The efficacy and safety of eculizumab in patients and the role of C5 polymorphisms

Hendrikus Bernhard Bouwman [1,*], **Henk-Jan Guchelaar** [2,*]

[1] Leiden University, Rapenburg 70, 2311 EZ Leiden, the Netherlands
[2] Clinical Pharmacy and Toxicology, Leiden University Medical Center, PO Box 9600, 2300 RC Leiden, the Netherlands

Hendrikus Bernhard Bouwman completed his Master's in the Bio-Pharmaceutical Sciences track at Leiden University and also holds a Master's degree in data science at Utrecht University. His Master's theses were on developing a differential expression analysis method and on modeling cross-resistance patterns in *Pseudomonas* species.

Henk-Jan Guchelaar is Professor of Clinical Pharmacy at the Leiden University Medical Center (LUMC) and the faculty of Math & Natural Sciences of the University of Leiden (LACDR). He is head of the department of Clinical Pharmacy & Toxicology of the LUMC and program leader of the research program 'Personalised Therapeutics'. Guchelaar is also a board member of division 4 of the LUMC.

Eculizumab is an orphan drug with indications for extremely rare autoimmune disorders. It is primarily prescribed for use in patients with paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome; but is also highly effective in the treatment of myasthenia gravis, among others. By binding to the C5 protein in the complement system, eculizumab effectively inhibits cellular hemolysis and autoimmune reactions. Despite this effective treatment, some patients reported no improvement in symptoms. Genetic sequencing revealed three distinct C5 mutations in the non-responders and these polymorphisms appeared to be most prevalent among Japanese, Korean and African populations. Here, we present an overview of the current and potential future applications of eculizumab, as well as the disadvantages of eculizumab treatment in patients with C5 polymorphisms.

Keywords: Eculizumab; C5; polymorphism; mutation; paroxysmal nocturnal hemoglobinuria; PNH; atypical hemolytic uremic syndrome; aHUS

Introduction

Developed as a recombinant humanized monoclonal antibody, eculizumab received its first FDA approval in 2007 under the trade name Soliris®.[(p1)] The drug received orphan drug status, because it is indicated for two extremely rare diseases: paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). In PNH, a genetic mutation leads to the undesired activation of the complement system, resulting in the destruction of red blood cells and other blood components. In aHUS, uncontrolled complement activation damages blood vessels, particularly in the kidneys, leading to severe kidney dysfunction. In addition to its original indications, eculizumab has expanded its use to other complement-system-mediated disorders such as neuromyelitis optica spectrum disorder (NMOSD) and myasthenia gravis (MG). NMOSD is an autoimmune disease that primarily affects the central nervous

* *Corresponding authors. Bouwman, H.B. (h.b.bouwman@umail.leidenuniv.nl), Guchelaar, H.-J. (h.j.guchelaar@lumc.nl).*

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system and MG is characterized by muscle weakness owing to autoantibodies. In both conditions, eculizumab has been shown to reduce disease activity and to improve patient quality of life.

Eculizumab's mechanism of action focuses on targeting the complement system. It does so by binding to complement protein C5, effectively inhibiting its cleavage into C5a and C5b. This, in turn, prevents the formation of the membrane attack complex (MAC), which is responsible for cell lysis and inflammation. By inhibiting this cascade, eculizumab halts the uncontrolled complement activation seen in PNH and aHUS, ultimately reducing the damaging effects of the immune system and alleviating the associated life-threatening complications. Although eculizumab demonstrated favorable responses in the vast majority of the patient population, an open-label pilot study in Japan (AEGIS) discovered a subgroup of patients that did not respond to eculizumab treatment. Genetic sequencing the non-responders revealed two polymorphisms in the C5 gene. The first polymorphism was found in 3% of the Japanese population, and the second polymorphism was found in one Argentinian patient of Asian descent. The polymorphisms in the C5 gene were located in the region where eculizumab binds, which renders eculizumab unable to bind to the C5 protein. Here, we present an overview of the current and potential future applications of eculizumab, as well as the disadvantages of eculizumab treatment in patients with C5 polymorphisms.

Indications and clinical use

Paroxysmal nocturnal hemoglobinuria

The primary and initial indication of eculizumab is the treatment of the life-threatening blood disease PNH. The prevalence of this ultra rare disease in the USA varied from 12 to 13 cases per 1 000 000 people, and its mean age of diagnosis was found to be 50 years.[p2] PNH is a complement-system-mediated disease that causes excessive hemolysis, caused by a deficiency in regulatory complement proteins.[p3] The vast majority of cases are caused by a somatic mutation in the *PIGA* gene of a hematopoietic stem cell, which is responsible for the synthesis of the cell surface protein anchor glycosylphosphatidylinositol (GPI).[p4] The absence of the GPI anchor protein results in a lack of inhibitory complement proteins CD55 and CD59, along with a variety of other proteins. CD55 functions by limiting the excessive cleavage of C3 in the complement system, and the reduction in this inhibitory protein results in the increased activation of downstream complement components. CD59 acts as a defense mechanism, inhibiting the formation of the MAC to protect cells from MAC-mediated damage. These proteins ensure precise immune responses while protecting healthy cells from unintended harm.[p5] In PNH patients, the deficiency of CD55 results in excessive C3 convertase proteins that then activate complement factors C3 and C5 and initiate the formation of MACs. The lack of CD59 results in the unchecked formation of MACs on the PNH erythrocytes and ultimately causes hemolysis of the blood cells.[p6],[p7] Although PNH is a chronic disease, the degree of hemolysis is often exacerbated by stress, trauma or inflammation.[p6]

Without adequate treatment of complement inhibitors, the 10-year survival rate of PNH patients is 65% and the main complications arising from the condition are pancytopenia and thrombosis. Corticosteroids reduce hemolysis in various patients, but this treatment causes long-term toxicity.[p8] In 2007, eculizumab was approved for treatment of PNH, and this has significantly improved patient survival rates up to a similar level of the survival rate of the general population.[p9],[p10] This high efficacy is achieved by the binding of eculizumab to the C5 complement which enables the blocking of the damaging complement system cascade.[p11] Treatment with eculizumab improves the hematological response significantly in 63% of patients, such that no blood transfusions are required anymore.[p12] Additionally, eculizumab was found to significantly improve renal function in PNH patients with chronic kidney disease (CKD) stages 1–4.[p13]

Atypical hemolytic uremic syndrome

The ultra-rare disease aHUS is a type of complement-mediated thrombotic microangiopathy (TMA), and it is primarily caused by dysregulation of the complement system. It is even rarer than PNH with a prevalence of five per 1 000 000 in the general population and, although the disease is found in all age groups, 40% of patients develop aHUS before the age of 18.[p14],[p15] In aHUS, the complement system is overactivated, leading to uncontrolled inflammation, blood vessel damage and a cascade of destructive events, particularly in the kidneys. Excessive activity of the immune system in aHUS emerges owing to a dysregulation in the complement system. The function of complement regulatory proteins can be compromised either through loss-of-function mutations in genes such as complement factor H (CFH), CFI, cluster of differentiation 46 (CD46) and thrombomodulin (THBD) or the function of the complement system can be stimulated by gain-of-function mutations in the CFB or C3 genes.[p16],[p17],[p18],[p19],[p20] The presentation of aHUS shares key clinical features with typical infection-related HUS, including microangiopathic hemolytic anemia, thrombocytopenia and acute kidney failure. However, aHUS can also lead to more-severe complications like CNS abnormalities, including altered consciousness, seizures or focal neurologic deficits, peripheral gangrene, dilated cardiomyopathy or cardiorespiratory arrest.[p16],[p19],[p21] Although aHUS is a rare condition, when it is triggered some common factors associated with its development include infections with H1N1, varicella and cytomegalovirus or diarrheal illnesses caused by a norovirus or bacteria.[p22],[p23],[p24] Other triggers include certain medications, cancer or pregnancy.[p16],[p25]

Historically, aHUS was treated with plasma exchanges or infusions and it was linked to high rates of morbidity and mortality.[p26] However, plasma exchanges do not affect the underlying problem, merely maintaining hematological parameters. In 2011, eculizumab was approved by the FDA and EMA for aHUS treatment and this significantly improved patient lives by inhibiting the underlying mechanism of aHUS. The first trials of eculizumab for aHUS observed up to 94% of trial participants discontinuing plasma exchanges or infusions, indicating a great improvement over historical treatment options.[p27] A prospective study of pediatric patients demonstrated that plasma exchanges or infusions were superfluous after eculizumab

treatment and 82% of the patients did not require renal dialysis anymore.[p21] Open-label studies with adult patients demonstrated similar results with significantly improved estimated glomerular filtration rate (eGFR) after 26 weeks and up to 79% of patients discontinuing kidney dialysis.[p28],[p29]

