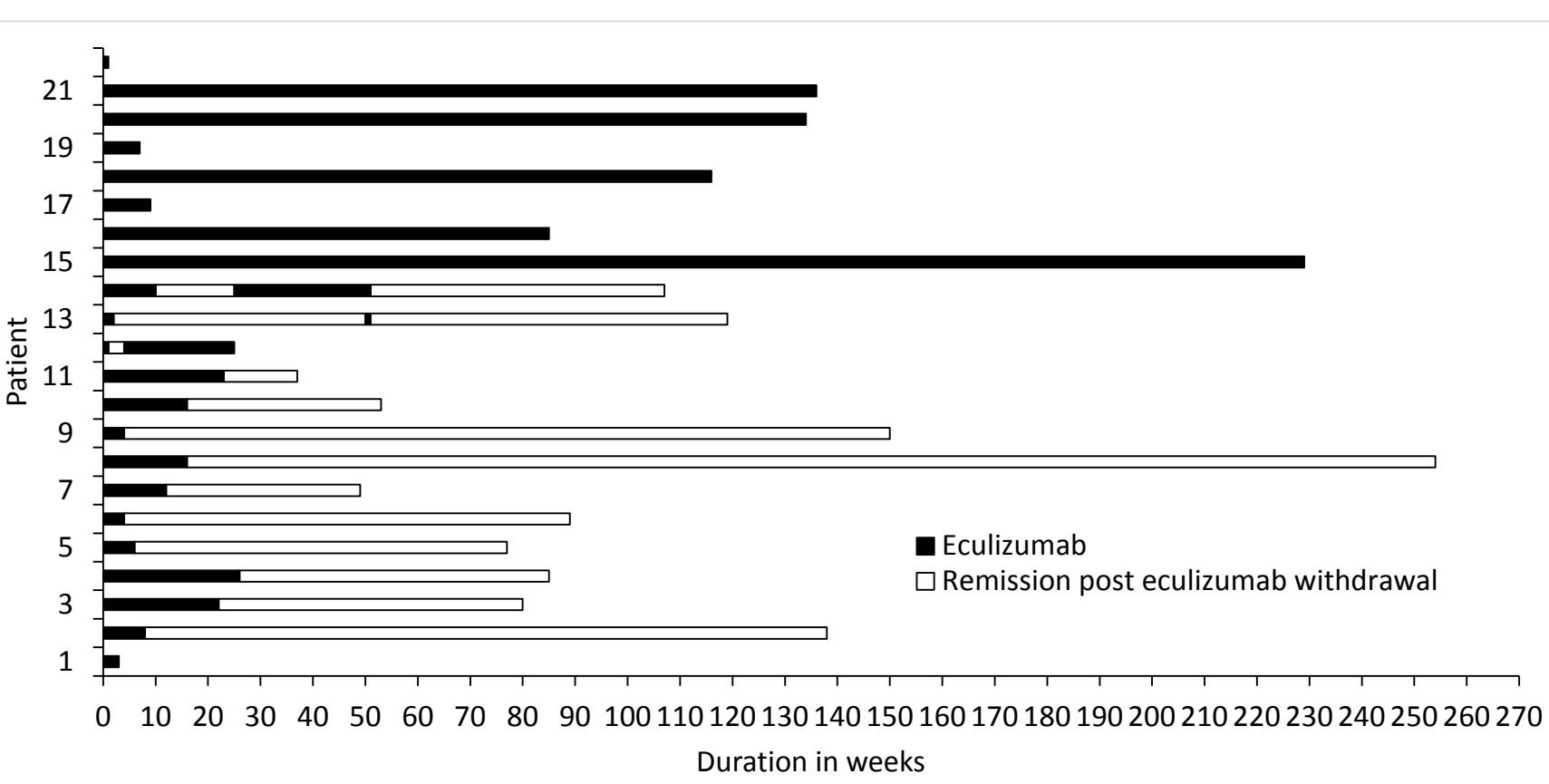
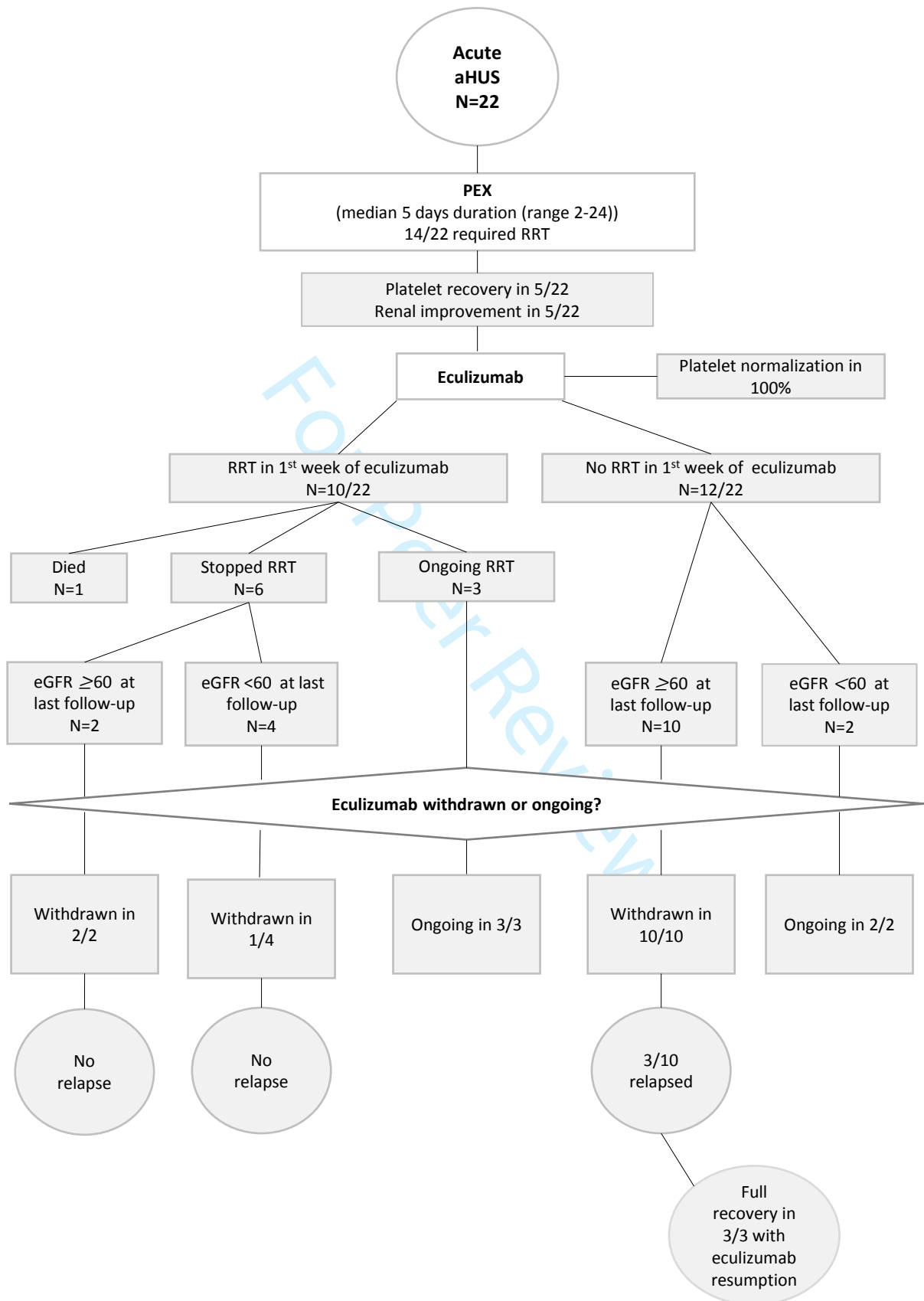


Atypical haemolytic uraemic syndrome in the eculizumab era: presentation, response to treatment and evaluation of an eculizumab withdrawal strategy.

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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
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3 **Atypical haemolytic uraemic syndrome in the eculizumab era: presentation, response to treatment**
4 **and evaluation of an eculizumab withdrawal strategy.**
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8 **Short Title: Eculizumab in aHUS**
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11 **Authors:** Lucy Neave¹, Daniel P Gale², Simon Cheesman³, Raakhee Shah³, Marie Scully⁴
12
13

14
15 ¹ Department of Haematology, University College London Hospitals NHS Foundation Trust, London
16 UK
17

18 ² UCL Centre for Nephrology, University College London, London UK
19

20 ³ Department of Pharmacy, University College London Hospitals NHS Foundation Trust, London UK
21

22 ⁴ Department of Haematology, UCLH, Cardiometabolic programme- NIHR UCLH/UCL BRC, London,
23 UK
24
25

26
27 **Corresponding author:**
28
29

30 Professor Marie Scully, Department of Haematology, University College London Hospital, 5th Floor
31 Central, 250 Euston Road, London NW1 2PG, UK.
32

33 Telephone +44 203 447 9884 Fax +44 203 447 9145
34

35 Email m.scully@ucl.ac.uk
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41 **Key words:**
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43 aHUS, atypical haemolytic uraemic syndrome, eculizumab, withdrawal, TMA
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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/1.73m²). At median duration of follow-up of 85 weeks (range 4-255), 54.5% had eGFR \geq 60 ml/min/1.73m², 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 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

