Review

DOI: 10.5582/irdr.2014.01001

Current treatment of atypical hemolytic uremic syndrome

Bernard S. Kaplan*, Rebecca L. Ruebner, Joann M. Spinale, Lawrence Copelovitch

Division of Pediatric Nephrology, Department of Pediatrics, The Children's Hospital of Philadelphia, and The Perelman School of Medicine at The University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Summary **Tremendous advances have been made in understanding the pathogenesis of atypical Hemolytic Uremic Syndrome (aHUS), an extremely rare disease. Insights into the molecular biology of aHUS resulted in rapid advances in treatment with eculizumab (Soliris®, Alexion Pharmaceuticals Inc.). Historically, aHUS was associated with very high rates of mortality and morbidity. Prior therapies included plasma therapy and/or liver transplantation. Although often life saving, these were imperfect and had many complications. We review the conditions included under the rubric of aHUS: *S. pneumoniae* HUS (SpHUS), inborn errors of metabolism, and disorders of complement regulation, emphasizing their differences and similarities. We focus on the clinical features, diagnosis, and pathogenesis, and treatment of aHUS that results from mutations in genes encoding alternative complement regulators, SpHUS and HUS associated with inborn errors of metabolism. Mutations in complement genes, or antibodies to their protein products, result in unregulated activity of the alternate complement pathway, endothelial injury, and thrombotic microangiopathy (TMA). Eculizumab is a humanized monoclonal antibody that inhibits the production of the terminal complement components C5a and the membrane attack complex (C5b-9) by binding to complement protein C5a. This blocks the proinflammatory and cytolytic effects of terminal complement activation. Eculizumab use has been reported in many case reports, and retrospective and prospective clinical trials in aHUS. There have been few serious side effects and no reports of tachphylaxis or drug resistance. The results are very encouraging and eculizumab is now recognized as the treatment of choice for aHUS.**

Keywords: Alternate pathway of complement, atypical hemolytic uremic syndrome, DGPE deficiency, eculizumab, hemolytic uremic syndrome, *S. pneumoniae* hemolytic uremic syndrome, Cbl deficiency, thrombotic microangiopathy

1. Introduction

The hemolytic uremic syndromes (HUS) (*1,2*) as defined by Gasser *et al*. consist of the triad of acute hemolytic anemia with fragmented red blood cells (microangiopathic hemolytic anemia), thrombocytopenia, and acute kidney injury. The histopathological lesions in the kidneys and other organs are referred to as a thrombotic microangiopathy (TMA). The term "atypical" was first used to differentiate patients with diarrhea-associated HUS from those without prodromal diarrhea (*3*). We think that the term aHUS should now be used for the 10% of HUS cases not caused by inborn errors of metabolism, Shiga toxin *Escherichia Coli* (STEC) or *S. pneumoniae*. HUS (SpHUS). HUS is not a discrete entity but a group of conditions (*1*) with many causes and pathogenic mechanisms. Although it is now clear that HUS and thrombotic thrombocytopenic purpura (TTP) are completely different clinico-pathological entities, there are cases, more often in adults than in children that can be difficult to distinguish clinically (*4*). However, it is important to note that patients with TTP usually have ≤ 5% of normal ADAMTS13 levels (*5*). HUS may be may be acquired or inherited; some patients have an environmental trigger and a genetic mutation (Table 1).

The most important acquired causes of HUS include serotypes of *Enterohemorrhagic Escherichia coli* O157:H7 (*6*) and O104:H4 (*7*). Approximately 90% of

**Address correspondence to:*
Dr. Bernard S. Kaplan, The Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104, USA.
E-mail: kaplanb@email.CHOP.edu

Table 1. Causes of HUS

Acquired causes
E. escherichia coli 0157:H7; O104:H4 (*6,7*)
Shigella dysenteriae type 1 (*8*)
Invasive *S. pneumoniae* infection (*9*)
HIV infection (*10*)*
Cyclosporine, quinine, ticlopidine, clopidogrel (*10*)
Systemic lupus (*10*)
Malignancies (*10*)
Solid organ transplants (*10*)
Hematopoietic stem cell transplants (*11*)
Inherited causes
Atypical HUS with demonstrated mutations
Mutations in genes encoding complement regulators -CFH, CFI, and MCP (*12,13*)
Gain-of-function mutations in genes encoding complement activators C3 and complement factor B (*14*)
Mutations in thrombomodulin (*15*)
Antibodies to CHF - DEAP HUS (*16*)
Atypical HUS without demonstrated mutations - approximately 30% of cases
Inborn abnormalities of metabolism
Cobalamin C deficiency (*17*)
Methionine synthase deficiency (*18*)
Coenzyme Q (succinate coenzyme Q reductase (complex II) deficiency (*19*)
Folate deficiency (*20*)
Mutations in DGKE (encoding diacylglycerol kinase ε) (*21*)
Combined abnormalities
E. coli 0157:H7 and CHF, MCP mutations (*22*)
S. pneumoniae and CFI, CFH, THBD mutations (*23*)
Hematopoietic stem cell transplants (*11*)
CblC deficiency and CFH mutation (*24*)
Cisplatin-induced aHUS and a heterozygous *CD46* splice site mutation (*25*)

* Summarized in reference (*10*).

pediatric cases of HUS follow a diarrheal illness caused by shiga-like toxin producing bacteria [STEC] that result in STEC HUS (*4*). This was previously called diarrheal, D+ or typical HUS. The acute mortality rate is under 5% in children, and, although about 70% achieve full long-term recovery a small number progress to end-stage kidney disease (ESKD) (*26*). In contrast to aHUS, there is no known genetic basis for STEC HUS *per se* and STEC HUS does not typically recur before or after renal transplantation. However, a yet unknown number of patients with definite STEC HUS may also have CFH or membrane cofactor protein (MCP) mutations and therefore are at risk for post-transplant recurrences of HUS (*22*). In addition, the increasing evidence that the alternate pathway of complement may have a role in the pathogenesis of STEC HUS (*27*) is beyond the scope of this review.

2. *S. pneumoniae* HUS

Although SpHUS does not clearly fit under the rubric of aHUS, new studies may change this perception because complement dysregulation may be occurring in at least some cases of SpHUS (*23*). SpHUS (*9,28*) can be defined as acute hemolytic anemia, thrombocytopenia and acute kidney injury in a patient with invasive *S. pneumoniae* infection. The most accepted theory of the pathogenesis of SpHUS involves exposure of the Thomsen-Freidrenreich antigen (TF antigen) (*28*). Neuraminidase can also disrupt complement factor H (CFH) binding sites by desialyation. As a result, CFH cannot bind properly to C3 convertase on cell surfaces, leading to complement activation and cell injury (*29*). Abnormal CFH activity is another potential mechanism (*30*). Serotype 3 expresses Hic protein, a binding inhibitor for Factor H and serotype 2 produces pneumococcal surface protein C which may bind CFH and complement (*30*). Therefore, if CFH cannot inhibit the activity of the alternative complement cascade, there may be unchecked complement activation and cell damage. In addition, some patients also have mutations in complement factor I (CFI), CFH, and thrombomodulin (*THBD*) genes that result in severe complement dysregulation (*6*). Plasmapheresis theoretically removes a causative factor such as neuraminidase or anti-TF antibodies or replaces a deficient factor but there is insufficient evidence to support the use of plasmapheresis in SpHUS. There are no rigorous studies on the use of eculizumab in the treatment of SpHUS (*31*) although the theoretical risk of inhibiting complement activity in patients with invasive pneumococcal disease cannot be overlooked.

