# Clinical promise of next-generation complement therapeutics

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Abstract | The complement system plays a key role in pathogen immunosurveillance and tissue homeostasis. However, subversion of its tight regulatory control can fuel a vicious cycle of inflammatory damage that exacerbates pathology. The clinical merit of targeting the complement system has been established for rare clinical disorders such as paroxysmal nocturnal haemoglobinuria and atypical haemolytic uraemic syndrome. Evidence from preclinical studies and human genome-wide analyses, supported by new molecular and structural insights, has revealed new pathomechanisms and unmet clinical needs that have thrust a new generation of complement inhibitors into clinical development for a variety of indications. This review critically discusses recent clinical milestones in complement drug discovery, providing an updated translational perspective that may guide optimal target selection and disease-tailored complement intervention.

Pattern recognition receptors
A wide spectrum of soluble or membrane-bound proteins present on cells of the innate immune system that specifically recognize molecular signatures derived from the surface or interior of microbial cells, termed pathogen-associated molecular patterns, or distinct structures on artificial surfaces or altered host cells, termed damage-associated molecular patterns, to trigger a proinflammatory response that aims to respectively contain the microbial challenge or a maladaptive inflammatory response that may lead to tissue damage.

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*For extreme diseases, extremely targeted treatments are most efficacious (Hippocrates of Kos, Aphorisms)*

The innate immune response comprises humoral and cell-based host defence systems that act as signalling gateways, in both the vasculature and distant tissues, for the prompt recognition and elimination of foreign intruders[1]. Through the coordinated activation of the endothelial barrier, the recruitment of phagocytic cells and the release of proinflammatory mediators, these innate immune systems effectively thwart infectious threats and clear aged or damaged cells to maintain homeostasis[2]. However, when regulatory control is lost as a result of genetic or acquired aberrations, this protective response can turn against host surfaces, self-perpetuating a vicious cycle of inflammation and tissue damage[1].

Activation of the complement system represents a highly conserved mechanism by which innate immunity senses and triages danger signals that disturb tissue homeostasis[3,4]. It involves the recognition of molecular signatures typically present on immune complexes, damaged cells or microbial pathogens (for example, distinct carbohydrate profiles) by soluble pattern recognition receptors that form the triggering complexes in a proteolytic cascade consisting of three canonical pathways (that is, the classical, lectin and alternative pathways of complement, termed CP, LP and AP, respectively)[5,6] (FIG. 1). These highly ordered interactions lead to the formation of the central 'workhorses' of the system, the C3 and C5 convertases[3,6,7]. These convertases are multiprotein assemblies that acquire short-lived enzymatic properties and further amplify the complement response by producing bioactive fragments of the key proteins C3 and C5, which decorate target surfaces (opsonization) and promote phagocytic, inflammatory and immunomodulatory processes[4]. Further activation of the terminal (lytic) pathway leads to the elimination of complement-opsonized cells through direct membrane attack complex (MAC)-mediated lysis (FIG. 1).

Today, the traditional perception of complement is being redefined through the advent of new high-resolution and high-throughput technologies and the availability of more sophisticated molecular tools and disease models[4]. What was first described by Jules Bordet and Paul Ehrlich in the late 19th century as an auxiliary system that simply augmented the antimicrobial action of antibodies is now being perceived as a multitasking innate immune system that carries out unprecedented immunomodulatory and homeostatic functions at the interface of innate and adaptive immunity[3,8,9]. This multifaceted role is achieved through elaborate protein–protein interactions, conformational transitions and robust signalling crosstalk with other innate immune pathways, including extracellular and intracellular pattern recognition systems and vascular host defence systems such as the contact and coagulation cascades[4,10].