Myasthenia gravis

MG is a relatively rare autoimmune disorder affecting neuromuscular junctions. Its prevalence is estimated at 150 to 200 cases per 1 000 000 individuals worldwide.[p30] It commonly emerges in adulthood, with a bimodal age distribution in women peaking at 30 and 50 years old. The incidence of the disease in males constantly increases with age, with most cases between 60 and 89 years of age.[p31] MG is characterized by IgG autoantibodies that are targeting components of the neuromuscular junction, particularly acetylcholine receptors (AChR), muscle-specific kinase (MuSK) or lipoprotein-receptor-related protein 4 (LRP4).[p32],[p33],[p34] The main mechanism in AChR-antibody-positive (Ab+) patients is the binding of the antibody to the AChR protein causing the activation of the complement cascade, leading to the formation of the MACs, and ultimately damaging the postsynaptic membrane and degrading of the synaptic folds, including voltage-gated sodium channels.[p35] This leads to reduced postsynaptic potential and muscle fiber depolarization manifesting itself in muscle weakness and fatigue. The mechanism of MuSK Ab+ MG patients is different because MuSK antibodies are functionally monovalent, meaning that they cannot crosslink antigens of similar classes. This results in the MuSK antibodies not activating the complement system but merely blocking the phosphorylation of the protein and disrupting the Agrin-Lrp4-MuSK-Dok-7 signaling pathway.[p36] The mechanism of MG patients with anti-LRP4 antibodies is also suggested to operate by blocking MuSK activation; but because these antibodies belong to the IgG1 subclass they are also able to activate the complement system.[p37],[p38] Because the mechanisms of AChR Ab+ and LRP4 Ab+ subgroups of MG patients rely on the complement system activation, they can be successfully treated with complement inhibitors such as eculizumab. The severity of MG has been related to the level of C5a, indicating that a blockade of C5 can halt the complement cascade that disrupts the neuromuscular communication.[p39] Although the initial trials reported no significant improvement of the primary endpoint (MG-ADL scores), they found it improved symptoms and alleviated disease progression.[p40],[p41] Later studies discovered that treatment sustained for 2 years yielded fast and sustained improvements in muscle strength and quality of life of MG patients.[p42],[p43] The cessation of treatment should be treated with caution, because the severe worsening of symptoms has been observed after eculizumab discontinuation.[p44] Results from the REGAIN open study suggested that most MG patients demonstrated signs of improvements after 12 weeks but in some cases responses could only be observed with longer treatment.[p45]

AQP4-IgG+ neuromyelitis optica spectrum disorder

NMOSD is a rare autoimmune disease of the CNS characterized by severe attacks of inflammation and demyelination. It affects ~3.9–10.0 in 100 000 people and the disease is up to nine-times more common in women.[p46],[p47] NMOSD primarily affects the optic nerves and spinal cord, leading to severe visual impairment and paralysis. In recent years, the discovery of a specific biomarker, aquaporin-4 immunoglobulin G (AQP4-IgG), has revolutionized our understanding and diagnosis of this condition.[p48] AQP4-IgG antibodies target the aquaporin-4 water channel protein, which is predominantly expressed on the astrocytic endfeet in the CNS. This binding triggers an immune response and leads to inflammation and damage to astrocytes, oligodendrocytes and neurons.[p49] The formation of lesions in distinct brain regions with high AQP-4 expression is also one of the defining characteristics of AQP4-IgG+ NMOSD.[p50],[p51],[p52] The clinical course of NMOSD is highly variable, with relapses occurring unpredictably, and the triggers for these relapses can include infections, surgery or pregnancy. As a result, diagnoses rely on clinical presentation, neuroimaging and the presence of AQP4-IgG antibodies in the serum.[p53] Therapeutic strategies for NMOSD primarily aim to prevent relapses and manage symptoms.[p54] Acute attacks are often treated with high-dose corticosteroids and plasma exchange.[p55] Long-term maintenance treatment usually consists of immunosuppressive agents like rituximab, mitoxantrone and eculizumab and these have shown efficacy in reducing relapse rates and severity.[p56],[p57],[p58] Complement inhibitors are effective in the prevention of neuronal injury because AQP4-IgG has been demonstrated to activate the complement cascade.[p59] In PREVENT, a randomized double-blind phase III clinical trial, eculizumab reduced the symptoms and disease progression significantly in up to 96% of the patients. Only 3% of the patients experienced relapse compared with 43% in the placebo control group. In addition, patients reported no worsening of their disabilities as well as a significant increase in quality of life during the median follow-up period of 2.8 years.[p58],[p60],[p61],[p62]

Off-label uses

Besides the four abovementioned FDA and EMA licensed indications, eculizumab has been reported to be effective for several off-label uses, one of which being the treatment of severe COVID-19 cases. Severe COVID-19 infections led to extensive lung damage and organ failures and several parts of the complement system have been implicated in the pathogenesis.[p63],[p64],[p65] Several small-scale studies treated severe COVID-19 patients with eculizumab and reported significant improvements in respiratory symptoms and radiographic pulmonary lesions, chronic complications and mortality.[p66],[p67],[p68] These studies suggest the possibility of eculizumab treatment in COVID-19 patients with severe respiratory diseases. Besides COVID-19, eculizumab has been used in Phase II trials for the autoimmune disease Guillan–Barré. This rare and progressive disease causes damage to the peripheral nerves and their myelin insulation in a complement-mediated manner. As a complement inhibitor, eculizumab was tested in a small randomized controlled group of 34 patients. Although significant improvements were observed, the results did not reach the predefined clinical endpoints.[p69] Another off-label use of eculizumab is the treatment of C3 glomerulopathy, an ultra-rare disease caused by the dysregulation of the alternative pathway that ultimately leads to renal fail-

ure. Small-scale cohorts of C3 glomerulopathy that were started on eculizumab reported mixed results. One study in 2018 found stable outcomes for four out of seven patients, whereas a later study reported only a good response for five out of 11 patients.[p70],[p71]

Mechanism of action

The complement system is a crucial part of the innate immune system. It consists of a complex network of soluble proteins and membrane-bound receptors the primary functions of which are to recognize and eliminate pathogens, immune complexes and damaged host cells. Complement activation occurs through three distinct pathways: the classical, alternative and lectin pathways. Regardless of the initiation pathway, all pathways converge at one central event, known as the C3 convertase formation. The C3 convertase enzymatically cleaves the C3 protein into C3a and C3b. C3b further contributes to the formation of C5 convertases, which are then responsible for cleaving C5 into its active fragments: C5a and C5b. C5 is a pivotal protein in the complement cascade. Once activated, it undergoes enzymatic cleavage into C5a and C5b. C5a serves as a potent inflammatory mediator, attracting immune cells to the site of infection or injury. By contrast, C5b can associate with C6, C7, C8 and C9 to initiate the formation of the MAC, a multiprotein complex that can puncture and destroy cell membranes. Although this process is crucial for eliminating pathogens, atypical or uncontrolled complement activation can lead to extensive tissue damage.

Eculizumab, developed from the murine antibody 5G1.1, achieves its therapeutic effect by binding specifically to the C5 protein. This binding interaction is highly specific to the N-terminal region of the α-chain of the C5 protein. Specifically, eculizumab has been introduced to peptide fragments derived from the human C5 protein that contained the KSSKC motif (residues 879–883) within the alpha2-macroglobulin-like domain 7 (MG7). This motif is found distal to the location where the C5-convertase binds and proteolytically cleaves C5 into C5a and C5b.[p72] The structural basis of eculizumab binding was elucidated by X-ray crystal studies of C5 and it was suggested that the binding of C5 to the noncatalytic subunit of C5-convertase undergoing steric hindrance after eculizumab binding was the suggested mechanism for complement inhibition.[p73] Additionally, several polymorphisms in this region (residue 885) have been reported to significantly alter the efficacy of eculizumab, providing further evidence that the antibody binds in this region.[p74],[p75] The binding of eculizumab to this region prevents C5 from undergoing cleavage and activation, effectively neutralizing the complement protein.[p11]

By binding to C5, eculizumab disrupts the cascade of complement activation as can be seen in Figure 1. This prevents the formation of C5a, the potent inflammatory mediator. C5a normally attracts immune cells, such as neutrophils and macrophages, to the site of complement activation, leading to inflammation and potential tissue damage. Eculizumab's inhibition of C5a production thus helps dampen the excessive inflammation seen in complement-mediated diseases. Eculizumab's interaction with C5 also has another important consequence: it prevents the formation of the MAC. By inhibiting the cleavage of C5 into C5b, eculizumab effectively halts the initiation of the MAC assembly process, protecting host cells from destruction. Eculizumab has a very high specificity to human C5 (30–120 pM), with a much lower affinity for the protein of other species. This was suggested to be the result of a Trp917Ser substitution in a large variety of other species.[p73] Although eculizumab has a highly specific binding affinity for C5, some evidence suggests that it can alter the serum proteometabolome. The inhibition of C5 results in downregulated leukotrienes through arachidonate 5-lipoxygenase (ALOX5) modulation, which can result in altered regulation of immune cell activity and bronchoconstriction.[p76]

Pharmacokinetics

Because eculizumab is a monoclonal human antibody, it shares many kinetic characteristics with other antibodies. The administration of eculizumab occurs through weekly intravenous infusions of 25–45 min in the loading phase, followed by biweekly infusions in the maintenance phase.[p77] The mean volume of distribution was estimated to be in the range of 80–110.3 ml/kg, indicating that it is primarily distributed in the plasma.[p78],[p79],[p80] The mean concentration of eculizumab in the CSF was measured in a small sample and the ratio between serum and CSF was reported to be 5000:1, indicating that eculizumab does not easily cross the blood–brain barrier.[p58]

Eculizumab is an IgG antibody, and it is metabolized by continual nonspecific pinocytosis by endothelial cells, similar to other IgG antibodies. This process happens with bound and unbound C5 proteins in the plasma. After pinocytosis, eculizumab can either be degraded in the lysosomes or it can be recycled via the binding to the neonatal Fc receptor (FcRn). However, the eculizumab–C5 complex is probably not dissociated efficiently in the endosomes, pointing toward the primary metabolism through target-mediated drug disposition (TMDD).[p81] The EMA and FDA reported no known active eculizumab metabolites.[p1],[p77] The half-life of eculizumab varies significantly with studies reporting a range between 97 and 378 h.[p78],[p80] Because the molecular size of eculizumab is rather large (148 kDa) it is unlikely to be excreted in urine. However, in patients with high degrees of proteinuria significant levels of eculizumab (56 μg/ml) were measured.[p82],[p83] The clearance rate of eculizumab was estimated to be in the range of 0.13–0.31 ml/h/kg.[p78],[p79],[p80] Owing to the highly intra-individual variance of clearance and volume of distribution, it is recommended that the treatment plans are tailored to individual patients. Individualized approaches to eculizumab have been demonstrated to be cost-effective and patient friendly.[p79],[p80],[p84]

