



## ORIGINAL ARTICLE

# Eculizumab prevents thrombotic microangiopathy in patients with atypical haemolytic uraemic syndrome in a long-term observational study

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## ABSTRACT

**Background.** Eculizumab, a terminal complement inhibitor, is approved for atypical haemolytic uraemic syndrome (aHUS) to inhibit complement-mediated thrombotic microangiopathy (TMA).

**Methods.** In five parent studies, eculizumab effectively prevented TMA and improved renal and haematologic outcomes in patients with aHUS; therefore, these patients could enrol in this long-term, prospective, observational and multicentre study. The primary endpoint was the TMA manifestation rate off and on eculizumab post-parent study. *Post hoc* analyses evaluated rates during labelled versus non-labelled dosing regimens, and in those with versus without identified complement abnormalities. Serious targeted treatment-emergent adverse events (TEAEs) were evaluated.

**Results.** Of 87 patients in the current study, 39 and 76 had off- and on-treatment periods, respectively; 17 (44%) with off periods reinitiated eculizumab. TMA manifestation rate per 100 patient-years was 19.9 off and 7.3 on treatment [hazard ratio (HR), 4.7;  $P = 0.0008$ ]; rates were highest off treatment and lowest during labelled regimens. TMA manifestations with hospitalizations/serious AEs occurred more frequently off versus on treatment. TMA rates were higher among patients with identified complement abnormalities (HR, 4.5;  $P = 0.0082$ ). Serious targeted TEAEs occurred at similar rates off and on treatment.

**Conclusions.** As expected, patients with aHUS have increased risk of TMA manifestations after discontinuation of eculizumab or in the setting of non-labelled eculizumab dosing. Collectively, results show that maintaining eculizumab treatment minimizes risk of TMA, particularly in patients with identified complement abnormalities. Future studies are

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needed to further characterize TMA and longer term outcomes on labelled or non-labelled eculizumab regimens and after discontinuation of treatment.

**Keywords:** atypical haemolytic uraemic syndrome, complement, discontinuation, eculizumab, observational study, thrombotic microangiopathy

## INTRODUCTION

Atypical haemolytic uraemic syndrome (aHUS) is a rare, genetic, potentially life-threatening disease predominantly caused by uncontrolled activation of the alternative complement pathway [1–3]. Abnormalities in complement genes or autoantibodies to complement proteins are identified in ~50–70% of patients [2, 3]. Complement dysregulation leads to persistent cleavage of C5 to the prothrombotic, pro-inflammatory anaphylatoxin C5a and to C5b, which initiates formation of the prothrombotic and cytolytic C5b-9 and ultimately causes injury, activation and lysis of endothelial cells, leucocytes and platelets [1, 4]. The resultant thrombotic microangiopathy (TMA) is typically characterized by microangiopathic haemolytic anaemia, thrombocytopenia and acute renal failure, and frequently includes extrarenal complications [2, 3].

Patients who remain untreated are at lifelong risk of renal impairment or failure, organ dysfunction and premature death [2, 3]. Eculizumab (Soliris<sup>®</sup>, Alexion Pharmaceuticals, Inc., New Haven, CT, USA), a humanized monoclonal antibody that inhibits C5a, C5b and C5b-9 formation by binding to C5, is the first and only approved treatment for patients with aHUS [5, 6]. The efficacy and safety of eculizumab have been demonstrated in four prospective, multicentre clinical studies [7–10] and a retrospective study [11].

Current regulatory guidance notes potential risk of TMA following discontinuation of eculizumab [5, 6]. Additional evidence for TMA manifestations occurring after discontinuation is limited to case studies [12–25], two retrospective studies [26, 27] and a small prospective observational study [28, 29]. Together, TMA manifestations were documented in 26/82 patients (32%) who discontinued eculizumab [12–29]. An analysis [30] from the eculizumab clinical trial programme determined that severe TMA manifestations occurred in 12/61 patients (20%) who chose to discontinue treatment.

This is the single largest prospective, observational study of the consequences following eculizumab discontinuation in aHUS. In an interim analysis, TMA manifestation rates off and on eculizumab in patients with aHUS were evaluated. *Post hoc* analyses based on a revised, more stringent definition of TMA, during labelled versus non-labelled regimens and by complement abnormality status, also were conducted. In addition, the safety of long-term eculizumab is reported.

## MATERIALS AND METHODS

### Study design and patients

This is a long-term, prospective, observational and multicentre study (NCT01522170) of patients with aHUS who were treated with eculizumab in any of five previous clinical studies (parent studies): four prospective studies (NCT00844545/NCT00844844 and NCT00838513/NCT00844428 [7, 8], NCT01193348 [9], NCT01194973 [10]) and one retrospective study (NCT01770951 [11]). Patients who participated in a parent study were eligible for the current study, regardless of whether they completed or

discontinued from the parent study or were on eculizumab at the time of enrolment. Patients could withdraw from the current study at any time. The protocol was approved by an institutional review board or independent ethics committee at each participating centre and the study was conducted in accordance with International Council for Harmonisation Guidelines and the Declaration of Helsinki. All patients and/or parents/guardians provided written informed consent before entry into the current study.