3. Inborn errors of metabolism

Inborn errors of metabolism complicated by HUS

(Table 1) are even more rare than aHUS and have a different pathogenesis. However, the report of chronic HUS associated with CblC deficiency as well as a mutation in the CFH gene indicates that both should be screened for in certain cases (*17*). The importance of differentiating these etiologies is exemplified in a report of Cbl deficiency associated with HUS in a 20-year-old (*32*). This is an informative case not only because of the age of presentation but also because eculizumab was used and Cbl deficiency was only diagnosed when he failed to respond to eculizumab.

CblC HUS disease: Inheritance is autosomal recessive inheritance. Usually presents in the neonatal period with vomiting, poor sucking, failure to thrive, lethargy, hypotonia, thrombocytopenia, microangiopathic hemolytic anemia and renal injury (*17*). It can also present during the first year of life, in childhood, and rarely in adults who have ataxia, cognitive impairment, and psychosis. CblC HUS may result from endothelial damage induced by hyperhomocysteinemia, impairment of the nitric oxide-dependent inhibition of platelet aggregation, or the procoagulant state of the endothelium leading to the formation of microthrombi. Blood homocysteine levels are high, urinary levels of homocystine and methylmalonic acid are increased, and there are increased propionylcarnitine levels and an increased C3/acetylcarnitine ratio. Treatment with parenteral hydroxycobalamin in combination with folic acid and betaine can reverse the renal injury (*17*).

Diacylglycerol kinase ε (DGKE) and HUS: Recessive loss-of-function mutations in the gene DGKE can cause HUS (*21,33,34*) not associated with activation of alternate complement pathway activation. Recessive mutations in DGKE were detected by exome sequencing in nine unrelated kindreds (*21*). Twenty-two percent of the siblings of the affected probands also had HUS and carried two affected alleles, demonstrating high penetrance. The HUS usually presented in the first year with multiple episodes and often progressed to ESKD by the second decade. DGKE mutations accounted for 27% of HUS cases in the first year of life and 50% of familial forms in this age group. Three patients developed nephrotic syndrome within 3 to 5 years of diagnosis (*33*). Renal biopsies showed chronic TMA, a membranoproliferative pattern, and podocyte foot process effacement, consistent with nephrotic syndrome. Of note, a membranoproliferative glomerulopathy also occurs in aHUS with the CFH mutation (*35*). Patients with DGKE mutations did not benefit from eculizumab or plasma treatments. Six patients had renal transplants without recurrences of HUS.

4. aHUS caused by dysregulation of the alternate complement pathway

aHUS is diagnosed by clinical and laboratory features and by the exclusion of other causes of HUS and TTP (Table 1). The incidence of aHUS is about 1 or 2 cases per 1,000,000/year. In the future it is likely that the term aHUS will be restricted to this subset of HUS caused by mutations in genes that regulate the alternate complement pathway. Most importantly, these are the patients that may benefit from eculizumab. Patients with aHUS present at any time of year, and may have had a family member with aHUS with or without a similar age of onset (*36-39*). They rarely have bloody diarrhea, often have an insidious onset, and tend to have severe arterial hypertension and a relapsing course. Extrarenal manifestations, mainly of the myocardium and central nervous system occur in a fifth of aHUS patients (*40-42*). Peripheral gangrene (*40*), gangrenous areas of the skin (*41*), retinal involvement, and cerebral artery stenosis and stroke (*42*) are serious but rare complications of aHUS. Most patients have an inexorable progression to ESKD. In those with a CFH mutation, 60-70% die or develop ESKD after their first presentation (*39*). The disease frequently recurs after renal transplantation (*43*).

Pathogenesis of aHUS: Acute and/or chronic uncontrolled dysregulation and/or excessive activation of the alternative pathway of complement is central to the pathogenesis of the sporadic and familial forms of aHUS (*12,13,44*). Activation of the complement cascade through the alternative pathway results in the generation of C3 convertase complexes that mediate the cleavage of C3 to C3a and C3b (*45*). The alternative pathway is initiated by deposition of preformed C3b on substrates such as bacteria and cell membranes, including erythrocytes. C3b is continuously available due to the interaction of C3 with water, a process called complement tick-over. C3b is necessary for the amplification and progression of the complement cascade through all pathways of activation and serves as a key immunoprotective and immunoregulatory molecule. The components of complement upstream of C5 are essential for microbial opsonization and immune complex clearance. All pathways of complement activation converge at the cleavage of the terminal complement protein C5 leading to the generation of molecules with pro-inflammatory and cell lytic properties. Targeted blockade at C5 with eculizumab therefore prevents the deleterious properties of terminal complement activation while preserving the immunoprotective and immunoregulatory functions of proximal complement (*46*).

The pathogenesis of aHUS is associated with dysregulation of the alternative complement pathway, with predisposing mutations, copy number variations, or polymorphisms in complement genes (*47*). Endothelial injury is also central to the pathogenesis of aHUS with exposure of the subendothelial matrix that becomes a target for complement activation. CFH from plasma may play a role in down-regulating complement activation on extracellular matrix and endothelial cells (*13*).

Approximately 70% of aHUS cases are associated with excessive complement activation in the microvasculature caused by known abnormalities affecting components of the alternative complement pathway (*13*). Normally, CFH, complement factor I (CFI), and membrane cofactor protein (MCP) regulate the activity of the C3 convertase on the cell surface and extracellular membranes and inhibit complement amplification. aHUS that is caused by loss-of-function mutations in CFH, MCP, and CFI are nonsense or missense mutations.

Mutations in the CFH gene occur in approximately 15-20% of aHUS patients, and autoantibodies against CFH are detected in approximately 10% of the patients (*48*). Most described mutations and autoantibodies affect short consensus repeats (SCR) 19-20 of CFH and disturb the physiological interaction of CFH with its ligands, in particular with C3b and endothelial cells (*49*). Five CFH-related genes (*CFHR1* to *CFHR5*) are located adjacent to the *CFH* gene on the long arm of chromosome 1 (*49*). This gene cluster is prone to rearrangements because of sequence homologies, and such genomic rearrangements may lead to hybrid genes or deletion of *CFHR1*, *CFHR3*, or *CFHR4*, all of which have been associated with aHUS. The deletion of CFHR1 and/or CFHR3 is strongly associated with the development of autoantibodies in aHUS. CFHR1 binds to C3b and C5 and regulates the C5 convertase and the terminal complement pathway. The CFHR1*B variant is associated with an increased risk for aHUS. The aHUS phenotype can result from antibodies to Factor H, but DEAP-HUS (deficiency of CFHR plasma proteins and factor H) refers specifically to the combination of an acquired autoantibody to Factor H and a genetic factor which, in most cases, is absence of the CFHR1 and CFHR3 proteins in plasma (*49*).