Notably, complement activation not only provides rapid protection from infectious challenge but can also slide to the 'dark side', becoming a driver or exacerbator of pathology in a plethora of inflammatory or autoimmune diseases[11]. When fluid-phase and surface-directed complement regulation is intact, complement proteins maintain their immunosurveillance function by sparing healthy cells. Their deleterious side, however, is exposed

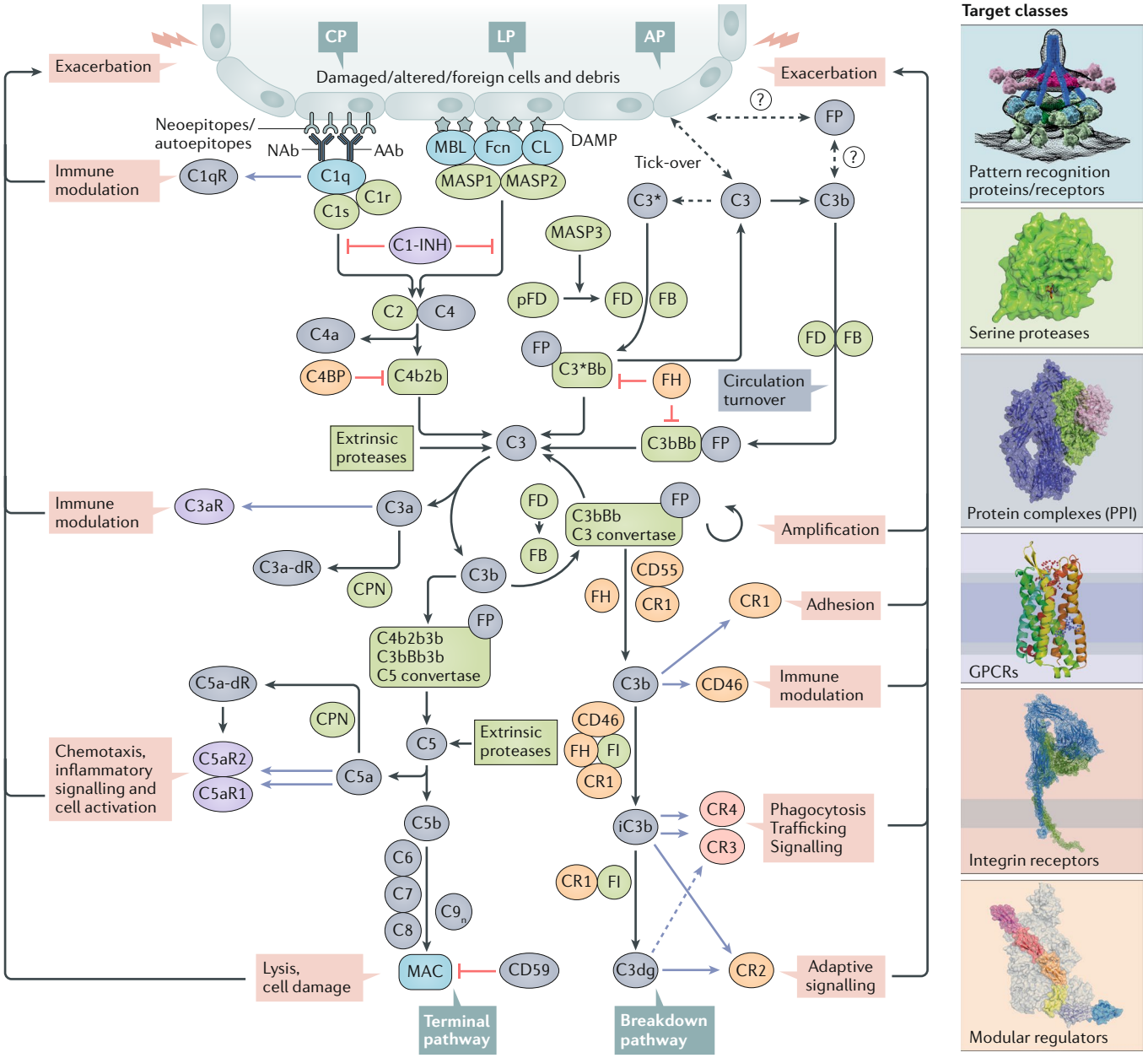


Fig. 1 | **Simplified scheme of the complement cascade with disease-relevant effector functions and major drug target classes.** Whereas complement typically exerts its various effector functions for defensive purposes, damaged host cells or foreign surfaces (for example, transplants and biomaterials) may trigger the cascade and cause clinical complications. For example, binding of natural antibodies (NAbs) to neoepitopes, released, for example, after hypoxia, or of auto-antibodies (AAbs) to auto-antigens may initiate the classical pathway (CP). Other damage-associated molecular patterns (DAMPs) can trigger the CP directly or invoke the lectin pathway (LP) via any of its pattern-recognition receptors, that is, mannose-binding lectin (MBL), ficolins (Fcns) or certain collectins (CLs). Either event leads to the formation of CP/LP C3 convertases (C4b2b), which in turn cleave C3 to generate the anaphylatoxin C3a and the opsonin C3b. Hydrolysis of C3 (depicted as C3*; also termed C3($H_2O$)), which may either occur spontaneously (tick-over) or upon surface contact, provides a low-level activation of the alternative pathway (AP) by forming an initial AP C3 convertase (C3*Bb) with Factors B and D (FB and FD); thereby released C3b may assemble further AP C3 convertases (C3bBb), deposit directly on surfaces or, potentially, be recruited to select surfaces by the modulator properdin (FP). Surface-deposited C3b is the driving force of an amplification loop that leads to rapid opsonization with C3b. Increasing density of C3b favours the formation of CP/LP or AP C5 convertases (C4b2b3b and C3bBb3b, respectively) that cleave C5 to generate the potent chemoattractant and immune modulator of C5a and C5b, the initial component of the membrane attack complex (MAC), which may cause lysis, cell damage and/or signalling events. In a parallel breakdown pathway, complement regulators such as Factor H (FH), complement