The current study consequently includes both prospective and retrospective data collection. Retrospective data were obtained from the date each patient ended participation in the parent study until the date of signed informed consent for the current study. All patients who received at least one infusion of eculizumab during the parent study and had signed consent forms for the current study were included in the analysis. Identification of complement abnormalities occurred during the parent studies and included analysis of complement factor I (CFI), complement factor B (CFB), complement factor H (CFH), membrane cofactor protein (MCP) and C3 mutations, complement factor H-related proteins 1-3 (CFHR1-3) deletions/polymorphisms and CFH autoantibodies [7, 9–11]. Patients received meningococcal vaccination in the parent studies [7, 9, 10] and were revaccinated according to country guidelines. The cut-off date for this interim analysis was 28 March 2015.

TMA manifestations were neither defined nor collected uniformly in the parent studies; thus, this analysis includes outcomes reported in this ongoing observational study only (i.e. beginning at the end of the parent study). Data were collected four times annually in both the retrospective and prospective portions of the current study.

### Treatment

After completion of the parent study and entry into the current study, the labelled dosing regimen of eculizumab was defined as that specified in the prescribing information approved by regulatory authorities [5, 6], and other dosing schedules (i.e. decreased or increased dosages, shortened or extended dosing intervals) were permitted and classified as non-labelled regimens. In this study, the first on-treatment period was defined as: from the date of the first infusion in the current study (i.e. beginning at the end of the parent study) through 3 weeks after the last infusion of eculizumab, or until the patient discontinued from the study, or data cut-off (whichever occurred first). The first off-treatment period was defined as: from 3 weeks after the last infusion of eculizumab within the current study until the patient restarted eculizumab therapy, discontinued from the study or data cut-off (whichever occurred first). Subsequent on- and off-treatment periods were defined similarly. Patient groups were not mutually exclusive; individual patients could be represented in both groups.

### Endpoints

Primary endpoint was the rate of TMA manifestations (defined in Table 1) in the current study off and on treatment. *Post hoc*

Table 1. Per-protocol definition of TMA manifestations (any  $\geq 1$  listed criteria)

Type/severity	Criteria
Laboratory value change only <sup>a</sup>	The occurrence of a change in $\geq 1$ laboratory value <sup>b</sup> : <ul style="list-style-type: none"> <li>• Platelet count decrease <math>\geq 25\%</math> compared with baseline<sup>c</sup> and <math>&lt; \text{LLN}</math></li> <li>• Increase in SCr or LDH level <math>\geq 25\%</math> compared with baseline<sup>c</sup> and <math>&gt; \text{ULN}</math></li> </ul>
Clinical signs and symptoms of TMA <sup>d</sup>	Clinical signs and symptoms of TMA considered definitely related to aHUS, including: <ul style="list-style-type: none"> <li>• Thrombosis</li> <li>• Seizure</li> <li>• Decreased renal function</li> <li>• Proteinuria (new or worse compared with baseline and <math>&gt; 1+</math> or <math>&gt; 30</math> mg/dL)</li> <li>• Haematuria (new or worse compared with baseline and <math>&gt; 50</math> RBC/HPF)</li> <li>• Increased haemolytic anaemia</li> <li>• Biopsy-proven TMA</li> <li>• Other (e.g. extrarenal TMA manifestations including confusion, cardiovascular abnormalities, pericarditis, gastrointestinal symptoms and diarrhoea)</li> </ul>
Intervention <sup>d</sup>	The patient received PE/PI, dialysis, blood transfusions or renal transplant due to a TMA manifestation

<sup>a</sup>As determined by changes in laboratory parameters with ongoing follow-up.

<sup>b</sup>Measurements were required to be confirmed by a second measurement  $\geq 28$  days apart with no interruption.

<sup>c</sup>Baseline was defined for each on period as the last laboratory value during the preceding off period, and for each off period as the last value during the preceding on period.

<sup>d</sup>As determined at the discretion of the investigator.

HPF, high-powered field; LDH, lactate dehydrogenase; LLN, lower limit of normal; PE/PI, plasma exchange/plasma infusion; RBC, red blood cells; SCr, serum creatinine; ULN, upper limit of normal.

analyses evaluated TMA manifestation rates off and on treatment when TMA manifestations based on only a single laboratory value change were excluded; in patients receiving labelled compared with non-labelled eculizumab regimens; and in patients with and without identified complement abnormalities. Safety endpoints included assessment of serious targeted treatment-emergent adverse events (TEAEs; predefined as incidence of serious infection, meningococcal infection, sepsis, renal impairment or leucopaenia), as well as any serious AE (SAE).

