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**Review**

The renaissance of complement diagnostics and therapeutics has introduced precision medicine into a widened field of complement-mediated diseases. In particular, complement-mediated diseases (or complementopathies) with ongoing or published clinical trials of complement inhibitors include paroxysmal nocturnal hemoglobinuria, cold agglutinin disease, hemolytic uremic syndrome, nephropathies, HELLP syndrome, transplant-associated thrombotic microangiopathy, antiphospholipid antibody syndrome, myasthenia gravis, and neuromyelitis optica. Recognizing that this field is rapidly expanding, we aim to provide a state-of-the-art review of (a) current understanding of complement biology for the clinician, (b) novel insights into complement with potential applicability to clinical practice, (c) complement in disease across various disciplines (hematology, nephrology, obstetrics, transplantation, rheumatology, and neurology), and (d) the potential future of precision medicine. Better understanding of complement diagnostics and therapeutics will not only facilitate physicians treating patients in clinical practice but also provide the basis for future research toward precision medicine in this field.

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# Complementopathies and precision medicine

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**The renaissance of complement diagnostics and therapeutics has introduced precision medicine into a widened field of complement-mediated diseases. In particular, complement-mediated diseases (or complementopathies) with ongoing or published clinical trials of complement inhibitors include paroxysmal nocturnal hemoglobinuria, cold agglutinin disease, hemolytic uremic syndrome, nephropathies, HELLP syndrome, transplant-associated thrombotic microangiopathy, antiphospholipid antibody syndrome, myasthenia gravis, and neuromyelitis optica. Recognizing that this field is rapidly expanding, we aim to provide a state-of-the-art review of (a) current understanding of complement biology for the clinician, (b) novel insights into complement with potential applicability to clinical practice, (c) complement in disease across various disciplines (hematology, nephrology, obstetrics, transplantation, rheumatology, and neurology), and (d) the potential future of precision medicine. Better understanding of complement diagnostics and therapeutics will not only facilitate physicians treating patients in clinical practice but also provide the basis for future research toward precision medicine in this field.**

## Introduction

The notion of precision or personalized medicine was introduced in 1999 by Francis Collins based on expected consequences of the Human Genome Project (1). Since then, numerous projects have tried to incorporate genetic and functional disease identities into diagnostic and therapeutic potentials across various disciplines (2). Despite unmet expectations especially in public health issues, precision medicine has expanded, along with a tremendous expansion of complement therapeutics. Indeed, the renaissance of complement therapeutics has led to the recognition of a wide range of complement-mediated disorders, also called “complementopathies” (3). This term has been proposed for disorders in which complement dysregulation drives disease pathogenesis, and complement inhibition has the potential to abate the disease course (4).

Recognizing that this field is rapidly expanding, we aim to provide a state-of-the-art review comprising (a) current understanding of complement biology for the clinician, (b) novel insights into complement with potential applicability to clinical practice, (c) complement in disease across various disciplines (hematology, nephrology, neurology, obstetrics, transplantation, and rheumatology), and (d) our perspective on the future development of precision medicine for complementopathies.

## Current understanding of complement biology for the clinician

More than 50 soluble and membrane-bound proteins form the complement system, providing innate defense against microbes and mediating inflammatory responses (5, 6). The complement cascade is activated by the classical, alternative, and lectin pathways. Importantly, the alternative pathway of complement serves as an ampli-

fication loop for the lectin and classical pathways, accounting for roughly 80% of complement activation products (7).

The classical pathway is mainly activated by antibody-antigen complexes recognized via complement component C1q. Among antibody isotypes, IgM is the most effective in activating complement. Activation of complement with the four subclasses of IgGs varies as a function of steric hindrance by the Fab arms in the approach of C1q to the IgG CH2 sites (IgG3>IgG1>IgG2>IgG4) (8). Besides antibodies, C1q also binds directly to certain epitopes from microorganisms or apoptotic cells and to cell surface molecules, such as acute-phase proteins that bind to pathogens or affected cells and activate complement (9, 10). C1q subsequently cleaves C1r, which activates C1s protease. Then, C1s cleaves C4 and C2, leading to the formation of classical pathway C3 convertase (C4bC2a). C3 convertase cleaves C3, generating the anaphylatoxin C5a and C5 convertase (C4bC2aC3b), which cleaves C5 into C5a and C5b, which initiate the terminal pathway of complement. A schematic of proximal and terminal complement activation is shown in Figure 1.

In the terminal pathway of complement, C5b binds to C6, generating C5b-6, which in turn binds to C7, creating C5b-7. C5b-7 is able to insert into lipid layers of the membrane (11). Once there, C5b-7 binds C8 and C9, forming a complex that unfolds in the membrane and binds several C9 molecules, thereby forming the membrane attack complex (MAC).