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3 membrane cofactor protein (*CD46*), are demonstrated in 40-60% of affected individuals (Fremeaux-
4 Bacchi, *et al* 2013, Noris and Remuzzi 2009, Schaefer, *et al* 2018). Factor H autoantibodies (Dragon-
5 Durey, *et al* 2005, Hofer, *et al* 2014) and mutations in *DGKE* (encoding diacylglycerol kinase ϵ)
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7 (Lemaire, *et al* 2013) are rare causes usually seen in children.
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13 Historically, the prognosis has been poor, with incomplete response rates to plasma exchange (PEX),
14 and rates of end stage renal disease (ESRD) or death as high as 50-77% after 3-5 years (Fremeaux-
15 Bacchi, *et al* 2013, Noris, *et al* 2010, Schaefer, *et al* 2018). However efficacy of the humanised
16 monoclonal anti-C5 antibody eculizumab was demonstrated in open label phase II trials (Fakhouri, *et*
17 *al* 2016, Greenbaum, *et al* 2016, Legendre, *et al* 2013, Licht, *et al* 2015a), inducing haematological
18 remission, improving or stabilizing renal function, and preventing graft failure following renal
19 transplant. US Food and Drug Administration and European Medicines Agency approvals were
20 granted in 2011, and the National Health Service has funded the drug in England since 2013 under
21 the coordination of the National aHUS Service.
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31 At a cost of over £300 000 a year per patient, treatment carries significant financial burdens, as well
32 as potential risks, including bacterial meningitis (Fakhouri, *et al* 2016), so the challenge now is to
33 confirm that the drug is used in the most effective way possible, including determining the optimum
34 treatment schedule. Whilst large-scale registries and prospective studies are needed to definitively
35 address such questions [and are underway (Licht, *et al* 2015b)], in the short-term, retrospective
36 cohort analyses are informative, though few have been published at present (Cataland, *et al* 2014,
37 Cunningham, *et al* 2017, Fakhouri, *et al* 2014, Krishnappa, *et al* 2018, Sheerin, *et al* 2016).
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46 We describe a cohort of 22 patients presenting to a UK TMA referral centre with acute aHUS and
47 treated with eculizumab over a 6-year period. We highlight the presenting features and responses to
48 eculizumab. We also assess the outcomes of a strategy of eculizumab withdrawal after achieving a
49 complete or near-complete response.
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55 **Patients and Methods**

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58 All adult patients prescribed at least one dose of eculizumab for an acute presentation of aHUS in a
59 single institution were retrospectively identified. Of 34 patients presenting with aHUS between 2012
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3 and 2018, 9 responded to PEX only, 3 went on to receive an alternative C5 inhibitor on a clinical trial,
4 while 22 patients received eculizumab. All 22 of these patients were included in the analysis,
5 including those who were no longer followed up in our institution. Data regarding presenting clinical
6 features, response to therapy and long-term outcomes of the aHUS episode was collected from the
7 medical records as part of a service review, and analysed anonymously. All investigations had been
8 performed as part of routine care.
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15 In all cases, PEX with solvent detergent-treated plasma was initiated on admission. Renal
16 replacement therapy (RRT) was commenced if indicated. The diagnosis of aHUS was made according
17 to international consensus criteria (Scully, *et al* 2017) on the basis of: (i) presence of TMA (direct
18 antiglobulin test-negative haemolytic anaemia with schistocytes on blood film and
19 thrombocytopenia); (ii) exclusion of severe ADAMTS13 deficiency [(ADAMTS13 activity by
20 fluorescence resonance energy transfer (Kokame, *et al* 2005) >10 IU/dL]; and (iii) exclusion of
21 secondary TMAs (demonstration of normal coagulation screen, negative autoimmune serology,
22 negative lupus anticoagulant and antiphospholipid antibody screening and negative reference
23 laboratory STEC stool/serological investigations in all diarrhoeal cases, with imaging to exclude
24 malignancy if indicated). End-organ damage was assessed via serum biochemistry, spot urine
25 protein:creatinine ratio (UPCR), renal ultrasound, cardiac troponin I, electrocardiogram and, in
26 selected cases, brain imaging. Renal biopsy was performed in cases of incomplete renal recovery
27 where there was diagnostic uncertainty (N=2).
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39 Following National aHUS Service approval to commence eculizumab, PEX was discontinued just prior
40 to the first dose. Intravenous eculizumab was administered weekly for 4 weeks at a dose of 900 mg,
41 followed by 1200 mg fortnightly starting on week 5. Meningococcal vaccination (against subtypes
42 ACWY and B) was administered prior to initiation of eculizumab, followed by antibiotic prophylaxis
43 (ciprofloxacin initially, followed by penicillin V) for the duration of therapy. If patients required
44 ongoing RRT they were referred to their local renal centre. Following discharge, patients continued
45 to receive eculizumab as an outpatient (and, in some cases, at home), with fortnightly blood and
46 urine monitoring.
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Withdrawal of eculizumab was considered in all patients who achieved complete haematological response [platelet count >150 x10⁹/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

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3 opinion and patient preference, after a discussion of risks, benefits and available evidence. All
4 patients who were offered the option to stop treatment elected to do so. Monitoring for relapse
5 after withdrawal included symptoms review, full blood and reticulocyte counts, LDH, serum
6 creatinine/eGFR and urinalysis/UPCR, initially fortnightly, but then at increasing time intervals and
7 ultimately 6-monthly. All patients had access to a 24-h telephone helpline in the event of concerning
8 symptoms in the interim.
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15 Complement genotyping, performed via the National aHUS Service, included direct sequencing of
16 coding exons of *CFH*, *CFI*, *CD46*, *C3* and *CFB*, and multiplex ligation-dependent probe amplification
17 (MLPA) analysis for deletions and duplications of *CFH*, *CFI*, *CD46*, *CFHR1* and *CFHR3*. Screening for
18 factor H autoantibodies was also undertaken in the majority of cases.
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23 Results

24 **Summary of patients treated**

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

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52 Gastrointestinal symptoms were common at presentation, affecting 64%, and were most commonly
53 nausea and vomiting (36%), and abdominal pain (32%). STEC-negative diarrhoea was present in 23%
54 of cases. Forty-one percent had neurological manifestations: seizure N=2; headache with or without
55 visual disturbance N=4; transient diplopia N=1; transient facial and/or limb weakness N=2. Other
56 presenting symptoms included: dark urine/ altered urine output (23%); bleeding/purpura (14%);
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3 jaundice (9%); lethargy/malaise (5%). Three patients were diagnosed following detection of
4 laboratory abnormalities in pregnancy. Thirteen patients were hypertensive at admission (though 3
5 had pre-existing hypertension).
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11 Median nadir platelet count was $23 \times 10^9/l$ (range $7-85 \times 10^9/l$) and median nadir haemoglobin (Hb)
12 was $70g/l$ (range $62-103 g/l$). Median presenting LDH was $1704 iu/l$ (range $582-4621 iu/l$, normal
13 range $135-214 iu/l$). Reticulocytosis was notably absent in 45.5% of patients at presentation, though
14 in all but one case this subsequently developed. Bilirubin remained normal in 22.7% of patients.
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21 Median presenting creatinine was $323.5 \mu mol/l$ (range $80-1153$, normal range $49-92$). Proteinuria
22 was demonstrated by urinalysis or UPCR in all cases. The median UPCR (N=17) was $199.5 mg/mmol$
23 (range $69->7000$, normal range $0-13$); it was $>300 mg/mmol$ for 8/17 (47%). Median nadir eGFR was
24 $11.5 ml/min/1.73m^2$ (range $3-57$), and 64% of patients (N=14) required RRT during the acute
25 episode. Admission to the intensive care unit (ITU) was required by 14/22 (64%) patients, 4 of whom
26 were intubated and ventilated. Whilst there were no overt cardiac manifestations at presentation,
27 77% (N=17) had elevated cardiac troponin T (median $64.5 ng/l$, range $17-397$, normal range $0-14$).
28 Despite neurological symptoms in 41%, brain magnetic resonance imaging was abnormal only in 3
29 cases (14%) (infarction/small vessel changes).
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39 Clear triggers were identified in 50% of cases: pregnancy/postpartum in 4 (Patients 4, 5, 7 and 14 in
40 Table I); influenza in 2; lower respiratory tract infections in 3; campylobacter diarrhoea in 1 (this was
41 believed to be a trigger rather than the cause of the HUS, given that the TMA persisted despite
42 resolution of the infection). Patient 10 initially presented with gallstone cholecystitis 5 months
43 postpartum, but a frank TMA picture quickly evolved (along with post-ERCP pancreatitis) and the
44 suspicion was that the gallstones resulted from a low grade postpartum HUS, as she had been noted
45 to be hypertensive and proteinuric peripartum, with intermittent abdominal pain, malaise and
46 nausea ever since.
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55 ADAMTS13 activity on presentation was within the normal range for 86.3% (n=19) patients, and
56 slightly low in 13.7% (n=3). Median activity was $72 iu/dl$ (range $56-91$, normal range $60-146$).
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Response to plasma exchange

All 22 patients commenced PEX at admission, with a median PEX duration of 5 days (range 2-24). Platelet normalization ($>150 \times 10^9/l$) 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 $eGFR >90 \text{ ml/min/1.73m}^2$ 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 $\geq 150 \times 10^9/l$, 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 $eGFR 49 \text{ ml/min/1.73m}^2$; range 22->80). The time for the

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3 creatinine to reach a new baseline generally depended on the extent of renal impairment, ranging
4 from 14 days to as long as 17 months.
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10 Twelve of 21 patients made a complete or near-complete renal recovery (to eGFR ≥ 60
11 ml/min/1.73m² in 11/12 and eGFR 55 ml/min/1.73m² in Patient 8 who was 66 years old; resolution
12 of proteinuria in 7/12), after a median of 23.5 days of eculizumab (range 14-51). 7 of those 12
13 patients (58%) had required RRT at presentation (duration 1-3 days in 6/7, but 68 days in Patient 11).
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19 Four of 21 patients had residual CKD with eGFR 25-60 ml/min/1.73m² after eculizumab duration
20 119-232 weeks, two of whom (50%) had required RRT at initiation (duration 21 days and 6 months).
21 Three of 21 patients remained on RRT and eculizumab at last follow-up, after 3, 7 and 85 weeks of
22 eculizumab. One further patient (Patient 17) stopped RRT after 7 weeks but, due to transfer of care
23 (while still on eculizumab), renal outcomes are unknown.
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30 The final patient died during the acute admission (Patient 22). She required intubation and
31 ventilation at presentation, for reduced consciousness and agitation, but had been extubated and
32 was clinically improving, though still on RRT, when she suffered an unexpected cardiac arrest on day
33 5 of eculizumab. Whilst Patient 19 is also known to have died, following transfer to another
34 institution after 7 weeks of eculizumab and RRT, the timing and circumstances of the death are
35 unknown, and therefore cannot be reliably attributed to aHUS.
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44 ***Predictors of renal response***