## Statistical methods

Time to first TMA manifestation was defined as time from the start of the current study (i.e. end of the parent study) to first TMA manifestation during the current study. Patients who did not have a TMA manifestation were censored at data cut-off or study discontinuation, whichever occurred first. Time to first TMA manifestation was analysed using Cox proportional hazards models with treatment status as a time-dependent explanatory variable and complement abnormality status as a covariate. Hazard ratios (HRs) and P values were obtained for comparisons off and on treatment and between identified and no identified complement abnormality subgroups.

## RESULTS

### Patients and exposure

Overall, 130 patients were enrolled in the parent studies. By the data cut-off for this analysis, 87 patients had enrolled in the current study. Of these, 39 (45%) had off-treatment periods whereas 76 (87%) had on-treatment periods. Seventeen patients (44%) with off-treatment periods reinitiated eculizumab; of these, 14 (82%) remained on therapy once they reinitiated (Figure 1). Age, frequency of complement abnormalities and kidney transplant status were not different between patients with ongoing eculizumab therapy versus those who discontinued (Table 2). Twenty-two patients (25%) had renal transplants, including

eight patients (21%) who discontinued eculizumab and 14 (29%) on ongoing eculizumab. A median (range) of 11.0 (0.0–230.0) plasma exchanges or plasma infusions were used by the overall population before eculizumab initiation, including 7.0 (0.0–64.0) in patients who discontinued eculizumab and 13.3 (0.0–230.0) in patients who never discontinued eculizumab. Dialysis was required by 29/87 patients (33%) before parent study enrolment, and use was more frequent in those who discontinued eculizumab (39%) compared with those who never discontinued eculizumab (29%). Including parent studies, patients had a total median (range) of 45.9 (1.3–86.9) months of eculizumab exposure. In the current study, median (range) follow-up was 20.1 (0.7–79.5) months off and 26.1 (0.7–64.2) months on treatment.

Compared with patients who continued on eculizumab, those who discontinued had a shorter interval from initial aHUS diagnosis, as well as the most recent pretreatment TMA manifestation, to first-ever eculizumab dose in the parent study. Patients who discontinued also presented with lower estimated glomerular filtration rates (eGFRs) and were more frequently dialysis dependent at initiation of eculizumab (Table 2).

Non-labelled eculizumab regimens were received by 33/87 patients (38%) during the current study. Of these, 11 always received non-labelled doses and 22 had periods of labelled and non-labelled regimens. Median (range) duration of therapy was 25.7 (0.5–60.4) months during labelled and 14.3 (0.4–64.3) months during non-labelled regimens. Dosing higher than labelled accounted for 0.4% of the total patient-years for non-labelled regimens.

### TMA manifestations

When using the per-protocol definition (Table 1), 28 TMA manifestations occurred. This included 14 TMA manifestations in 11/39 patients (28%) off treatment and 14 TMA manifestations in 10/76 patients (13%) on treatment (Tables 3 and 4). TMA manifestation rate per 100 patient-years was 19.9 off and 7.3 on treatment (63% lower; HR, 4.7;  $P=0.0008$ ; Table 5).