## Activation of the alternative pathway of complement

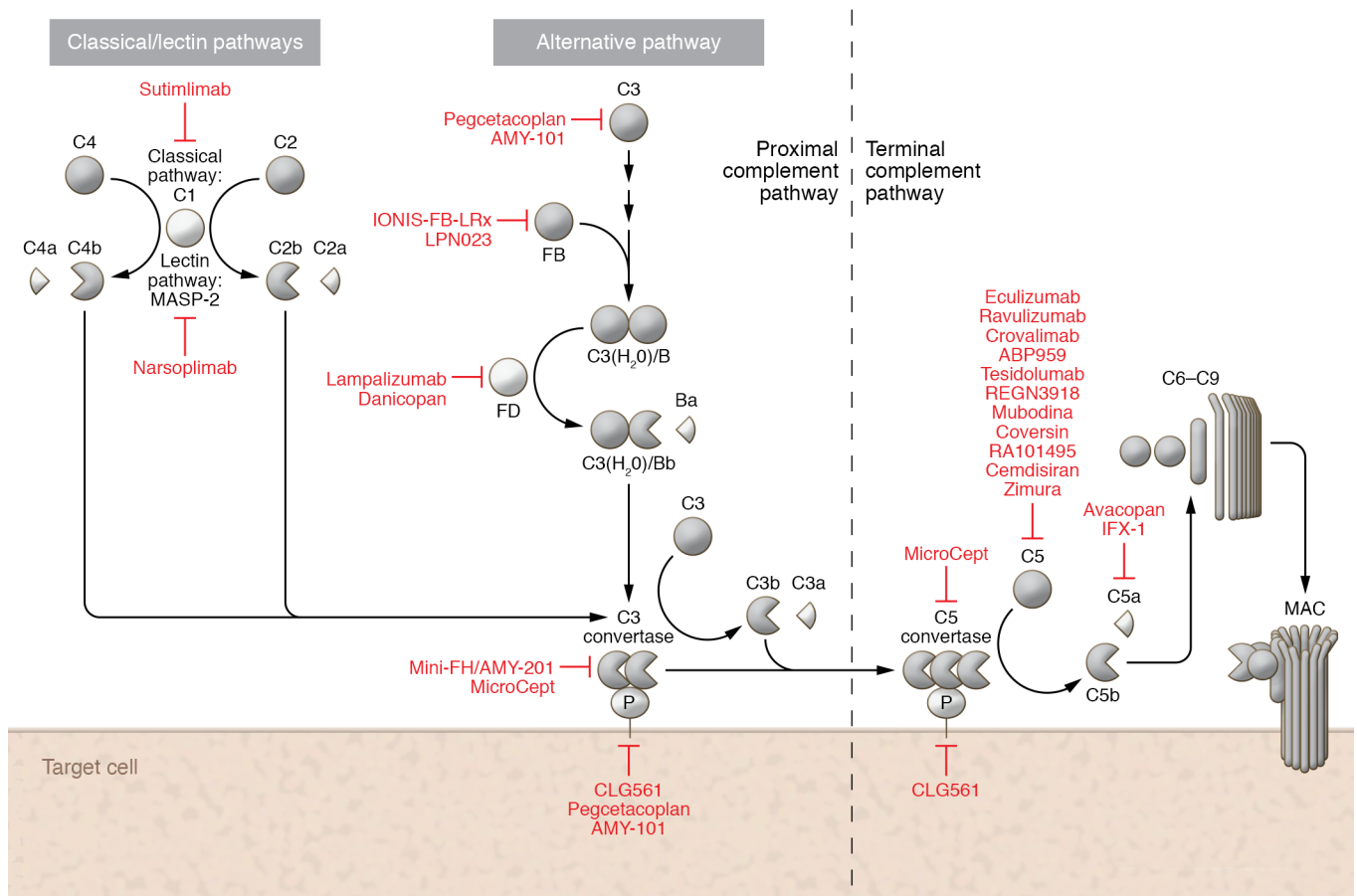
The alternative pathway of complement (APC) is summarized by Figure 2. The APC is continuously activated at low levels through slow spontaneous hydrolysis of C3, which forms C3(H<sub>2</sub>O). This process is called “tickover.” Therefore, the APC can be activated on any surface that has the ability to amplify complement, including the surface of bacteria, apoptotic, and necrotic cells (12). The activated C3(H<sub>2</sub>O) binds factor B, generating C3(H<sub>2</sub>O)B. Factor B is subsequently cleaved by factor D, generating the fluid-phase APC C3 convertase, or C3(H<sub>2</sub>O)Bb. C3 convertase then catalyzes the cleavage

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**Figure 1. Targets of complement inhibitors in various stages of clinical development for complement-mediated disorders.** Complement-targeting compounds are shown in red and indicate the step of the complement pathway they target. From left to right: sutimlimab inhibits C1s of the classical pathway; narsoplimab inhibits mannose-binding protein-associated serine protease 2 (MASP-2) of the lectin pathway; pegcetacoplan (formerly APL-2) and AMY-101 inhibit C3 and C3 convertase activity; IONIS-FB-LRx and LPN023 inhibit factor B; lampalizumab and danicopan inhibit factor D; mini-FH/AMY-201 inhibits alternative pathway C3 convertase; CLG561 inhibits properdin; MicroCept inhibits C3 and C5 convertases; eculizumab, ravulizumab, crovalimab, ABP959, tesidolumab, REGN3918, mubodina, coversin, RA101495, cemdisiran, and zimura inhibit C5; and avacopan inhibits C5a receptor; and IFX-1 inhibits C5a.