receptor 1 (CR1) or CD55 destabilize convertases and impair the amplification loop. Some regulators also serve as essential cofactors for the Factor I (FI)-mediated degradation of C3b to iC3b (that is, FH, CR1 and CD46) and C3dg (that is, CR1). These opsonin fragments modulate immune functions, including cell adhesion and activation, phagocytosis, cell signalling and stimulation of B cells and follicular dendritic cells. FH is the major fluid-phase regulator of the AP, whereas C4b-binding protein (C4BP) and C1 esterase inhibitor (C1-INH) control CP/LP activation, carboxypeptidase N (CPN) modulates anaphylatoxin activity and CD59 impairs MAC formation. Many complement proteins belong to major drug target classes, such as serine proteases or G protein-coupled receptors (GPCR), and/or engage in protein–protein interactions (PPIs) that may be modulated with therapeutic antibodies. MASP, mannose-binding lectin-associated serine protease; pFD, pro-FD. Top right image adapted with permission from REF.[226], Science/AAAS.

Constrained peptides
A new class of peptide molecules whose supramolecular structure is constrained into a particular conformation via intramolecular covalent bonds that endow these peptides with biochemical and/or physicochemical properties amenable to drug development.

RNA aptamers
Single-stranded RNA-based biopolymer sequences selected from a large, random sequence pool by virtue of their ability to bind a molecular target with high selectivity.

when damaged cells or artificial surfaces are sensed by complement pattern recognition receptors or when host surfaces are not sufficiently protected by regulatory molecules that, under steady state conditions, drive the C3b breakdown cycle, preventing AP amplification and further C3 fragment deposition[12] (FIG. 1). In such cases, persistent assembly of surface-bound C3 and C5 convertases fuels effector generation and inflammatory tissue damage. In fact, excessive or deregulated complement activation has been linked to the pathogenesis of a wide spectrum of disorders ranging from acute inflammatory conditions (for example, sepsis, ischemic stroke and antibody-mediated graft rejection), ocular pathologies and periodontal diseases to chronic disorders, including cancer, autoimmune and age-related neurodegenerative diseases, renal disorders, and chronic haemolytic conditions with a thromboinflammatory signature[1,13–15].