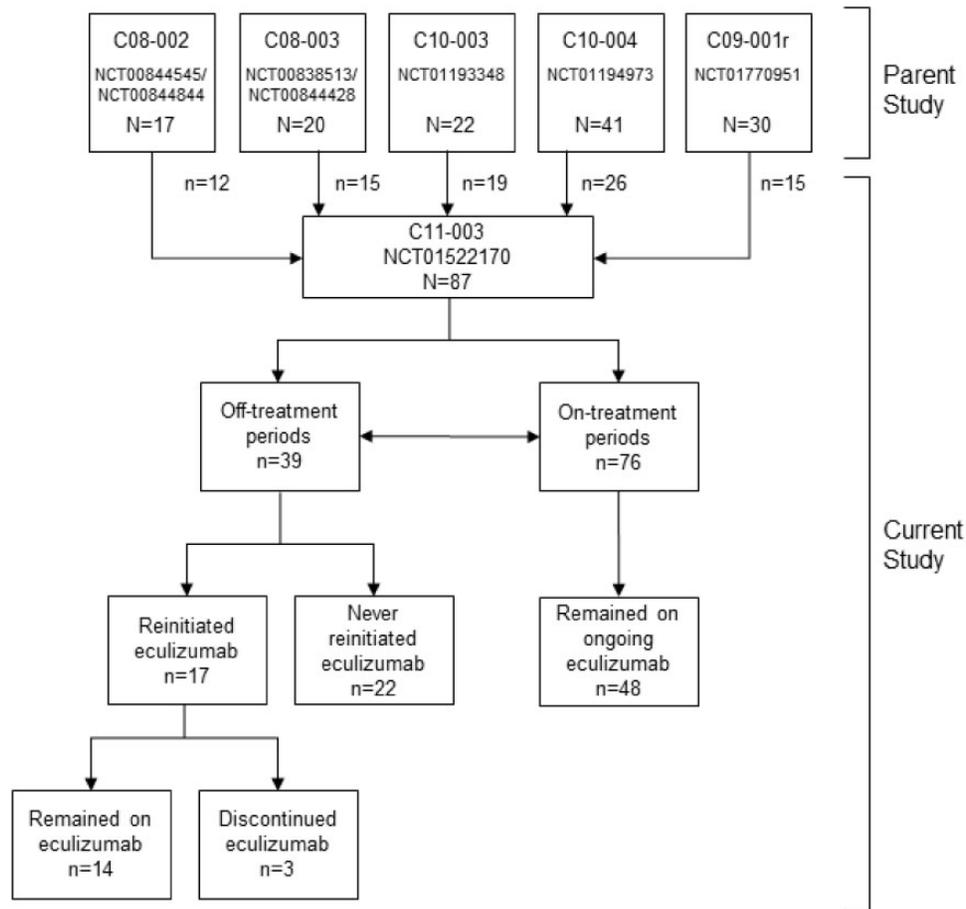


FIGURE 1: Patient disposition.

Patients treated with non-labelled and labelled regimens had TMA manifestation rates of 12.1 and 5.2 per 100 patient-years, respectively (39% and 74% lower, respectively, versus off treatment).

### Characteristics of TMA manifestations

TMA manifestations off treatment were more frequently associated with multiple laboratory criteria for TMA, clinical signs and symptoms of TMA, interventions, SAEs and/or hospitalizations (Tables 3 and 4). Eleven of 14 TMA manifestations (79%) in patients off treatment included multiple laboratory TMA criteria and/or an intervention, compared with 2/14 TMA manifestations (14%) on treatment. Patients required hospitalization during 9/14 TMA manifestations (64%) off treatment and 3/14 TMA manifestations (21%) on treatment.

### TMA manifestation rate excluding TMAs based on single laboratory value changes

An abnormality in a single laboratory value may not be considered as TMA clinically; therefore, a *post hoc* analysis was performed to better reflect TMA evaluation in clinical practice. TMA defined by a change from baseline in a single laboratory value occurred in 3/14 TMA manifestations (21%) off treatment and 12/14 TMA manifestations (86%) on treatment (Tables 3 and 4). When using this definition, 11 TMA manifestations occurred in 8/39 patients (21%) off treatment and two TMA

manifestations occurred in 2/76 patients (3%) on treatment. The TMA manifestation rate per 100 patient-years was 15.6 and 1.0 on treatment (94% decrease; HR, 16.8;  $P=0.0010$ ) (Table 5).

### TMA manifestations by complement abnormality status

The majority of patients who reported TMA manifestations as defined per protocol had complement abnormalities (Tables 3 and 4), particularly in those with *CFH* [10/17 (59%)] and *CFI* mutations [4/17 (24%)]. Rates were higher for patients with identified complement abnormalities compared with no identified complement abnormalities (HR, 4.5;  $P=0.0082$ ).

### Safety

Overall, treatment with eculizumab was well tolerated. The occurrence of serious targeted TEAEs during the current study was similar off and on treatment (Table 6). Two patients from the parent retrospective study reported meningococcal infections during the current study; both were determined to be probably related to eculizumab. Both patients were treated and recovered while continuing eculizumab on schedule. Diagnoses/underlying conditions associated with reported serious targeted TEAEs of renal impairment that did not meet criteria for TMA included new kidney transplant, renal graft rejection, multiorgan failure, dehydration, infection and interstitial tubulopathy. One adult patient, who received non-labelled dosing during the current study,