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46 Whilst recognizing the limitations of a retrospective cohort analysis, it is noticeable that patients
47 with a final eGFR < 60 ml/min/1.73m² after treatment, had significantly higher presenting creatinine
48 levels than those who recovered eGFR to ≥ 60 ml/min/1.73m² [median 520 μ mol/l (range 236-1153)
49 vs median 219.5 μ mol/l (range 80-402), $p = 0.026$ (Mann-Whitney test, $U = 12.5$)]. There was no
50 significant correlation between renal outcome and peak UPCR, nadir platelet count, nadir Hb, peak
51 LDH or time to eculizumab (Table II).
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59 ***Complement abnormalities***

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3 Complement genetic abnormalities were identified in 40.9 % (9/22) of patients, involving *CD46*
4 (N=4), *CFH* (N=3), *CFI* (N=2), *C3* (N=1) and *CFB* (N=1). Two patients (9%) had abnormalities in 2 genes
5 (Table I).
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11 Table III compares key clinical features of patients with normal and abnormal genetic screening.
12 There was little obvious difference in severity of presentation or renal outcomes, though numbers
13 are small and, for two cases in the abnormal genetic screening group and one in the normal group,
14 care was transferred to other institutions before ultimate renal outcomes were known.
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21 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)],
22 and this remained permanently or intermittently low despite clinical remission on eculizumab in 4/9
23 cases, suggesting poor correlation with disease activity. Low C3 was not a predictor of mutation
24 status: 3/9 with low presenting C3 were subsequently found to have complement genetic
25 abnormalities compared to 6/13 with normal C3.
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32 Factor H autoantibody screening was performed in 59% of cases, and antibodies were not detected
33 in any patients, though some samples were convalescent.
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39 ***Withdrawal of eculizumab***

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41 Eculizumab was withdrawn in all 12 of the patients who made a complete or near complete renal
42 response, after a median 11 weeks (range 1-26). The one patient who had normal renal function but
43 persistent thrombocytopenia when eculizumab was initiated (Patient 9) stopped therapy after 4
44 weeks (following a complete haematological response), bringing the total number of patients in
45 whom eculizumab was withdrawn to 13/22.
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52 The remaining 8/22 patients (excluding Patient 22 who died after 1 dose) remained on eculizumab
53 therapy at last follow-up due to incomplete renal recovery. The median duration of therapy at last
54 follow-up was 21.5 weeks (range 3-227).
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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 ($139 \times 10^9/l$) 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 $<30 \times 10^9/l$, LDH >1000 iu/l and creatinine 200-300 $\mu\text{mol/l}$, 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,

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

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11 The outcomes of all patients are summarised in Figure 2
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19 **Discussion**

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21 Whilst the retrospective nature of this cohort is a limitation, its size is comparable to the original
22 prospective phase 2 trials, and 'real world' outcome data in this ultra-rare disease is scarce, so the
23 findings are of value.
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29 The presenting features of the cohort reiterate some important characteristics of aHUS: end organ
30 involvement is not necessarily confined to the kidneys (Cataland and Wu 2014, Noris and Remuzzi
31 2009); neurological and gastrointestinal symptoms are common (Jamme, *et al* 2017, Schaefer, *et al*
32 2018); and whilst severe thrombocytopenia and mild renal impairment are more common in TTP
33 (Cataland, *et al* 2012, Coppo, *et al* 2010), they do not exclude aHUS (Phillips, *et al* 2016) (nadir
34 platelet count was $<30 \times 10^9/l$ in 59% of patients, and peak creatinine was $<200 \mu\text{mol/l}$ in 14%). It is
35 possible that our cohort is skewed to the less severe end of the renal spectrum renally, as cases
36 presenting with severe renal impairment are often referred direct to nephrology, but in fact the
37 proportion requiring RRT (64%) in the acute phase is similar to larger cohorts (Fakhouri, *et al* 2016,
38 Sheerin, *et al* 2016). The 41% prevalence of complement genetic abnormalities is also in keeping
39 with the existing literature, as is the finding of pregnancy as a common trigger (Fakhouri, *et al* 2010).
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50 In terms of novel findings, nearly half of patients had nephrotic range proteinuria at presentation
51 despite this not classically being associated with aHUS, and previous reports tending to be in
52 children or cases with secondary causes (Noris, *et al* 2015). Whilst cardiovascular manifestations are
53 reported (Noris and Remuzzi 2014), this is the first demonstration to our knowledge of a high
54 prevalence (77%) of asymptomatic cardiac troponin elevation, suggesting frequent subclinical
55 cardiac involvement (although renal impairment may have contributed to elevated troponin).
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6 To summarise outcomes, at a median follow-up of 85 weeks (range 4-255), following a median
7 duration of initial course of eculizumab of 11 weeks (range 1-227), 100% of patients showed
8 resolution of thrombocytopenia and 81% showed improvement in eGFR (median increase in eGFR 49
9 ml/min/1.73m²; range 22->90). Of the 14/22 patients who initially required RRT, 10 became dialysis
10 independent. At last follow-up, 54.5 % had eGFR \geq 60 ml/min/1.73m², 27% had CKD with eGFR <60
11 ml/min/1.73m² but not requiring RRT, 14% were on RRT and 4.5% had died. It should be noted that 2
12 of the 3 patients requiring RRT at last follow-up had their care transferred to another institution
13 after only 3 and 7 weeks' of eculizumab, after which time there may have been some renal recovery.
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22 The renal outcomes compare very favourably with data from the pre-eculizumab era [rates of ESRD
23 or death 50-77% after 3-5 years (Fremeaux-Bacchi, *et al* 2013, Noris, *et al* 2010, Schaefer, *et al*
24 2018)], but also to previously published outcomes with eculizumab. Of the three Phase 2 trials in
25 adults (Fakhouri, *et al* 2016, Legendre, *et al* 2013, Licht, *et al* 2015a), our cohort is most comparable
26 to the 41 patients reported by Fakhouri *et al* (2016), who were not required to be plasma dependent
27 or refractory and who generally received eculizumab early in the acute phase (although the
28 proportion of relapses and renal transplants was higher in the Phase 2 cohort). Thirty-eight of 41
29 patients received the intended 26 weeks' of treatment, by which time point 98% achieved platelet
30 normalisation. Fifty-four percent showed an increase in eGFR of >15 ml/min/1.73m² by 26 weeks,
31 and 15% were dialysis-dependent at 26 weeks, from 58% at baseline and 46% at initiation of
32 eculizumab.
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44 The renal outcomes of our cohort are also comparable to published retrospective cohorts
45 (Cunningham, *et al* 2017, Fakhouri, *et al* 2014, Gediz, *et al* 2016, Mallett, *et al* 2015, Sheerin, *et al*
46 2016). In a French series of 19 adult patients (Fakhouri, *et al* 2016), 63% required RRT at diagnosis,
47 while at last follow-up (range 4-22 months, treatment ongoing in 74%), 16% required RRT, 37% had
48 CKD and 47% had normal renal function. In a US cohort (N=52), 35% required dialysis prior to
49 eculizumab, and 21% at 3 months (Cunningham, *et al* 2017). Of 23 incident patients in an analysis of
50 the first year of the national specialised service in England (Sheerin, *et al* 2016), 15 (65%) required
51 dialysis at eculizumab initiation, of whom 8 were able to stop dialysis after a duration of 1-30 weeks.
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3 Whilst there are potential confounders, it is notable that a lower presenting creatinine was a
4 significant predictor of a better renal response in this cohort. The same was not true in a post-hoc
5 analysis of pooled data from the 4 prospective trials (Walle, *et al* 2017). The finding that mutation
6 status did not affect the likelihood of renal recovery adds to existing data in this regard (Fakhouri, *et*
7 *al* 2016, Sheerin, *et al* 2016, Walle, *et al* 2017).
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14 If and when to withdraw eculizumab is an important and debated question, which has not yet been
15 addressed prospectively as treatment was continued for the duration of the Phase 2 trials in the
16 majority of cases. The 23% relapse rate post-withdrawal seen in this cohort is in keeping with 20-
17 31% relapse rates reported in the largest four case series of patients who stopped in stable
18 remission (a total of 86 cases) (Ardissino, *et al* 2014, Ardissino, *et al* 2015, Fakhouri, *et al* 2017,
19 Merrill, *et al* 2017, Wijnsma, *et al* 2017). The time frame of relapse within 1 year is also comparable.
20 There was a suggestion from two cohorts (Ardissino, *et al* 2015, Fakhouri, *et al* 2017), as in ours, that
21 those with a mutation, especially *CFH* mutations, were more likely to relapse.
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31 Whilst relapses did occur, all three patients who relapsed made rapid and complete recoveries with
32 re-initiation of eculizumab, without needing PEX or RRT. The withdrawal strategy therefore led to no
33 long-term adverse effects for these patients, or on outcomes of the cohort as a whole, given that the
34 overall outcomes were comparable to those of the Phase 2 trial (Fakhouri, *et al* 2016), despite 59%
35 of our cohort not receiving indefinite treatment.
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42 Whilst the vast majority of published cases of relapse post-eculizumab withdrawal made a full
43 recovery after early re-initiation of eculizumab (Ardissino, *et al* 2015, Ardissino, *et al* 2014, Fakhouri,
44 *et al* 2017, Merrill, *et al* 2017, Wijnsma, *et al* 2017) a recent review (Macia, *et al* 2017) cites two
45 cases in which re-initiation of eculizumab did not prevent deterioration to end stage renal failure
46 (though timing to re-initiation is not given for one case, and the other case involves a patient who
47 only received one dose initially). The potential benefits however are undeniable: just over 750 doses
48 of eculizumab have been avoided in this cohort to date, with associated reduced risk of adverse
49 effects (including meningococcal infection), reduced hospital attendances and service delivery
50 burdens, and drug cost savings of over £11 million. Whilst a randomised controlled trial is needed to
51 definitively assess the safety of eculizumab withdrawal, in the absence of such data this cohort adds
52 to the growing body of evidence in support of such a strategy. Monitoring post-withdrawal, patient
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3 education regarding potential symptoms of relapse, and pathways to ensure timely re-initiation of
4 eculizumab in the event of relapse, are all vital however. In addition, our current approach is to give
5 a minimum of 6 months' therapy before considering withdrawal, given that this data showed a trend
6 towards a higher relapse risk following shorter durations of initial therapy.
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12 Whilst the efficacy of eculizumab in aHUS is clear, many questions still remain to be answered with
13 definitive prospective data, including the feasibility of dose tapering and stopping, how best to
14 monitor disease activity, predictors of response, and whether therapy could be targeted to those
15 who benefit most. Developing diagnostics to accurately differentiate aHUS from other TMAs is also
16 key to ensure that eculizumab is used appropriately and in a timely fashion, to ensure maximum
17 therapeutic benefit.
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26 **Author contributions:**

27 LN collected and analysed the data and wrote the manuscript. DG reviewed and wrote the
28 manuscript. SC and RS assisted with data collection and reviewed the manuscript. MS reviewed and
29 wrote the manuscript.
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43 **Disclosure and competing interests statement:**

44 LN and RS have no competing interests to declare.
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47 DPG has received honoraria from Alexion. SC has received speaker's fees from Alexion. MS has
48 received honoraria and speaker's fees from Alexion.
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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., Burtsey, 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.