Adverse drug reactions

Although this therapeutic agent has demonstrated significant efficacy in managing rare and life-threatening conditions, it is not without its side effects. As a complement inhibitor, eculizumab targets the innate immune system and this leaves users of the drug susceptible to certain infections such as *Neisseria*, pneumonia and bacteremia.[p78],[p85] Eculizumab is generally well tolerated and safe and the most common side effects reported include hemoglobin decrease, fatigue, pyrexia, headache,

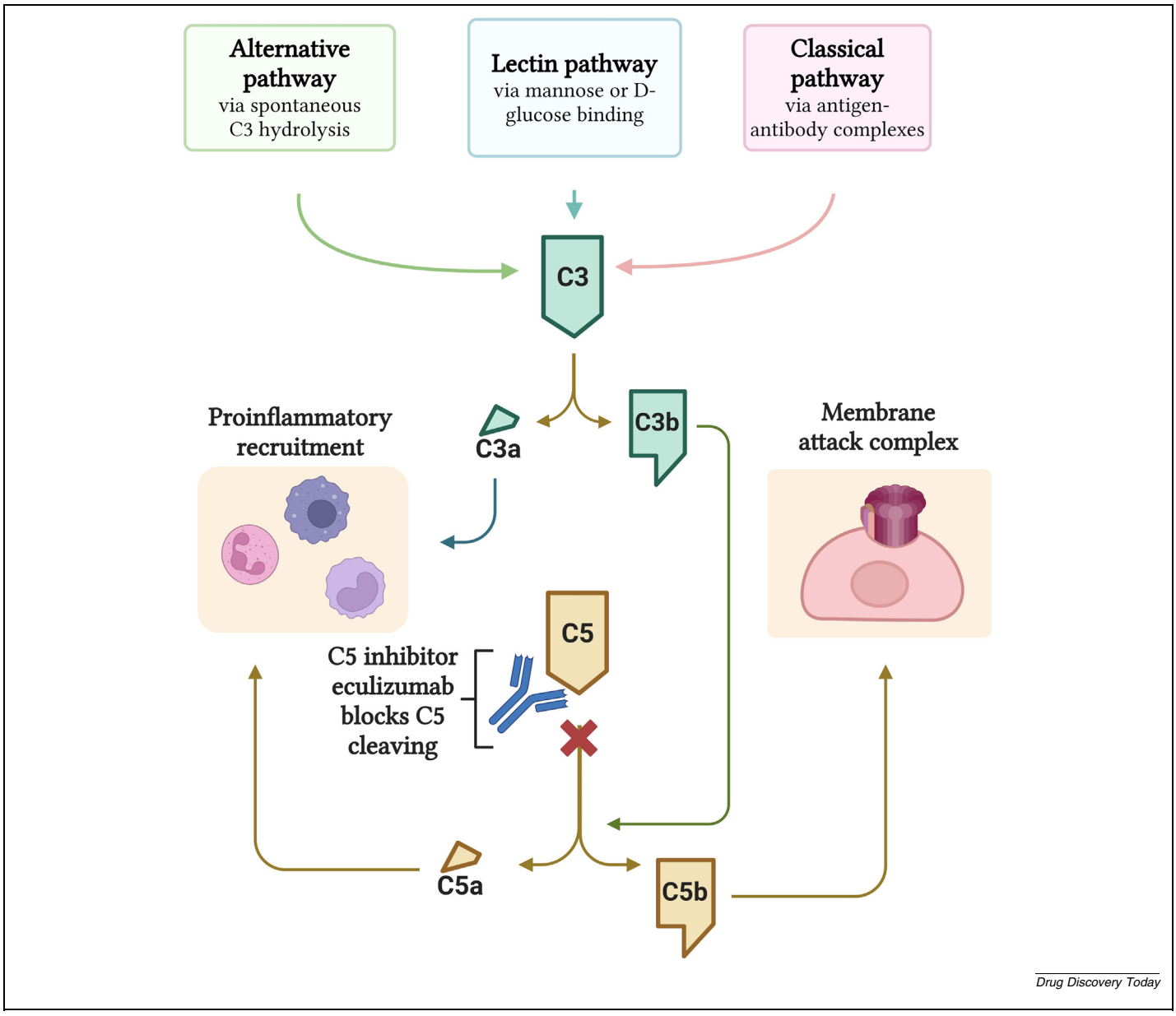

FIGURE 1

Eculizumab's mechanism of action. All three complement pathways activate C3 which is then hydrolyzed into C3a and C3b. Although C3a induces cytokines and attracts proinflammatory cells, C3b activates C5 convertase which cleaves C5 into C5a and C5b. Eculizumab binds to C5 so that cleavage into the subcomponents is prevented by steric hindrance. Ultimately, eculizumab inhibits the MAC formation resulting in the diminished hemolysis of cells. The figure was generated with BioRender.

decreased platelet counts, nausea and vomiting.[(p13),(p86),(p87)] An allergic infusion reaction was observed in one patient of the MG trial for eculizumab, necessitating the discontinuation of treatment.[(p78)]

One of the most serious side effects of eculizumab is the susceptibility to a variety of infections and the inhibited immune system results in a significant higher mortality rate in eculizumab users compared with non-users.[(p86)] Patients on complement inhibitors are especially at risk for encapsulated bacteria such as meningococcal strains or pneumonia because the complement system can be activated by the capsulated polysaccharides.[(p78),(p85)] The incidence of meningococcal disease caused by *Neisseria meningitidis* is up to 1000-times higher in eculizumab users and pairs with a high fatality rate, often despite being vaccinated with the meningococcal vaccine.[(p88),(p89),(p90)] The inhibition of the serum's bactericidal properties results from the inhibition of C5a release, which is needed for upregulation of the phagocytosis of meningococci.[(p91)] The *Neisseria gonorrhoeae* bacteria very rarely enter the bloodstream but in one case study they reported 89% of eculizumab patients with gonococcal infections resulting in disseminated gonococcal infections.[(p92),(p93)] Other infections caused by non-meningococcal and non-gonococcal *Neisseria* species are usually noninvasive and commensal but, in several

cases, they have been reported to hospitalize eculizumab users.[p94] Besides bacterial infections, the use of eculizumab also unexpectedly predisposed patients to systemic fungal infections; but a recent study suggests that C5 plays a crucial part in the antifungal defense through the regulation of C5a–C5aR1 signaling in phagocytes.[p86],[p95]

Hepatoxicity has been rarely reported in the adult population of eculizumab users but several cases have been reported in pediatric aHUS patients. Although hepatitis has been reported in 50% of patients with anti-CFH autoantibody-mediated aHUS, it has been associated with eculizumab dosing and not aHUS progression in five case reports of children.[p96],[p97],[p98] The mechanism for hepatotoxicity with eculizumab use has not yet been understood but the complement C5 has been reported to be important in liver regeneration and defense reactions of Kupffer cells and hepatocytes in animal models.[p99],[p100] Another indirect adverse effect caused by eculizumab treatment in PNH patients is hyperferritinemia. Recent reports have demonstrated an iron overload in the reticuloendothelial system (RES) of PNH patients treated with eculizumab.[p101],[p102],[p103] This is hypothesized to be caused by a decline in renal iron loss and due to ongoing extravascular hemolysis. Physicians should take into consideration that PNH patients on eculizumab will not require iron supplementation anymore after achieving stable and sufficient iron levels.[p104],[p105]

Genetic polymorphisms affect the response to eculizumab

Since the release of eculizumab, the vast majority of eculizumab users responded well to the treatment, but several studies have identified a small proportion of non-responders for MG, aHUS and PNH.[p45],[p74],[p106],[p107] Therapeutic proteins such as humanized recombinant molecules often elicit some degree of antibody response, which, in the case of eculizumab, could significantly impact the treatment efficacy.[p108],[p146] However, long-term studies of eculizumab users reported no clinically significant human anti-human antibody (HAHA) responses to treatment.[p109],[p110],[p111],[p112] More commonly, PNH patients do not achieve a complete response to eculizumab because of extravascular hemolysis (EVH).[p113] Eculizumab inhibits only the complement pathway at the C5 level, causing uninhibited opsonization by the selective binding of C3 particles to PNH red blood cells with phenotype CD59−. The variability in response to eculizumab could be explained by differences in expression of CR1 or factor-H-binding sites on red blood cells.[p114] Despite increased anemia and transfusion dependence, all patients with increased EVH still reported reduction in transfusion independence.[p105],[p114],[p115],[p116] Another mechanism for ineffective eculizumab treatment is resistance through C5 polymorphisms. Rare genetic variations in the C5 gene have been associated with a poor response to eculizumab.[p74],[p75],[p117],[p118]

In the AEGIS trial of 2011, researchers first reported two Japanese patients with remarkedly high lactase dehydrogenase (LDH), a marker for intravascular hemolysis, in the blood. The LDH concentration was unchanged despite sustained eculizumab concentration in the serum.[p119],[p120] A follow-up report by Nishimura *et al.* in 2014 sought to investigate the molecular basis for the poor response in Japanese PNH patients. The blood of the two patients in the AEGIS trial was further analyzed and hemolysis indeed remained exceedingly high, similarly to the LDH concentration predicted in the earlier report. An *in vitro* experiment demonstrated no discernable inhibition of hemolysis with serum eculizumab concentrations as high as 2000 μg/ml, where a healthy control and a good responder to eculizumab required only 6.25 μg/ml and 12.5 μg/ml for complete inhibition of hemolytic activity. Interestingly, the N19-8 antibody that binds to C5 in a different position to eculizumab demonstrated an inhibition of complement-mediated hemolysis at similar concentrations for the healthy control, as well as the good and poor eculizumab responders.