Table 2. Demographic and baseline clinical characteristics in the parent studies

Characteristic	Ongoing eculizumab (n = 48)	Discontinued (n = 39)	Total (n = 87)
Age at first-ever infusion of eculizumab, years			
Median (range)	24.0 (0.0–65.0)	21.0 (0.0–80.0)	22.0 (0.0–80.0)
<12, n (%)	13 (27)	11 (28)	24 (28)
12 to <18, n (%)	6 (13)	5 (13)	11 (13)
≥18, n (%)	29 (60)	23 (59)	52 (60)
Age at entry to current study, years			
Median (range)	27.5 (2.5–67.6)	25.1 (1.6–81.6)	26.0 (1.6–81.6)
<12, n (%)	10 (21)	11 (28)	21 (24)
12 to <18, n (%)	3 (6)	0 (0)	3 (3)
≥18, n (%)	35 (73)	28 (72)	63 (72)
Female, n (%)	30 (63)	23 (59)	53 (61)
Complement gene mutation or autoantibody, n (%)	31 (65)	20 (51)	51 (59)
CFH <sup>a</sup>	12 (25)	8 (21)	20 (23)
CFH autoantibodies <sup>b</sup>	6 (13)	1 (3)	7 (8)
C3	6 (13)	1 (3)	7 (8)
CD46 (MCP)	2 (4)	5 (13)	7 (8)
CFI	3 (6)	4 (10)	7 (8)
C3, CFHR3-CFHR1	1 (2)	0	1 (1)
CD46 (MCP), CFI	1 (2)	0	1 (1)
CFB	0	1 (3)	1 (1)
Identified CFHR1, CFHR3 deletion	0	1 (3)	1 (1)
Time from last pretreatment aHUS manifestation to first-ever dose of eculizumab, median (range), months	2.3 (0.0–47.4)	0.5 (0.0–19.2)	0.9 (0.0–47.4)
Family history of aHUS, n (%)	8 (17)	8 (21)	16 (18)
Time from aHUS diagnosis to first-ever dose of eculizumab, median (range), months	18.4 (0.0–313.3)	0.9 (0.0–178.1)	4.0 (0.0–313.3)
History of TMA manifestations, n (%)			
Single	29 (60)	27 (69)	56 (64)
Multiple	19 (40)	12 (31)	31 (36)
Prior renal transplant, n (%)	14 (29)	8 (21)	22 (25)
PE/PI sessions per patient, median (range)	13.3 (0.0–230.0)	7.0 (0.0–64.0)	11.0 (0.0–230.0)
Dialysis at baseline <sup>c</sup> , n (%)	14 (29)	15 (39)	29 (33)
eGFR <sup>d</sup> at baseline, median (range), mL/min/1.73 m <sup>2</sup>	21.2 (8.4–128.3)	12.1 (5.3–105.5)	18.9 (5.3–128.3)

<sup>a</sup>Includes patients with additional abnormalities [i.e. C3, CD46 (MCP), CFI, CFHR1, CFHR3, and CFHR3-CFHR1].

<sup>b</sup>Includes patients with additional abnormalities (i.e. CFHR1, CFHR3, CFHR3-CFHR1).

<sup>c</sup>Dialysis at baseline was defined as any dialysis that occurred within 7 days prior to or 14 days following the first eculizumab dose in the parent study.

<sup>d</sup>eGFR was defined as 10 mL/min/1.73 m<sup>2</sup> when a patient was on dialysis.

PE/PI, plasma exchange/plasma infusion.

died due to severe intensive care complications and severe multi-organ dysfunction after gastrointestinal haemorrhage, lithiasic cholecystitis and severe sepsis, which were determined to be unrelated to eculizumab.

## DISCUSSION

Results from this interim analysis of a non-randomized, prospective observational study demonstrate that rates of TMA manifestations in patients with aHUS were 2.7-fold higher off compared with on eculizumab (63% lower), despite longer follow-up on treatment. TMA manifestation rates were lowest during labelled dosing regimens (74% lower than off treatment), higher during non-labelled regimens (39% lower than off treatment) and highest off treatment.

The per-protocol definition of TMA manifestations was broad, including changes in laboratory values, clinical signs and symptoms of TMA related to aHUS, and/or interventions related to TMA. Thus, reported TMA per this definition represented varying degrees of clinical deterioration. Importantly, there is no single,

agreed-upon definition of TMA based upon a single laboratory value in clinical practice. TMA manifestations off treatment were associated with multiple laboratory criteria, clinical sequelae (e.g. renal impairment and acute renal failure), SAEs, hospitalizations and/or required interventions (e.g. transfusion) in 13/14 cases (93%). In contrast, TMA manifestations on treatment typically comprised changes in single laboratory values with no clinical signs/symptoms. Therefore, *post hoc* analyses were conducted to provide insights using a more stringent TMA definition that we believe more closely defines TMA in the setting of aHUS. When TMA manifestations based only on changes in single laboratory values were excluded, the rate off treatment was 15.6-fold higher than on treatment. These results taken together could suggest worse outcomes for patients who discontinue eculizumab, although it is possible that changes in single laboratory values may signal subclinical disease processes.

Patients with identified complement abnormalities had statistically significantly higher TMA rates than patients with no identified abnormalities. CFH and CFI mutations were predominant among patients who experienced TMA, regardless of treatment status. This finding is consistent with previous