Pentraxin 3 (PTX3) is a soluble pattern recognition molecule expressed by endothelial cells and upregulated under inflammatory conditions. PTX3 activates complement, but it also binds CFH. Native CFH, factor H-like protein 1, and factor H-related protein 1 (CFHR1) bind to PTX3 and that PTX3-bound CFH and factor H-like protein 1 maintain their complement regulatory activities (*47*). PTX3, when bound to extracellular matrix, recruited functionally active CFH. aHUS-associated CFH mutations in the binding site caused reduced CFH binding to PTX3. Seven of nine analyzed anti-factor H autoantibodies isolated from aHUS patients inhibited the interaction between CFH and PTX3, and five autoantibodies also inhibited PTX3 binding to CFHR1. In addition, the aHUS-associated CFHR1*B variant showed reduced binding to PTX3 in comparison with CFHR1*A. Therefore, the interactions of PTX3 with complement regulators were impaired by certain mutations and autoantibodies affecting CFH and CFHR1. Kopp *et al* suggested that this could result in enhanced local complement-mediated inflammation, endothelial cell activation, and damage in aHUS (*47*).

The prevalence of CFH autoantibodies was studied in the Newcastle cohort of 142 aHUS patients (*50*). CFH autoantibodies were found in 13 individuals less than 11 years of age. In ten patients there were no copies of *CFHR1*, and in three patients there were two. In three patients with no copies of *CFHR1*, there was one copy of *CFHR3*, and these individuals had a novel deletion incorporating *CFHR1* and *CFHR4*. Mutations were identified in five patients: one in CFH, one in CFI, one in CD46, and two in C3. This emphasizes that multiple concurrent factors may be necessary in individual patients for disease manifestation. A high prevalence of deletions in CFH-related genes 3 and 1 (*delCFHR3-CFHR1*) and CFH autoantibodies were found in patients with HSCT-TMA; these were not detected in HSCT without TMA (*11*).

In addition to the inactivating mutations in genes encoding complement regulators (CFH, CFI, and MCP) there are also gain-of-function mutations in genes encoding the complement activators (C3 and complement factor B [CFB]) (*44*). aHUS mutations in CFB and C3 cause enhanced formation of C3 convertase or an increase in its resistance to inactivation by complement regulators. Mutations in the gene encoding thrombomodulin, a membrane-bound glycoprotein with anticoagulant properties that modulates complement activation on cell surfaces, also result in aHUS (*15*). About 20% of aHUS patients have mutations in more than one gene.

Clinical aHUS does not necessarily develop in all patients with a genetic mutation. Half of the family members with a mutation in one of the genes do not have clinical aHUS. Therefore, a second hit may be required to develop aHUS. This may be a trigger such as an infection, vaccinations, or pregnancy; or it may be an additional genetic variant (modifier) that increases the risk of developing the disease. Common at-risk genetic variants (single nucleotide polymorphisms and haplotype blocks) in *CFH*, *CD46*, and *CFHR1* might act as susceptibility factors for the development of aHUS. Therefore, the presence of a rare genetic variant (mutation), a common at-risk genetic variant (single nucleotide polymorphisms and haplotype blocks), and a trigger, may be necessary for the disease to occur. Modifiers such as CD46 and FHL-1 may determine the kidney phenotype of patients who present with homozygous CFH deficiency (*51*). On the other hand, patients with STEC HUS who do poorly or who have an unusual outcome such as a recurrence of anemia, thrombocytopenia, and neurological involvement may have CFH or other mutations (*22*).

Although the prognosis of aHUS is usually poor, some patients with the clinical features of aHUS but without known complement abnormalities have a favorable prognosis similar to diarrhea-associated disease (*52*). Patients with CFH mutations have the worst long-term prognosis with 73% progressing to

ESKD five years after diagnosis. For patients with *CFI* and *MCP* mutations, the percentage of ESKD is 50% and 38%, respectively. Thirty two percent of patients with aHUS with no identified genetic mutations progress to ESKD within five years (*53*). The post-transplantation recurrence rate is 76% in aHUS patients with CFH mutations, and 80% lose their grafts within one year of transplantation (*54*). Patients with CFI mutations also do poorly. Post-transplant recurrence occurs in 20% of patients with MCP-mutations. The risk of recurrences is not known for aHUS patients with CFB or C3 mutations. In the absence of identification of mutations in any of these genes, the risk of recurrence is 30%.

Supportive treatment of aHUS: Treatment includes blood transfusions, dialysis if indicated, and blood pressure control. Patients on dialysis may develop malignant hypertension, and bilateral nephrectomy may be needed to achieve blood pressure control in some of these patients. Traditionally, it is important to start plasma exchange (PE) or plasma infusion (PI) within 24 hours of diagnosis pending the results of mutation analysis (*55*). Many clinicians now advocate starting with eculizumab in cases in which the diagnosis of aHUS is more certain (a non-simultaneous family history of HUS, recurrent HUS, or hypocomplementemia at presentation). The rationale for PE and PI is to replace absent or mutated circulating complement regulators, such as CFH. However, the pathogenesis of HUS induced by the CFH mutation is incompletely understood. Many mutations are heterozygous, suggesting either a dominant negative effect or haplotype insufficiency. PI is likely to overcome the latter but not the former. PE also has the advantage of removing antibodies to CFH if they are the source of the problem. At least two patients with an isolated MCP (CD46) dysfunction have responded to plasma exchange (*56*). However, mutated CFB that permits excessive complement activation may be removed by PE. The exact frequency and duration of PE or PI are arbitrary and depend on the clinical responses.

The discovery of the abnormal molecular mechanisms that cause aHUS has enabled the development of a classification based on pathogenetic mechanisms as well as the clinical phenotypes. The genetic basis of aHUS may result in a new nomenclature, for example, CFH HUS, CFI HUS, DEAP HUS, *etc*. More importantly, the discovery of the molecular mechanisms has resulted in the use of eculizumab, a novel and specific therapy. Increased understanding of the molecular mechanisms responsible for the development of aHUS are the basis for the development of national and international guidelines for the investigation and treatment of this disease (*57*).

5. Management of aHUS (Table 2)

Eculizumab treatment of aHUS: Eculizumab (Soliris®, Alexion Pharmaceuticals Inc. Cheshire, CT, USA) is a humanized monoclonal antibody that inhibits the production of the terminal complement components and the membrane attack complex C5b-9 by binding to complement C5. This blocks the proinflammatory and cytolytic effects of terminal complement activation (*46*). Ideal outcome criteria for the use of eculizumab would be cessation of acute hemolysis, normalization of low platelet counts, stabilization or improvement in renal function, prevention of recurrences prior to and after renal transplant, reduction in mortality rate, normalization of complement proteins, and drug safety. Eculizumab treatment achieved all these outcomes in nearly all patients in the trials

Table 2. Current management of aHUS

Fresh frozen plasma and apheresis
While awaiting confirmatory tests
If TTP is a plausible diagnosis
Eculizumab
Treatment of choice if available
Liver and kidney transplant
If eculizumab is unavailable
May not be successful
Liver and kidney transplant plus short-term eculizumab
Precise criteria need to be established
If cost of long-term eculizumab is prohibitive

FDA approval of eculizumab for the treatment of aHUS was based on data from two prospective Phase 2 open-label clinical trials in adolescent and adult patients with aHUS, and a third retrospective study in children, adolescents, and adults with aHUS. Legendre *et al* (*58*) reported the combined results of two prospective trials. A total of 37 patients (17 in trial 1 and 20 in trial 2) received eculizumab for a median of 64 and 62 weeks, respectively. Eculizumab resulted in increases in the platelet count and 80% of the patients achieved a TMA event-free status. Eculizumab was associated with time-dependent increases in the estimated glomerular filtration rate (eGFR). Importantly, in trial 1, dialysis was discontinued in four of five patients. Furthermore, earlier intervention with eculizumab was associated with significantly greater improvement in the eGFR. Eculizumab was also associated with improvement in health-related quality of life. There were no cumulative toxicity of therapy, or meningococcal infections, through the follow-up period. Long-term eculizumab treatment over three years demonstrated ongoing inhibition of TMA with few side-effects in pediatric patients (*59*).

