DIAGNOSTIC CHALLENGES IN THROMBOTIC MICROANGIOPATHIES

Summary of Presentations from the Alexion-Sponsored Symposium, held at the 51st ERA–EDTA Congress, Amsterdam, the Netherlands, on 1st June 2014

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MEETING SUMMARY

The Alexion Satellite Symposium provided an introduction to thrombotic microangiopathies (TMA) by Prof Dirk Kuypers, who described atypical haemolytic uraemic syndrome (aHUS) as a rare but severe disease that causes TMA and can result in organ failure. Prof Josep Campistol presented two patient cases to illustrate the need to make a differential diagnosis between aHUS, thrombotic thrombocytopaenic purpura (TTP), and Shiga toxin-related-HUS (STEC-HUS). Prof Christophe Legendre then described aHUS clinical management, introduced eculizumab as the only approved treatment for aHUS, and provided an overview of the efficacy and safety data from recent clinical trials.

An Introduction to TMAs

Professor Dirk Kuypers

TMA is mostly caused by three separate conditions, summarised in Figure 1 and described here:

1. The disease can be directly triggered by uncontrolled complement activation[1,2] as with aHUS. There are currently six important CF mutations described that are responsible for dysregulated complement activation in patients with aHUS.[3,4] Although recent reports have identified the genetic underlying condition for aHUS in around 60–68% of patients,[5,6] the remaining patients do not currently have any identifiable genetic mutation and are a target for future research. A low percentage of patients can develop antibodies against factor H, which also results in uncontrolled complement activation.

2. TMA can also be associated with reduced activity of a disintegrin and metalloproteinase with thrombospondin Type 1 motif, 13, (ADAMTS13). ADAMTS13 is a cleavage molecule that acts upon ultra-large von Willebrand factors (vWF) and is related to the development of TTP.

3. The third major cause of TMA is Shiga toxin-related-HUS, which is caused following a Shiga toxin producing *Escherichia coli* infection (STEC-HUS).[7] Of all haemolytic uraemic syndromes, 90% are STEC-HUS and 10% are aHUS.