- 1
2
3 Gediz, F., Payzin, B.K., Ecemis, S., Guler, N., Yilmaz, A.F., Topcugil, F. & Berdeli, A. (2016) Efficacy and
4 safety of eculizumab in adult patients with atypical hemolytic uremic syndrome: A single
5 center experience from Turkey. *Transfus Apher Sci*, **55**, 357-362.
- 6 Greenbaum, L.A., Fila, M., Ardissino, G., Al-Akash, S.I., Evans, J., Henning, P., Lieberman, K.V.,
7 Maringhini, S., Pape, L., Rees, L., van de Kar, N.C., Vande Walle, J., Ogawa, M., Bedrosian, C.L.
8 & Licht, C. (2016) Eculizumab is a safe and effective treatment in pediatric patients with
9 atypical hemolytic uremic syndrome. *Kidney Int*, **89**, 701-711.
- 10 Hofer, J., Giner, T. & Jozsi, M. (2014) Complement factor H-antibody-associated hemolytic uremic
11 syndrome: pathogenesis, clinical presentation, and treatment. *Semin Thromb Hemost*, **40**,
12 431-443.
- 13 Jamme, M., Raimbourg, Q., Chauveau, D., Seguin, A., Presne, C., Perez, P., Gobert, P., Wynckel, A.,
14 Provot, F., Delmas, Y., Mousson, C., Servais, A., Vrigneaud, L., Veyradier, A., Rondeau, E. &
15 Coppo, P. (2017) Predictive features of chronic kidney disease in atypical haemolytic uremic
16 syndrome. *PLoS One*, **12**, e0177894.
- 17 Jokiranta, T.S. (2017) HUS and atypical HUS. *Blood*, **129**, 2847-2856.
- 18 Kokame, K., Nobe, Y., Kokubo, Y., Okayama, A. & Miyata, T. (2005) FRETS-VWF73, a first fluorogenic
19 substrate for ADAMTS13 assay. *Br J Haematol*, **129**, 93-100.
- 20 Krishnappa, V., Gupta, M., Elrifai, M., Moftakhar, B., Ensley, M.J., Vachharajani, T.J., Sethi, S.K. &
21 Raina, R. (2018) Atypical Hemolytic Uremic Syndrome: A Meta-Analysis of Case Reports
22 Confirms the Prevalence of Genetic Mutations and the Shift of Treatment Regimens. *Ther*
23 *Apher Dial*, **22**, 178-188.
- 24 Legendre, C.M., Licht, C., Muus, P., Greenbaum, L.A., Babu, S., Bedrosian, C., Bingham, C., Cohen
25 , D.J., Delmas, Y., Douglas, K., Eitner, F., Feldkamp, T., Fouque, D., Furman, R.R., Gaber,
26 O., Herthelius, M., Hourmant, M., Karpman, D., Lebranchu, Y., Mariat, C., Menne, J.,
27 Moulin, B., Nürnberger, J., Ogawa, M., Remuzzi, G., Richard, T., Sberro-Soussan, R.,
28 Severino, B., Sheerin, N.S., Trivelli, A., Zimmerhackl, L.B., Goodship, T. & Loirat, C. (2013)
29 Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic-Uremic Syndrome. *New*
30 *England Journal of Medicine*, **368**, 2169-2181.
- 31 Lemaire, M., Fremeaux-Bacchi, V., Schaefer, F., Choi, M., Tang, W.H., Le Quintrec, M., Fakhouri, F.,
32 Taque, S., Nobili, F., Martinez, F., Ji, W., Overton, J.D., Mane, S.M., Nurnberg, G., Altmuller,
33 J., Thiele, H., Morin, D., Deschenes, G., Baudouin, V., Llanas, B., Collard, L., Majid, M.A.,
34 Simkova, E., Nurnberg, P., Rioux-Leclerc, N., Moeckel, G.W., Gubler, M.C., Hwa, J., Loirat, C.
35 & Lifton, R.P. (2013) Recessive mutations in DGKE cause atypical hemolytic-uremic
36 syndrome. *Nat Genet*, **45**, 531-536.
- 37 Licht, C., Greenbaum, L.A., Muus, P., Babu, S., Bedrosian, C.L., Cohen, D.J., Delmas, Y., Douglas, K.,
38 Furman, R.R., Gaber, O.A., Goodship, T., Herthelius, M., Hourmant, M., Legendre, C.M.,
39 Remuzzi, G., Sheerin, N., Trivelli, A. & Loirat, C. (2015a) Efficacy and safety of eculizumab in
40 atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int*,
41 **87**, 1061-1073.
- 42 Licht, C., Ardissino, G., Ariceta, G., Cohen, D., Cole, J.A., Gasteyger, C., Greenbaum, L.A., Johnson, S.,
43 Ogawa, M., Schaefer, F., Vande Walle, J. & Fremeaux-Bacchi, V. (2015b) The global aHUS
44 registry: methodology and initial patient characteristics. *BMC Nephrol*, **16**, 207.
- 45 Macia, M., de Alvaro Moreno, F., Dutt, T., Fehrman, I., Hadaya, K., Gasteyger, C. & Heyne, N. (2017)
46 Current evidence on the discontinuation of eculizumab in patients with atypical haemolytic
47 uraemic syndrome. *Clin Kidney J*, **10**, 310-319.
- 48 Mallett, A., Hughes, P., Szer, J., Tuckfield, A., Van Eps, C., Cambell, S.B., Hawley, C., Burke, J.,
49 Kausman, J., Hewitt, I., Parnham, A., Ford, S. & Isbel, N. (2015) Atypical haemolytic uraemic
50 syndrome treated with the complement inhibitor eculizumab: the experience of the
51 Australian compassionate access cohort. *Intern Med J*, **45**, 1054-1065.
- 52 Merrill, S.A., Brittingham, Z.D., Yuan, X., Moliterno, A.R., Sperati, C.J. & Brodsky, R.A. (2017)
53 Eculizumab cessation in atypical hemolytic uremic syndrome. *Blood*, **130**, 368-372.
- 54
55
56
57
58
59
60

- 1
2
3 Noris, M. & Remuzzi, G. (2009) Atypical hemolytic-uremic syndrome. *N Engl J Med*, **361**, 1676-1687.
- 4 Noris, M. & Remuzzi, G. (2014) Cardiovascular complications in atypical haemolytic uraemic
5 syndrome. *Nature Reviews Nephrology*, **10**, 174.
- 6 Noris, M., Caprioli, J., Bresin, E., Mossali, C., Pianetti, G., Gamba, S., Daina, E., Fenili, C., Castelletti, F.,
7 Sorosina, A., Piras, R., Donadelli, R., Maranta, R., van der Meer, I., Conway, E.M., Zipfel, P.F.,
8 Goodship, T.H. & Remuzzi, G. (2010) Relative role of genetic complement abnormalities in
9 sporadic and familial aHUS and their impact on clinical phenotype. *Clin J Am Soc Nephrol*, **5**,
10 1844-1859.
- 11
12 Noris, M., Mele, C. & Remuzzi, G. (2015) Podocyte dysfunction in atypical haemolytic uraemic
13 syndrome. *Nature Reviews Nephrology*, **11**, 245.
- 14 Phillips, E.H., Westwood, J.P., Brocklebank, V., Wong, E.K., Tellez, J.O., Marchbank, K.J., McGuckin, S.,
15 Gale, D.P., Connolly, J., Goodship, T.H., Kavanagh, D. & Scully, M.A. (2016) The role of
16 ADAMTS-13 activity and complement mutational analysis in differentiating acute thrombotic
17 microangiopathies. *J Thromb Haemost*, **14**, 175-185.
- 18
19 Schaefer, F., Ardissino, G., Ariceta, G., Fakhouri, F., Scully, M., Isbel, N., Lommele, A., Kupelian, V.,
20 Gasteyger, C., Greenbaum, L.A., Johnson, S., Ogawa, M., Licht, C., Vande Walle, J. &
21 Fremeaux-Bacchi, V. (2018) Clinical and genetic predictors of atypical hemolytic uremic
22 syndrome phenotype and outcome. *Kidney Int*, **94**, 408-418.
- 23
24 Scully, M., Cataland, S., Coppo, P., de la Rubia, J., Friedman, K.D., Kremer Hovinga, J., Lammler, B.,
25 Matsumoto, M., Pavenski, K., Sadler, E., Sarode, R. & Wu, H. (2017) Consensus on the
26 standardization of terminology in thrombotic thrombocytopenic purpura and related
27 thrombotic microangiopathies. *J Thromb Haemost*, **15**, 312-322.
- 28
29 Sheerin, N.S., Kavanagh, D., Goodship, T.H. & Johnson, S. (2016) A national specialized service in
30 England for atypical haemolytic uraemic syndrome-the first year's experience. *Qjm*, **109**, 27-
31 33.
- 32
33 Walle, J.V., Delmas, Y., Ardissino, G., Wang, J., Kincaid, J.F. & Haller, H. (2017) Improved renal
34 recovery in patients with atypical hemolytic uremic syndrome following rapid initiation of
35 eculizumab treatment. *J Nephrol*, **30**, 127-134.
- 36
37 Wijnsma, K.L., Duineveld, C., Volokhina, E.B., van den Heuvel, L.P., van de Kar, N. & Wetzels, J.F.M.
38 (2017) Safety and effectiveness of restrictive eculizumab treatment in atypical haemolytic
39 uremic syndrome. *Nephrol Dial Transplant*, **33**, 635-645.
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Table I: Key clinical features of each of the 22 patients.