The largest prospective trial to-date of eculizumab in adult patients with aHUS was an open-label, single-arm, multinational trial that enrolled 41 adult patients (*60*).

Data from clinical trials were presented at the annual meeting of the American Society of Nephrology in 2013. Each trial demonstrated the clinical benefits

of eculizumab for the treatment of aHUS. There were no deaths in any of the trials during the duration of treatment. Two patients had meningococcal infections. Adverse events were headache, diarrhea and peripheral edema. The largest prospective trial of eculizumab was an open-label, single-arm, multinational trial of 41 adult patients (*60*). Eculizumab significantly improved renal function with a mean increase in eGFR from baseline of 29 mL/min/1.73 m^2. Most importantly, 20 of the 24 patients who were on dialysis at baseline discontinued dialysis by week 26.

A three-year update of the results of eculizumab in 20 aHUS patients with a long duration of disease and chronic kidney damage who had previously received prolonged PE/PI showed promising results. Despite long disease duration and CKD eculizumab led to improvements in hematologic and renal function over years (*61*).

We previously reviewed 32 case reports of eculizumab treatment of aHUS (*62*) to highlight important clinical observations that may be missed in epidemiological reviews. We reviewed the reports of 20 children 18 days to 17 years, and 12 adults 18 to 50 years. Twelve patients had no identified mutations or DEAP. Mutations were detected for CFH in 15, CFI in two, C3 in two, and MCP in one patient respectively. Eculizumab was given for up to 36 months. Renal function improved in four patients who received a single infusion of eculizumab, but each subsequently progressed to ESKD. Eculizumab was used in five cases aHUS relapse in native kidneys. In each case there was hematological recovery within 7-10 days. In three cases, there was also recovery in renal function, and these patients avoided dialysis. Eculizumab was continued as maintenance therapy. In the fourth case, there was improvement in renal function after a single dose of eculizumab. Eculizumab was used again in a subsequent relapse but there was progression to anuria, and eculizumab was stopped after hemodialysis was started. Eculizumab was used successfully in patients with aHUS refractory to, or allergic to plasma therapy. Eculizumab appeared to alter the course of a 28-day-old with aHUS refractory to plasma therapy. This infant was on mechanical ventilation and continuous renal replacement therapy and had multiple intestinal perforations and leg skin necrosis (*63*). Within 48 hours the patient recovered from acute kidney injury with complete hematologic remission and was disease-free after 14 months while on eculizumab, 300 mg every 3 weeks. No genetic cause was found for the aHUS.

Eculizumab has successfully treated post-transplant recurrences of aHUS (*64-66*). TMA was treated post-transplant in 14 patients and at the time of transplant in three cases. Eculizumab was used in a 34-year-old female who presented seven days post-simultaneous pancreas and kidney transplant with acute renal allograft dysfunction, thrombocytopenia, and microangiopathic hemolytic anemia. Renal biopsy revealed acute antibody-mediated rejection (AMR) and TMA. The clinical and laboratory manifestations partly responded to treatment with daily PE and intravenous immunoglobulin but resolved rapidly and completely on eculizumab (*67*). Eculizumab has been used pre-emptively for renal transplant in aHUS (*68*) and prophylactically after renal transplantation (*43,54,69*). The best results in terms of normalizing renal function were obtained when eculizumab was given as soon as possible after onset of aHUS (*43*). Eculizumab also successfully treated skin necrosis in aHUS (*41*).

The optimum maintenance doses and dosing schedules have not been determined, but severe aHUS was maintained in remission with sustained improved renal function on a reduced dose of eculizumab, 600 mg every 2 weeks (*70*). Eculizumab rescued a highly sensitized 13-year-old female who developed severe steroid-, ATG- and plasmapheresis-resistant AMR with TMA one week after a second kidney transplant despite previous desensitization therapy with immunoglobulin infusions (*71*).

Treatment of DEAP-HUS: There is no consensus regarding the optimal treatment of DEAP-HUS. DEAP-HUS is associated with a diarrheal prodrome in up to 53% of patients, and therefore initiation of appropriate therapies is frequently delayed. Despite delay in initiating plasma therapy, three cases all remitted with plasma therapy and normal renal function was restored (*16*). Long-term remission has also been achieved in cases of aHUS with anti-factor H antibodies treated with cyclophosphamide (*72*). Eculizumab rescue therapy resulted in a dramatic improvement in a patient deficient in the CFH-related protein 3/1 (CFHR3/1) involved in the pathogenesis of aHUS caused by CFH autoantibodies (*71*).

Eculizumab in pregnancy: Eculizumab was used safely in a 26-year-old woman with aHUS caused by a homozygous mutation in CFH who relapsed at 17 weeks of gestation. Eculizumab was started at 26 weeks, was well tolerated, induced a remission, and resulted in the delivery of a healthy neonate (*73*).

Safety information: Life-threatening and fatal meningococcal infections have occurred in patients treated with eculizumab. The prescribing physician must comply with the most current Advisory Committee on Immunization Practices (ACIP) recommendations for meningococcal vaccination in patients with complement deficiencies. Patients must be immunized with a meningococcal vaccine at least two weeks prior to administering the first dose of eculizumab unless the risks of delaying eculizumab therapy outweigh the risks of developing a meningococcal infection. Patients must be monitored for early signs of meningococcal infections and immediately evaluated and treated if infection is suspected. Bouts *et al* noted that according to the medication guide of the U.S. Food

and Drug Administration, a tetravalent unconjugated polysaccharide vaccine (serogroups A, C, Y, W135) must be provided at least two weeks before the first dose of eculizumab but that this approach is not sufficient for prevention in many countries, because none of the available vaccines contains a serogroup B antigen (*74*). A humoral immune response to conjugate Men C vaccination may be mounted and maintained despite chronic kidney disease, kidney transplantation, immunosuppressive drugs, and eculizumab. However, the authors stressed it was unclear whether serologically defined protective serum bactericidal antibody (SBA) titers mediate true protection from invasive *meningococcal* disease in an immunocompromised patient especially with treatment with a complement inhibitor, and that close monitoring of SBA titers seemed mandatory in their patient (*75*).

Dosing schedules and laboratory monitoring: Eculizumab is administered as an intravenous infusion. The recommended dosing for adult patients with aHUS is 900 mg weekly for the first 4 weeks, followed by 1200 mg weekly one week later, and 1200 mg every 2 weeks thereafter. The dosage regimen for pediatric patients is based upon body weight. Early signs of TMA include a decrease in platelet count and increases in serum LDH and serum creatinine levels. Patients should be followed for signs of TMA by monitoring serial platelet counts, serum LDH, and creatinine during eculizumab therapy and following discontinuation of eculizumab.