To identify the genetic variants responsible for the differences in treatment response, all 41 exons of the C5 gene were sequenced, after which they identified a single missense heterozygous mutation in exon 21. The mutation c.2654G→A, predicting p.Arg885His, was found in 11 poor-responding patients out of the 345 Japanese eculizumab users in the AEGIS study (3.2%), and in none of the control patients with good eculizumab response. A similar allele frequency was found in 288 healthy Japanese people (3.5%) but the mutation was found less frequently in Han Chinese (0.8%), British (0%) and Mexican (0%) populations. An Argentinian patient responding poorly to eculizumab treatment with a new mutation c.2653C→T (predicting p.Arg885Cys) was reported but this mutation was not detected in patients of Chinese Han, British or Mexican origin. The mechanism of poor response was confirmed by generating recombinant nonmutant human C5 (rC5) and mutant C5 (rC5m). Eculizumab blocked the classical pathway lysis with rC5 in C5-depleted serum and was not able to block the pathway in serum with rC5m as expected. Additionally, no detected binding of eculizumab to rC5m was reported even with concentrations up to 1 μM.[p74]

In 2015, Langemeijer *et al.* presented the first non-Asian case report of a 30-year-old Caucasian man with PNH who was started on eculizumab 4 years after diagnosis. Although the patient sustained sufficient drug levels and no HAHA antibodies were discovered in the serum, he still suffered from severe hemolysis with high LDH levels, extreme fatigue and muscle dystonia. The symptoms seemed to improve initially. However, several months into the treatment after an episode of extreme hemolysis, and one day after receiving 900 mg eculizumab, treatment was discontinued. The C5 exons were sequenced and, similarly to the AEGIS non-responders, a heterozygous mutation was found in the predicted eculizumab-binding region (c.2653C>A).

This missense mutation is at a different position as the polymorphism in the Japanese population but it codes for the same amino acid in the C5 protein, predicting p.Arg885Ser.[p74] This polymorphism was probably inherited from the father because it was reported in the DNA of the healthy father and not in his healthy mother. A CH50 assay was used to determine the total complement activity in serum and this confirmed *in vitro* the diminished response to eculizumab when compared to a control with nonmutant C5. In the non-responding patient doses of 30 μg/ml and higher resulted in >70% decrease in CH50 concentrations compared to the control. The serum of the normal control patient resulted in a complete inhibition of complement activity at eculizumab concentration of 30 μg/ml and higher, suggesting that this polymorphism still enables some limited eculizumab binding and complement inhibition. After the failed treatment of eculizumab, the patient enrolled in a Phase II study of coversin with successful results (ClinicalTrials.gov, NCT03427060).[p75],[p121]

Another case study by Goodship *et al.* in 2017 reported a 1-year-old male child with hematopoietic stem cell transplantation-associated thrombotic microangiopathy (HSCT-TMA). The patient was started on eculizumab because a significant portion of HSCT-TMA patients will have factor H autoantibodies, which has a well-established connection with aHUS.[p122],[p123] The treatment was stopped after no clinical improvements were reported after 10 weeks, after which DNA samples were screened for mutations. Together with a heterozygous CFH mutation associated with familial aHUS they reported a mutation in p.Arg885His, previously found in the Japanese population.[p74] Following the results of the DNA screening of the parents, they discovered that the mutation was inherited from the patient's healthy father. The CH50 assay demonstrated that the complement activity was only 35% of normal after 2 weeks of eculizumab treatment. Consequently, this patient was started on coversin and initially responded well to treatment (similar to the case report by Langemeijer *et al.*) but eventually died after discontinuing coversin owing to limited supply.[p75],[p117]

Finally, an Israeli poor-responder to eculizumab was reported to have a novel C5 mutation c.2422 G>A, which predicts p.Val808Ile, but it is currently unclear whether this mutation affects eculizumab's efficacy.[p124] Although the C5 polymorphisms leading to no eculizumab response were discovered in higher frequency, population studies of eculizumab users reported no significant differences in drug efficacy between Asian and non-Asian patients.[p125],[p126]

Besides the reported C5 mutations that influence the eculizumab response, Chen *et al.* described two mutations that influence the functioning of C5. The rs17611 C>T mutation in C5 was linked to increased C5a production, which might require an increase in eculizumab dosage to curb the increased complement activation. The rs2269067 GG genotype has been associated with increased C5 serum concentrations. Interestingly, this mutation did not influence C5a concentrations and it was associated with a diminished susceptibility to sepsis, suggesting an anti-inflammatory effect that might require lower eculizumab doses in patients with this mutation.[p127],[p128] Several mutations leading to functional C5 deficiency in people with African heritage have been reported in literature.[p129],[p130],[p131] These loss-of-function mutations are associated with increased infections and, because they cause a natural deficiency in C5, it is expected that these mutations confer an inherent protective effect against diseases that require eculizumab treatment.

Since the release of eculizumab, multiple noninferior C5 inhibitor alternatives have been developed and released for such as ravulizumab and zilucoplan.[p118],[p132] Because these therapies have been approved for some of the four main indications mentioned, we will briefly go over the effects of C5 polymorphism in ravulizumab and zilucoplan. In 2019, a fourth report of a patient with the c.2654G→A mutation was reported by Lee *et al.* in an open-label Phase III study comparing the effects of eculizumab and ravulizumab – a new C5 inhibitor. The comparison study employed 121 participants for eculizumab and 125 for ravulizumab for a total of 246 participants. In the subgroup of 129 Asian participants, one patient from South Korea presented with the p.Arg885His heterozygous missense mutation that caused poor eculizumab binding. Although the patient was in the ravulizumab group, there was no clinical response reported, suggesting that ravulizumab binds to a similar C5 region as eculizumab.[p118] Zilucoplan is a C5 cleavage inhibitor that has been recently indicated for MG. It has been shown to inhibit hemolysis induced by the p.Arg885His C5 variant which rendered eculizumab ineffective, making this a suitable treatment for gMG patients with C5 polymorphisms.[p133]

In total, three different types of C5 polymorphisms have been discovered that bring about poor responses to eculizumab treatment. The exact mechanism leading to the poor eculizumab response has not been studied in detail yet; but in 2016 Schatz-Jakobsen *et al.* suggested a possible mechanism for this phenomenon. They generated a Fab fragment identical to the sequence present in eculizumab and allowed binding with C5 to generate the Fab–C5 complex. The Fab–C5 complex was purified and then crystallized to generate the X-ray diffraction data needed to elucidate the structure of the complex. They found that the poor response of patients with an Arg885His or Arg885Cys polymorphism could be caused by a disruption in the binding location of C5 and eculizumab. The histidine or cysteine groups are probably too small to fill the arginine-binding pocket of eculizumab, leading to a loss of affinity. Additionally, the arginine is located close to a disulfide bridge resulting in a possibly perturbed disulfide bridge pattern owing to the Arg885Cys polymorphism. The altered disulfide bridge can disturb the MG7 domain integrity.[p73]

Population characteristics of common polymorphisms affecting eculizumab response

Various databases contain genetic variation data of healthy patients in a large variety of populations and can be used to estimate allele frequencies of the three known C5 polymorphisms in different populations and might help to decide whether or not testing for the C5 polymorphism should be considered (Box 1). The 1000G and PAGE databases contain genetic variation data from random populations whereas the ALFA and GnomAD databases contain aggregated genetic variation data from a variety of studies, with computed allele frequencies for several population groups. Additionally, the KRGDB, Korea1K, 54KJPN and KOVA databases sampled only participants from Japanese or Korean populations to analyze genetic variants in specific population groups. Finally, Nishimura *et al.* investigated C5 polymorphisms in a small group of DNA samples of Japanese, Chinese, British and Mexican origin. The allele frequency data were summarized and the weighted average was computed based on the sample sizes of the respective studies and is presented in Table 1.[(p74),(p134),(p135),(p136),(p137),(p138),(p139),(p140),(p141)] Surprisingly, the Finnish and African population have relatively high frequencies C5 polymorphisms even though no signals of poor response to eculizumab in patients from these populations have been reported. Given the low genotype frequencies found in the population and the fact that all patients that responded poorly to eculizumab were found to have only heterozygous mutations, the phenotype frequencies will be close to twice the genotype frequencies. In this review, the phenotype frequencies will be assumed to be the twofold multiplication of the genotype frequencies. All genotype frequencies with their sources can be found in Table A1.

Box 1 Genetic testing for C5 polymorphisms could be financially beneficial and improve patient care: a short consideration of the benefits of predictive testing in eculizumab users.

Given the high cost of eculizumab treatment, genetic screening for C5 mutations that render treatment ineffective could be cost-effective in certain populations. To date, no pharmacoeconomic studies have considered the economic benefits or cost–utility of predictive testing. Given the high treatment costs for eculizumab of up to €500 000 per year and a relatively long treatment time before switching to another treatment of up to 3 months, it could be financially feasible to start genetic screening in certain populations (EMA, 2023). Complications can arise during the ineffective treatment, leading to potential increased costs owing to hospital stays. Episodic crises are common in PNH, aHUS, MG and AQP4+ NMOSD patients and the first few weeks of treatment can be crucial to avoid complications that can lead to myocardial scars, organ failure, permanent disabilities and death.[(p13),(p142),(p143),(p144)] As a result, genetic testing should not cause treatment delays because this will also be harmful for the patient. The economic benefits depend on the cost of alternative treatments, however, and currently the alternative treatments such as pegcetacoplan are similar in price to eculizumab.[(p145)] Another factor that could negatively influence the benefits of genetic screening is that the eculizumab user group is small owing to the rarity of the indicated diseases. Even if it is financially beneficial, it might not be worth changing hospital treatment policies when the likelihood of patients with C5 mutations is so limited.