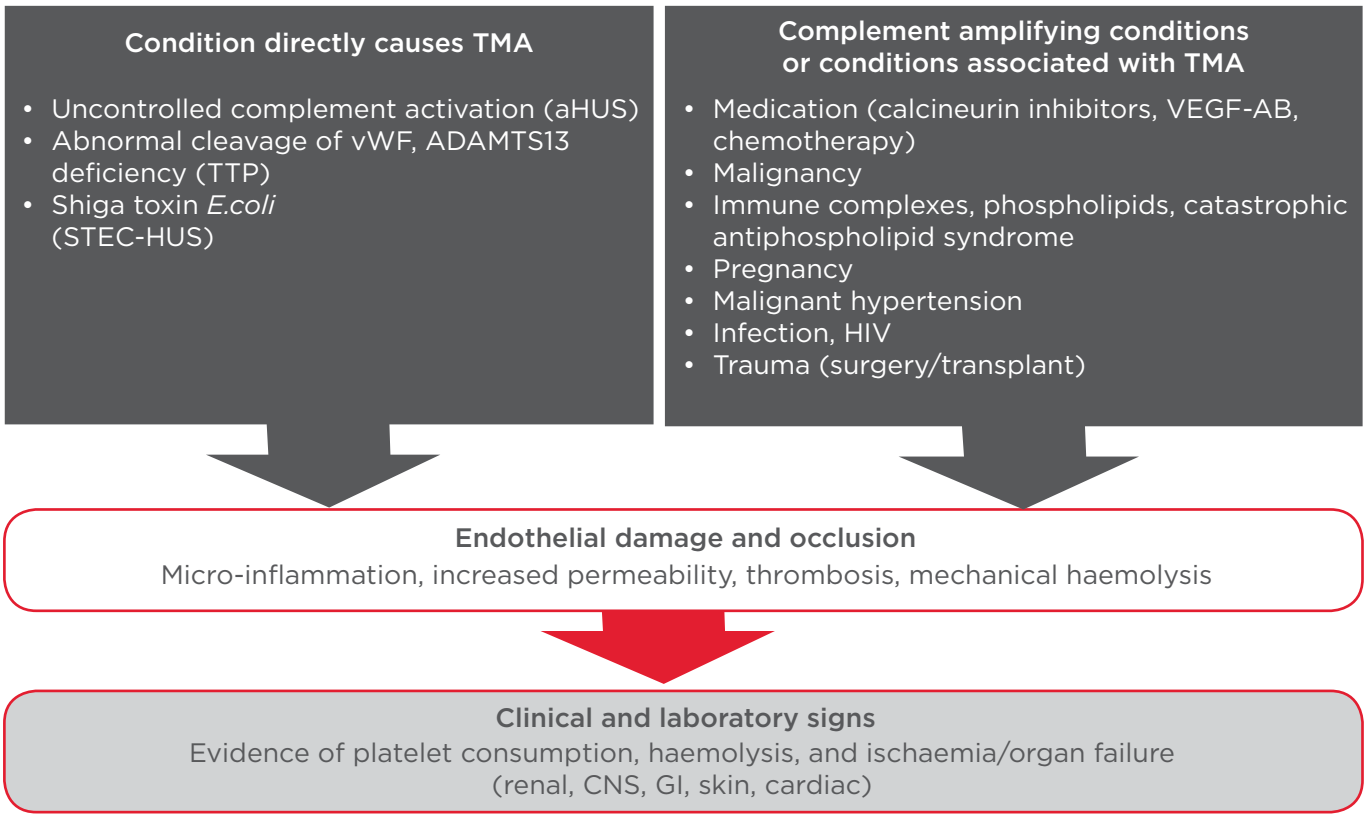

Figure 1: Pathogenesis of TMA.[1–5]

ADAMTS13: a disintegrin and metalloproteinase with thrombospondin Type 1 motif 13; aHUS: atypical haemolytic uraemic syndrome; STEC-HUS: Shiga toxin-producing E. coli haemolytic uraemic syndrome; TMA: thrombotic microangiopathy; TTP: thrombocytopaenic purpura; vWF: von Willebrand factor.

From Dirk Kuypers, presentation at the Alexion Satellite Symposium.

Additional conditions that can be associated with the development of TMA - but where TMA is not an integral part of the disease - are TMA due to calcineurin inhibitors, vascular endothelial growth factor antibodies, and chemotherapy along with malignancies, catastrophic antiphospholipid syndrome, pregnancy, malignant hypertension, infection, HIV, and trauma such as surgery or transplants.[8] As these conditions can amplify complement and endothelial damage; they may in addition precipitate TMA in patients with a predisposition to aHUS.

Although the causes of TMA can differ, there are similarities in regards to clinical symptoms.[9] The Shiga toxins produced by STEC result in damage to the endothelium, thereby activating complement and platelets.[10] Genetic mutations in complement regulatory proteins lead to chronic uncontrolled complement activation, which damages the endothelium and activates platelets,[11] whilst TTP from defective activity of ADAMTS13 allows the formation of ultra-large vWF multimers binding platelets and formation of blood clots. Across all three types of TMA the endothelial damage and occlusion with micro-inflammation and increased permeability cause thrombosis in potentially any organ, which is shown through evidence of platelet consumption, haemolysis, ischaemia, and eventually target organ failure.[12]

Although it is established that aHUS has a significant effect on renal function and development of end-stage renal disease (ESRD), aHUS is a systemic disease that can involve many systems and organs[3,13–16] including the cardiovascular,[11] gastrointestinal,[17] skin,[18] and central nervous system (CNS).[19] The outcome of aHUS is poor: 56% of adult patients either die or have ESRD within the first year after diagnosis, despite plasma exchange (PE) or plasma infusion (PI).[6] Regarding patients with aHUS who have had renal transplants,[20] TMA generally presents again rapidly after transplantation, associated with a mortality rate of 7%, and 50% graft failure within 5 years post transplant.[14]

In summary, TMA is a serious rapidly progressing condition that can lead to severe, irreversible organ damage with considerable morbidity and mortality. TMA can be caused by several conditions that should be managed differently for best patient outcomes. Differential diagnosis of TMA can be very difficult as symptoms are overlapping between conditions, thus it is important to rapidly identify cause of TMA and initiate appropriate treatment.