Table 3. Reported TMA manifestations in patients off treatment with eculizumab

Patient	Patient demographics and clinical characteristics			Eculizumab therapy <sup>a</sup>				Criteria achieved for TMA manifestation	SAE/hospitalization <sup>b</sup>	Eculizumab reinitiation <sup>c</sup>
	Sex	Patient age (years)	Complement abnormality	Eculizumab duration before discontinuation (months)	Duration of discontinuation before TMA manifestation (months)	TMA manifestation based on single lab criterion				
1	Male	9	CFH	6	1.3	No	↑Scr, ↓LDH	No	Yes	
2	Male	11	CFI	5	17	No	↑Scr, ↓LDH, ↓platelets	Yes	Yes	
3	Male	4	CFH	0.7	3.7	No	↑Scr, ↓LDH, ↓platelets, transfusion	Yes	Yes	
4	Male	38	CFH	14	2.5	No	↓platelets, ↓renal function, ↓haemolytic anaemia	No	Yes	
5	Female	25	MCP	27	2.6	Yes	↑Scr	Yes	Yes	
6	Female	39	CFI	37	7	Yes	↓platelets	Yes	Yes	
7	Female	84	None identified	3	28	No	↑LDH, ↓platelets	Yes	No	
8	Male	26	MCP	3	42	No	Signs of reactivation of TMA with clinical repercussion	No	No	
9	Male	27	None identified	6	18	No	↑Scr, transfusion	Yes	No	
10	Female	73	CFH autoantibodies, CFHR1-3	6	34	No	↑Scr, transfusion	Yes	Yes	
11	Female	20	CFH, C3	50	2	No	↓Haptoglobin	Yes	Yes	
				34	17	Yes	↑Scr	No	No	
				6	2	No	↓Platelets, acute renal failure	No	Yes	

<sup>a</sup>During the current study only (i.e. excluding the parent study).

<sup>b</sup>Before or during TMA manifestation.

<sup>c</sup>Seventeen of 39 patients (43.6%) reinitiated eculizumab after discontinuation of therapy. Median (range) time to reinitiation was 2.6 (0.7–69.3) months. Fourteen of 17 patients (82%) continue on therapy after reinitiation. Hb, haemoglobin; LDH, lactate dehydrogenase; Scr, serum creatinine.

Table 4. Reported TMA manifestations in patients on treatment with eculizumab

Patient demographics and clinical characteristics				Eculizumab therapy <sup>a</sup>				
Patient	Sex	Patient age (years)	Complement abnormality	Treatment duration (months)	Labelled regimen <sup>b</sup>	TMA manifestation based on single lab criterion	Criteria achieved for TMA manifestation	SAE/hospitalization <sup>c</sup>
1	Female	4	None identified	26	No	Yes	↑SCr	No
2	Female	31	CFH	28	Yes	Yes	↓Platelets	Yes
3	Female	44	CFH	20	Yes	Yes	↑LDH	No
4	Female	12	CFH	59	Yes	Yes	↑SCr	No
5	Female	44	CFI	19 <sup>d</sup>	Yes	Yes	↓Platelets	Yes
6	Male	27	MCP	51	Yes	Yes	↑LDH	No
				60	No	Yes	↑LDH	No
7	Male	49	CFI	39	Yes	No	Dialysis	Yes
8	Female	10	CFH	56	No	Yes	↑SCr	No
				61	No	No	↑SCr, ↓platelets, ↑Proteinuria	No
9	Male	15	CFH	41	No	Yes	↓Platelets	No
				44	No	Yes	↑SCr	No
				53	No	Yes	↑SCr	No
10	Male	20	None identified	3	Yes	Yes	↑LDH	No

<sup>a</sup>During the current study only (i.e. excluding the parent study).

<sup>b</sup>No TMA manifestations occurred while patients received dosing higher than approved by regulatory authorities.

<sup>c</sup>Before or during TMA manifestation.

<sup>d</sup>The patient's first on-treatment period was 19 months, followed by an off-treatment period of 31 months and another on-treatment period of 10 months.

LDH, lactate dehydrogenase; SCr, serum creatinine.

Table 5. TMA manifestation rates

Parameter	Eculizumab treatment status		Eculizumab dosing		Excluding single laboratory change criterion	
	Off treatment (n = 39)	On treatment (n = 76)	Non-labelled regimen (n = 33)	Labelled regimen (n = 65)	Off treatment (n = 39)	On treatment (n = 76)
Patients with manifestation, n (%)	11 (28)	10 (13)	4 (12)	7 (11)	8 (21)	2 (3)
Total number of manifestations	14	14	7	7	11	2
Total patient-years	70.5	192.8	57.9	135.0	70.5	192.8
TMA manifestation rate/100 patient-years	19.9	7.3	12.1	5.2	15.6	1.0
Fold change in rate <sup>a</sup>	2.7	Ref	2.3	Ref	15.6	Ref
Per cent change compared with off treatment <sup>b</sup> (%)	Ref	-63	-39	-74	Ref	-94
HR (P value) <sup>c</sup>	4.7 (P = 0.0008)	Ref	1.3 (P = 0.7000) <sup>d</sup>	Ref	16.8 (P = 0.0010)	Ref

<sup>a</sup>Off treatment compared with on treatment (overall) or non-labelled compared with labelled regimen for the same analysis.

<sup>b</sup>On treatment (overall), non-labelled or labelled regimen compared with off treatment for the same analysis.

<sup>c</sup>HRs were based on Cox proportional hazards model of time to first TMA manifestation, with treatment status as a time-dependent explanatory variable and complement abnormality status as a covariate.

<sup>d</sup>Compared with the labelled dosing regimen of eculizumab.