TABLE 1

Aggregated allele frequencies for each of the three C5 polymorphisms linked to a poor eculizumab response in literature: the allele frequencies are aggregated with the study sample size weighted average

Population	p.Arg885His (%)	p.Arg885Cys (%)	p.Arg885Ser (%)
Global	0.0385	0.0191	0.000697
African	0	0.0211	0
East Asian	0.428	0	0
Japanese	1.83	0	0
Korean	1.28	0	0
European	0.00515	0.000962	0.00145
Finnish	0.00847	0.00282	0
Latino	0.00847	0.00282	0

Concluding remarks

Eculizumab is a highly effective and innovative drug in patients with PNH, aHUS, MG and AQP4+ NMOSD. Despite the high overall effectiveness of this drug, certain populations were discovered to have missense mutations in the C5 gene that led to a poor eculizumab response. Here, all known cases of C5 mutations in literature that were linked to a poor eculizumab response were identified. Furthermore, the allele frequency data were aggregated from all major public genome databases for the three known C5 polymorphisms that led to ineffective eculizumab treatment: p.Arg885His, p.Arg885Cys and p.Arg885Ser. Albeit relatively rare, these mutations have the highest prevalence in the Japanese and Korean populations. Interestingly, two more populations were identified with relatively high C5 mutations leading to poor eculizumab response that have not been reported in literature yet, namely the Finnish and African ethnicities.

Conflicts of interest

All authors declare that they have no conflicts of interest with any organization or entity with any financial interest in the subject matter or materials discussed in this manuscript.

CRediT authorship contribution statement

Hendrikus Bernhard Bouwman: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization, Writing – review & editing. **Henk-Jan Guchelaar:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization.

Data availability

The data was obtained from public sourced that are referenced in the article.

Appendix

Table A1
An overview of the population frequency data used to generate Table 1

Study	Reference	Mutation	Population	Count	Size	Frequency
GnomAD	GRCh37	Arg885His	asian_east	75	19952	0.003759
GnomAD	GRCh37	Arg885His	japanese	4	152	0.026316
GnomAD	GRCh37	Arg885His	korean	47	3816	0.012317
GnomAD	GRCh37	Arg885His	european	9	154322	5.83E−05
GnomAD	GRCh37	Arg885His	finnish	3	25124	0.000119
GnomAD	GRCh37	Arg885His	latino	3	35436	8.47E−05
GnomAD	GRCh37	Arg885Ser	european	1	113764	8.79E−06
GnomAD	GRCh37	Arg885Cys	african	5	24966	0.0002
GnomAD	GRCh37	Arg885Cys	european	1	129198	7.74E−06
GnomAD	GRCh37	Arg885Cys	latino	1	35440	2.82E−05
GnomAD	GRCh38	Arg885His	asian_east	28	5202	0.005383
GnomAD	GRCh38	Arg885His	european	3	78616	3.82E−05
GnomAD	GRCh38	Arg885His	finnish	1	10606	9.43E−05
GnomAD	GRCh38	Arg885Cys	african	9	41430	0.000217
GnomAD	GRCh38	Arg885Cys	european	1	78632	1.27E−05
GnomAD	GRCh38	Arg885Cys	global	11	140212	0.000078
GnomAD	GRCh38	Arg885His	global	97	391642	0.000248
PAGE	GRCh38	Arg885His	global	99	78698	0.001258
1000G	GRCh37	Arg885His	global	9	4999	0.0018
1000G	GRCh37	Arg885His	asian_east	9	999	0.009009
1000G	GRCh37	Arg885His	chinese	2	414	0.004831
1000G	GRCh37	Arg885His	japanese	7	201	0.034826
54KJPN	GRCh38	Arg885His	japanese	1979	108604	0.018222
KOREAN	GRCh38	Arg885His	korean	34	2922	0.011636
Korea1K	GRCh38	Arg885His	korean	27	1832	0.014738
KOVA	GRCh38	Arg885His	korean	136	10516	0.012933
Nishimura	GRCh38	Arg885His	chinese	1	240	0.008333
Nishimura	GRCh38	Arg885His	japanese	10	576	0.035714
ALFA	GRCh38	Arg885Ser	european	1	26588	3.91E−05
ALFA	GRCh38	Arg885Ser	global	1	35426	2.82E−05
GnomAD	GRCh37	Arg885Ser	global	1	251478	3.98E−06
GnomAD	GRCh37	Arg885Cys	global	7	282884	0.000248
GnomAD	GRCh37	Arg885His	global	88	282884	0.000311
GnomAD	GRCh37	Arg885Ser	global	1	251478	3.98E−06
GnomAD	GRCh37	Arg885Cys	global	7	282884	0.000248
GnomAD	GRCh37	Arg885His	global	88	282884	0.000311

Study refers to the study from which the data was obtained, reference refers to the reference genome, mutation refers to the type of mutation, population denotes the population group in which the mutation was found. Count refers to the number of mutations found in this population, size is the total study size and mutation frequency is calculated using the count and size. All references can be found in the references for Table 1.