In view of their prominent pathogenic involvement, diverse complement effectors have gained attention in recent years as tractable targets for therapeutic modulation and as scaffolds for drug development[16–19]. Over a decade ago, the clinical approval of the first complement-specific drug, eculizumab (Soliris, Alexion), for the treatment of the haemolytic disorder paroxysmal nocturnal haemoglobinuria (PNH) marked an important milestone in complement drug discovery[20]. Eculizumab's approval came as a culmination of earlier studies in the 1980s that had spearheaded the development of C5-based therapies (that is, monoclonal antibody (mAb) BB5.1) and led to the first proof-of-efficacy preclinical studies in inflammatory disease models[21,22]. Clinical application of eculizumab was soon extended to patients with atypical haemolytic uraemic syndrome (aHUS) (in 2011), a thrombotic microangiopathy of renal significance fuelled by AP dysregulation[23]. In 2017, Soliris was granted clinical approval for the treatment of refractory generalized myasthenia gravis (gMG) in patients positive for acetylcholine receptor autoantibodies, thus further validating therapeutic complement modulation in the clinical setting[24]. In addition to anti-C5 therapy, complement drug discovery has also benefited from the clinical approval of recombinant and plasma-purified preparations of C1 esterase inhibitor (C1-INH), a plasma serine protease inhibitor that has broad specificity against the CP and LP as well as the coagulation, kinin and fibrinolytic cascades[25]. C1-INH (Cinryze) received US Food and Drug Administration (FDA) approval for use in patients with hereditary angioedema in 2008. While not a strictly complement-specific agent, C1-INH has provided an efficacious supplementation therapy for hereditary angioedema and has also contributed to the launch of several clinical trials addressing the combined therapeutic targeting of the CP and LP in human disease.

The launch of anti-C5 therapy provided proof of efficacy for complement modulation in human diseases and raised confidence in this approach, thereby ushering a new era in complement drug discovery. Unprecedented progress in the structural resolution of multidomain proteins, protein modules and multiprotein assemblies involved in target recognition and AP amplification[26], supported by genome-wide association studies, molecular insights from disease models and clinical observations, has rekindled the interest of pharmaceutical companies in developing drug candidates that target multiple components of the cascade[16,18].

Structure-guided drug design has culminated in a series of drug leads with high selectivity and improved inhibitory potency that are steadily advancing closer to the clinic. Of note, the complement system features almost all druggable target classes, including serine proteases, G protein-coupled receptors and, thanks to the success of therapeutic antibodies, protein–protein interactions[18] (FIG. 1). Alongside this diversity of targets lies an equally diverse arsenal of clinically developed therapeutics, ranging from small molecules and constrained peptides to small-interfering RNAs, antibodies and RNA aptamers[16,18]. Overcoming translational hurdles such as the saturation of abundant plasma proteins, pharmacokinetic (PK) issues and poor drug bioavailability, these new drug leads offer the opportunity to intercept disease-promoting complement activities upstream of C5 and even centrally, at the level of C3. In certain indications, broader inhibition of complement at the level of C3 may warrant investigation, leveraging clinical benefits over existing therapies, and C3-targeted therapeutics are now being evaluated in long-awaited phase II/III trials.

Despite the progression of drug leads to late-stage clinical development, almost a decade after the launch of eculizumab, the complement drug market still lacks approved therapeutics against alternate targets in the cascade. Of even greater concern, high expectations raised by a drug candidate developed for ocular indications (that is, the Factor D (FD)-targeting mAb lampalizumab) failed to translate into meaningful clinical responses in phase III trials[27], indicating that individual challenges related to drug bioavailability or efficacy remain to be addressed. The recent FDA approval of a long-acting version of eculizumab (ALXN1210/ravulizumab, Ultomiris, Alexion), which features an extended plasma residence and only requires administration every 8 weeks instead of biweekly, is an important step in improving patient management but cannot be considered a bona fide new drug entry in the field[28–30]. With the current clinical availability of only a single complement-specific drug, which evidently cannot cater for all clinical indications, the need for disease-tailored therapeutic approaches is becoming ever more urgent.