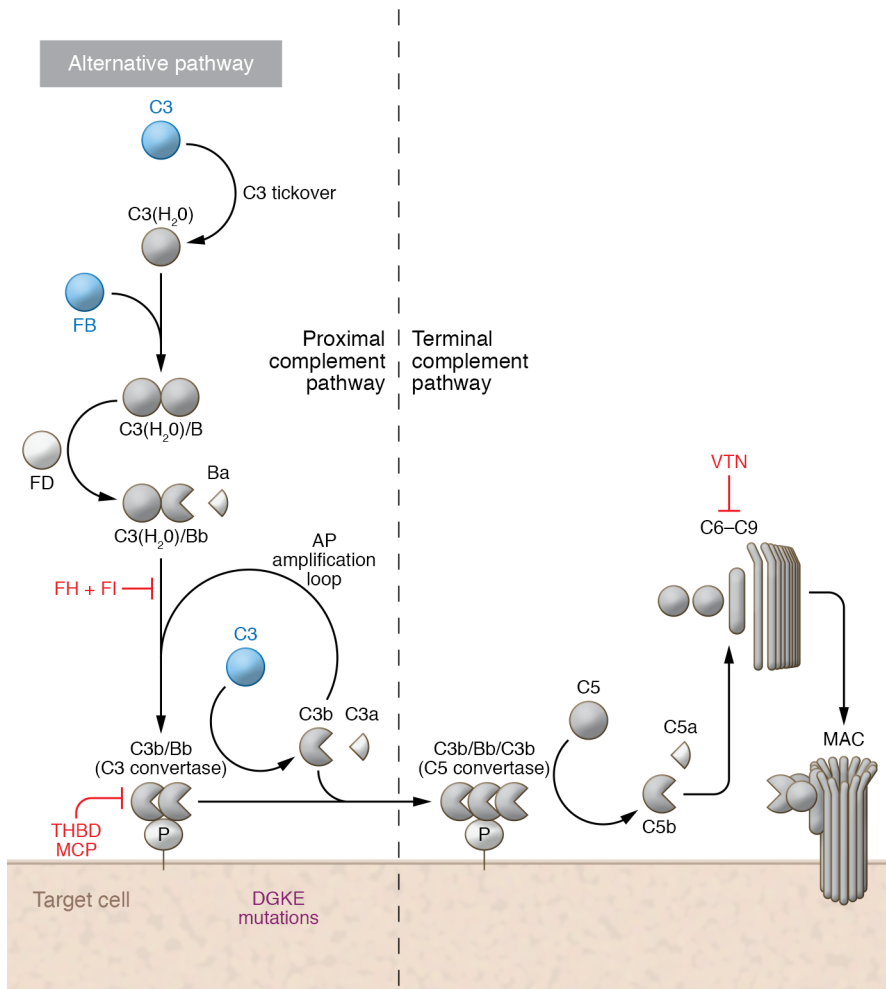
of additional C3 molecules to generate C3a and C3b, which attach to cell surfaces (13). This initiates the amplification loop, where C3b pairs with factor B on cell surfaces and bound factor B is cleaved by factor D to generate a second (surface-phase) APC C3 convertase (C3bBb). Membrane-bound C3 convertase then cleaves additional C3 to generate more C3b deposits, closing the amplification loop. The binding and cleavage of an additional C3 molecule to C3 convertase forms the APC C5 convertase (C3bBbC3b) that cleaves C5 to C5a and C5b. C5b initiates the terminal complement pathway that forms the MAC, as described above. The process, from initial spontaneous C3 activation through amplification, is depicted in Figure 1. Both C3 and C5 APC convertases are stabilized by properdin (also known as factor P) (14), which also serves as a selective pattern recognition molecule for de novo C3 APC convertase assembly (12). Properdin is the only known positive regulator of complement. It increases the activity of C3 and C5 convertases, which amplify C3b deposition on cell surfaces (15).

#### Activation of the lectin pathway of complement

Lectin pathway activation is initiated by mannose-binding lectins (MBLs) that recognize carbohydrate structures on the surfaces of

microbes, such as viruses, protozoan parasites, fungi, and various bacteria (16, 17). Other pattern recognition molecules involved in lectin pathway activation are ficolins and collectin 11 (18). These molecules act through MBL-associated serine proteases (MASPs), which generate the C3 convertase (C4bC2a) in a process similar to that of the classical pathway.

Other mechanisms of complement activation have been postulated, including the interaction with the coagulation cascade (discussed below), and heme-induced complement activation (19). Indeed, a plethora of experimental studies have shown that heme interacts with classical and alternative complement pathways (20). This heme-induced complement activation may be relevant in diseases with intravascular hemolysis. Among them, paroxysmal nocturnal hemoglobinuria (PNH) and complement-mediated hemolytic uremic syndrome (CM-HUS) are well-known models of complement activation and will be further discussed below. In others, such as sickle cell disease, the role of complement activation is currently being investigated (21). Excessive complement activation is physiologically prevented by complement-regulatory proteins. Membrane-bound or soluble complement regulators that are relevant to complement-mediated diseases are summarized in Table 1.



**Figure 2. Mutations in complement regulators involved in complement-mediated diseases.** Complement activation leads to C3 activation and C3 convertase formation on C3-opsonized surfaces, culminating in pronounced C3 fragment deposition on complement-targeted surfaces (proximal complement). In the presence of increased surface density of deposited C3b, the terminal complement is triggered, leading to membrane attack complex (MAC) formation on the surface of target cell. Complement pathway dysregulation results from loss-of-function mutations in regulatory factors (i.e., factor H [FH], factor I [FI], thrombomodulin [THBD], and vitronectin [VTN]) shown in red, gain-of-function mutations (i.e., C3 and factor B [FB]) shown in blue, and DGKE mutations in purple, indicating their unknown effect on complement cascade.

## Insights into complement potentially applicable to clinical practice

### Immune function of complement

Complement was first recognized to modulate adaptive immunity in the 1970s (22). Since then, a number of studies have investigated molecular pathways of complement interaction with B and T cells (23). Complement modulates innate immune responses by sensing danger signals and interacting with Toll-like receptors (TLRs) (24). Novel pathways have linked complement-mediated signaling with the paracrine and autocrine activation of T cells, and complement proteins have also been implicated in shaping T cell fate by acting at the intracellular level, as extensively reviewed by Reis et al. (24). Another emerging feature of complement is the regulation of cell metabolism extending from adipocytes to liver and pancreas (25). Complement also modulates metabolic pathways in immune cells (26), suggesting that inflammation could be restrained by targeting of specific complement proteins. As a result, experimental studies have investigated complement in the immune modulation of diverse inflammatory diseases, including asthma, arthritis, and solid cancer (27–29).

In addition to cells traditionally considered part of the immune system, complement was also recently shown to interact with platelets, which are currently characterized as an innate

immune cell (30). Accumulating data suggest that complement and platelets interact during the early cellular and molecular events that promote atherogenesis (31). Interestingly, signaling pathways between platelets and complement act on endothelial cells, affecting their pro-atherogenic features (32). These data suggest the potential involvement of complement in a wide spectrum of diseases associated with atherogenesis.