Adverse reactions: Administration of eculizumab may result in infusion reactions, including anaphylaxis or other hypersensitivity reactions. In clinical trials, no patients experienced an infusion reaction that required discontinuation of eculizumab. Eculizumab infusion should be interrupted and appropriate supportive measures should be instituted if signs of cardiovascular instability or respiratory compromise occur. The most frequently reported adverse reactions in aHUS single arm, prospective trials, (≥ 15% combined per patient incidence) were hypertension, upper respiratory tract infection, diarrhea, headache, anemia, vomiting, nausea, urinary tract infection, and leukopenia. The effect of withdrawal of anticoagulant therapy during eculizumab treatment has not been established. Therefore, treatment with eculizumab should not alter anticoagulant management.

Alternative therapies for aHUS: No other medications or monoclonal drugs are currently available for the prevention or treatment of aHUS. Genetic counseling should be offered. Pre-implantation diagnosis should be discussed. Plasma therapy, either with regular infusions of fresh frozen plasma and or PE has been the mainstay of treatment prior to the use of eculizumab (*76*) and may be helpful especially in patients with no identified complement mutation or in whom TTP is suspected (*5*). Plasma therapy has reduced the high mortality rate and prevented relapses but is far from optimal. There are no randomized controlled trials but there are guidelines for its use (*55*). Initial treatment with intensive PE should be initiated as soon as possible. The aim is to remove mutant complement proteins and factor H autoantibodies, and to provide fresh frozen plasma containing normal CFH as replacement fluid. Guidelines for chronic treatment are based on the risk of uncontrolled aHUS recurrence. The half-life of CFH is six days, and this provides a rationale for giving regular infusions every 1-2 weeks. Some patients become refractory to plasma, or have allergic reactions or have complications from the procedure. Renal transplant is associated with a very high incidence of recurrent aHUS and graft loss despite the use of plasma therapy (*43*).

Liver transplant alone or combined liver-kidney transplants have been used successfully in patients with aHUS, as CFH is synthesized in the liver. In contrast to the poor results of isolated kidney transplantation prior to eculizumab, the outcome of isolated liver or combined liver-kidney transplantation have been

Table 3. What is the optimum long-term treatment of aHUS?

Long-term eculizumab alone	Isolated kidney with chronic eculizumab	Combined liver – kidney transplant
Lower short-term risk	Lower short-term risk	Higher short-term mortality
Few known side-effects Long-term side-effects unknown	Long-term outcomes unknown	Long-term outcomes stable over 10-20 years
Long-term dependence on eculizumab to prevent recurrences of aHUS	Long-term dependence on eculizumab to prevent recurrences of aHUS	aHUS recurrence unlikely
No need for immunosuppressive agents	Complications of immunosuppressive agents	? Fewer complications of immunosuppressive agents
	Chronic rejection	? Reduced risk of chronic rejection
IV infusion every 2 weeks	IV infusion every 2 weeks	Better lifestyle with no infusions
Limited worldwide availability	Limited worldwide availability	More widely available But scarce liver plus kidney availability
Extremely expensive	Extremely expensive	Less expensive

Table 4. Summary of the value of eculizumab in aHUS

Positive effects
No deaths in any trial
Few serious side effects
Stabilizes hematological abnormalities
Can improve neurological abnormalities
Stabilizes renal abnormalities
Can reverse acute renal injury
Can improve eGFR
Patients may become dialysis-independent
Effective regardless of the type of detected mutation
Effective in aHUS without detected mutation
Effective in cases with DEAP
Prevents recurrent episodes pre-transplant
Prevents post-transplant recurrence
Rescues critically ill patients before and after renal transplant
Success in plasmapheresis-resistant AMR
Can be used safely in pregnancy
Negative effects
Rare cases of meningitis
Expensive
Not universally available

successful (*77*). It is likely that aHUS patients with CFI, CFB, or C3 mutations, which are also synthesized in the liver, could be treated similarly. However, combined liver-kidney transplantation may be associated with a poor outcome as a result of premature liver failure secondary to uncontrolled complement activation; concurrent treatment with eculizumab has overcome this problem (*78*). In fact, liver-kidney transplantation should not be carried out without a preparative regimen for complement regulation such as plasma therapy or eculizumab. All five children known to have undergone liver transplant without such preparation suffered fatal complications (*79*). Alternatively, a simple transplant with eculizumab therapy could be offered as a less invasive option. This concept was implemented by using eculizumab and PE in successful combined liver-kidney transplantation in a patient with CFH aHUS (*79*). The

Table 5. A tentative approach to investigation and treatment
(A). Neonates and infants

Causes	Investigations	Management
Alternate pathway of complement mutations	Serum C3 Serum CH50 (CHF, CHI, *etc*. if available)	Start plasma infusions immediately Start eculizumab as soon as possible If C3 is low and CH50 is high continue eculizumab and stop plasma
DGKe mutation	DGKe mutation if available	Stop plasma and eculizumab if no response (*81*) No specific treatment
Congenital TTP	ADAMTS13	Stop eculizumab if ADAMTS 13 < 5% Continue plasma infusions
CblC	Plasma amino acids Plasma homocystein Blood methylmalonic acid or plasma acylcarnitine	Stop plasma infusions and eculizumab if positive for CblC Low protein diet, betaine, methionine, and subcutaneous vitamin B12

(B). Children, adolescents and adults

Causes	Investigations	Management
STEC HUS (Management is clearer if there are severe bloody diarrhea and an epidemic of HUS)	Positive *E. coli* or shiga toxin – no further investigations	Dialysis if indicated No proven indications for plasma or plasmapheresis
TTP (Management is clearer if there is severe central nervous system disease and less severe renal disease)	ADAMTS13 ADAMTS 13 antibodies	Start plasma infusions and plasmapheresis immediately Continue plasma alone if ADAMTS 13 < 5% and negative for antibodies Continue plasmapheresis if ADAMTS 13 < 5% and positive antibodies to ADAMTS13
Alternate pathway of complement mutations (Management is clearer if there is minimal diarrhea, previously affected family member or a recurrence)	Serum C3 Serum CH50 (CHF, CHI, *etc*. if available)	Start plasma infusions immediately Start eculizumab as soon as possible If C3 is low and CH50 is high continue eculizumab and stop plasma Stop plasma once eculizumab is started Pre-emtive ue of eculizumab if a renal transplant is done See Table 3
DEAP HUS	Serum C3 Serum CH50 (CHF, CHI, *etc*. if available) Antibodies to CFH	Start plasma infusions immediately Start eculizumab as soon as possible If antibodies are detected, continue eculizumab and stop plasmapheresis
SpHUS (Management is clearer if there is invasive disease – pneumonia, meningitis)	Positive culture for *S. pneumoniae* Invasive *S. pneumoniae* infection	Dialysis if indicated

pros and cons of chronic treatment with eculizumab alone, kidney transplant plus chronic eculizumab treatment, and a combined liver and kidney transplant (with preparatory eculizumab treatment, are summarized in Table 3 (modified from *Ref. 80*).

Living-related kidney donation is contraindicated for aHUS patients with mutations in CFH, CFI, CFB, and C3 and in patients with aHUS in whom no mutations have been detected.