References

1. Dmytrijuk A, Robie-Suh K, Cohen MH, Rieves D, Weiss K, Pazdur R. FDA report: eculizumab (Soliris) for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Oncologist*. 2008;13:993–1000. https://doi.org/10.1634/THEONCOLOGIST.2008-0086.
2. Jalbert JJ, Chaudhari U, Zhang H, Weyne J, Shammo JM. Epidemiology of PNH and real-world treatment patterns following an incident PNH diagnosis in the US. *Blood*. 2019;134(Supplement_1):3407. https://doi.org/10.1182/BLOOD-2019-125867.
3. Bessler M et al. Paroxysmal nocturnal haemoglobinuria (PNH) is caused by somatic mutations in the PIG-A gene. *EMBO J*. 1994;13:110–117. https://doi.org/10.1002/J.1460-2075.1994.TB06240.X.
4. Takeda J et al. Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. *Cell*. 1993;73:703–711. https://doi.org/10.1016/0092-8674(93)90250-T.
5. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. The complement system and innate immunity. Published online 2001. Accessed September 10, 2023. https://www.ncbi.nlm.nih.gov/books/NBK27100/.
6. Brodsky RA. Paroxysmal nocturnal hemoglobinuria. *Blood*. 2014;124:2804. https://doi.org/10.1182/BLOOD-2014-02-522128.
7. Brodsky RA, Hu R. PIG-A mutations in paroxysmal nocturnal hemoglobinuria and in normal hematopoiesis. *Leuk Lymphoma*. 2006;47:1215–1221. https://doi.org/10.1080/10428190600555520.
8. Socié G et al. Paroxysmal nocturnal haemoglobinuria: Long-term follow-up and prognostic factors. *Lancet*. 1996;348:573–577. https://doi.org/10.1016/S0140-6736(95)12360-1.
9. Kelly RJ et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood*. 2011;117:6786–6792. https://doi.org/10.1182/BLOOD-2011-02-333997.
10. 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. https://doi.org/10.1038/NBT1344.
11. Thomas TC et al. Inhibition of complement activity by humanized anti-C5 antibody and single-chain Fv. *Mol Immunol*. 1996;33:1389–1401. https://doi.org/10.1016/S0161-5890(96)00078-8.
12. Debureaux PE et al. Hematological response to eculizumab in paroxysmal nocturnal hemoglobinuria: application of a novel classification to identify unmet clinical needs and future clinical goals. *Blood*. 2019;134(Supplement_1):3517. https://doi.org/10.1182/BLOOD-2019-125917.
13. Hillmen P et al. Long-term effect of the complement inhibitor eculizumab on kidney function in patients with paroxysmal nocturnal hemoglobinuria. *Am J Hematol*. 2010;85:553–559. https://doi.org/10.1002/AJH.21757.
14. Wühl E et al. Renal replacement therapy for rare diseases affecting the kidney: an analysis of the ERA-EDTA Registry. *Nephrol Dial Transplant*. 2014;29(Suppl 4):iv1–iv8. https://doi.org/10.1093/NDT/GFU030.
15. Fremeaux-Bacchi V et al. Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide french series comparing children and adults. *Clin J Am Soc Nephrol*. 2013;8:554–562. https://doi.org/10.2215/CJN.04760512/-/DCSUPPLEMENTAL.
16. Zhang K, Lu Y, Harley KT, Tran MH. Atypical hemolytic uremic syndrome: a brief review. *Hematol Rep*. 2017;9:62–67. https://doi.org/10.4081/HR.2017.7053.
17. Noris M 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. https://doi.org/10.2215/CJN.02210310.
18. Maga TK, Nishimura CJ, Weaver AE, Frees KL, Smith RJH. Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. *Hum Mutat*. 2010;31. https://doi.org/10.1002/HUMU.21256.
19. Westra D et al. Genetic disorders in complement (regulating) genes in patients with atypical haemolytic uraemic syndrome (aHUS). *Nephrol Dial Transplant*. 2010;25:2195–2202. https://doi.org/10.1093/NDT/GFQ010.
20. Sellier-Leclerc AL et al. Differential impact of complement mutations on clinical characteristics in atypical hemolytic uremic syndrome. *J Am Soc Nephrol*. 2007;18:2392–2400. https://doi.org/10.1681/ASN.2006080811.
21. Greenbaum LA et al. Eculizumab is a safe and effective treatment in pediatric patients with atypical hemolytic uremic syndrome. *Kidney Int*. 2016;89:701–711. https://doi.org/10.1016/J.KINT.2015.11.026.
22. Allen U, Licht C. Pandemic H1N1 influenza A infection and (atypical) HUS–more than just another trigger? *Pediatr Nephrol*. 2011;26:3–5. https://doi.org/10.1007/S00467-010-1690-Z.
23. Çaltik A, Akyüz SG, Erdogan Ö, Demircin G. Hemolytic uremic syndrome triggered with a new pandemic virus: influenza A (H1N1). *Pediatr Nephrol*. 2011;26:147–148. https://doi.org/10.1007/S00467-010-1649-0.
24. Kwon T et al. Varicella as a trigger of atypical haemolytic uraemic syndrome associated with complement dysfunction: two cases. *Nephrol Dial Transplant*. 2009;24:2752–2754. https://doi.org/10.1093/NDT/GFP166.
25. Fakhouri F et al. Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. *J Am Soc Nephrol*. 2010;21:859. https://doi.org/10.1681/ASN.2009070706.
26. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. *https://doi.org/101056/NEJMra0902814*. 2009;361:1676–1687. https://doi.org/10.1056/NEJMRA0902814.
27. Legendre CM et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. *N Engl J Med*. 2013;368:2169–2181. https://doi.org/10.1056/NEJMOA1208981.
28. Licht C et al. Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int*. 2015;87:1061. https://doi.org/10.1038/KI.2014.423.
29. Fakhouri F et al. Terminal complement inhibitor eculizumab in adult patients with atypical hemolytic uremic syndrome: a single-arm, open-label trial. *Am J Kidney Dis*. 2016;68:84–93. https://doi.org/10.1053/J.AJKD.2015.12.034.
30. Phillips LH. The epidemiology of myasthenia gravis. *Ann N Y Acad Sci*. 2003;998:407–412. https://doi.org/10.1196/ANNALS.1254.053.
31. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in Myasthenia Gravis. *BMC Neurol*. 2010;10. https://doi.org/10.1186/1471-2377-10-46.
32. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. *Ann Neurol*. 2011;69:418–422. https://doi.org/10.1002/ANA.22312.
33. McConville J et al. Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. *Ann Neurol*. 2004;55:580–584. https://doi.org/10.1002/ANA.20061.
34. Fambrough DM, Drachman DB, Satyamurti S. Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. *Science*. 1973;182:293–295. https://doi.org/10.1126/SCIENCE.182.4109.293.
35. Morgan BP, Chamberlain-Banoub J, Neal JW, Song W, Mizuno M, Harris CL. The membrane attack pathway of complement drives pathology in passively induced experimental autoimmune myasthenia gravis in mice. *Clin Exp Immunol*. 2006;146:294–302. https://doi.org/10.1111/J.1365-2249.2006.03205.X.
36. Huijbers MG et al. MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. *Proc Natl Acad Sci U S A*. 2013;110:20783–20788. https://doi.org/10.1073/PNAS.1313944110/-/DCSUPPLEMENTAL/PNAS.201313944SI.PDF.
37. Rivner MH et al. Clinical features of LRP4/agrin-antibody-positive myasthenia gravis: a multicenter study. *Muscle Nerve*. 2020;62:333–343. https://doi.org/10.1002/MUS.26985.
38. Shen C et al. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. *J Clin Invest*. 2013;123:5190–5202. https://doi.org/10.1172/JCI66039.
39. Aguirre F et al. C3, C5a and anti-acetylcholine receptor antibody as severity biomarkers in myasthenia gravis. *Ther Adv Neurol Disord*. 2020;13. https://doi.org/10.1177/1756286420935697.
40. Howard JF et al. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. *Muscle Nerve*. 2013;48:76–84. https://doi.org/10.1002/MUS.23839.
41. Howard JF et al. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. *Lancet Neurol*. 2017;16:976–986. https://doi.org/10.1016/S1474-4422(17)30369-1.
42. Mantegazza R et al. Consistent improvement with eculizumab across muscle groups in myasthenia gravis. *Ann Clin Transl Neurol*. 2020;7:1327. https://doi.org/10.1002/ACN3.51121.
43. Muppidi S et al. Long-term safety and efficacy of eculizumab in generalized myasthenia gravis. *Muscle Nerve*. 2019;60:14–24. https://doi.org/10.1002/MUS.26447.