### Complement and thrombosis

Complement and coagulation were once considered entirely independent pathways; however, it is now clear there is close interaction. Many of the key enzymes in both pathways are serine proteases. Complement-driven diseases, such as PNH, are characterized by a high thrombosis rate that is abrogated by complement inhibition, to the extent that anticoagulation is no longer needed (33).

The underlying mechanisms of complement-mediated thrombosis are not fully clarified, but thrombosis is a prominent clinical feature of all complementopathies. There are multiple proposed mechanisms of complement and thrombosis interactions, as thoroughly reviewed by Hill et al. (34). Direct interactions between complement and coagulation are mediated by C5a (35) and coagulation factors (i.e., thrombin, plasmin, and coagulation factors FXa and FXIa), which can activate complement (36–40). Throm-

**Table 1. Complement regulators relevant to complement-mediated disorders**

Type	Regulator	Mechanism of regulation	Disorder
Membrane bound	CD55	Accelerates the decay of cell surface-bound C3 and C5 convertases	PNH
	CD59	Inhibits pore formation of MAC	PNH
	CD46	Accelerates decay of C3 convertases; inactivates C3b to iC3b	CM-HUS; glomerulopathies
	Thrombomodulin	Regulates factor I-mediated C3b inactivation	CM-HUS; glomerulopathies
	Complement receptor 1	Accelerates decay of C3 and C5 convertases; cofactor for factor I	CM-HUS; glomerulopathies; CAPS
	Vitronectin	Inhibits MAC formation	CM-HUS
Soluble	Factor H	Disrupts the APC C3 convertase; cleaves C3b as a cofactor of factor I	CM-HUS; glomerulopathies; HELLP
	Factor H-related proteins	Bind to C3b	CM-HUS; glomerulopathies; HELLP; CAPS
	Factor I	Cleaves cell-bound or fluid-phase C3b and C4b	CM-HUS; glomerulopathies; HELLP
	Factor B	Generates the APC C3 convertase	CM-HUS; glomerulopathies
	Factor D	Cleaves factor B	CM-HUS; glomerulopathies

APC, alternative pathway of complement; CAPS, catastrophic antiphospholipid antibody syndrome; CM-HUS, complement-mediated hemolytic uremic syndrome; HELLP, hemolysis, elevated liver enzymes, and low platelets (syndrome); MAC, membrane attack complex; PNH, paroxysmal nocturnal hemoglobinuria.

bin was also recently shown to act as a potential C5a convertase in vitro, generating C5<sub>1</sub> and C5b<sub>1</sub> (41).

Indirect effects of complement on thrombosis have also been observed in hemolytic anemias (42). Recent evidence suggests that heme-induced thromboinflammation is significantly attenuated by C5 inhibition, with additional benefits observed when C5 inhibition is combined with an inhibitor of the TLR coreceptor CD14 (43). In addition, cholesterol crystals can induce coagulation activation via complement-mediated expression of tissue factor (44). This novel notion of thromboinflammation is expected to play a central role in a wide spectrum of disorders, ranging from thrombotic microangiopathies to autoimmune diseases (45).

## Complement in disease

The inability to regulate complement drives the pathophysiology of a variety of diseases that cross multiple medical specialties. These disorders are often associated with specific mutations or autoantibodies that drive complement-mediated end-organ damage. Increasingly, pharmacologic complement inhibition of these pathways mitigates end-organ damage, which lays the foundation for precision medicine in complementopathies. Specific complement inhibitors at different sites of the complement cascade, similar to what exists in coagulation, will soon be widely available. Since these entities are diagnosed and treated by different medical specialties, this section of the Review will focus specifically on disease characteristics and diagnostic and therapeutic features that concern the complement cascade. Table 2 summarizes disorders in which complement inhibition has been shown to be beneficial.

## Hematology

### Paroxysmal nocturnal hemoglobinuria

**Complement activation.** Paroxysmal nocturnal hemoglobinuria (PNH) is a clonal hematopoietic stem cell disorder caused by somatic mutations in *PIGA* that lead to the absence of glycosylphosphatidylinositol-anchored (GPI-anchored) proteins on the surfaces of affected cells (33). Two of the missing GPI-anchored proteins (CD55 and CD59) are complement-regulatory proteins (46, 47).

Because of their absence from erythrocyte membranes, hemolysis in PNH is primarily due to APC activation. Before 1990, diagnosis of PNH was based on the Ham, or acidified serum, test that was described in the 1930s (48). This test is based on the susceptibility of PNH cells to acidified serum, which serves as an APC activator (48). Thus, incubation of PNH erythrocytes with acidified serum leads to hemolysis that is not observed in normal erythrocytes. Today, PNH is diagnosed by flow cytometry. Fluoresceinated monoclonal antibodies against GPI-anchored proteins and/or fluorescein-labeled proaerolysin (FLAER) are used to detect the absence of GPI-anchored proteins from the surface of cells in peripheral blood (49, 50). Without therapy, the median survival is roughly 15–20 years; the leading cause of death is thrombosis, highlighting the important link between complement and thrombosis (51, 52).

**Complement inhibition.** Complement inhibition is the treatment of choice for PNH patients with severe hemolytic anemia and/or thrombosis. There are two FDA-approved drugs: eculizumab (approved in 2007) and ravulizumab (approved in 2019). Both monoclonal antibodies bind C5 and sterically hinder cleavage of C5 by the C5 convertase. This blocks the generation of the proinflammatory C5a molecule and MAC formation (53, 54). Ravulizumab has the advantage of 4-fold longer half-life, but otherwise the drugs are noninferior (55, 56). Recently, ravulizumab has shown sustained 1-year safety and efficacy (57), as well as decreased breakthrough hemolysis (58). Both drugs stop intravascular hemolysis, eliminate or reduce the need for blood transfusion, improve quality of life, and markedly attenuate the thrombosis risk. Ravulizumab is currently the drug of choice given its long half-life and more convenient dosing.