Conclusions

There has been demonstrable success of eculizumab treatment of aHUS in many case reports, and in retrospective and prospective clinical trials (Table 4). Eculizumab can stabilize hematological and renal abnormalities of aHUS. It is equally efficacious in aHUS cases regardless of demonstration of a detected mutation, but this statement needs to be tempered in cases of apparent aHUS who have an inborn error of metabolism. Eculizumab has also been efficacious in aHUS cases with DEAP.

The results of the prospective trials are increasingly encouraging. Patients with severe or chronic renal TMA have a poorer outcome than those with early and less severe TMA but may benefit from the drug. It is not established whether family members, without clinical evidence for aHUS but with a demonstrated mutation, should be given eculizumab. However, a compelling argument can be made for administering eculizumab to pre-symptomatic pregnant females with a known mutation or an affected family member. This also applies to a post-renal transplant patient with aHUS.

Traditionally, PE/PI was instituted immediately in all patients with an aHUS phenotype and this can still be done while awaiting the results of tests or availability of eculizumab. However, eculizumab should be started in patients with no response to PE/PI within a few days or in those cases in which the presumptive diagnosis of aHUS is more certain. The optimum doses and dosing schedules are established and it is apparent that long-term use is mandatory.

After much consideration we offer a tentative approach to the management of patients with HUS or TTP (Tables 5A and 5B). We recognize that eculizumab and sophisticated investigations are not universally available and that the cost of the drug may be prohibitive. We also recognize that some patients with STEC HUS (those with alternate pathway mutations) may benefit from eculizumab but this is beyond the scope of this review. Indications for eculizumab use in SpHUS need to be established. The discovery of the inherited cause of HUS caused by recessive mutations in DGKE (*11,12*) is extremely important because this condition does not respond to treatment with eculizumab. Equally important are the unknown number of cases of SpHUS (*18*), Cbl HUS (*29*) and STEC HUS (*16,51*) that are associated with mutations in alternate complement pathway regulating genes.

References

1. Kaplan BS, Drummond KN. The hemolytic-uremic syndrome is a syndrome. N Engl J Med. 1978; 298:964-966.
2. Gasser C, Gautier E, Steck A, Siebenmann RE, Oechslin R. Hemolytic-uremic syndrome: Bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. Schweiz Med Wochenschr. 1955; 85:905-909. (in German)
3. Barnard PJ, Kibel M. The haemolytic-uraemic syndrome of infancy and childhood. A report of eleven cases. Cent Afr J Med. 1965; 11:31-34.
4. Zheng XL, Wu HM, Shang D, Falls E, Skipwith CG, Cataland SR, Bennett CL, Kwaan HC. Multiple domains of ADAMTS13 are targeted by autoantibodies against ADAMTS13 in patients with acquired idiopathic thrombotic thrombocytopenic purpura. Haematologica. 2010; 95:1555-1562.
5. Scully M, Goodship T. How I treat thrombotic thrombocytopenic purpura and atypical haemolytic uraemic syndrome. Br J Haematol. 2014;164: 759-766.
6. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis. 1985; 151:775-782.
7. Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, Peters G, Karch H. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: A microbiological study. Lancet Infect Dis. 2011; 11:671-676.
8. Koster FT, Boonpucknavig V, Sujaho S, Gilman RH, Rahaman MM. Renal histopathology in the hemolytic-uremic syndrome following shigellosis. Clin Nephrol. 1984; 21:126-133.
9. Klein PJ, Bulla M, Newman RA, Müller P, Uhlenbruck G, Schaefer HE, Krüger G, Fisher R. Thomsen-Friedenreich antigen in haemolytic-uraemic syndrome. Lancet. 1977; 2:1024-1025.
10. Kaplan BS, Meyers KE, Schulman SL. The pathogenesis and treatment of hemolytic uremic syndrome. J Am Soc Nephrol. 1998; 9:1126-1133.
11. Jodele S, Fukuda T, Vinks A, Mizuno K, Laskin BL, Goebel J, Dixon BP, Teusink A, Pluthero FG, Lu L, Licht C, Davies SM. Eculizumab therapy in children with severe hematopoietic stem cell transplantation-associated thrombotic microangiopathy. Biol Blood Marrow Transplant. 2014; 20:518-525.
12. Buddles MR, Donne RL, Richards A, Goodship J, Goodship TH. Complement factor H gene mutation associated with autosomal recessive atypical hemolytic uremic syndrome. Am J Hum Genet. 2000; 66:1721-1722.
13. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009; 361:1676-1687.
14. Frémeaux-Bacchi V, Miller EC, Liszewski MK, *et al*. Mutations in complement C3 predispose to development of atypical hemolytic uremic syndrome. Blood. 2008; 112:4948-4952.
15. Delvaeye M, Noris M, De Vriese A, Esmon CT, Esmon