Clinical Cases: Differential Diagnosis of TMA

Professor Josep Campistol

Prof Josep Campistol presented the differential diagnosis of two clinical cases in order to illustrate the interesting but difficult aspects of identifying the cause of TMA. The first case was a 31-year-old female with no relevant medical background who consulted a community hospital for severe frontal headache that had lasted 1 week. She was admitted to the emergency room and found to be severely hypertensive, approximately 220/120 mmHg. The patient had severe and acute renal failure (ARF), with a serum creatinine of 3.9 mg/dL; severe anaemia, 9.5 g/L haemoglobin; haematocrit around 27%; increased lactate dehydrogenase (LDH: 1200 UI/ml); and also low platelets (~65x10^9/L). The patient was initially diagnosed with probable TMA and then transferred to the university hospital.

At the university hospital the hypertension had raised to 230/120 mmHg, with a Grade 3 hypertensive retinopathy. She was oliguric at that time, with a serum creatinine around 4 mg/dL and proteinuria on the dipstick. The haemoglobin and haematocrit levels were low, along with increased LDH, platelets around 60x10^9/L and importantly, negative Coombs with schistocytes in the peripheral blood. Transaminases were normal at that time, with normal aspartate aminotransferase (AST) test and alanine aminotransferase (ALT) test, so the finding was typical of a Grade 3 hypertensive retinopathy with some small haemorrhages. As the young female had severe ARF, haemolytic anaemia, and thrombocytopaenia, along with severe frontal headache and retinopathy, this presented the typical triad of TMA.

Rapid differential diagnosis of TMA needs to be performed as the therapeutic options differ depending on the cause.[21] For the patient case described, the three important differential diagnoses were TTP, aHUS, and a malignant hypertension-associated TMA. ADAMTS13 would be the initial test advised for the differential diagnosis of the patient, especially to define between TTP and aHUS that can be problematic in adult patients with TMA. As the ADAMTS13 test is sometimes not rapidly available, it has been suggested that a serum creatinine level and platelet count at presentation might help distinguish between aHUS and TTP. In general, TTP patients demonstrate serum creatinine lower than 2.2 mg/dL, a platelet count lower than 30x10^9/L, and antinuclear antibodies <2.26 mg/L.[22,23]

A renal biopsy was performed, and is generally recommended at a later stage in order to confirm the disease and define the prognosis, reversibility, and severity of the disease. However, there is often a high risk of performing the procedure in thrombocytopaenic patients, and the usefulness of the procedure is debatable at the diagnosis stage. The biopsy demonstrated typical TMA with a severe glomerular thrombin, severe endothelial proliferation, and severe kidney involvement with acute tubular necrosis (ATN) and vascular involvement. Inflammation, hypoxia, and ischaemia with the development of ATN were also apparent. Regarding the diagnostic merit of organ involvement, aHUS often affects kidneys (also 10-15% of TTP patients) but can involve other organs. So whilst there is generally an organ privilege, differential diagnosis of various forms of TMA cannot be based solely upon organ involvement.

The patient's ADAMTS13 test showed normal activity of 55%. As an ADAMTS13 activity value of <5% indicates TTP combined with the patient's creatinine value of 3.9 mg/dL and platelets >30x10^9/L, it was determined that the patient was at high risk of having aHUS. Whilst genetic analysis can be useful, treatment has to be initiated before the results are determined due to the time required for the analysis. The C3, C4, and factor H complement levels were shown to be normal, along with the factor H related proteins and the remaining genetic factors. A lower activity of factor I (60%) was identified and a mutation was later identified in factor I.

The patient was initially managed with PE over 6 months and showed a transient recovery of renal function, demonstrated by a serum creatinine concentration of 2.5 mg/dL. 2 months after cessation of dialysis the patient developed another episode