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15	16	17	18	19	20	21	22	23	24	25	26	27	28
29	30	31	32	33	34	35	36	37	38	39	40	41	42
43	44	45	46	47	48	49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80	81	82	83	84
85	86	87	88	89	90	91	92	93	94	95	96	97	98
99	100	101	102	103	104	105	106	107	108	109	110	111	112
15	51, M	No	VUCS <i>CFH</i> (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	
16	41, F	No	No	7	10	3 days	No	8 (7)	No	eGFR >60	Withdrawn	Ongoing remission (130 weeks)	
17	40, F	No	VUCS <i>CD46</i> (c.389+5G>A)	32	12	2 days	Yes	22 (14)	No	eGFR >60	Withdrawn	Ongoing remission (58 weeks)	
18	35, F	No	No	54	8	1 day	Yes	26 (16)	No	eGFR >60	Withdrawn	Ongoing remission (59 weeks)	
19	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	
20	42, M	Yes: previous 'relapsing TTP'	Pathogenic mutation in <i>CD46</i> (c.175 C>T p.(Arg59*))	13	49	No	No	4 (5)	No	eGFR >60	Withdrawn	Ongoing remission (85 weeks)	
21	23, F	No	No	62	14	2 days	No	12 (9)	No	eGFR >60	Withdrawn	Ongoing remission (37 weeks)	
22	66, F	No	No	14	13	3 days	Yes	16 (11)	No	eGFR 50-60	Withdrawn	Ongoing remission with stable eGFR (238 weeks)	

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3			<i>CFI</i> likely pathogenic variant (c.1246A>C p.(Ile416Leu))									
4												
5												
6												
7	33, M	No	No	85	11	No	No	116 weeks	No	eGFR 30-40	Ongoing	n/a
8												
9	50, M	No	No	22	8	Ongoing at nearly 8 weeks*	No	7 weeks (6 doses)*	No	Requiring RRT*	Ongoing*	n/a
10												Subsequently died, circumstances unknown
11												
12	31, F	No	No	41	8	No	Yes	134	2 possible subacute episodes (mild isolated thrombocytopenia)	eGFR 30-40	Ongoing	n/a
13												
14												
15												
16												
17	33, F	No	No	23	6	16 days	No	136	1 possible mild relapse	eGFR 50-60	Ongoing	n/a
18												
19	67, F	No (but 'HELLP' in pregnancy)	Pathogenic mutation in <i>CFI</i> (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
20												
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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.

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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, $\times 10^9/\text{l}$	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.

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3 Figure legends
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5 Figure 1: Duration of eculizumab (black bars) or remission following eculizumab withdrawal (white
6 bars) in weeks for each patient (1-22).
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9 First course of eculizumab was ongoing at last follow-up in Patients 1, 15-21. Patient 22 died 4 days
10 following first eculizumab dose. Eculizumab was withdrawn in first remission in Patients 2-14.
11 Patients 2-11 remained in remission at last follow-up. Patients 12-14 relapsed post withdrawal and
12 restarted eculizumab. Durations of therapy including number of doses are also given in Table I.
13

14 Figure 2: Outcomes of all 22 patients
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16 CM-HUS: complement-mediated haemolytic uraemic syndrome; eGFR: estimate glomerular filtration
17 rate (ml/min/1.73m²); PEX: plasma exchange; RRT: renal replacement therapy.
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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 Neave¹, Daniel P Gale², Simon Cheesman³, Raakhee Shah³, Marie Scully⁴

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

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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/1.73m²). At median duration of ~~follow~~ follow-up of 85 weeks (range 4-255), 54.5% had eGFR ≥60 ml/min/1.73m², 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

1
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3 result of an environmental trigger in a genetically susceptible individual is regarded as the key
4 abnormality, leading to endothelial and platelet activation, and consequently TMA (Jokiranta 2017).
5 Pathogenic mutations in genes encoding complement factors H, B, I and 3 (*CFH*, *CFB*, *CFI*, *C3*), and
6 membrane cofactor protein (*CD46*), are demonstrated in 40-60% of affected individuals (Fremeaux-
7 Bacchi, *et al* 2013, Noris and Remuzzi 2009, Schaefer, *et al* 2018). Factor H autoantibodies (Dragon-
8 Durey, *et al* 2005, Hofer, *et al* 2014) and mutations in *DGKE* (encoding diacylglycerol kinase ϵ)
9 (Lemaire, *et al* 2013) are rare causes usually seen in children.

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

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

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

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3 Withdrawal of eculizumab was considered in all patients who achieved complete haematological
4 response (~~platelets-platelet count~~ $>150 \times 10^9/l$ and normal ~~lactate dehydrogenase (LDH)~~] and
5 complete or near-complete renal recovery [~~estimated glomerular filtration rate (eGFR)~~ back to
6 baseline without significant proteinuria~~)-].~~]. The decision to stop ~~eculizumab~~ was made based on
7 consensus clinician opinion and patient preference, after a discussion of risks, benefits and available
8 evidence. All patients who were offered the option to stop treatment elected to do so. Monitoring
9 for relapse after withdrawal included symptoms review, ~~full blood and reticulocyte counts, lactate~~
10 ~~dehydrogenaseLDH~~, serum creatinine/eGFR and urinalysis/~~U-PCR~~, initially fortnightly, but then at
11 increasing time intervals and ultimately 6-monthly. All patients had access to a ~~24-24-hour~~ telephone
12 helpline in the event of concerning symptoms in the interim.

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

30 Results

35 Summary of patients treated

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39 Of the 22 patients identified, 68% were female. Median age at presentation was 32 years (range 16-
40 67). All presented acutely with TMA~~-~~; 21/22 were *de novo* presentations, whilst one patient had 4
41 previous episodes of 'relapsing TTP' but ADAMTS13 analysis performed for the first time on the
42 index admission excluded TTP and led to a diagnosis of aHUS. All had native kidneys, and none were
43 known to have chronic kidney disease (CKD) prior to presentation. Median duration of admission at
44 our institution was 14 days (range 6-55), though 5 patients were discharged to local renal units for
45 ongoing dialysis and 1 was repatriated to his local hospital. Median duration of ~~follow-follow-up~~
46 from initiation of eculizumab was 85 weeks (range 4-255), excluding a patient who died on day 5. 5
47 ~~Five~~ patients had ongoing outpatient management transferred to other centres (at 3, 7, 9, 37 and
48 124 weeks ~~post-post~~-initiation of eculizumab, respectively) but data inclusive to those timepoints
49 was included in the analysis. Key clinical information for all 22 patients is summarised in Table I.

Presenting features of aHUS

~~G~~Gastrointestinal 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 23×10^9 ~~/L~~ (range 7-85 $\times 10^9$ ~~/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 ~~umol~~umol ~~/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/1.73m² (range 3-57), and 64% of patients (N=14) required ~~renal replacement therapy~~RRT during the acute episode. ~~14/22 (64%) required Admission 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~~, 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~~); 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

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3 persisted despite resolution of the infection). Patient 10 initially presented with gallstone
4 cholecystitis 5 months postpartum, but a frank TMA picture quickly evolved (along with post-ERCP
5 pancreatitis) and the suspicion was that the gallstones resulted from a low grade postpartum aHUS,
6 as she had been noted to be hypertensive and proteinuric peripartum, with intermittent abdominal
7 pain, malaise and nausea ever since.
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14 ADAMTS13 activity on presentation was within the normal range for 86.3% (n=19) patients, and
15 slightly low in 13.7% (n=3). Median activity was 72 iu/dl (range 56-91, normal range 60-146).
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18 19 20 21 **Response to plasma exchange**

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25 All 22 patients commenced ~~plasma exchange (PEX)~~ from at admission, with a median PEX duration
26 of 5 days (range 2-24). Platelet normalization ($>150 \times 10^9/L$) was achieved in 5/22 (range of PEX
27 duration 5-18 days), with improvement in platelet count without normalization in 10/22 (range of
28 PEX duration 2-24 days). Renal recovery to eGFR >90 ml/min/1.73m² was seen in 1 patient during
29 PEX (Patient 9, PEX duration 24 days), but with incomplete platelet response. ~~4~~Four of 22 patients
30 came off RRT during PEX but 7/22 remained RRT-dependent, while 3/22 required initiation of RRT.
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39 **Response to eculizumab**

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43 The median time from admission to initiation of eculizumab was 6 days (range 2-38 (with delays in
44 the latter case due to funding issues due to non-UK nationality)). Figure 1 illustrates the duration
45 of therapy in all cases.
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52 In terms of haematological response, all patients who were still thrombocytopenic at initiation
53 (N=17) achieved sustained platelet counts $\geq 150 \times 10^9/L$, after a median of 5 days (range 2-15). LDH
54 normalised after median 22 days (range 3-74) for 14/16 patients for whom data was available, while
55 1 patient (patient 4) has persistently elevated LDH (but no other features of persistent TMA), and 1
56 patient already had a normal LDH at eculizumab initiation. Haemoglobin normalization occurred
57 after median 43 days (range 11-211) in 17/18 patients for whom data was available, while 1 patient
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3 remains anaemic after 90 weeks of treatment, attributed to RRT-dependency and iron deficiency.

4 ~~20/Twenty of~~ 22 (86%) patients maintained a normal platelet count for the duration of therapy.

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6 Patient 20 had two episodes of mild thrombocytopenia on therapy, with no other evidence of TMA,
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8 which resolved without any change to the eculizumab regime. Patient 22 developed a mild
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10 thrombocytopenia with elevated LDH (but stable renal function) 8 months into therapy in the
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12 context of a urinary tract infection with systemic features. Eculizumab was given 2 days early and all
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14 parameters normalised within 3 days.

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18 In terms of renal response, renal function was maintained in the one patient who had normal renal
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20 function at eculizumab initiation (~~Patient Patient~~ 9). Of the 21 patients who had abnormal renal
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22 function at initiation of eculizumab, none showed renal deterioration on eculizumab and 17/21
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24 (81%) showed improvement in eGFR (median increase in eGFR 49 ml/min/1.73m²; (range 22->80)).
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26 The time for the creatinine to reach a new baseline generally depended on the extent of renal
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28 impairment, ranging from 14 days to as long as 17 months.