This review critically discusses recent developments in complement drug discovery, highlighting the opportunities and challenges leading up to the successful clinical translation of new therapeutics. Emphasis is placed on major disease areas in which complement-based drug candidates are advancing through clinical trials such as for acute or transient indications with prominent complement involvement and chronic haematological, renal and ocular inflammatory diseases. Embracing the clinical potential of complement modulation in cancer and neurodegenerative diseases, we briefly discuss the preclinical findings, translational efforts and therapeutic prospects offered by complement inhibitors in these emerging new indications (BOX 1).

### Acute or transient complement interception

Therapeutic modulation of complement in disease settings that entail acute or transiently increased complement activation has garnered considerable attention by both research groups and pharmaceutical companies[18]. Shutting down complement's activity in a well-defined and narrow time window appears to be a low-risk approach that could afford maximal therapeutic benefit from a clinical perspective, particularly in view of complement's key role in immunosurveillance and pathogen clearance[31]. Indeed, complement inhibitors targeting multiple components of the cascade are being developed for indications associated with an overactive or deregulated complement response that can drive thromboinflammatory activation on ischemic tissue following reperfusion, on biomaterial surfaces coming in close contact with blood in extracorporeal circuits or medical implants, or in donor organs through various stages of the transplantation process[32,33] (an overview of major targeted conditions is provided in FIG. 2).

#### *Ischaemia–reperfusion (I/R) organ injury*

Inappropriate complement activation on host surfaces exposed to I/R has been well documented in several preclinical models, and therapeutic intervention has shown promising results ameliorating key pathological markers[34]. Complement activation is thought to predominantly involve the lectin and alternative pathways by virtue of the recognition of certain carbohydrate signatures (for example, terminal mannose or fucose)[35] on ischemic endothelial tissue by mannose-binding lectin or collectin 11 (REF.[36]) and the capacity of the AP to amplify complement deposition on infarcted tissue[37] (FIG. 2b). The tight linkage of the LP and AP is made apparent by the ability of the LP-associated protease, mannose-binding lectin-associated serine protease 3 (MASP3), to act as the enzyme that supplies mature FD to the circulation through the cleavage of pro-FD[38] (FIG. 1), further supporting their overlapping pathogenic involvement in I/R injury. The CP has also been implicated in the pathogenesis of I/R injury through the binding of natural IgM to certain neoantigens exposed on ischemic tissue[39]. The role of the terminal pathway has been debated, with conflicting results, in preclinical models of ischemic stroke[37].

I/R injury is the major cause of delayed graft function (DGF) in transplanted kidneys, an early event significantly affecting the graft's long-term function and survival[40]. Use of C1-INH, a broad serine protease inhibitor with activity against CP-associated and LP-associated proteases, attenuated inflammatory markers and prevented complement deposition in a porcine model of kidney I/R injury[40]. Following up on these promising results, a phase II trial has evaluated the effect of C1-INH (Berinert) in reducing I/R damage in recipients of deceased donor kidney transplants, who are at high risk for DGF[41] (TABLE 1).

Earlier efforts to target complement in the ischemic heart were translated into a phase II clinical trial in patients undergoing cardiopulmonary bypass that evaluated the efficacy of TP10/CDX1135 (Celldex), a recombinant form of soluble complement receptor 1 inhibiting the CP and AP C3/C5 convertases[42,43] (TABLE 1). TP10 treatment resulted in a reduction in myocardial infarction in male patients undergoing cardiopulmonary bypass, suggesting that central inhibition at the level of C3 could likely offer broader protection to ischemic organs by shutting down early C3 opsonization of ischemic tissues[44]. However, limited efficacy and the observed gender specificity of TP10's activity restricted the true translational value of this trial[45],