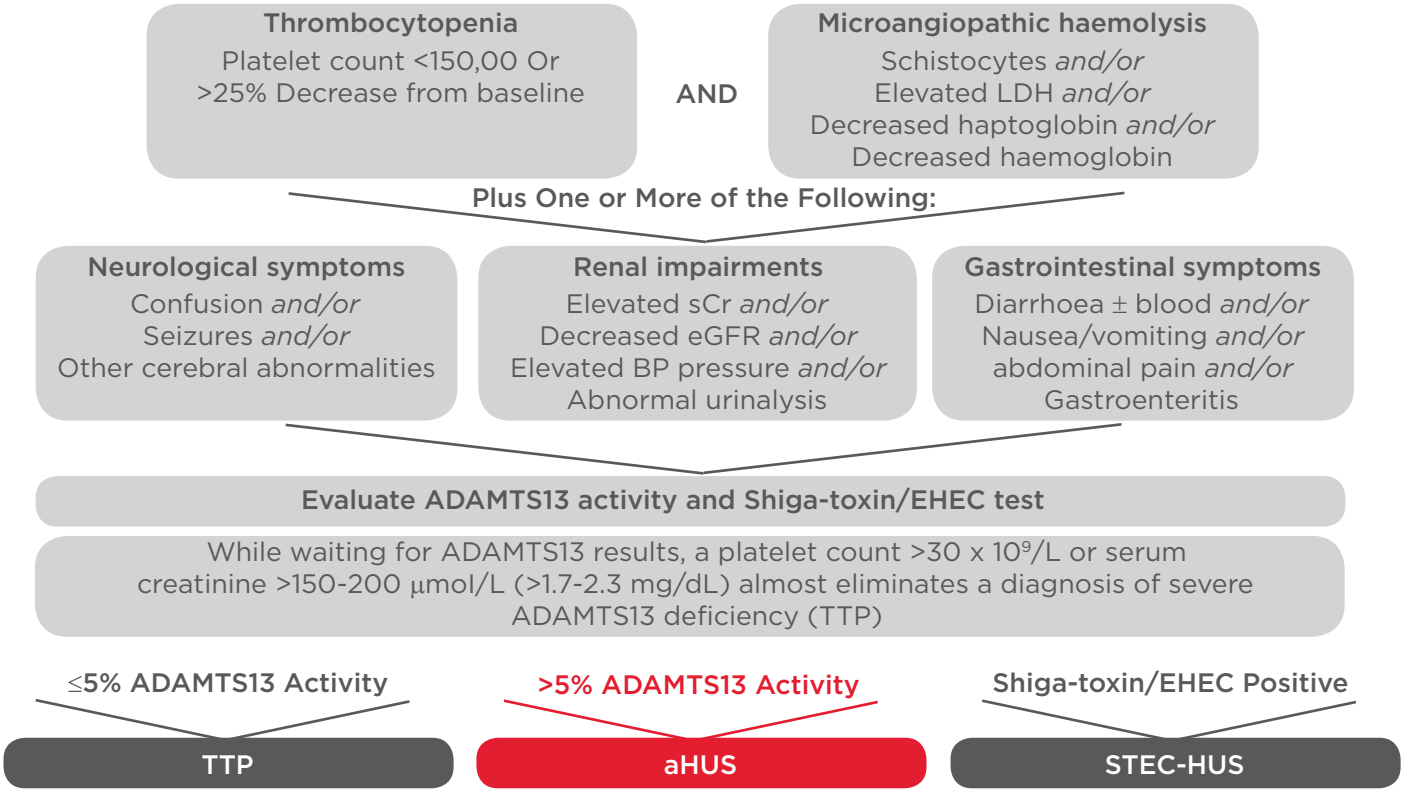

Figure 2: Differential diagnosis for TMAs: aHUS, TTP, and STEC-HUS.[2,17,34]
ADAMTS13: a disintegrin and metalloproteinase with thrombospondin Type 1 motif 13; aHUS: atypical haemolytic uraemic syndrome; BP: blood pressure; LDH: lactate dehydrogenase; sCR: serum creatinine; eGFR: estimated glomerular filtration rate; STEC-HUS: Shiga toxin-producing E. coli haemolytic uraemic syndrome; TTP: thrombocytopaenic purpura; EHEC: enterohaemorrhagic *E. coli*.
From Josep Campistol presentation at the Alexion Satellite Symposium.

of TMA with ARF, with a serum creatinine of 6 mg/dL, haemoglobin of 8 g/L, and platelets at 65x109/L. At that point the patient was started on eculizumab. 1 year after eculizumab initiation the patient was in good condition: renal function and haemoglobin were within normal values of 1.2 and 12.5 g/L, respectively, there was very mild proteinuria of 250 mg/24 hour, and a fairly normal platelet count of 175x10^9/L. Eculizumab was effective in reversing the second TMA with ARF as well as preventing further TMA in a patient with factor I mutation.