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31 ~~12/Twelve of~~ 21 patients made a complete or near-complete renal recovery (to eGFR ≥60
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33 ml/min/1.73m² in 11/12 and eGFR 55 ml/min/1.73m² in Patient 8 who was 66 years old; resolution
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35 of proteinuria in 7/12), after a median of 23.5 days of eculizumab (range 14-51). 7 of those 12
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37 patients (58%) had required RRT at presentation (duration 1-3 days in 6/7, but 68 days in Patient 11).

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41 ~~4/Four of~~ 21 patients had residual CKD with eGFR 25-60 ml/min/1.73m² after eculizumab duration
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43 119-232 weeks, ~~2 of these 4 patients~~ two of whom (50%) had required RRT at initiation (duration
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45 21 days and 6 months). ~~Three of 21 patients~~ 3/21 remained on RRT and eculizumab at last follow-
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47 up, after 3, 7 and 85 weeks of eculizumab. One further patient (Patient 17) stopped RRT after 7
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49 weeks but, due to transfer of care (while still on eculizumab), renal outcomes are unknown.

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52 The final patient died during the acute admission (Patient 22). She required intubation and
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54 ventilation at presentation, for reduced consciousness and agitation, but had been extubated and
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56 was clinically improving, though still on RRT, when she suffered an unexpected cardiac arrest on day
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58 5 of eculizumab. Whilst Patient 19 is also known to have died, following transfer to another
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3 institution after 7 weeks of eculizumab and RRT, -the timing and circumstances of the death are
4 unknown, and therefore cannot be reliably attributed to aHUS.
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9 **Predictors of renal response**

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14 Whilst recognizing the limitations of a retrospective cohort analysis, it is noticeable that patients
15 with a final eGFR <60 ml/min/1.73m² after treatment, had significantly higher presenting creatinine
16 levels than those who recovered eGFR to ≥60 ml/min/1.73m² ~~(median 520 umol/L (range 236-~~
17 ~~1153) vs median 219.5 umol/L (range 80-402), p = 0.026 (Mann-Whitney test, U= 12.5)).~~ There
18 was no significant correlation between renal outcome and ~~peak urine protein:creatinine ratio (UPCR),~~
19 nadir platelet count, nadir Hb, peak LDH, or time to eculizumab, ~~as shown in~~ (Table II).
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28 **Complement abnormalities**

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32 Complement genetic abnormalities were identified in 40.9 % (9/22) of patients, involving *CD46*
33 (N=4), *CFH* (N=3), *CFI* (N=2), *C3* (N=1) and *CFB* (N=1). ~~Two~~ patients (9%) had abnormalities in 2
34 genes. ~~Details are given in~~ (Table I).
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40 Table III compares key clinical features of patients with normal and abnormal genetic screening.
41 There was little obvious difference in severity of presentation or renal outcomes, though numbers
42 are small and, for two cases in the abnormal genetic screening group, and one in the normal group,
43 care was transferred to other institutions before ultimate renal outcomes were known.
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50 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))~~,
51 and ~~this remained permanently or intermittently low despite clinical remission on eculizumab in~~
52 ~~4/9 cases, suggesting poor correlation with disease activity. Low C3 was not a predictor of mutation~~
53 ~~status: 3/9 with low presenting C3 were subsequently found to have complement genetic~~
54 ~~abnormalities compared to 6/13 with normal C3.~~
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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 ~~follow~~ 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).

~~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 ($139 \times 10^9/\text{L}$) on routine ~~follow~~ 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 ~~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 $<30 \times 10^9/\text{L}$, LDH $>1000 \text{ -iu}/\text{L}$ and creatinine $200\text{-}300\text{umol}/\text{L}$, -after defined triggers (a viral infection 3 months postpartum, and flu A,

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3 respectively). Re-initiation of eculizumab in both cases on day 1 led to rapid full recovery, without
4 need for PEX or RRT, and discharge home after 7 and 10 days, respectively. Eculizumab was
5 subsequently stopped again in both cases (after 2 doses in Patient 13 and 6 months in Patient 14),
6 and they remain in remission 17 and 14 months later, respectively.
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13 In terms of predictors of relapse after stopping, there was a significantly higher risk of relapse in
14 those with a complement genetic abnormality, than those without (3 of out 5 with mutations
15 relapsed versus 0 out of 8 without, $p=0.035$ (Fisher exact test)). The genetic abnormalities in the 3
16 patients who relapsed are detailed in Table I, but included abnormalities in *C3* and *CFH* in Patient 12;
17 in *CFB* in Patient 13; and in *CD46* in Patient 14. In addition, the duration of initial treatment in those
18 who relapsed tended to be shorter than in those who did not (median 2 weeks (range 1-10) vs 14
19 weeks (4-26). *C3* levels were not predictive of relapse as the three patients who did relapse had
20 consistently normal *C3* levels.
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29 **Adverse events**

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

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48 The outcomes of all patients are summarised in Figure 2
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55 **Discussion**

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3 Whilst the retrospective nature of this cohort is a limitation, its size is comparable to the original
4 prospective phase 2 trials, and 'real world' outcome data in this ultra-rare disease is scarce, so the
5 findings are of value.
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11 The presenting features of the cohort reiterate some important characteristics of aHUS: end organ
12 involvement is not necessarily confined to the kidneys (Cataland and Wu 2014, Noris and Remuzzi
13 2009); neurological and gastrointestinal symptoms are common (Jamme, *et al* 2017, Schaefer, *et al*
14 2018); and whilst severe thrombocytopenia and mild renal impairment are more common in TTP
15 (Cataland, *et al* 2012, Coppo, *et al* 2010), they do not exclude aHUS (Phillips, *et al* 2016) (nadir
16 platelet count was $<30 \times 10^9/L$ in 59% of patients, and peak creatinine was $<200 \mu\text{mol/L}$ in 14%). It
17 is possible that our cohort is skewed to the less severe end of the renal spectrum renally, as cases
18 presenting with severe renal impairment are often referred direct to nephrology, but in fact the
19 proportion requiring RRT (64%) in the acute phase is similar to larger cohorts (Fakhouri, *et al* 2016,
20 Sheerin, *et al* 2016). The 41% prevalence of complement genetic abnormalities is also in keeping
21 with the existing literature, as is the finding of pregnancy as a common trigger (Fakhouri, *et al* 2010).
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33 In terms of novel findings, nearly half of patients had nephrotic range proteinuria at presentation
34 despite this not classically being associated with aHUS, and previous reports tending to be in
35 children or cases with secondary causes (Noris, *et al* 2015). Whilst cardiovascular manifestations are
36 reported (Noris and Remuzzi 2014), this is the first demonstration to our knowledge of a high
37 prevalence (77%) of asymptomatic cardiac troponin elevation, suggesting frequent subclinical
38 cardiac involvement (although renal impairment may have contributed to elevated troponin).
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46 To summarise outcomes, at a median follow-up of 85 weeks (range 4-255), following a median
47 duration of initial course of eculizumab of 11 weeks (range 1-227), 100% of patients showed
48 resolution of thrombocytopenia and 81% showed improvement in eGFR (median increase in eGFR 49
49 ml/min/1.73m^2 ; (range 22->90)). Of the 14/22 patients who initially required RRT, 10
50 became dialysis independent. At last follow-up, 54.5 % had $\text{eGFR} \geq 60 \text{ ml/min/1.73m}^2$, 27% had
51 CKD with $\text{eGFR} < 60 \text{ ml/min/1.73m}^2$ but not requiring RRT, 14% were on RRT, and 4.5% had died. It
52 should be noted that 2 of the 3 patients requiring RRT at last follow-up had their care transferred
53 to another institution after only 3 and 7 weeks' of eculizumab, after which time there may have
54 been some renal recovery.
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6 The renal outcomes compare very favourably with data from the pre-eculizumab era ([rates of ESRD
7 or death 50-77% after 3-5 years (Fremeaux-Bacchi, *et al* 2013, Noris, *et al* 2010, Schaefer, *et al*
8 2018)), (Fremeaux-Bacchi, *et al* 2013, Noris, *et al* 2010, Schaefer, *et al* 2018)], but also to previously
9 published outcomes with eculizumab. Of the three Phase 2 trials in adults (Fakhouri, *et al* 2016,
10 Legendre, *et al* 2013, Licht, *et al* 2015b5a), our cohort is most comparable to the 41 patients ~~in~~
11 reported by the third (Fakhouri, *et al* (2016), who were not required to be plasma dependent or
12 refractory and who generally received eculizumab early in the acute phase (although the proportion
13 of relapses and renal transplants was higher in the Phase 2 cohort). ~~38/Thirty-eight of~~ 41 patients
14 received the intended 26 weeks' of treatment, by which time point 98% achieved platelet
15 normalisation. ~~54%~~ Fifty-four percent showed an increase in eGFR of >15 ml/min/1.73m² by 26
16 weeks, and 15% were ~~dialysis-dialysis~~-dependent at 26 weeks, from 58% at baseline and 46% at
17 initiation of eculizumab.

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29 The renal outcomes of our cohort are also comparable to published retrospective cohorts
30 (Cunningham, *et al* 2017, Fakhouri, *et al* 2014, Gediz, *et al* 2016, Mallett, *et al* 2015, Sheerin, *et al*
31 2016). In a French series of 19 adult patients (Fakhouri, *et al* 2016), 63% required RRT at diagnosis,
32 while at last follow-up (range 4-22 months, treatment ongoing in 74%), 16% required RRT, 37%
33 had CKD₂, and 47% had normal renal function. In a US cohort (N=52), 35% required dialysis prior to
34 eculizumab, and 21% at 3 months (Cunningham, *et al* 2017). Of 23 incident patients in an analysis of
35 the first year of the national specialised service in England (Sheerin, *et al* 2016), 15 (65%) required
36 dialysis at eculizumab initiation, of whom 8 were able to stop dialysis after a duration of 1-30 weeks.

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

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56 If and when to withdraw eculizumab is an important and debated question, which has not yet been
57 addressed prospectively as treatment was continued for the duration of the Phase 2 trials in the
58 majority of cases. The 23% relapse rate post-post-withdrawal seen in this cohort is in keeping with
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3 20-31% relapse rates reported in the largest four case series of patients who stopped in stable
4 remission (a total of 86 cases) (Ardissino, *et al* 2015~~2014~~, Ardissino, *et al* 2015~~4~~, Fakhouri, *et al* 2017,
5 Merrill, *et al* 2017, Wijnsma, *et al* 2017). The time frame of relapse within 1 year is also comparable.
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7 There was a suggestion from two cohorts (Ardissino, *et al* 2015, Fakhouri, *et al* 2017), as in ours, that
8 those with a mutation, especially CFH mutations, were more likely to relapse, ~~especially CFH~~
9 mutations.