Ref, reference value.

observational studies of the natural history of aHUS [2, 3]. In a small observational study, Ardissino *et al.* [28, 29] also noted particular risk for TMA in patients who chose to discontinue eculizumab with CFH mutations, and CFI mutations to a lesser extent. In a retrospective study of eculizumab discontinuation in a French cohort (n = 38) [27], all 12 patients (32%) with TMA post-eculizumab discontinuation had rare or novel CFH or MCP variants; both were independent risk factors for TMA following discontinuation. However, patients with transplant, on chronic dialysis or 'secondary' aHUS were excluded from the French cohort. Results of the current analysis, which are from the single largest prospective study of TMA risk following

eculizumab discontinuation, reinforce previous findings that patients who discontinued eculizumab were at greater risk for TMA compared with patients on treatment, and particularly those with identified complement abnormalities. In this study, patients without identified abnormalities were at a significantly lower yet distinct risk for TMA after eculizumab discontinuation. However, genetic analyses were performed during the parent studies several years ago; thus, it is possible that novel mutations not known at that time have been left unrecognized.

Overall, characteristics of patients with TMA manifestations were highly heterogeneous with respect to age, treatment

Table 6. Serious targeted TEAEs reported in the current study

TEAE	Off treatment <sup>a</sup> (n = 39)	On treatment (n = 76)
Any serious targeted TEAEs, rate per 100 patient-years	15.6 (11)	14.0 (27)
Renal impairment <sup>b</sup>	11.3 (8)	8.3 (16)
Infection, other	2.8 (2)	2.1 (4)
Infection, septic	1.4 (1)	2.1 (4)
Infection, meningococcal	0.0 (0)	1.0 (2)
Leucopaenia	0.0 (0)	0.5 (1)

Data are presented as n (%).

<sup>a</sup>Data were obtained retrospectively between the end of the parent study and enrolment in the current study for patients off treatment and on treatment.

<sup>b</sup>TEAEs of renal impairment were evaluated and reported by each investigator, and no set definition was used.

duration and duration of eculizumab before onset of TMA. Age, frequency of complement abnormalities and kidney transplant status did not differ between patients who discontinued versus remained on eculizumab. However, patients who discontinued appeared to have poorer renal function at baseline in the parent study and initiated eculizumab more rapidly. Such patients may have had clinically significant renal improvement with eculizumab, since they initiated treatment in a rapid manner [31], followed by the clinical decision to discontinue treatment after recovery. Discontinuation of therapy was not randomized, potentially allowing selection bias for continuing versus discontinuing eculizumab. In the previously cited studies [26–28], not all patients with aHUS were included. In the current study, all patients who met inclusion criteria for the parent studies (including those with dialysis and renal transplants) were allowed to enrol. Further studies are needed to identify patient characteristics potentially associated with increased risk for TMA after eculizumab discontinuation.

Notably, 17/39 patients (44%) reinitiated eculizumab following a period of discontinuation, including 9/11 patients (82%) who had TMA manifestations while off treatment. After reinitiation, 14/17 patients (82%) continued eculizumab. Longer term evaluation may provide additional insight as to clinical outcomes associated with therapy stops and restarts.

Collectively, the current results reinforce the need for ongoing treatment with eculizumab to minimize risk of TMA in patients with aHUS, particularly those with an identified complement abnormality. Although thorough genetic testing informs prognosis, additional considerations when optimizing treatment strategy include the patient's unique clinical situation, age, TMA and family histories, as well as recognition of the complex and unpredictable nature of aHUS. For individual patients in whom discontinuation of eculizumab is being considered, clinicians would be well advised to consult an expert centre in the field while ensuring that the patient: (i) has been treated for a sufficiently long period to ensure maximal organ function recovery; (ii) can be monitored closely for signs and/or symptoms of TMA; and (iii) has immediate access to eculizumab so treatment can be restarted at the first signs and/or symptoms of TMA [27, 32].

As was observed in the parent studies [7–10], eculizumab was generally well tolerated in the current study. Rates of serious targeted TEAEs, including infections, reported off and on treatment were similar. In particular, rates of renal impairment were relatively high both off and on treatment, but commonly

associated with new kidney transplantation and existing graft failure. Two patients reported meningococcal infections during the current study. Both recovered and there were no changes in eculizumab dosing. Frequency of meningococcal infections [2/87 patients (2%)] is similar to that from the overall parent trial programme of eculizumab in aHUS [two cases/100 total patients (2%)] [7–10]. Overall, the reported meningococcal infection rate in patients treated with eculizumab is 0.3 events/100 patient-years [33]. Regulatory guidance for eculizumab includes increased susceptibility to meningococcal infection [5, 6]. Patients should be counselled in order to fully understand potential benefits and risks of treatment, early signs of meningococcal disease and processes for seeking immediate medical care. Risks of potentially severe complications, including meningococcal infection, should be considered during the decision-making process regarding initiating treatment or discontinuing eculizumab. Long-term evaluations of the eculizumab safety profile will be included in future analyses from the Global aHUS Registry [34].