The second clinical case was a common but difficult situation; a 41-year-old female with post-partum TMA and no prior familial or personal relevant medical records. 4 days after her first uneventful birth in June 2012, the patient was admitted to the emergency room for asthenia, generalised oedema, and hypertension. ARF was indicated by high serum creatinine of 3.3 mg/dL that had been normal before delivery. Severe anaemia with haemoglobin lower than 5 g/L was also present, along with elevated LDH of around 3,000 UI/mL and severe thrombocytopenia as indicated by a platelet count of 35x10^9/L. The Coombs test was negative. There was presence of schistocytes in the peripheral blood and also normal AST/ALT, so the patient was initially diagnosed with TMA. A recent study has shown that in pregnant women who develop a TMA during pregnancy most will be affected with severe ADAMTS13 deficiency (TTP), whilst aHUS is the most prevalent form of TMA that presents after birth.[24]

After transfer to the university hospital the patient was still hypertensive (175/100 mmHg), with a serum creatinine of 6 mg/dL that indicated quite severe ARF, anaemia, and severe increase of LDH. Platelet count at presentation was >30x10^9/L and serum creatinine was above 2.2 mg/dL thus strongly indicating low risk of TTP. Results were negative for Shiga toxin and demonstrated a normal ADAMTS13 activity of 45%. A diagnosis of postpartum aHUS was given and the patient was started on eculizumab 48 hours after admission.

1 year later the patient was in good condition with a normal health status; normal blood function, haemoglobin, and platelets with negative proteinuria, which demonstrated the effectiveness of early initiation of eculizumab in a female patient affected with aHUS.

In summary, the algorithm of the differential diagnosis of TMAs, as shown in Figure 2, suggest that if there is thrombocytopaenia and microangiopathic anaemia, and evidence of organ damage like renal impairment, neurological symptoms, and/or gastrointestinal symptoms, the Shiga toxin test should be used to exclude the presence of STEC-HUS, whilst the ADAMTS13 test can differentiate between TTP (≤5% activity) and aHUS (>5% activity). If it takes a while for the results of the ADAMTS13 activity, consider that very severe thrombocytopaenia along with moderate renal failure can be indicative of TTP, whilst severe renal failure with platelets above $30x10^9$/L would indicate aHUS.

Management of aHUS in 2014

Professor Christophe Legendre

Previous treatment management guidelines for aHUS published in 2009 recommended PE or PI at presentation and during the first month of aHUS, for both children and adults.[25,26] After 5 days of daily PE, aHUS was considered resistant if the platelet counts were still below $150x10^9$,[3] or if serum creatinine had not decreased by >25% or the haemolysis was persisting. A French cohort study[6] reported a mortality rate of 8% in children (n=89) and 2% in adults (n=125) during the first year after aHUS diagnosis, of whom 39% and 80% received PE or PI at the first episode, respectively. However, incidence of end-stage renal failure or death at 5 years was 36% in children and 64% for adults, clearly indicating that the current treatment options were not very effective.

In 2011 eculizumab was the first approved treatment for aHUS in paediatric and adult patients.[27] Eculizumab is a monoclonal humanised anti-C5 antibody that selectively targets the terminal complement activation, C5, blocking the cleavage to C5a and C5b but leaving the proximal functions of the complement active[27-29] so that there is still weak anaphylatoxin action,[30] immune complex clearance, and microbial opsonisation.[29,31]

The eculizumab clinical development programme is extensive for an orphan disease, with 100 patients enrolled in prospective clinical trials.[32-39] Two prospective 26-week clinical trials enrolled adult and adolescent patients ≥12 years of age (study C08-003 enrolled 20 patients, study C08-002 enrolled 17 patients[32]).[36,37] Two additional prospective trials were in children (study C10-003, 22 patients)[33] and adults (study C10-004, 41 patients),[34] whilst study C09-001 generated retrospective data from 15 paediatric patients <12 years of age.[35] Patients from all the trials described could enlist on to the long-term follow-up study C11-003 for at least 5 years,[38] or will be monitored through the aHUS registry that is running at least until 2023.[36]

According to the inclusion criteria for the two pivotal prospective studies (C08-003 and C08-002) patients needed to receive prior PE/PI. Patients had either a long duration of aHUS and chronic kidney disease (CKD) as in study C08-003, or aHUS with progressing TMA as in study C08-002.[32,34] The third adult (≥18 years) prospective trial (C10-004) enrolled patients with a broad disease presentation, severe renal failure with no specification for PE/PI prior to enrolment. None of the three adult prospective trials required genetic mutations, polymorphism or presence of autoantibodies for inclusion, but the tests were performed during the trial by one central laboratory.