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16 Whilst relapses did occur, all three patients who relapsed made rapid and complete recoveries with
17 re-initiation of eculizumab, without needing PEX or RRT. The withdrawal strategy therefore led to no
18 ~~long-long~~ term adverse effects for these patients, nor on outcomes of the cohort as a whole, given
19 that the overall outcomes were comparable to those of the Phase 2 trial (Fakhouri, *et al* 2016),
20 despite 59% of our cohort not receiving indefinite treatment.
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28 Whilst the vast majority of published cases of relapse ~~post-post~~-eculizumab withdrawal made a full
29 recovery after early re-initiation of eculizumab (Ardissino, *et al* 2015, Ardissino, *et al* 2014, Fakhouri,
30 *et al* 2017, Merrill, *et al* 2017, Wijnsma, *et al* 2017) a recent review (Macia, *et al* 2017) cites two
31 cases in which re-initiation of eculizumab did not prevent deterioration to ESRF end stage renal
32 failure (though timing to re-initiation is not given for one case, and the other case involves a patient
33 who only received one dose initially). The potential benefits however are undeniable: just over 750
34 doses of eculizumab have been avoided in this cohort to date, with associated reduced risk of
35 adverse effects (including meningococcal infection), reduced hospital attendances and service
36 delivery burdens, and drug cost savings of over £11 million. Whilst a randomised controlled trial is
37 needed to definitively assess the safety of eculizumab withdrawal, in the absence of such data this
38 cohort adds to the growing body of evidence in support of such a strategy. Monitoring ~~post-post-~~
39 withdrawal, patient education regarding potential symptoms of relapse, and pathways to ensure
40 timely re-initiation of eculizumab in the event of relapse, are all vital however. In addition, our
41 current approach is to give a minimum of 6 months' therapy before considering withdrawal, given
42 that this data showed a trend towards a higher relapse risk following shorter durations of initial
43 therapy.
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57 Whilst the efficacy of eculizumab in aHUS is clear, many questions still remain to be answered with
58 definitive prospective data, including the feasibility of dose tapering and stopping, how best to
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3 monitor disease activity, predictors of response, and whether therapy could be targeted to those
4 who benefit most. Developing diagnostics to accurately differentiate aHUS from other TMAs is also
5 key to ensure that eculizumab is used appropriately and in a timely fashion, to ensure maximum
6 therapeutic benefit.
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13 **Author contributions:**
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16 LN collected and analysed the data and wrote the manuscript. DG reviewed and wrote the
17 manuscript. SC and RS assisted with data collection and reviewed the manuscript. MS reviewed and
18 wrote the manuscript.
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34 **Disclosure and competing interests statement:**
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38 LN and RS have no completing interests to declare.
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40 DPG has received honoraria from Alexion. SC has received speaker's fees from Alexion. MS has
41 received honoraria and speaker's fees from Alexion.
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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., Ruggenti, 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.](#)

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3 Fakhouri, F., Fila, M., Provot, F., Delmas, Y., Barbet, C., Chatelet, V., Rafat, C., Cailliez, M., Hogan, J.,
4 Servais, A., Karras, A., Makdassi, R., Louillet, F., Coindre, J.P., Rondeau, E., Loirat, C. &
5 Fremeaux-Bacchi, V. (2017) Pathogenic Variants in Complement Genes and Risk of Atypical
6 Hemolytic Uremic Syndrome Relapse after Eculizumab Discontinuation. *Clin J Am Soc*
7 *Nephrol*, **12**, 50-59.
- 8
9 ~~Fakhouri, F., Hourmant, M., Campistol, J.M., Cataland, S.R., Espinosa, M., Gaber, A.O., Menne, J.,
10 Minetti, E.E., Provot, F., Rondeau, E., Ruggenenti, P., Weekers, L.E., Ogawa, M., Bedrosian,
11 C.L. & Legendre, C.M. (2016) Terminal Complement Inhibitor Eculizumab in Adult Patients
12 With Atypical Hemolytic Uremic Syndrome: A Single-Arm, Open-Label Trial. *Am J Kidney Dis*,
13 **68**, 84-93.~~
- 14 ~~Fakhouri, F., Roumenina, L., Provot, F., Sallee, M., Caillard, S., Couzi, L., Essig, M., Ribes, D., Dragon-
15 Durey, M.A., Bridoux, F., Rondeau, E. & Fremeaux-Bacchi, V. (2010) Pregnancy-associated
16 hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc*
17 *Nephrol*, **21**, 859-867.~~
- 18
19 Fremeaux-Bacchi, V., Fakhouri, F., Garnier, A., Bienaime, F., Dragon-Durey, M.A., Ngo, S., Moulin, B.,
20 Servais, A., Provot, F., Rostaing, L., Burtey, S., Niaudet, P., Deschenes, G., Lebranchu, Y.,
21 Zuber, J. & Loirat, C. (2013) Genetics and outcome of atypical hemolytic uremic syndrome: a
22 nationwide French series comparing children and adults. *Clin J Am Soc Nephrol*, **8**, 554-562.
- 23 Gediz, F., Payzin, B.K., Ecemis, S., Guler, N., Yilmaz, A.F., Topcugil, F. & Berdeli, A. (2016) Efficacy and
24 safety of eculizumab in adult patients with atypical hemolytic uremic syndrome: A single
25 center experience from Turkey. *Transfus Apher Sci*, **55**, 357-362.
- 26
27 Greenbaum, L.A., Fila, M., Ardissino, G., Al-Akash, S.I., Evans, J., Henning, P., Lieberman, K.V.,
28 Maringhini, S., Pape, L., Rees, L., van de Kar, N.C., Vande Walle, J., Ogawa, M., Bedrosian, C.L.
29 & Licht, C. (2016) Eculizumab is a safe and effective treatment in pediatric patients with
30 atypical hemolytic uremic syndrome. *Kidney Int*, **89**, 701-711.
- 31 Hofer, J., Giner, T. & Jozsi, M. (2014) Complement factor H-antibody-associated hemolytic uremic
32 syndrome: pathogenesis, clinical presentation, and treatment. *Semin Thromb Hemost*, **40**,
33 431-443.
- 34
35 Jamme, M., Raimbourg, Q., Chauveau, D., Seguin, A., Presne, C., Perez, P., Gobert, P., Wynckel, A.,
36 Provot, F., Delmas, Y., Mousson, C., Servais, A., Vrigneaud, L., Veyradier, A., Rondeau, E. &
37 Coppo, P. (2017) Predictive features of chronic kidney disease in atypical haemolytic uremic
38 syndrome. *PLoS One*, **12**, e0177894.
- 39 Jokiranta, T.S. (2017) HUS and atypical HUS. *Blood*, **129**, 2847-2856.
- 40 Kokame, K., Nobe, Y., Kokubo, Y., Okayama, A. & Miyata, T. (2005) FRETS-VWF73, a first fluorogenic
41 substrate for ADAMTS13 assay. *Br J Haematol*, **129**, 93-100.
- 42
43 Krishnappa, V., Gupta, M., Elrifai, M., Moftakhar, B., Ensley, M.J., Vachharajani, T.J., Sethi, S.K. &
44 Raina, R. (2018) Atypical Hemolytic Uremic Syndrome: A Meta-Analysis of Case Reports
45 Confirms the Prevalence of Genetic Mutations and the Shift of Treatment Regimens. *Ther*
46 *Apher Dial*, **22**, 178-188.
- 47 Legendre, C.M., Licht, C., Muus, P., Greenbaum, L.A., Babu, S., Bedrosian, C., Bingham, C., Cohen,
48 D.J., Delmas, Y., Douglas, K., Eitner, F., Feldkamp, T., Fouque, D., Furman, R.R., Gaber, O.,
49 Herthelius, M., Hourmant, M., Karpman, D., Lebranchu, Y., Mariat, C., Menne, J.,
50 Moulin, B., Nürnberg, J., Ogawa, M., Remuzzi, G., Richard, T., Sberro-Soussan, R.,
51 Severino, B., Sheerin, N.S., Trivelli, A., Zimmerhackl, L.B., Goodship, T. & Loirat, C. (2013)
52 Terminal Complement Inhibitor Eculizumab in Atypical Hemolytic-Uremic Syndrome. *New*
53 *England Journal of Medicine*, **368**, 2169-2181.
- 54
55 Lemaire, M., Fremeaux-Bacchi, V., Schaefer, F., Choi, M., Tang, W.H., Le Quintrec, M., Fakhouri, F.,
56 Taque, S., Nobili, F., Martinez, F., Ji, W., Overton, J.D., Mane, S.M., Nurnberg, G., Altmuller,
57 J., Thiele, H., Morin, D., Deschenes, G., Baudouin, V., Llanas, B., Collard, L., Majid, M.A.,
58 Simkova, E., Nurnberg, P., Rioux-Leclerc, N., Moeckel, G.W., Gubler, M.C., Hwa, J., Loirat, C.
- 59
60

- & 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. (2015a15b) 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.

- 1
2
3 Sheerin, N.S., Kavanagh, D., Goodship, T.H. & Johnson, S. (2016) A national specialized service in
4 England for atypical haemolytic uraemic syndrome-the first year's experience. *Qjm*, **109**, 27-
5 33.
6
7 Walle, J.V., Delmas, Y., Ardissino, G., Wang, J., Kincaid, J.F. & Haller, H. (2017) Improved renal
8 recovery in patients with atypical hemolytic uremic syndrome following rapid initiation of
9 eculizumab treatment. *J Nephrol*, **30**, 127-134.
10
11 Wijnsma, K.L., Duineveld, C., Volokhina, E.B., van den Heuvel, L.P., van de Kar, N. & Wetzels, J.F.M.
12 (2017) Safety and effectiveness of restrictive eculizumab treatment in atypical haemolytic
13 uremic syndrome. *Nephrol Dial Transplant*, **33**, [635-645](#).
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Table I: Key clinical features of each of the 22 patients.