Terminal complement inhibition at C5 (downstream of CD55) in PNH usually results in mild to moderate extravascular hemolysis. This is because PNH red cells are also CD55 deficient, leading to unimpaired C3b opsonization and subsequent formation of C3 fragments that result in extravascular hemolysis in the liver and spleen (59). Moreover, complement-amplifying conditions (e.g., pregnancy, surgery, infections) can lead to a high density of surface C3b molecules that cause steric hindrance and decrease binding of eculizumab/ravulizumab to C5, ultimately causing breakthrough



**Table 2. Disorders in which complement inhibition is beneficial**

Disorder	Mechanism of complement activation	Complement pathway implicated	Proof of benefit from complement inhibition
Age-related macular degeneration	Genetic variants of complement-regulatory proteins	Alternative	Phase III clinical trials
ANCA vasculitis	C5a-mediated effects of complement-activating autoantibodies	Alternative/classical	Phase III clinical trial
Antiphospholipid antibody syndrome	Genetic variants of complement-regulatory proteins	Alternative/classical	Case reports/series
Atypical hemolytic uremic syndrome	Genetic variants of complement-regulatory proteins; autoantibodies	Alternative	Approved treatment
Cold agglutinin disease	Complement-activating antibodies	Classical	Phase III clinical trials
Glomerulopathies	Genetic variants of complement-regulatory proteins; autoantibodies	Alternative/classical	Phase II clinical trials
HELLP syndrome	Genetic variants of complement-regulatory proteins	Alternative	Case reports
Myasthenia gravis	Complement-activating antibodies	Classical	Approved treatment
Neuromyelitis optica	Complement-activating antibodies	Alternative/classical	Approved treatment
Paroxysmal nocturnal hemoglobinuria	GPI anchor deficiency	Alternative	Approved treatment
Periodontitis	Local microbially induced complement activation	Alternative	Phase II clinical trial
Transplant-associated TMA	Genetic variants of complement-regulatory proteins	Alternative	Phase III clinical trial

Disorders are listed alphabetically. ANCA, anti-neutrophil cytoplasmic antibody; GPI, glycosylphosphatidylinositol; HELLP, hemolysis, elevated liver enzymes, and low platelets; TMA, thrombotic microangiopathy.

intravascular hemolysis (60). The only major adverse effect of C5 inhibition has been an expected increased risk of *Neisseria meningitidis* infection (0.5% risk annually) (61). The predictable toxicity from C5 inhibition and lack of other major end-organ toxicity are a testament to this precision medicine–based approach.

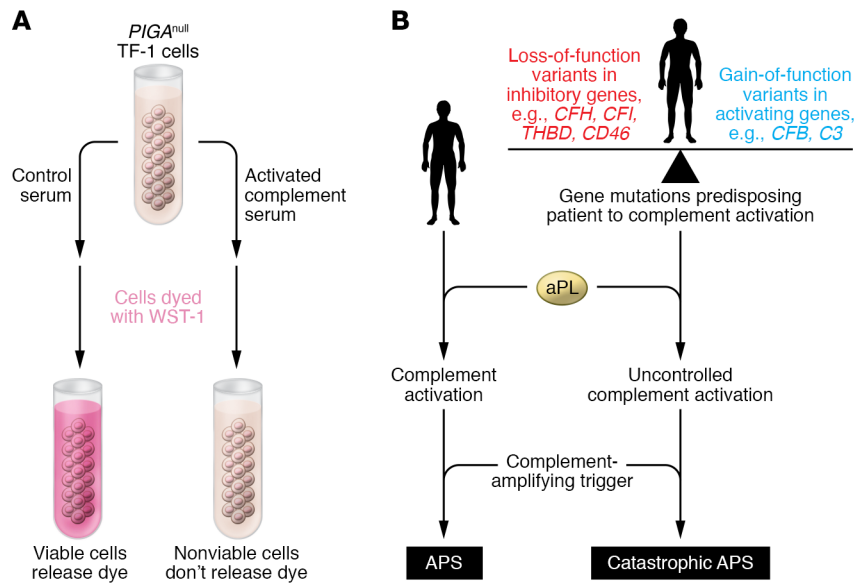
Novel complement inhibitors are in development, as summarized in Figure 1 (62–64). Among them, crovalimab is a subcutaneously administered monoclonal antibody that also targets C5 at a different epitope from eculizumab and ravulizumab. It is administered every 4 weeks and, in a phase I/II trial, was able to stop intravascular hemolysis in 10 treatment-naïve PNH patients (65). Inhibition of targets upstream of both CD59 and CD55, such as C3, factor D, and factor B, is even more precise and can block intravascular and extravascular hemolysis because it blocks C3 fragment accumulation on red blood cells (RBCs). In an open-label phase II trial of treatment-naïve patients, an oral factor D inhibitor (danicopan) resulted in hemoglobin improvement and elimination of intravascular hemolysis without evidence of C3-mediated extravascular hemolysis (66). In a separate study of 12 eculizumab-treated, transfusion-dependent PNH patients, danicopan was able to improve hemoglobin and eliminate the need for blood transfusions (67). In vitro studies suggest that danicopan preserves classical and lectin pathway activity against invasive pathogens (68). In addition, increased meningococcal killing in vaccinated volunteers has been observed in the presence of danicopan in contrast to anti-C5 inhibitors (69). Pegcetacoplan is a 15-amino acid cyclic peptide conjugated to polyethylene glycol that binds to C3 and prevents C3 and C5 cleavage by their respective convertases. In a phase Ib, open-label clinical study involving 6 transfusion-dependent, eculizumab-treated PNH patients, daily subcutaneous pegcetacoplan was well tolerated, improved hemoglobin, and stopped the need for transfusions (70). Thus, the treatment paradigm for PNH is likely to change toward a precision medicine model as these novel complement inhibitors enter the clinic.