Eculizumab dosage regimen were 900 mg per week during the first month and then 1,200 mg fortnightly, whereas eculizumab dosages for patients <18 years of age are based upon body weight.[35] Administration is through an intravenous (IV) infusion over 35 minutes, with prophylaxis against meningococcal infection through prior vaccination and antibiotic treatment for 2 weeks after the vaccination if given alongside eculizumab. Dose adjustment every 12 days is permitted, with TMA complications seen in five patients (n=18) following a missed dose (four of whom were reinitiated with eculizumab).[35]

After 26 weeks of eculizumab treatment across the prospective C08 trials in adult and adolescent patients, there was overall normalisation of platelets, no need for new PE in any patient, and haematological normalisation in ≥88% of the population. Additionally, patients within the C08-003 trial who had a longer duration of aHUS along with CKD and had previously been managed with PE/PI still demonstrated improved estimated

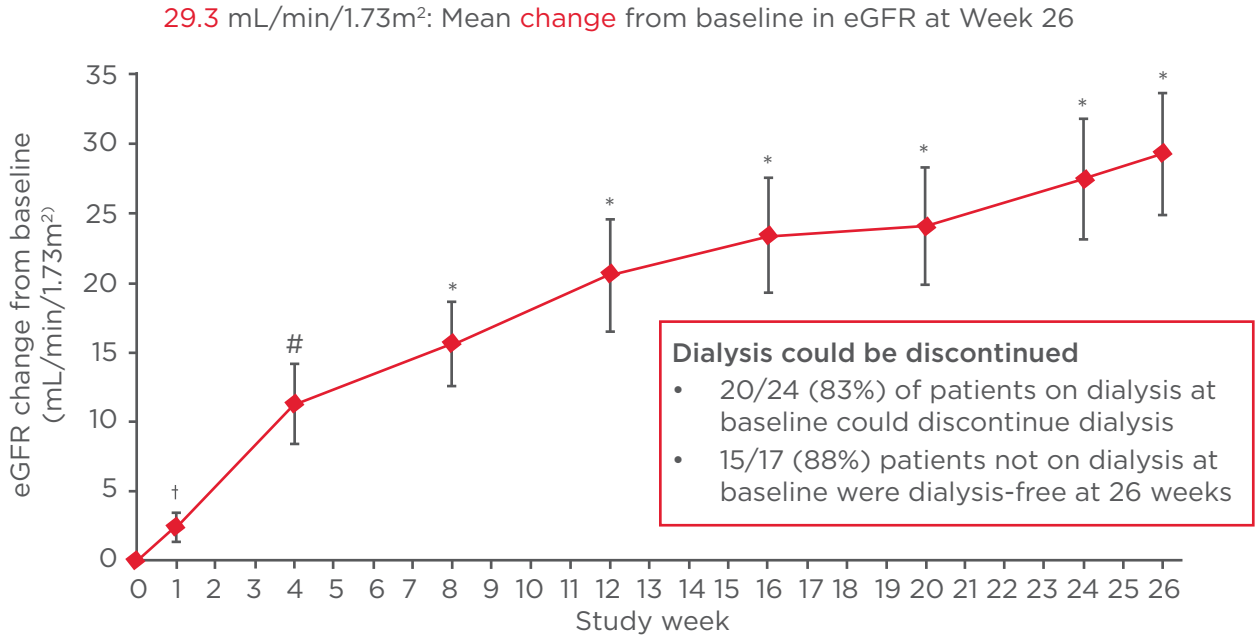

Figure 3: eGFR change from baseline (mL/min/1.73 m²) in patients on sustained eculizumab treatment.[36]
†p<0.05, #p<0.001, *p<0.0001.
eGFR: estimated glomerular filtration rate.
From Christophe Legendre, presentation at the Alexion Satellite Symposium.

glomerular filtration rate (eGFR), increasing by 6.1 and 7.2 mL/min/1.73 m² from baseline after 26 weeks and 2 years (median treatment duration 114 weeks), respectively.[32] Patients who had progressive TMA in the C08-002 trial also demonstrated improvements over 2 years, with an increased eGFR from baseline of 35.2 mL/min/1.73 m² after 2 years.[32] Patients treated within 1 month of clinical manifestation of TMA with eculizumab saw greater improvement in renal function compared with those treated between 1–4 months after TMA manifestation, whilst safety data demonstrated that the number of adverse events remained steady or declined with longer duration of eculizumab, along with no infection-related serious AEs. There was one death at 1.9 years of treatment, which was considered to be unrelated to eculizumab treatment.[32]