NL, Ferrell G, Del-Favero J, Plaisance S, Claes B, Lambrechts D, Zoja C, Remuzzi G, Conway EM. Thrombomodulin mutations in atypical hemolytic-uremic syndrome. N Engl J Med. 2009; 361:345-357.
16. Józsi M1, Licht C, Strobel S, Zipfel SL, Richter H, Heinen S, Zipfel PF, Skerka C. Factor H autoantibodies in atypical hemolytic uremic syndrome correlate with CFHR1/CFHR3 deficiency. Blood. 2008; 111:1512-1514.
17. Menni F, Testa S, Guez S, Chiarelli G, Alberti L, Esposito S. Neonatal atypical hemolytic uremic syndrome due to methylmalonic aciduria and homocystinuria. Pediatr Nephrol. 2012; 27:1401-1405.
18. Labrune P, Zittoun J, Duvaltier I, Trioche P, Marquet J, Niaudet P, Odièvre M. Haemolytic uraemic syndrome and pulmonary hypertension in a patient with methionine synthase deficiency. Eur J Pediatr. 1999; 158:734-739.
19. Micheletti MV, Lavoratti G, Gasperini S, Donati MA, Pela I. Hemolytic uremic syndrome and rhabdomyolysis in a patient with succinate coenzyme Q reductase (complex II) deficiency. Clin Nephrol. 2011; 76:68-73.
20. Watkins D, Schwartzentruber JA, Ganesh J, Orange JS, Kaplan BS, Nunez LD, Majewski J, Rosenblatt DS. Novel inborn error of folate metabolism: Identification by exome capture and sequencing of mutations in the MTHFD1 gene in a single proband. J Med Genet. 2011; 48:590-592.
21. Lemaire M, Frémeaux-Bacchi V, *et al*. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nat Genet. 2013; 45:531-536.
22. Alberti M, Valoti E, Piras R, Bresin E, Galbusera M, Tripodo C, Thaiss F, Remuzzi G, Noris M. Two patients with history of STEC-HUS, posttransplant recurrence and complement gene mutations. Am J Transplant. 2013; 13:2201-2206.
23. Szilágyi A, Kiss N, Bereczki C, Tálosi G, Rácz K, Túri S, Györke Z, Simon E, Horváth E, Kelen K, Reusz GS, Szabó AJ, Tulassay T, Prohászka Z. The role of complement in Streptococcus pneumoniae-associated haemolytic uraemic syndrome. Nephrol Dial Transplant. 2013; 28:2237-2245.
24. Guigonis V, Frémeaux-Bacchi V, Giraudier S, Favier R, Borderie D, Massy Z, Mougenot B, Rosenblatt DS, Deschênes G. Late-onset thrombocytic microangiopathy caused by cblC disease: Association with a factor H mutation. Am J Kidney Dis. 2005; 45:588-595.
25. Gilbert RD, Stanley LK, Fowler DJ, Angus EM, Hardy SA, Goodship TH. Cisplatin-induced haemolytic uraemic syndrome associated with a novel intronic mutation of CD46 treated with eculizumab. Clin Kidney J. 2013; 6:421-425.
26. Spinale JM, Ruebner RL, Copelovitch L, Kaplan BS. Long-term outcomes of Shiga toxin hemolytic uremic syndrome. Pediatr Nephrol. 2013; 28:2097-2105.
27. Noris M, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. Nat Rev Nephrol. 2012; 8:622-633.
28. Novak RW, Martin CR, Orsini EN. Hemolytic-uremic syndrome and T-cryptantigen exposure by neuraminidase-producing pneumococci: An emerging problem? Paediatr Pathol. 1983; 1:409-413.
29. Ault BH. Factor H and the pathogenesis of renal diseases. Pediatr Nephrol. 2000; 14:1045-1053.
30. Jarva H, Hellwage J, Jokiranta TS, Lehtinen MJ, Zipfel PF, Meri S. The group B streptococcal beta and pneumococcal Hic proteins are structurally related immune evasion molecules that bind the complement inhibitor factor H in an analogous fashion. J Immunol. 2004; 172:3111-3118.
31. Gilbert RD, Nagra A, Haq MR. Does dysregulated complement activation contribute to haemolytic uraemic syndrome secondary to Streptococcus pneumoniae? Med Hypotheses. 2013; 81:400-403.
32. Cornec-Le Gall E, Delmas Y, De Parscau L, Doucet L, Ogier H, Benoist JF, Fremeaux-Bacchi V, Le Meur Y. Adult-onset eculizumab-resistant hemolytic uremic syndrome associated with cobalamin C deficiency. Am J Kidney Dis. 2014; 63:119-123.
33. Ozaltin F, Li B, Rauhauser A, *et al*. DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. J Am Soc Nephrol. 2013; 24:377-384.
34. Quaggin SE. DGKE and atypical HUS. Nat Genet. 2013; 45:475-476.
35. Heinen S, Sanchez-Corral P, Jackson MS, Strain L, Goodship JA, Kemp EJ, Skerka C, Jokiranta TS, Meyers K, Wagner E, Robitaille P, Esparza-Gordillo J, Rodriguez de Cordoba S, Zipfel PF, Goodship TH. De novo gene conversion in the *RCA* gene cluster (1q32) causes mutations in complement factor H associated with atypical hemolytic uremic syndrome. Hum Mutat. 2006; 27:292-293.
36. Noris M, Caprioli J, Bresin E, *et al*. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010; 5:1844-1859.
37. Kaplan BS, Chesney RW, Drummond KN. Hemolytic uremic syndrome in families. N Engl J Med. 1975; 292:1090-1093.
38. Sullivan M1, Erlic Z, Hoffmann MM, Arbeiter K, Patzer L, Budde K, Hoppe B, Zeier M, Lhotta K, Rybicki LA, Bock A, Berisha G, Neumann HP. Epidemiological approach to identifying genetic predispositions for atypical hemolytic uremic syndrome. Ann Hum Genet. 2010; 74:17-26.
39. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011; 6:60.
40. Kaplan BS, Garcia CD, Chesney RW, Segar WE, Giugno K, Chem R. Peripheral gangrene complicating idiopathic and recessive hemolytic uremic syndromes. Pediatr Nephrol. 2000; 14:985-989.
41. Ardissino G, Tel F, Testa S, Marzano AV, Lazzari R, Salardi S, Edefonti A. Skin Involvement in Atypical Hemolytic Uremic Syndrome. Am J Kidney Dis. 2013; doi: 10.1053/j.ajkd.2013.09.020.
42. Ažukaitis K, Loirat C, Malina M, Adomaitienė I, Jankauskienė A. Macrovascular involvement in a child with atypical hemolytic uremic syndrome. Pediatr Nephrol. 2013.
43. Zuber J, Le Quintrec M, Sberro-Soussan R, Loirat C, Frémeaux-Bacchi V, Legendre C. New insights into postrenal transplant hemolytic uremic syndrome. Nat Rev Nephrol. 2011; 7:23-35.
44. Kavanagh D, Goodship TH, Richards A. Atypical hemolytic uremic syndrome. Semin Nephrol. 2013; 33:508-530.
45. Walport MJ. Complement. First of two parts. N Engl J Med. 2001; 344:1058-1066.
46. Rother RP, Rollins SA, Mojcik CF, Brodsky RA, Bell L. Discovery and development of the complement inhibitor eculizumab for the treatment of paroxysmal nocturnal