#### Cold agglutinin disease

**Complement activation.** Cold agglutinins are autoantibodies (typically IgM) that agglutinate RBCs at 4°C but may also act at

warmer temperatures. Cold agglutinin disease (CAD) is classified as either primary or secondary. Primary CAD is a clonal B cell lymphoproliferative disorder that is also called primary cold agglutinin-associated lymphoproliferative disease; it is distinct from lymphoplasmacytic lymphoma (MYD88 L265P negative), marginal zone lymphoma, and other low-grade lymphoproliferative diseases (71). Secondary CAD is a syndrome associated with a variety of infectious and neoplastic disorders (aggressive lymphomas, Hodgkin's lymphoma, carcinomas, etc.). Hemolysis is driven by activation of the classical complement pathway, resulting in opsonization and predominantly extravascular hemolysis (72). Cold agglutinins with high thermal amplitude bind to erythrocytes in acral parts of the circulation and often have specificity for the I antigen on RBCs. The IgM cold agglutinin (IgM-CA) antibody activates the classical complement pathway. C1 esterase activates C4 and C2, ultimately generating the C3 convertase, which cleaves C3 to C3a and C3b. Upon return to warmer portions of the circulation (~37°C), the IgM-CA dissociates from the cell surface, but C3b remains bound to the RBC. The C3b-coated RBCs are then sequestered by macrophages of the reticuloendothelial system, predominantly in the liver (extravascular hemolysis). C3b of the surviving RBCs is eventually cleaved, leaving a high number of circulating RBCs with C3d on the surface. Patients with CAD have increased early mortality and a high risk of thromboembolism.

**Complement inhibition.** Rituximab is often used as initial therapy for CAD; it leads to remission (median duration 1 year) in roughly 50% of patients (73). Sutimlimab is a humanized monoclonal antibody that binds to C1s and inhibits classical complement activation (Figure 1). A recent phase Ib trial of sutimlimab in patients with CAD demonstrated that weekly intravenous dosing for 4 weeks followed by biweekly dosing thereafter rapidly aborted complement C1s-mediated hemolysis and significantly increased hemoglobin levels, precluding the need for RBC transfusions (74). All patients responded to sutimlimab within a few weeks, with a median rise in hemoglobin of almost 4 g/dL. Sutimlimab does not affect the production of cold agglutinins or their binding to RBC antigens; thus, CAD patients may still experience acrocyanosis.



**Figure 3. Complementopathies in the clinic.** (A) Model of the modified Ham (mHam) test.  $PIGA^{null}$  (PNH-like) TF1 cells do not express CD55 and CD59 and are therefore susceptible to complement-mediated killing. Cells are incubated with patient and control sera, then with a WST-1 cell proliferation dye reagent (Roche). Nonviable cells do not release dye because of complement-mediated killing, resulting in differences in measured absorbance. The percentage of live cells is calculated as the ratio of sample absorbance relative to its heat-inactivated control, multiplied by 100. The percentage of nonviable cells is a measure of complement activation. (B) Proposed model for APS and CAPS. Recent studies suggest that aPLs induce complement activation in patients with complement-amplifying trigger(s), such as infection, surgery, or autoimmune disease, and cause thrombosis in APS. Patients who also have a pathogenic loss-of-function mutation in a complement-inhibitory factor (e.g., CFH, CFI, CD46, or THBD) or a gain-of-function mutation of a complement-activating factor (e.g., CFB, C3) are likely to be predisposed to uncontrolled complement activation. In the setting of a complement-amplifying trigger, aPL-induced complement activation could lead to disseminated thrombosis and ischemic multiorgan failure in CAPS. PIGA, phosphatidylinositol *N*-acetylglucosaminyltransferase subunit A; PNH, paroxysmal nocturnal hemoglobinuria; APS, antiphospholipid syndrome; CAPS, catastrophic antiphospholipid syndrome; aPL, antiphospholipid antibody.

More recently, results from the phase III trial of sutimlimab have demonstrated efficacy in primary endpoints (a composite of hemoglobin increase  $\geq 2$  g/dL or hemoglobin  $\geq 12$  g/dL at treatment assessment [average from weeks 23, 25, and 26] and transfusion avoidance from week 5 to week 26) and secondary endpoints (change from baseline in hemolytic markers and quality of life) (75). Thus, targeting of C1s with sutimlimab, a more precise target than CD20, will likely become standard therapy for CAD.

## Nephrology

### Atypical or complement-mediated hemolytic uremic syndrome

**Complement activation.** Atypical hemolytic uremic syndrome (aHUS) presents as a thrombotic microangiopathy (TMA) with the clinical triad of microangiopathic hemolytic anemia, thrombocytopenia, and organ damage (76) with preserved function of the disintegrin and metalloproteinase ADAMTS13. Among TMAs, aHUS has long served as an archetypal disease model of complement dysregulation. Recently, two published consensus documents have changed the terminology of TMAs from a model based on underlying disease to a pathophysiology-driven model (77, 78), introducing the term complement-mediated HUS

(CM-HUS). The prevalent “two-hit” hypothesis for CM-HUS pathogenesis is that genetic or acquired (e.g., anti-complement factor H autoantibodies) defects in complement regulation shape a predisposing phenotype toward excessive complement activation. This complement phenotype is then coupled to a second hit that propagates complement amplification (79, 80). Complement-amplifying conditions are often infections, autoimmunity, surgery, pregnancy, or cancer.