An important study limitation was its open-label and observational nature. Voluntary patient enrolment into this prospective study may have introduced selection bias because data are not available for patients who completed a parent study but did not consent to enrolment in the current study. In this analysis, 43/130 patients (33%) who enrolled in one of the parent studies had not continued into the current study. During the parent and current studies, which together included a median exposure of 45.9 months, withdrawal of eculizumab due to an AE was uncommon. One adult patient died due to multiorgan failure following a reduced dosing regimen. One paediatric patient discontinued eculizumab in the parent study due to agitation [9]. Three patients with previous renal transplants and poor renal function (eGFR <30 mL/min/1.73 m<sup>2</sup>) at the start of treatment discontinued eculizumab following reports of renal impairment in the current study; of these, one later restarted eculizumab and the other two received additional renal transplants. Additional studies are needed to further understand patient and physician rationale for discontinuing and reinitiating treatment.

Taken together, findings from this interim analysis suggest that patients with aHUS have an increased risk for TMA manifestations after discontinuation of eculizumab or during non-labelled regimens compared with labelled eculizumab dosing. These results support current regulatory guidance [5, 6] in noting potential risk for TMA following discontinuation of eculizumab. Evidence demonstrates that patients had a 63–94% lower risk of TMA on eculizumab therapy, depending on the definition used. Future analyses will allow for further characterization of TMA and evaluation of longer term outcomes on labelled or non-labelled regimens of eculizumab and after therapy discontinuation.

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### CONFLICT OF INTEREST STATEMENT

This interim analysis was sponsored by Alexion Pharmaceuticals, Inc. J.M. receives lecture and/or advisory fees from Alexion Pharmaceuticals, Inc., AstraZeneca, Berlin-Chemie, Daiichi Sankyo, Boehringer Ingelheim and Novartis. F.F. received fees for participation in advisory boards, experts' meetings and/or teaching courses from Alexion Pharmaceuticals, Inc. J.F.K. was a stockholder and employee of Alexion Pharmaceuticals, Inc. when the study was conducted. C.L. has received grant/research support and/or consultancy fees from Achillion Pharmaceuticals, Inc., Alexion Pharmaceuticals, Inc. and CSL Behring; has received honoraria from Alexion Pharmaceuticals, Inc. and CSL Behring; has submitted patents for CSL Behring and Finnegan, Henderson, Farabow, Garrett & Dunner; is a member of the Editorial Boards for *Kidney International*, *Nephrology Dialysis Transplantation* and *Pediatric Nephrology*; is a Steering Committee Member of the Alport Syndrome Treatments and Outcomes Registry (ASTOR) and an Appointed Member of the Safety Board of the European Treatment Trial for Alport Syndrome (EARLY PRO-TECT); is the aHUS International Registry Scientific Advisory Board Chair and HUS International Chair for Alexion Pharmaceuticals, Inc.; and has participated in the Eculizumab in Adolescent Patients With Plasma Therapy-Resistant aHUS (C08-002; NCT00844844), Eculizumab in Adolescent Patients With Plasma Therapy-Sensitive aHUS (C08-003; NCT00844428), and Eculizumab in Pediatric Patients With Atypical Hemolytic-Uremic Syndrome (C10-003; NCT01193348) clinical studies for Alexion Pharmaceuticals, Inc. E.E.M. has participated in the C10-004 adult interventional study (NCT01194973) and in the C11-003 observational, follow-up study (NCT01522170) of atypical haemolytic uraemic syndrome patients for Alexion Pharmaceuticals, Inc. C.M. was a stockholder and employee of Alexion Pharmaceuticals, Inc. when the study was conducted. F.P. has received honoraria from Alexion Pharmaceuticals, Inc. E.R. has received fees for participation in advisory boards, experts' meetings and/or teaching courses from Alexion Pharmaceuticals, Inc. N.S.S. has received research funding from GlaxoSmithKline plc. J.W. is a stockholder and employee of Alexion Pharmaceuticals, Inc. L.E.W. has received fees for participation in advisory boards from Alexion Pharmaceuticals, Inc. L.A.G. has received research funding for Emory University from Alexion Pharmaceuticals, Inc., for his participation in the Eculizumab in Pediatric Patients and aHUS International Registry clinical studies; has received grant/research support and/or consultancy fees from AbbVie Inc., Alexion Pharmaceuticals, Inc., Bristol Myers Squibb, Advicenne Pharmaceuticals, Mallinckrodt Pharmaceuticals, Otsuka America Pharmaceutical, Inc. and Vifor Pharma; has served as a member of a scientific advisory board for Alexion Pharmaceuticals, Inc., and as a member of data safety monitoring boards for Retrophin, Inc. and Relypsa Pharmaceuticals. Y.D. has no relevant financial relationships to disclose.

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