hemoglobinuria. Nat Biotechnol. 2007; 25:1256-1264.
47. Kopp A, Strobel S, Tortajada A, Rodríguez de Córdoba S, Sánchez-Corral P, Prohászka Z, López-Trascasa M, Józsi M. Atypical hemolytic uremic syndrome-associated variants and autoantibodies impair binding of factor h and factor h-related protein 1 to pentraxin 3. J Immunol. 2012; 189:1858-1867.
48. Kim JJ, McCulloch M, Marks SD, Waters A, Noone D. The clinical spectrum of hemolytic uremic syndrome secondary to complement factor H autoantibodies. Clin Nephrol. 2013.
49. Roumenina LT, Loirat C, Dragon-Durey MA, Halbwachs-Mecarelli L, Sautes-Fridman C, Fremeaux-Bacchi V. Alternative complement pathway assessment in patients with atypical HUS. J Immunol Methods. 2011; 365:8-26.
50. Moore I, Strain L, Pappworth I, Kavanagh D, Barlow PN, Herbert AP, Schmidt CQ, Staniforth SJ, Holmes LV, Ward R, Morgan L, Goodship TH, Marchbank KJ. Association of factor H autoantibodies with deletions of CFHR1, CFHR3, CFHR4, and with mutations in CFH, CFI, CD46, and C3 in patients with atypical hemolytic uremic syndrome. Blood. 2010; 115:379-387.
51. Wilson V, Darlay R, Wong W, Wood KM, McFarlane J, Schejbel L, Schmidt IM, Harris CL, Tellez J, Hunze EM, Marchbank K, Goodship JA, Goodship TH. Genotype/phenotype correlations in complement factor h deficiency arising from uniparental isodisomy. Am J Kidney Dis. 2013; 62:978-983.
52. Ruebner RL, Kaplan BS, Copelovitch L. A time for reappraisal of "atypical" hemolytic uremic syndrome: Should all patients be treated the same? Eur J Pediatr. 2012: 171:1519-1525.
53. Noris M, Bresin E, Mele C, Remuzzi G. In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K, editors. GeneReviews™ [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2013. (updated 2013 Aug 08)
54. Kavanagh D, Richards A, Goodship T, Jalanko H. Transplantation in atypical hemolytic uremic syndrome. Semin Thromb Hemost. 2010; 36:653-659.
55. Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C, Loirat C, Pecoraro C, Taylor CM, Van de Kar N, Vandewalle J, Zimmerhackl LB. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. European Paediatric Study Group for HUS. Pediatr Nephrol. 2009; 24:687-696.
56. Reid VL, Mullan A, Erwig LP. Rapid recovery of membrane cofactor protein (MCP; CD46) associated atypical haemolytic uraemic syndrome with plasma exchange. BMJ Case Rep. 2013; doi: 10.1136/bcr-2013-200980.
57. Bresin E, Rurali E, Caprioli J, Sanchez-Corral P, Fremeaux-Bacchi V, Rodriguez de Cordoba S, Pinto S, Goodship TH, Alberti M, Ribes D, Valoti E, Remuzzi G, Noris M; European Working Party on Complement Genetics in Renal Diseases. Combined complement gene mutations in atypical hemolytic uremic syndrome influence clinical phenotype. J Am Soc Nephrol. 2013; 24:475-486.
58. Legendre CM, Licht C, Muus P, *et al*. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013; 368:2169-2181.
59. Gaber AO, Loirat C, Greenbaum LA, Babu S, Furman RR, Sheerin NS, Cohen DJ, Eitner F, Delmas Y, Bedrosian CL, Legendre MC. Abstract: [SA-PO852] Eculizumab (ECU) Maintains Efficacy in Atypical Hemolytic Uremic Syndrome (aHUS) Patients (Pts) with Progressing Thrombotic Microangiopathy (TMA): 3-Year (Yr) Update.
60. Fakhouri F, Hourmant M, Campistol JM, *et al*. Eculizumab (ECU) inhibits thrombotic microangiopathy (TMA) and improves renal function in adult patients (pts) with atypical hemolytic uremic syndrome (aHUS). Presented at American Society of Nephrology (ASN) Kidney Week 2013, Atlanta, Ga., November 8, 2013. Abstract FR-OR057.
61. Delmas Y, Loirat C, Muus P, *et al*. Eculizumab (ECU) in atypical hemolytic uremic syndrome (aHUS) patients (pts) with long disease duration and chronic kidney disease (CKD): Sustained efficacy at 3 years. Presented at American Society of Nephrology (ASN) Kidney Week 2013, Atlanta, Ga., November 8, 2013. Abstract FR-PO536.
62. Kaplan BS, Ruebner RL, Copelovitch. An evaluation of the results of eculizumab treatment of atypical hemolytic uremic syndrome. Expert opinion Orphan Drugs. 2013; 2:167-176.
63. Ariceta G, Arrizabalaga B, Aguirre M, Morteruel E, Lopez-Trascasa M. Eculizumab in the treatment of atypical hemolytic uremic syndrome in infants. Am J Kidney Dis. 2012; 59:707-710.
64. Al-Akash SI, Almond PS, Savell VH Jr, Gharaybeh SI, Hogue C. Eculizumab induces long-term remission in recurrent post-transplant HUS associated with C3 gene mutation. Pediatr Nephrol. 2011; 26:613-619.
65. Chatelet V, Fremeaux-Bacchi V, Lobbedez T, Ficheux M, Hurault de Ligny B. Safety and long-term efficacy of eculizumab in a renal transplant patient with recurrent atypical hemolytic-uremic syndrome. Am J Transplant 2009; 9:2644-2645.
66. Larrea CF, Cofan F, Oppenheimer F, Campistol JM, Escolar G, Lozano M. Efficacy of eculizumab in the treatment of recurrent atypical hemolytic-uremic syndrome after renal transplantation. Transplantation. 2010; 89:903-904.
67. Chandran S, Baxter-Lowe L, Olson JL, Tomlanovich SJ, Webber A. Eculizumab for the treatment of de novo thrombotic microangiopathy post simultaneous pancreas-kidney transplantation – a case report. Transplant Proc. 2011; 43:2097-2101.
68. Nester C, Stewart Z, Myers D, Jetton J, Nair R, Reed A, Thomas C, Smith R, Brophy P. Pre-emptive eculizumab and plasmapheresis for renal transplant in atypical hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2011; 6:1488-1494.
69. Châtelet V, Lobbedez T, Frémeaux-Bacchi V, Ficheux M, Ryckelynck JP, Hurault de Ligny B. Eculizumab: Safety and efficacy after 17 months of treatment in a renal transplant patient with recurrent atypical hemolytic-uremic syndrome: Case report. Transplant Proc. 2010; 42:4353- 4355.
70. Ohanian M, Cable C, Halka K. Reduced dose maintenance eculizumab in atypical hemolytic uremic syndrome (aHUS): An update on a previous case report. Clin Pharmacol. 2011; 3:45-50.
71. Noone D, Al-Matrafi J, Tinckam K, Zipfel PF, Herzenberg AM, Thorner PS, Pluthero FG, Kahr WH, Filler G, Hebert D, Harvey E, Licht C. Antibody mediated rejection associated with complement factor

h-related protein 3/1 deficiency successfully treated with eculizumab. Am J Transplant. 2012; 12:2546-2553.
72. Sana G, Dragon-Durey MA, Charbit M, Bouchireb K, Rousset-Rouvière C, Bérard E, Salomon R, Frémeaux-Bacchi V, Niaudet P, Boyer O. Long-term remission of atypical HUS with anti-factor H antibodies after cyclophosphamide pulses. Pediatr Nephrol. 2014; 29:75- 83.
73. Ardissino G, Wally Ossola M, Baffero GM, Rigotti A, Cugno M. Eculizumab for atypical hemolytic uremic syndrome in pregnancy. Obstet Gynecol. 2013; 122:487-489.
74. Bouts A, Monnens L, Davin JC, Struijk G, Spanjaard L. Insufficient protection by Neisseria meningitidis vaccination alone during eculizumab therapy. Pediatr Nephrol. 2011; 26:1919-1920.
75. Zlamy M, Hofer J, Elias J, Vogel U, Frosch M, Jungraithmayr T, Zimmerhackl LB, Prelog M. Immunogenicity of meningococcus C vaccination in a patient with atypical hemolytic uremic syndrome (aHUS) on eculizumab therapy. Pediatr Transplant. 2012; 16:E246-E250.
76. Kim JJ, Goodship TH, Tizard J, Inward C. Plasma therapy for atypical haemolytic uraemic syndrome associated with heterozygous factor H mutations. Pediatr Nephrol. 2011; 26:2073-2076.
77. Saland JM, Ruggenenti P, Remuzzi G. Liver-kidney transplantation to cure atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2009; 20:940-949.
78. Weitz M, Amon O, Bassler D, Koenigsrainer A, Nadalin S. Prophylactic eculizumab prior to kidney transplantation for atypical hemolytic uremic syndrome. Pediatr Nephrol. 2011; 26:1325-1329.
79. Tran H, Chaudhuri A, Concepcion W, Grimm PC. Use of eculizumab and plasma exchange in successful combined liver-kidney transplantation in a case of atypical HUS associated with complement factor H mutation. Pediatr Nephrol. 2014; 29:477-480.
80. Saland J. Liver-kidney transplantation to cure atypical HUS: Still an option post-eculizumab? Pediatr Nephrol. 2014; 29:329-332.
81. Westland R, Bodria M, Carrea A, Lata S, Scolari F, Fremeaux-Bacchi V, D'Agati VD, Lifton RP, Gharavi AG, Ghiggeri GM, Sanna-Cherchi S. Phenotypic expansion of DGKE-associated diseases. J Am Soc Nephrol. 2014.

(Received January 10, 2014; Revised February 17, 2014; Accepted February 23, 2014)