The most recent prospective study of a broad adult aHUS population (C10-004) also led to improvements in eGFR, including 83% (20/24) of patients on dialysis at baseline who discontinued dialysis during the 26 week period of eculizumab treatment (Figure 3).[34] Eculizumab was again well tolerated with mostly mild-to-moderate AEs, apart from two patients who had meningococcal infections, both of whom recovered (one patient continued in the trial without interrupting eculizumab). The primary and secondary endpoints were met by most patients, including 73% (30/41) of patients who had a complete TMA response (platelet count >150x10⁹/L and LDH within the upper limit of normal, together with preserved renal function demonstrated by <25% increase from baseline in serum creatinine) after a median of 56 days, whilst 88% (36/41) of patients demonstrated haematological normalisation (platelet count and LDH normalisation) after a median of 55 days and 98% (40/41) of patients showed platelet count normalisation after 8 days (median).[34] As well as the proven efficacy of eculizumab for restoring renal function and preventing further TMA,[32-40] numerous case reports have shown that eculizumab also seems to play a role in the rescue of CNS involvement (Table 1), as patients who reported seizures among other symptoms were reported to show full recovery after 1–56 days.[41-47]

Recommended management for the first episode of aHUS in paediatric patients is to use eculizumab as a first-line treatment in order to avoid PE and central catheterisation and maximise outcomes.[35] As differential diagnosis of aHUS may be more complex in adults presenting for the first time PE is recommended whilst TTP is ruled out. It is recommended to switch to eculizumab if there is plasma resistance (either no constant trend

Table 1: Results of eculizumab use in patients with aHUS and central nervous system involvement.[43-49]

Author	Age (years)	Neurological manifestations	MRI	Time to eculizumab initiation (days)	Outcome
Pu 2013	85	Seizures, mental disturbances	ND	18	Improvement over 2 weeks Full recovery
Salem 2012	66	Seizures, mental disturbances, coma	Focal Lesions	3	Awoke and verbal after 8 weeks Nearly complete recovery
Beye 2013	64	Status epilepticus, focal defects, nystagmus, confusion	Normal CTS	9	Improvement within 24 hours Full recovery
Ohanian 2011	50	Seizures, unresponsiveness	Right parietal infarction	3	Improvement after 1 week Full recovery
Chaudhary 2014	20	Seizures, lethargy	ND	42	Slow initial improvement (sub-therapeutic doses) Full recovery after increase of dose
Gulleroglu 2013	11	Seizures, visual loss, confusion	Bilateral occipital and posterior parietal hyperdensities/ oedema	<1	Improvement after 4 days Full recovery after 1 month
Gulleroglu 2013	6	Seizures, visual loss	Bilateral occipital and posterior parietal hyperdensities	<1	Normal vision within 24 hours Full recovery after 5 weeks
Hu 2013	1.7	Seizures, haemiparesis, lethargy, unresponsiveness	Subtle bilateral anomalies	<1	Improvement over 3 weeks Full recovery with residual weakness of right thumb/index

ND: no data; CTS: computerised tomography scan.
From Christophe Legendre, presentation at the Alexion Satellite Symposium.

upwards of platelet count, no constant trend downwards of LDH, or no decrease of serum creatinine by ≥25% after five daily PE) or if there is TMA during tapering of treatment. When the diagnosis of aHUS is unequivocal, such as in patients with familial history, TMA in a previously diagnosed patient, eculizumab can be used as first-line within 24 hours or as soon as possible after diagnosis. It is not necessary to confirm and define a complement anomaly before starting eculizumab as it can be administered for any type of complement mutation including those without any yet identified mutations. However, it is still advised to obtain the anti-factor H antibody results rapidly and use genetic screening to inform further decisions.[35]

In conclusion, eculizumab is licensed as first-line therapy for aHUS and allows normalisation of haematological outcomes, renal function recovery, and improvement of extra-renal TMA such as that described in Table 1.[18,32,34,35,42,44,46-49] Earlier treatment has also been shown to improve renal

outcomes.[32,50,51] Eculizumab is well tolerated, but prevention of meningococcal infection is mandatory and long-term follow up of eculizumab treatment has yet to be reported.[48] Withdrawal could be considered on a case by case basis, however currently we do not have data to inform our decision, thus treatment duration is still a matter of discussion.

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