CM-HUS-associated mutations cause either loss of function of complement-regulatory proteins, including complement factor H (CFH), complement factor I (CFI), thrombomodulin (THBD), and CD46/membrane cofactor protein (MCP), or gain of function of complement-activating proteins, including complement factor B (CFB) and C3 (81). Although THBD may also act as a complement regulator (82), further studies are needed to confirm the roles of coagulation pathway proteins (83). A recent study also revealed mutations in *VTN*, which encodes the terminal complement inhibitor vitronectin, in CM-HUS patients (84). The only mutations in this disease that are not associated with complement dysregulation are found in diacylglycerol kinase- $\epsilon$  (DGKE) (85, 86). Figure 2 summarizes mutations in these complement-related proteins. These germline variants in genes that regulate the APC are present in about 50% of patients with CM-HUS (87, 88). Factor H autoantibodies may also be found in up to 10% of CM-HUS (89). The majority of these patients

lack CFHR1 and CFHR3, owing to homozygous deletion of the genomic region that expresses them (90). Sequencing results do not affect early treatment decisions given the acute presentation, the time it takes to get results, and the uncertainty regarding the relevance of some germline variants (91).

Traditional biomarkers used in clinical complement laboratories, such as hemolytic assays of classical and alternative pathway activity (CH-50 and AP-50, respectively) and ELISA of C3 concentration or APC activity (Wieslab), are not reliable for CM-HUS diagnosis (92). Soluble C5b-9 is not diagnostic for CM-HUS because values have a substantial overlap with other TMAs (93). Translational studies have also used C5b-9 deposition on endothelial cells to detect evidence of complement activation in patients with TMAs (94, 95). In vivo deposition of C5b-9 on dermal microvessels in the transplant setting has also been shown (96). In an effort to develop a rapid and reliable in vitro diagnostic assay for CM-HUS, the modified Ham test has been suggested, as described in Figure 3A (97). The latter can distinguish between CM-HUS and thrombotic thrombocytopenic purpura (TTP), but the assay is not yet available in clinical laboratories (97–99).

**Complement inhibition.** CM-HUS is an urgent life-threatening syndrome requiring prompt initiation of therapy (100). The

diagnosis is suspected in a patient with TMA who is Shiga toxin-negative with ADAMTS13 activity over 10%. Distinction between TTP and CM-HUS is important, as plasma exchange does not reliably arrest the complement-mediated organ damage occurring in CM-HUS (101). Improvements in platelet count and lactate dehydrogenase (LDH) are usually seen within days of eculizumab administration (102, 103). Kidney recovery may take several weeks to months (104). Eculizumab is administered intravenously every 7 days for the first 5 weeks and biweekly thereafter; however, the optimal duration of therapy is unclear (103, 105, 106). While early reports suggested that long-term/indefinite therapy is required, more recent reports suggest that eculizumab may be safely discontinued in many CM-HUS patients (107–109). Before eculizumab is discontinued, the patient should be in complete remission (normal platelet counts, LDH, and renal function) and potential complement-activating “triggers” should be controlled. In addition to the risk of meningitis mentioned in association with PNH, eculizumab hepatotoxicity has been reported in pediatric CM-HUS (110).

### Glomerulopathies

Glomerulopathies consist of a wide range of diseases in the majority of which complement plays a central role. C3 glomerulopathy (C3G) is characterized by APC activation leading to C3 deposition in the glomeruli (111). Recent studies have found complement-related mutations in C3G, similar to those of CM-HUS. Interestingly, mutations in C3G cause different protein changes and, therefore, different phenotypes compared with CM-HUS mutations (112). These discoveries, along with experimental models of complement dysregulation (113), have prompted studies of complement inhibitors in these patients. Indeed, eculizumab has been administered in case reports and series of C3G transplant recipients (114–116). However, since the principal defect is caused by proximal complement activation, specific blockade is expected to show higher efficacy. Ongoing clinical trials are examining the efficacy of specific blockade in C3G with narsoplimab, sutimlimab, danicopan, and avacopan (Figure 1), all of which block complement activation more proximally. Apart from C3G, IgA nephropathy, lupus nephritis, and membranous nephropathy are also under study with complement inhibitors (117).

### Obstetrics: HELLP syndrome

HELLP (hemolysis, elevated liver enzymes, and low platelets) syndrome usually arises in the third trimester of pregnancy and resolves shortly after delivery (118). Although its pathogenesis is not fully clear, endothelial dysfunction, partly mediated by complement, plays a central role. Fetal mortality approaches 30% when HELLP syndrome occurs early in the third trimester; maternal mortality may also approach 5% to 10%. Investigators have hypothesized that CM-HUS and HELLP syndrome may share a similar pathophysiology, because the clinical manifestations of hypertension, renal insufficiency, thrombocytopenia, elevated LDH, elevated aspartate aminotransferase, and even the presence of schistocytes are common to both disorders. Recent data using next-generation sequencing and functional complement assays in HELLP patients support this hypothesis (98, 99). Similar to CM-HUS, rare germline variants (variant allele frequency <1%) in genes regulating the APC (e.g., *C3*, *CFH*, *CFB*, *MCP*, etc.)

and/or activation of complement using the modified Ham test are found in up to 50% of patients with HELLP syndrome (98, 99). These data suggest that, as with CM-HUS, a large subset of HELLP syndrome is driven by an inability to regulate complement. The thrombocytopenia is consumptive, the hemolysis is mechanical, and the elevated “liver function tests” (LDH, bilirubin, and aspartate aminotransferase) are actually markers of intravascular hemolysis rather than intrinsic liver dysfunction. Germline mutations in genes that regulate the APC may predispose to HELLP syndrome. Complement levels normally rise after the second trimester of pregnancy and may serve as a complement amplifier, along with other factors (autoimmunity, infection, etc.) that contribute to vascular damage (119, 120). Complement levels decrease following delivery, possibly explaining why the disease typically resolves postpartum. There are now several case reports describing the use of eculizumab to treat HELLP syndrome, but this is not an FDA-approved use of the drug (121).