44. Uzawa A, Ozawa Y, Yasuda M, Kuwabara S. Severe worsening of myasthenic symptoms after the eculizumab discontinuation. *J Neuroimmunol*. 2020;349. https://doi.org/10.1016/J.JNEUROIM.2020.577424.
45. Howard JF, Karam C, Yountz M, O'Brien FL, Mozaffar T. Long-term efficacy of eculizumab in refractory generalized myasthenia gravis: responder analyses. *Ann Clin Transl Neurol*. 2021;8:1398. https://doi.org/10.1002/ACN3.51376.
46. Flanagan EP et al. Epidemiology of aquaporin-4 autoimmunity and neuromyelitis optica spectrum. *Ann Neurol*. 2016;79:775–783. https://doi.org/10.1002/ANA.24617.
47. Etemadifar M, Nasr Z, Khalili B, Taherioun M, Vosoughi R. Epidemiology of neuromyelitis optica in the world: a systematic review and meta-analysis. *Mult Scler Int*. 2015;2015:1–8. https://doi.org/10.1155/2015/174720.
48. Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med*. 2005;202:473–477. https://doi.org/10.1084/JEM.20050304.
49. Bradl M et al. Neuromyelitis optica: pathogenicity of patient immunoglobulin in vivo. *Ann Neurol*. 2009;66:630–643. https://doi.org/10.1002/ANA.21837.
50. Misu T et al. Loss of aquaporin 4 in lesions of neuromyelitis optica: distinction from multiple sclerosis. *Brain*. 2007;130:1224–1234. https://doi.org/10.1093/BRAIN/AWM047.
51. Roemer SF et al. Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. *Brain*. 2007;130:1194–1205. https://doi.org/10.1093/BRAIN/AWL371.
52. Pittock SJ, Lennon VA, Krecke K, Wingerchuk DM, Lucchinetti CF, Weinshenker BG. Brain abnormalities in neuromyelitis optica. *Arch Neurol*. 2006;63:390–396. https://doi.org/10.1001/ARCHNEUR.63.3.390.
53. Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology*. 2006;66:1485–1489. https://doi.org/10.1212/01.WNL.0000216139.44259.74.
54. Wingerchuk DM, Weinshenker BG. Neuromyelitis optica. *Curr Treat Options Neurol*. 2005;7:173–182. https://doi.org/10.1007/S11940-005-0010-6.
55. Watanabe S et al. Therapeutic efficacy of plasma exchange in NMO-IgG-positive patients with neuromyelitis optica. *http://dx.doi.org/101177/1352458506071174*. 2007;13:128–132. https://doi.org/10.1177/1352458506071174.
56. Cree BAC, Lamb S, Morgan K, Chen A, Waubant E, Genain C. An open label study of the effects of rituximab in neuromyelitis optica. *Neurology*. 2005;64:1270–1272. https://doi.org/10.1212/01.WNL.0000159399.81861.D5.
57. Weinstock-Guttman B et al. Study of mitoxantrone for the treatment of recurrent neuromyelitis optica (devic disease). *Arch Neurol*. 2006;63:957–963. https://doi.org/10.1001/ARCHNEUR.63.7.957.
58. Pittock SJ et al. Eculizumab in AQP4-IgG-positive relapsing neuromyelitis optica spectrum disorders: an open-label pilot study. *Lancet Neurol*. 2013;12:554–562. https://doi.org/10.1016/S1474-4422(13)70076-0.
59. Hinson SR et al. Molecular outcomes of neuromyelitis optica (NMO)-IgG binding to aquaporin-4 in astrocytes. *Proc Natl Acad Sci U S A*. 2012;109:1245–1250. https://doi.org/10.1073/PNAS.1109980108/SUPPL_FILE/PNAS.201109980SI.PDF.
60. Pittock SJ et al. Eculizumab in aquaporin-4–positive neuromyelitis optica spectrum disorder. *N Engl J Med*. 2019;381:614–625. https://doi.org/10.1056/NEJMOA1900866/SUPPL_FILE/NEJMOA1900866_DATA-SHARING.PDF.
61. Pittock SJ et al. Eculizumab monotherapy for NMOSD: data from PREVENT and its open-label extension. *Mult Scler*. 2022;28:480. https://doi.org/10.1177/13524585211038291.
62. Wingerchuk DM et al. Long-term safety and efficacy of eculizumab in aquaporin-4 IgG-positive NMOSD. *Ann Neurol*. 2021;89:1088. https://doi.org/10.1002/ANA.26049.
63. Wang D, Hu B, Hu C, et al.. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323:1061–1069. https://doi.org/10.1001/JAMA.2020.1585.
64. Gralinski LE et al. Complement activation contributes to severe acute respiratory syndrome coronavirus pathogenesis. *mBio*. 2018;9:1753–1771. https://doi.org/10.1128/MBIO.01753-18.
65. Magro C et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. *Translat Res*. 2020;220:1. https://doi.org/10.1016/J.TRSL.2020.04.007.
66. Giudice V et al. Combination of ruxolitinib and eculizumab for treatment of severe SARS-CoV-2-related acute respiratory distress syndrome: a controlled study. *Front Pharmacol*. 2020;11, 558265. https://doi.org/10.3389/FPHAR.2020.00857/BIBTEX.
67. Annane D et al. Eculizumab as an emergency treatment for adult patients with severe COVID-19 in the intensive care unit: a proof-of-concept study. *EClinicalMedicine*. 2020;28. https://doi.org/10.1016/j.eclinm.2020.100590.
68. Ruggenenti P et al. Eculizumab in patients with severe coronavirus disease 2019 (COVID-19) requiring continuous positive airway pressure ventilator support: retrospective cohort study. *PLoS One*. 2021;16. https://doi.org/10.1371/JOURNAL.PONE.0261113.
69. Misawa S et al. Safety and efficacy of eculizumab in Guillain-Barré syndrome: a multicentre, double-blind, randomised phase 2 trial. *Lancet Neurol*. 2018;17:519–529. https://doi.org/10.1016/S1474-4422(18)30114-5.
70. Welte T et al. Treating C3 glomerulopathy with eculizumab. *BMC Nephrol*. 2018;19. https://doi.org/10.1186/S12882-017-0802-4.
71. Welte T et al. Eculizumab as a treatment for C3 glomerulopathy: a single-center retrospective study. *BMC Nephrol*. 2023;24:1–9. https://doi.org/10.1186/S12882-023-03058-9/TABLES/2.
72. Evans MJ, *et al*. C5-specific antibodies for the treatment of inflammatory diseases. Published online June 7, 1995.
73. Schatz-Jakobsen JA, Zhang Y, Johnson K, Neill A, Sheridan D, Andersen GR. Structural basis for eculizumab-mediated inhibition of the complement terminal pathway. *J Immunol*. 2016;197:337–344. https://doi.org/10.4049/JIMMUNOL.1600280.
74. Nishimura J ichi et al. Genetic variants in C5 and poor response to eculizumab. *N Engl J Med*. 2014;370:632–639. https://doi.org/10.1056/NEJMOA1311084/SUPPL_FILE/NEJMOA1311084_DISCLOSURES.PDF.
75. Langemeijer S et al. C5 Polymorphism in a Dutch patient with paroxysmal nocturnal hemoglobinuria (PNH) and no Asian ancestry, resistant to eculizumab, but in vitro sensitive to coversin. *Blood*. 2015;126:1209. https://doi.org/10.1182/BLOOD.V126.23.1209.1209.
76. Nelke C et al. Eculizumab treatment alters the proteometabolome beyond the inhibition of complement. *JCI Insight*. 2023;8. https://doi.org/10.1172/JCI.INSIGHT.169135.
77. European Medicines Agency (EMA). *Assessment Report: Eculizumab*; 2013. Accessed September 21, 2023. www.ema.europa.eu.
78. European Medicines Agency (EMA). *Eculizumab: summary of product characteristics*. London: EMA; 2023. Published online 2023.
79. Ter Avest M et al. Proposal for individualized dosing of eculizumab in atypical haemolytic uraemic syndrome: patient friendly and cost-effective. *Nephrol Dial Transplant*. 2023;38:362–371. https://doi.org/10.1093/ndt/gfac056.
80. Wijnsma KL et al. Pharmacology, pharmacokinetics and pharmacodynamics of eculizumab, and possibilities for an individualized approach to eculizumab. *Clin Pharmacokinet*. 2019;58:859. https://doi.org/10.1007/S40262-019-00742-8.
81. Sheridan D et al. Design and preclinical characterization of ALXN1210: a novel anti-C5 antibody with extended duration of action. *PLoS One*. 2018;13. https://doi.org/10.1371/JOURNAL.PONE.0195909.
82. Volokhina EB et al. Sensitive, reliable and easy-performed laboratory monitoring of eculizumab therapy in atypical hemolytic uremic syndrome. *Clin Immunol*. 2015;160:237–243. https://doi.org/10.1016/J.CLIM.2015.05.018.
83. Wehling C et al. Monitoring of complement activation biomarkers and eculizumab in complement-mediated renal disorders. *Clin Exp Immunol*. 2017;187:304. https://doi.org/10.1111/CEI.12890.
84. Gatault P et al. Therapeutic drug monitoring of eculizumab: rationale for an individualized dosing schedule. *Mabs*. 2015;7:1205. https://doi.org/10.1080/19420862.2015.1086049.
85. Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. *Clin Microbiol Rev*. 1991;4:359. https://doi.org/10.1128/CMR.4.3.359.
86. Socié G et al. Eculizumab in paroxysmal nocturnal haemoglobinuria and atypical haemolytic uraemic syndrome: 10-year pharmacovigilance analysis. *Br J Haematol*. 2019;185:297. https://doi.org/10.1111/BJH.15790.
87. Kanakura Y et al. Long-term efficacy and safety of eculizumab in Japanese patients with PNH: AEGIS trial. *Int J Hematol*. 2013;98:406–416. https://doi.org/10.1007/S12185-013-1404-Y/METRICS.
88. Matsumura Y. Risk analysis of eculizumab-related meningococcal disease in Japan using the Japanese adverse drug event report database. *Drug Healthc Patient Saf*. 2020;12:207. https://doi.org/10.2147/DHPS.S257009.
89. McNamara LA, Topaz N, Wang X, Hariri S, Fox L, MacNeil JR. High risk for invasive meningococcal disease among patients receiving eculizumab (soliris) despite receipt of meningococcal vaccine. *Morbidity Mortality Week Rep*. 2017;66:734. https://doi.org/10.15585/MMWR.MM6627E1.
90. Nolfi-Donegan D et al. Fatal nongroupable neisseria meningitidis disease in vaccinated patient receiving eculizumab. *Emerg Infect Dis*. 2018;24:1561. https://doi.org/10.3201/EID2408.180228.
91. Konar M, Granoff DM. Eculizumab treatment and impaired opsonophagocytic killing of meningococci by whole blood from immunized adults. *Blood*. 2017;130:891. https://doi.org/10.1182/BLOOD-2017-05-781450.

