post-withdrawal, then restarted as prophylaxis during pregnancy
6	42, M	Yes: previous 'relapsing TTP'	Pathogenic mutation in *CD46* (c.175 C>T p.(Arg59*))	13	49	No	No	4 (5)	No	eGFR >60	Withdrawn	Ongoing remission (85 weeks)
7	23, F	No	No	62	14	2 days	No	12 (9)	No	eGFR >60	Withdrawn	Ongoing remission (37 weeks)
8	66, F	No	No	14	13	3 days	Yes	16 (11)	No	eGFR 50-60	Withdrawn	Ongoing remission with stable eGFR (238 weeks)

	16, M	No	No	14	24	No	Yes	4 (5)	No	eGFR >60	Withdrawn	Ongoing remission (146 weeks)
0	18, F	No	No	9	34	No	No	16 (11)	No	eGFR >60	Withdrawn	Ongoing remission (37 weeks)
1	17, F	No	No	61	10	10 weeks	Yes	23 (14)	No	eGFR >60	Withdrawn	Ongoing remission (14 weeks)*
2	26, M	No	Pathogenic mutation in *C3* (c.193A>C p.(Lys65Gln)); VUCS *CFH* (c.1548T>A p.(Asn516Lys))	17	57	No	No	1 (2)	No	eGFR >60	Withdrawn	Thrombocytopenia 3 weeks after withdrawal. Full recovery with eculizumab. Plan to stop after 6 months.
3	23, F	No	VUCS *CFB* (c.1112A>G(p.Asp371Gly))	20	12	No	No	2 (3)	No	eGFR >60	Withdrawn	Relapse 48 weeks after withdrawal. Full recovery with eculizumab (2 doses only). Ongoing remission 68 weeks later*.
4	20, F	No	Pathogenic mutation in *CD46* (c.286+2T>G in intron 2)	11	26	No	No	10 (8)	No	eGFR >60	Withdrawn	Relapse 15 weeks after withdrawal. Full recovery with eculizumab. Withdrawal again after 2 weeks. Ongoing remissio 56 weeks later
5	30, F	No	No	55	3	6 months	No	229	No	Best eGFR 25 (22 months post-stopping) Now 10-15.	Ongoing	n/a
6	47, M	No	VUCS *CD46* (c.472G>C p.(Glu158Gln))	16	22	Ongoing at 19 months	Yes	85	No	Requiring RRT	Ongoing	n/a
7	25, F	Yes, mother known aHUS	Pathogenic *CFHR1-CFH* hybrid gene;	24	7	7 weeks	No	9 weeks (7 doses)*	n/a	eGFR 30*	Ongoing*	n/a

			CFI likely pathogenic variant (c.1246A>C p.(Ile416Leu))										
8	33, M	No	No	85	11	No	No	116 weeks	No	eGFR 30-40	Ongoing	n/a
9	50, M	No	No	22	8	Ongoing at nearly 8 weeks*	No	7 weeks (6 doses)*	No	Requiring RRT*	Ongoing*	n/a Subsequently died, circumstances unknown
0	31, F	No	No	41	8	No	Yes	134	2 possible subacute episodes (mild isolated thrombocytopenia)	eGFR 30-40	Ongoing	n/a
1	33, F	No	No	23	6	16 days	No	136	1 possible mild relapse	eGFR 50-60	Ongoing	n/a
2	67, F	No (but 'HELLP' in pregnancy)	Pathogenic mutation in *CFI* (c.561delG p.(Ala219fs) in exon 4)	42	7	Ongoing when died (day 11)	Yes	Died 4 days after 1st dose	n/a	On RRT when died	n/a	n/a

All mutations are heterozygous.
* denotes care was transferred to another institution: clinical details given correspond to the last follow-up visit at our institution.
aHUS: atypical haemolytic uraemic syndrome; eGFR: estimate glomerular filtration rate; F: female; FH: family history; HELLP: haemolysis, elevated liver enzyme levels, and low platelet levels; M: male; n/a: not available; RRT: renal replacement therapy; TMA: thrombotic microangiopathy; TTP: thrombotic thrombocytopenic purpura; VUCS = variant of unknown clinical significance.

Table II: Comparison of baseline characteristics and time to eculizumab initiation for patients attaining complete and incomplete renal recoveries.

	eGFR>60 ml/min/1.73m² **at last follow-up**	**eGFR <60 ml/min/1.73m²** **at last follow-up**	**Test statistic** **P value**
Presenting creatinine, µmol	219.5 (80-402)	520 (236-1153)	U = 12.5* P = 0.026
Peak UPCR[#], mg/mmol	253 (69-1228)	145 (119->7000)	U= 18* P = 0.58
Nadir platelet count, $x10^9$/l	15.5 (9-62)	32 (14-85)	U= 21.5* P = 0.19
Nadir Hb, g/l	72 (41-103)	72 (59-78)	U = 30.5* P = 0.63
Peak LDH, iu/l	1408 (803-4621)	2184.5 (582-3704)	U = 31* P= 0.68
Time to eculizumab	≤ 7 days: N=10 > 7 days: N=2	≤ 7 days: N=3 > 7 days: N=3	P = 0.27**

All values are given as median (range) unless otherwise indicated.
Patients 1, 17, 19 and 22 are excluded from the analysis as care was transferred or death occurred before ultimate renal outcome was known.
All p values are two-tailed.
*Mann Whitney test
**Fisher exact test
[#]UPCR values were not available for 4 patients.

eGFR: estimate glomerular filtration rate; Hb: haemoglobin concentration; LDH: lactate dehydrogenase; UPCR: urine protein:creatinine ratio.

Table III: Comparison of clinical characteristics of patients with and without identified complement genetic abnormalities.

	Complement genetic abnormality detected (N=9)	**No complement genetic abnormality detected (N=13)**
C3 low at presentation	33% (N=3)	46% (N=6)
Nadir platelet count, x 10^9/l; median (range)	20 (11-42)	23 (7-85)
RRT during acute episode	55.6 % (N=5)	69.2% (N=9)
Peak UPCR, mg/mmol; median (range)**	660 (142-1406)	145 (69-1576)
Died during FU period	11.1% (N=1)	0[#]
Ongoing RRT at last FU	22.2% (N=2)*	7.7% (N=1)*
eGFR <60 ml/min/1.73m^2, not requiring RRT, at last FU	11.1% (N=1)*	38.4% (N=5)
eGFR ≥60 ml/min/1.73m^2, at last FU	55.5% (N=5)	53.4% (N=7)

* Final renal outcome unknown in 1 case (due to transfer of care to another institution)
** UPCR values were not available for 5 patients.
[#]Patient 19 (without a complement genetic abnormality) is known to have died after care was transferred to another institution, but the circumstances are unknown.

eGFR: estimate glomerular filtration rate; FU: follow-up; RRT: renal replacement therapy; UPCR: urine protein:creatinine ratio.

Figure legends

Figure 1: Duration of eculizumab (black bars) or remission following eculizumab withdrawal (white bars) in weeks for each patient (1-22).

First course of eculizumab was ongoing at last follow-up in Patients 1, 15-21. Patient 22 died 4 days following first eculizumab dose. Eculizumab was withdrawn in first remission in Patients 2-14. Patients 2-11 remained in remission at last follow-up. Patients 12-14 relapsed post withdrawal and restarted eculizumab. Durations of therapy including number of doses are also given in Table I.

Figure 2: Outcomes of all 22 patients

CM-HUS: complement-mediated haemolytic uraemic syndrome; eGFR: estimate glomerular filtration rate (ml/min/1.73m^2); PEX: plasma exchange; RRT: renal replacement therapy.