### Transplantation: transplant-associated thrombotic microangiopathy

Transplant-associated TMA (TA-TMA) is a potentially life-threatening complication of allogeneic hematopoietic cell transplantation (HCT) (122). Although it manifests with the clinical triad of a TMA, diagnosis is largely hindered by the high incidence of cytopenias and organ dysfunction in HCT recipients. Current diagnostic criteria have been criticized for their diagnostic sensitivity (123). Moving the field forward, a growing number of genetic and functional data suggest increased complement activation in both the adult and pediatric population of TA-TMA (124–126). Soluble C5b-9 levels were also incorporated into recently proposed severity criteria of TA-TMA aiming to facilitate early diagnosis and treatment (127).

Eculizumab treatment is increasingly used to treat both adult and pediatric patients with TA-TMA (128–132). Despite high response rates to eculizumab treatment that reach 93%, overall survival remains low (~30%) in early reports from the adult population (130, 131). However, a recent study of 64 pediatric TA-TMA patients has shown an increased 1-year survival of 66% in eculizumab-treated patients compared with 17% in a historic control group (132). Several issues remain to be further investigated: timing of initiation, proper patient selection, dosing, and duration of therapy in patients with transplants. Interestingly, a novel C5 inhibitor, coversin, was successfully used in a TA-TMA patient with a C5 variant that caused resistance to eculizumab treatment (133). Recently, a phase II single-arm, open-label study of an inhibitor of the lectin pathway, the MASP-2 inhibitor OMS721/narsoplimab, in 19 TA-TMA patients also reported increased median overall survival in comparison with a historical control of conventional treatment (347 vs. 21 days from TA-TMA diagnosis) (134). As a result, a phase III clinical trial is ongoing (Table 2).

### Rheumatology: antiphospholipid antibody syndrome

Antiphospholipid antibody syndrome (APS) is an acquired thrombophilia characterized by thrombosis affecting the venous or arterial vascular systems and/or obstetrical morbidity with the persistent presence of antiphospholipid antibodies, including lupus anticoagulant, anticardiolipin antibody, and anti- $\beta_2$ -glycopro-



tein-I (anti- $\beta$ 2GPI) (135). A severe form of APS characterized by widespread thrombosis and multi-organ failure developing over less than a week, termed catastrophic APS (CAPS), affects a subset (~1%) of APS patients. CAPS often presents as a TMA and has a fulminant course with more than 40% mortality despite the best available therapy (136, 137).

Complement activation has been shown in murine models of APS, suggesting a crucial role of complement in antiphospholipid antibody-mediated thrombosis (138–141) and obstetric (142–144) complications. Increased C5b-9 (145), Bb fragments, and C3a (146, 147) have been observed in APS sera (148). More recent data demonstrate that complement activation in APS is triggered by anti- $\beta$ 2GPI antibodies (149). A positive modified Ham test, as described in Figure 3A, was highly predictive for thrombotic events. Moreover, more than 50% of patients with CAPS harbor rare germline variants in complement-regulatory genes, similarly to CM-HUS and HELLP syndrome patients. This may explain the more severe CAPS phenotype, as demonstrated in Figure 3B (149). In line with these data, several reports have documented efficacy of eculizumab in refractory thrombotic APS (150) and CAPS (151–154). Finally, eculizumab prevented recurrence of APS and enabled renal transplantation in three APS patients (155). Thus, future studies of complement inhibition are indicated for severe forms of APS and CAPS.

## Neurology

### Myasthenia gravis

The majority of myasthenia gravis (MG) patients express acetylcholine receptor antibodies (AChR-Abs) (156). These antibodies bind C1q, activate the complement cascade, and ultimately lead to MAC generation. Initial evidence of complement activation in MG patients (157–160) has been confirmed in complement-deficient mouse models, suggesting a crucial role of MAC-mediating signals in MG (161–165). Complement was successfully targeted with passive and active experimental studies in MG (166). These data led to the phase III randomized double-blind placebo-controlled REGAIN trial in 125 patients with AChR-Ab-positive refractory generalized MG (167). Based on significant improvements in activities of daily living, muscle strength, and health-related quality of life, eculizumab received regulatory approval for treatment of these patients.

### Neuromyelitis optica spectrum disorder

Neuromyelitis optica spectrum disorder (NMOSD) is a rare disorder of the central nervous system traditionally considered an autoimmune inflammatory disease and treated mainly with immunosuppressive agents such as rituximab (168). Antibodies against aquaporin-4 (AQP4) are found in the majority of patients

(169, 170) and have been shown to activate complement in vitro and in vivo (171, 172). Complement-mediated death of neurons near astrocytes was mitigated by complement inhibition (173). In this context, upregulation of the complement regulator CD55 has reduced NMOSD pathology (174).

A phase II study of eculizumab in 14 patients has shown the potential of the drug to prevent relapses (175). These results have been confirmed in the most recent randomized, double-blind, time-to-event trial in 143 AQP4-positive patients (176). It should be noted, however, that eculizumab did not improve measures of disability progression, suggesting that long-term administration needs to be evaluated in light of two additional clinical trials of immunotherapeutic agents in patients with NMOSD (177).

## Conclusion and future perspectives

Over the past few decades, our understanding of complement and precision medicine has evolved. Terminal complement inhibition is currently the mainstay of treatment for complement-mediated disorders, or complementopathies, across multiple medical specialties. Potentially novel indications span various disciplines, including hematology, nephrology, obstetrics, transplantation, rheumatology, and neurology. Complement involvement has been speculated in a wide range of entities that have not been described in detail in this Review, such as age-related macular degeneration (178), hyperhemolysis syndrome (21), neurodegenerative diseases (62), periodontitis (179), and anti-neutrophil cytoplasmic antibody (ANCA) vasculitis (180). Improvements in genetic and functional assays coupled with numerous novel and highly specific complement inhibitors will only increase the personalized approach to treating complementopathies.

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