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15	16	17	18	19	20	21	22	23	24	25	26	27	28
29	30	31	32	33	34	35	36	37	38	39	40	41	42
43	44	45	46	47	48	49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64	65	66	67	68	69	70
71	72	73	74	75	76	77	78	79	80	81	82	83	84
85	86	87	88	89	90	91	92	93	94	95	96	97	98
99	100	101	102	103	104	105	106	107	108	109	110	111	112
113	114	115	116	117	118	119	120	121	122	123	124	125	126
127	128	129	130	131	132	133	134	135	136	137	138	139	140
141	142	143	144	145	146	147	148	149	150	151	152	153	154
155	156	157	158	159	160	161	162	163	164	165	166	167	168
169	170	171	172	173	174	175	176	177	178	179	180	181	182
183	184	185	186	187	188	189	190	191	192	193	194	195	196
197	198	199	200	201	202	203	204	205	206	207	208	209	210
211	212	213	214	215	216	217	218	219	220	221	222	223	224
225	226	227	228	229	230	231	232	233	234	235	236	237	238
239	240	241	242	243	244	245	246	247	248	249	250	251	252
253	254	255	256	257	258	259	260	261	262	263	264	265	266
267	268	269	270	271	272	273	274	275	276	277	278	279	280
281	282	283	284	285	286	287	288	289	290	291	292	293	294
295	296	297	298	299	300	301	302	303	304	305	306	307	308
309	310	311	312	313	314	315	316	317	318	319	320	321	322
323	324	325	326	327	328	329	330	331	332	333	334	335	336
337	338	339	340	341	342	343	344	345	346	347	348	349	350
351	352	353	354	355	356	357	358	359	360	361	362	363	364
365	366	367	368	369	370	371	372	373	374	375	376	377	378
379	380	381	382	383	384	385	386	387	388	389	390	391	392
393	394	395	396	397	398	399	400	401	402	403	404	405	406
407	408	409	410	411	412	413	414	415	416	417	418	419	420
421	422	423	424	425	426	427	428	429	430	431	432	433	434
435	436	437	438	439	440	441	442	443	444	445	446	447	448
449	450	451	452	453	454	455	456	457	458	459	460	461	462
463	464	465	466	467	468	469	470	471	472	473	474	475	476
477	478	479	480	481	482	483	484	485	486	487	488	489	490
491	492	493	494	495	496	497	498	499	500	501	502	503	504
505	506	507	508	509	510	511	512	513	514	515	516	517	518
519	520	521	522	523	524	525	526	527	528	529	530	531	532
533	534	535	536	537	538	539	540	541	542	543	544	545	546
547	548	549	550	551	552	553	554	555	556	557	558	559	560
561	562	563	564	565	566	567	568	569	570	571	572	573	574
575	576	577	578	579	580	581	582	583	584	585	586	587	588
589	590	591	592	593	594	595	596	597	598	599	600	601	602
603	604	605	606	607	608	609	610	611	612	613	614	615	616
617	618	619	620	621	622	623	624	625	626	627	628	629	630
631	632	633	634	635	636	637	638	639	640	641	642	643	644
645	646	647	648	649	650	651	652	653	654	655	656	657	658
659	660	661	662	663	664	665	666	667	668	669	670	671	672
673	674	675	676	677	678	679	680	681	682	683	684	685	686
687	688	689	690	691	692	693	694	695	696	697	698	699	700
701	702	703	704	705	706	707	708	709	710	711	712	713	714
715	716	717	718	719	720	721	722	723	724	725	726	727	728
729	730	731	732	733	734	735	736	737	738	739	740	741	742
743	744	745	746	747	748	749	750	751	752	753	754	755	756
757	758	759	760	761	762	763	764	765	766	767	768	769	770
771	772	773	774	775	776	777	778	779	780	781	782	783	784
785	786	787	788	789	790	791	792	793	794	795	796	797	798
799	800	801	802	803	804	805	806	807	808	809	810	811	812
813	814	815	816	817	818	819	820	821	822	823	824	825	826
827	828	829	830	831	832	833	834	835	836	837	838	839	840
841	842	843	844	845	846	847	848	849	850	851	852	853	854
855	856	857	858	859	860	861	862	863	864	865	866	867	868
869	870	871	872	873	874	875	876	877	878	879	880	881	882
883	884	885	886	887	888	889	890	891	892	893	894	895	896
897	898	899	900	901	902	903	904	905	906	907	908	909	910
911	912	913	914	915	916	917	918	919	920	921	922	923	924
925	926	927	928	929	930	931	932	933	934	935	936	937	938
939	940	941	942	943	944	945	946	947	948	949	950	951	952
953	954	955	956	957	958	959	960	961	962	963	964	965	966
967	968	969	970	971	972	973	974	975	976	977	978	979	980
981	982	983	984	985	986	987	988	989	990	991	992	993	994
995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008
1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022
1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036
1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050
1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064
1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078
1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092
1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106
1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120
1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134
1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148
1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162
1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176
1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190
1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204
1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218
1219	1220	1221	1222	1223	1224	1225	1226	1227	1228	1229	1230	1231	1232
1233	1234	1235	1236	1237	1238	1239	1240	1241	1242	1243	1244	1245	1246
1247	1248	1249	1250	1251	1252	1253	1254	1255	1256	1257	1258	1259	1260
1261	1262	1263	1264	1265	1266	1267	1268	1269	1270				

1												
2												
3			<u>CFI likely pathogenic variant (c.1246A>C p.(Ile416Leu))</u>									
4												
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6												
7	<u>33, M</u>	<u>No</u>	<u>No</u>	<u>85</u>	<u>11</u>	<u>No</u>	<u>No</u>	<u>116 weeks</u>	<u>No</u>	<u>eGFR 30-40</u>	<u>Ongoing</u>	<u>n/a</u>
8												
9	<u>50, M</u>	<u>No</u>	<u>No</u>	<u>22</u>	<u>8</u>	<u>Ongoing at nearly 8 weeks*</u>	<u>No</u>	<u>7 weeks (6 doses)*</u>	<u>No</u>	<u>Requiring RRT*</u>	<u>Ongoing*</u>	<u>n/a</u>
10												
11												
12	<u>31, F</u>	<u>No</u>	<u>No</u>	<u>41</u>	<u>8</u>	<u>No</u>	<u>Yes</u>	<u>134</u>	<u>2 possible subacute episodes (mild isolated thrombocytopenia)</u>	<u>eGFR 30-40</u>	<u>Ongoing</u>	<u>n/a</u>
13												
14												
15												
16												
17	<u>33, F</u>	<u>No</u>	<u>No</u>	<u>23</u>	<u>6</u>	<u>16 days</u>	<u>No</u>	<u>136</u>	<u>1 possible mild relapse</u>	<u>eGFR 50-60</u>	<u>Ongoing</u>	<u>n/a</u>
18												
19	<u>67, F</u>	<u>No (but 'HELLP' in pregnancy)</u>	<u>Pathogenic mutation in CFI (c.561delG p.(Ala219fs) in exon 4)</u>	<u>42</u>	<u>7</u>	<u>Ongoing when died (day 11)</u>	<u>Yes</u>	<u>Died 4 days after 1st dose</u>	<u>n/a</u>	<u>On RRT when died</u>	<u>n/a</u>	<u>n/a</u>
20												
21												
22												

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
<u>Presenting creatinine, μmol</u>	<u>219.5 (80-402)</u>	<u>520 (236-1153)</u>	<u>U = 12.5*</u> <u>P = 0.026</u>
<u>Peak UPCR[#], mg/mmol</u>	<u>253 (69-1228)</u>	<u>145 (119->7000)</u>	<u>U= 18*</u> <u>P = 0.58</u>
<u>Nadir platelet count, x10⁹/l</u>	<u>15.5 (9-62)</u>	<u>32 (14-85)</u>	<u>U= 21.5*</u> <u>P = 0.19</u>
<u>Nadir Hb, g/l</u>	<u>72 (41-103)</u>	<u>72 (59-78)</u>	<u>U = 30.5*</u> <u>P = 0.63</u>
<u>Peak LDH, iu/l</u>	<u>1408 (803-4621)</u>	<u>2184.5 (582-3704)</u>	<u>U = 31*</u> <u>P= 0.68</u>
<u>Time to eculizumab</u>	<u>≤ 7 days: N=10</u> <u>> 7 days: N=2</u>	<u>≤ 7 days: N=3</u> <u>> 7 days: N=3</u>	<u>P = 0.27**</u>

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)
<u>C3 low at presentation</u>	<u>33% (N=3)</u>	<u>46% (N=6)</u>
<u>Nadir platelet count, x 10⁹/l; median (range)</u>	<u>20 (11-42)</u>	<u>23 (7-85)</u>
<u>RRT during acute episode</u>	<u>55.6 % (N=5)</u>	<u>69.2% (N=9)</u>
<u>Peak UPCR, mg/mmol; median (range)**</u>	<u>660 (142-1406)</u>	<u>145 (69-1576)</u>
<u>Died during FU period</u>	<u>11.1% (N=1)</u>	<u>0[#]</u>
<u>Ongoing RRT at last FU</u>	<u>22.2% (N=2)*</u>	<u>7.7% (N=1)*</u>
<u>eGFR <60 ml/min/1.73m², not requiring RRT, at last FU</u>	<u>11.1% (N=1)*</u>	<u>38.4% (N=5)</u>
<u>eGFR ≥60 ml/min/1.73m², at last FU</u>	<u>55.5% (N=5)</u>	<u>53.4% (N=7)</u>

* 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.

1
2
3 Figure legends
4

5 Figure 1: Duration of eculizumab (black bars) or remission following eculizumab withdrawal (white
6 bars) in weeks for each patient (1-22).
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9 First course of eculizumab was ongoing at last follow-up in Patients 1, 15-21. Patient 22 died 4 days
10 following first eculizumab dose. Eculizumab was withdrawn in first remission in Patients 2-14.
11 Patients 2-11 remained in remission at last follow-up. Patients 12-14 relapsed post withdrawal and
12 restarted eculizumab. Durations of therapy including number of doses are also given in Table I.
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14 Figure 2: Outcomes of all 22 patients
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16 CM-HUS: complement-mediated haemolytic uraemic syndrome; eGFR: estimate glomerular filtration
17 rate (ml/min/1.73m²); PEX: plasma exchange; RRT: renal replacement therapy.
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