92. Crew PE et al. Disseminated gonococcal infections in patients receiving eculizumab: a case series. *Clin Infect Dis*. 2019;69:596–600. https://doi.org/10.1093/CID/CIY958.
93. Saito M et al. Disseminated gonococcal infection associated with eculizumab therapy for paroxysmal nocturnal hemoglobinuria: a case report and literature review. *Int J Gen Med*. 2020;13:403. https://doi.org/10.2147/IJGM.S255298.
94. Crew PE, McNamara L, Waldron PE, McCulley L, Jones SC, Bersoff-Matcha SJ. Unusual neisseria species as a cause of infection in patients taking eculizumab. *J Infect*. 2019;78:113–118. https://doi.org/10.1016/J.JINF.2018.10.015.
95. Desai JV et al. C5a-licensed phagocytes drive sterilizing immunity during systemic fungal infection. *Cell*. 2023;186:2802–2822.e22. https://doi.org/10.1016/J.CELL.2023.04.031.
96. Hayes W, Tschumi S, Ling SC, Feber J, Kirschfink M, Licht C. Eculizumab hepatotoxicity in pediatric aHUS. *Pediatr Nephrol*. 2015;30:775–781. https://doi.org/10.1007/S00467-014-2990-5/METRICS.
97. Dragon-Durey MA et al. Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol*. 2010;21:2180–2187. https://doi.org/10.1681/ASN.2010030315.
98. Oruc A et al. Hepatotoxicity associated with eculizumab in a patient with atypical hemolytic uremic syndrome. *Nefrologia*. 2018;38:448–450. https://doi.org/10.1016/J.NEFRO.2017.10.001.
99. Schieferdecker HL, Schlaf G, Jungermann K, Götze O. Functions of anaphylatoxin C5a in rat liver: direct and indirect actions on nonparenchymal and parenchymal cells. *Int Immunopharmacol*. 2001;1:469–481. https://doi.org/10.1016/S1567-5769(00)00038-2.
100. Mastellos D, Papadimitriou JC, Franchini S, Tsonis PA, Lambris JD. A novel role of complement: mice deficient in the fifth component of complement (C5) exhibit impaired liver regeneration. *J Immunol*. 2001;166:2479–2486. https://doi.org/10.4049/JIMMUNOL.166.4.2479.
101. Risitano AM et al. From perpetual haemosiderinuria to possible iron overload: iron redistribution in paroxysmal nocturnal haemoglobinuria patients on eculizumab by magnetic resonance imaging. *Br J Haematol*. 2012;158:415–418. https://doi.org/10.1111/J.1365-2141.2012.09145.X.
102. Lukina K et al. Iron overload in patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2018;132:1051. https://doi.org/10.1182/BLOOD-2018-99-114654.
103. Waheed A, Kuter DJ. Iron overload after complement inhibitor treatment of paroxysmal nocturnal hemoglobinuria. *Am J Hematol*. 2021;96:E235–E237. https://doi.org/10.1002/AJH.26187.
104. Schaap CC, *et al*. Effect of eculizumab on iron metabolism in transfusion-independent patients with paroxysmal nocturnal hemoglobinuria. Published online 2023. https://doi.org/10.1097/HS9.0000000000000878.
105. Höchsmann B, Leichtle R, von Zabern I, Kaiser S, Flegel WA, Schrezenmeier H. Paroxysmal nocturnal haemoglobinuria treatment with eculizumab is associated with a positive direct antiglobulin test. *Vox Sang*. 2012;102:159–166. https://doi.org/10.1111/J.1423-0410.2011.01530.X.
106. Katyal N, Govindarajan R. Poor response to eculizumab in Caucasian patients with treatment refractory generalized myasthenia gravis: a case series. *RRNMF Neuromusc J*. 2023;4:21–25. https://doi.org/10.17161/RRNMF.V4I2.18489.
107. Kulasekararaj AG et al. Phase 2 study of danicopan in patients with paroxysmal nocturnal hemoglobinuria with an inadequate response to eculizumab. *Blood*. 2021;138:1928. https://doi.org/10.1182/BLOOD.2021011388.
108. Mosch R, Guchelaar HJ. Immunogenicity of monoclonal antibodies and the potential use of HLA haplotypes to predict vulnerable patients. *Front Immunol*. 2022;13. https://doi.org/10.3389/FIMMU.2022.885672.
109. Hillmen P et al. Long-term safety and efficacy of sustained eculizumab treatment in patients with paroxysmal nocturnal haemoglobinuria. *Br J Haematol*. 2013;162:62–73. https://doi.org/10.1111/BJH.12347.
110. Hillmen P et al. Assessment of human antihuman antibodies to eculizumab after long-term treatment in patients with paroxysmal nocturnal hemoglobinuria. *Am J Hematol*. 2016;91:E16–E17. https://doi.org/10.1002/AJH.24280.
111. Hillmen P et al. The Complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2006;355:1233–1243. https://doi.org/10.1056/NEJMOA061648/SUPPL_FILE/NEJM_HILLMEN_1233SA1.PDF.
112. Brodsky RA et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2008;111:1840–1847. https://doi.org/10.1182/BLOOD-2007-06-094136.
113. Shammo J et al. Low rate of clinically evident extravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria treated with a complement C5 inhibitor: Results from a large, multicenter, US real-world study. *J Blood Med*. 2022;13:425. https://doi.org/10.2147/JBM.S361863.
114. Risitano AM et al. Complement fraction 3 binding on erythrocytes as additional mechanism of disease in paroxysmal nocturnal hemoglobinuria patients treated by eculizumab. *Blood*. 2009;113:4094–4100. https://doi.org/10.1182/BLOOD-2008-11-189944.
115. Lin Z et al. Complement C3dg-mediated erythrophagocytosis: implications for paroxysmal nocturnal hemoglobinuria. *Blood*. 2015;126:891–894. https://doi.org/10.1182/BLOOD-2015-02-625871.
116. Hill A et al. Eculizumab prevents intravascular hemolysis in patients with paroxysmal nocturnal hemoglobinuria and unmasks low-level extravascular hemolysis occurring through C3 opsonization. *Haematologica*. 2010;95:567. https://doi.org/10.3324/HAEMATOL.2009.007229.
117. Goodship THJ et al. Use of the complement inhibitor Coversin to treat HSCT-associated TMA. *Blood Adv*. 2017;1:1254. https://doi.org/10.1182/BLOODADVANCES.2016002832.
118. Lee JW et al. Ravulizumab (ALXN1210) vs eculizumab in adult patients with PNH naive to complement inhibitors: the 301 study. *Blood*. 2019;133:530. https://doi.org/10.1182/BLOOD-2018-09-876136.
119. Kato GJ et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood*. 2006;107:2279. https://doi.org/10.1182/BLOOD-2005-06-2373.
120. Kanakura Y et al. Safety and efficacy of the terminal complement inhibitor eculizumab in Japanese patients with paroxysmal nocturnal hemoglobinuria: the AEGIS clinical trial. *Int J Hematol*. 2011;93:36–46. https://doi.org/10.1007/S12185-010-0748-9/FIGURES/3.
121. Mahon FX et al. Successful treatment of a PNH patient non-responsive to eculizumab with the novel complement C5 inhibitor coversin (nomacopan). *Br J Haematol*. 2020;188:334–337. https://doi.org/10.1111/BJH.16305.
122. Moore I et al. 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. https://doi.org/10.1182/BLOOD-2009-05-221549.
123. Jodele S et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. *Blood*. 2013;122:2003. https://doi.org/10.1182/BLOOD-2013-05-501445.
124. Ueda Y et al. Current status and optimal management of eculizumab poor-responders due to C5 polymorphisms. *Blood*. 2018;132:2323. https://doi.org/10.1182/BLOOD-2018-99-111230.
125. Ishiyama K et al. Results from multinational phase 3 studies of ravulizumab (ALXN1210) versus eculizumab in adults with paroxysmal nocturnal hemoglobinuria: subgroup analysis of Japanese patients. *Int J Hematol*. 2020;112:466–476. https://doi.org/10.1007/S12185-020-02934-6.
126. Kim HJ et al. Eculizumab in Asian patients with anti-aquaporin-IgG-positive neuromyelitis optica spectrum disorder: a subgroup analysis from the randomized phase 3 PREVENT trial and its open-label extension. *Mult Scler Relat Disord*. 2021;50. https://doi.org/10.1016/J.MSARD.2021.102849.
127. Xu D, Hou S, Jiang Y, et al.. Complement C5 gene confers risk for acute anterior uveitis. *Invest Ophthalmol Vis Sci*. 2015;56:4954–4960. https://doi.org/10.1167/IOVS.15-16645.
128. Chen K, Lin Y, Liu Y, et al.. Investigation of association of complement 5 genetic polymorphisms with sepsis and sepsis-induced inflammatory responses. *J Inflamm Res*. 2021;14:6461. https://doi.org/10.2147/JIR.S340446.
129. Pfarr N et al. Linking C5 deficiency to an exonic splicing enhancer mutation Accessed October 15, 2023. *J Immunol*. 2005;174:4172–4177 http://genes.mit.edu/burgelab/.
130. Franco-Jarava C, Comas D, Orren A, Hernández-González M, Colobran R. Complement factor 5 (C5) p.A252T mutation is prevalent in, but not restricted to, sub-Saharan Africa: implications for the susceptibility to meningococcal disease. *Clin Exp Immunol*. 2017;189:226. https://doi.org/10.1111/CEI.12967.
131. Wang X et al. Inherited human complement C5 deficiency: nonsense mutations in exons 1 (Gln1 to stop) and 36 (Arg1458 to stop) and compound heterozygosity in three African-American families. *J Immunol*. 1995;154:5464–5471. https://doi.org/10.4049/jimmunol.154.10.5464.
132. Syed YY. Ravulizumab: a review in atypical haemolytic uraemic syndrome. *Drugs*. 2021;81:587–594. https://doi.org/10.1007/S40265-021-01481-6.
133. Howard JF et al. Zilucoplan: an investigational complement C5 inhibitor for the treatment of acetylcholine receptor autoantibody-positive generalized myasthenia gravis. *Expert Opin Investig Drugs*. 2021;30:483–493. https://doi.org/10.1080/13543784.2021.1897567.
134. Lee S et al. Korean Variant Archive (KOVA): a reference database of genetic variations in the Korean population. *Sci Rep*. 2017;7. https://doi.org/10.1038/S41598-017-04642-4.

135. Matise TC et al. The next PAGE in understanding complex traits: design for the analysis of population architecture using genetics and epidemiology (PAGE) study. *Am J Epidemiol*. 2011;174:849–859. https://doi.org/10.1093/AJE/KWR160.
136. Jeon S et al. Korean Genome Project: 1094 Korean personal genomes with clinical information. *Sci Adv*. 2020;6. https://doi.org/10.1126/SCIADV.AAZ7835.
137. Jung KS et al. KRGDB: the large-scale variant database of 1722 Koreans based on whole genome sequencing. *Database*. 2020;2020:146. https://doi.org/10.1093/DATABASE/BAZ146.
138. Chen S et al. A genome-wide mutational constraint map quantified from variation in 76,156 human genomes. *bioRxiv*. 20222022.03.20.485034. https://doi.org/10.1101/2022.03.20.485034. Published online October 10, 2022.
139. Phan, L. *et al*. ALFA: Allele Frequency Aggregator. Published online March 10, 2020. www.ncbi.nlm.nih.gov/snp/docs/gsr/alfa/.
140. Tadaka S et al. 3.5KJPNv2: an allele frequency panel of 3552 Japanese individuals including the X chromosome. *Hum Genome Variat*. 2019;6:1–9. https://doi.org/10.1038/s41439-019-0059-5.
141. Auton A et al. A global reference for human genetic variation. *Nature*. 2015;526:68–74. https://doi.org/10.1038/nature15393.
142. Hill A et al. Under-recognized complications in patients with paroxysmal nocturnal haemoglobinuria: raised pulmonary pressure and reduced right ventricular function. *Br J Haematol*. 2012;158:409–414. https://doi.org/10.1111/J.1365-2141.2012.09166.X.
143. Mealy MA et al. Long-term disability in neuromyelitis optica spectrum disorder with a history of myelitis is associated with age at onset, delay in diagnosis/preventive treatment, MRI lesion length and presence of symptomatic brain lesions. *Mult Scler Relat Disord*. 2019;28:64. https://doi.org/10.1016/J.MSARD.2018.12.011.
144. Ryan M, Donato BMK, Irish W, Gasteyger C, L'Italien G, Laurence J. Economic impact of early-in-hospital diagnosis and initiation of eculizumab in atypical haemolytic uraemic syndrome. *Pharmacoeconomics*. 2020;38:307–313. https://doi.org/10.1007/S40273-019-00862-W.
145. Fishman J, Wilson K, Drzewiecka A, Pochopień M, Dingli D. The cost–effectiveness of pegcetacoplan in complement treatment-naïve adults with paroxysmal nocturnal hemoglobinuria in the USA. *J Comp Eff Res*. 2023230055-232042. https://doi.org/10.57264/CER-2023-0055/SUPPL_FILE/SUPPLEMENTARY. Published online October 31, 2023.
146. Koren E, Zuckerman L, Mire-Sluis A. Immune responses to therapeutic proteins in humans – clinical significance, assessment and prediction. *Curr Pharm Biotechnol*. 2002;3:349–360. https://doi.org/10.2174/1389201023378175.