Box 1 | **Targeting complement in cancer and neurodegenerative diseases**

**Cancer immunotherapy**

Cancer-associated inflammation is steadily appreciated as a driver of tumorigenesis[234]. While traditionally considered an auxiliary system for enhancing tumour antibody-mediated effector responses, complement is now increasingly perceived as a proinflammatory driver of tumour progression via immunosuppressive functions in the tumour microenvironment[96,235]. The capacity of complement proteins, such as C3 fragments, C3a–C3aR or C5a–C5aR1, to downregulate antitumour T cell responses through recruitment and/or activation of myeloid-derived suppressor cells, regulatory T cells or M2 tumour-associated macrophages has propelled translational research to exploit these targets therapeutically[235–237]. Preclinical studies in syngeneic models of lung, skin and colon cancer have indicated the clinical promise of combining immune checkpoint blockade (anti-programmed cell death 1/programmed cell death 1 ligand 1) with C5a–C5aR1 targeting to enhance antitumour $CD8^+$ T cell responses[238,239]. Prompted by these observations, Innate Pharma has instigated a phase I/II study (STELLAR-001) in which the safety and efficacy of durvalumab (an anti-programmed cell death 1 ligand 1 monoclonal antibody) is being tested in combination with IPH5401 (an anti-C5aR1 monoclonal antibody) in patients with selected solid tumours[240] (NCT03665129) (TABLE 1). Moreover, C3 inhibition is also emerging as a viable approach to achieve broader interception of immunosuppressive pathways in the tumour microenvironment[96]. This notion was recently validated in a human ex vivo epithelial ovarian cancer model in which compstatin Cp40 abrogated the suppressor phenotype of ascites-primed neutrophils, thereby offering a handle to potentiate antitumour T cell responses in epithelial ovarian cancer[241]. Results from ongoing trials will be informative regarding the clinical benefit of complement inhibition in multimodal cancer immunotherapy, but there is still a long way to go regarding the validation of complement biomarkers for patient staging or prognosis[96,235]. Broadening our understanding of the mechanisms driving complement activation during cancer evolution and metastasis will be instrumental in designing effective complement-targeted immunotherapies.

**Neurodegenerative disorders**

Accumulating evidence from preclinical models, genetic and epidemiological studies, and human biomarker profiling have implicated multiple complement effectors (C1q, C3, C3a C5a and C5b-9) in age-related synaptic impairment and microglia-driven neuroinflammatory and demyelinating pathologies of the central nervous system (CNS), including Alzheimer disease, Parkinson disease, multiple sclerosis and neuromyelitis optica[242–245]. The pathogenic role of complement in advanced stages of neurodegeneration and cognitive decline is likely countered by its beneficial housekeeping role in the early stages of the removal of toxic β-amyloid oligomers, neurofibrillary tangles and damaged neurons[242]. This dual role of complement, along with the low penetration of the blood–brain barrier (BBB) by systemically delivered therapeutics, poses a challenge in designing clinical studies. By itself, the optimal therapeutic window for administering complement inhibitors in chronic CNS diseases will be hard to define. Nevertheless, studies employing the C5aR1 antagonists PMX205/PMX53 in multiple pathologies have suggested a sustained therapeutic benefit of C5aR1 blockade in the inflamed CNS[246–248] and point to the capacity of small-sized inhibitors to penetrate the leaky BBB and home into the brain at pharmacologically effective doses. To date, human data regarding neurological diseases have been scarce, but the anti-C5 mAb eculizumab has reversed pathology in a pilot study of patients with neuromyelitis optica, a demyelinating disease characterized by BBB disruption and degeneration of the optic nerve and spinal cord[249] (TABLE 1). Finally, the fine-tuning roles of complement during basal neurogenesis, neural progenitor migration and CNS development should not be disregarded when chronic therapeutic regimens are being designed that might affect the developing fetus[250,251].

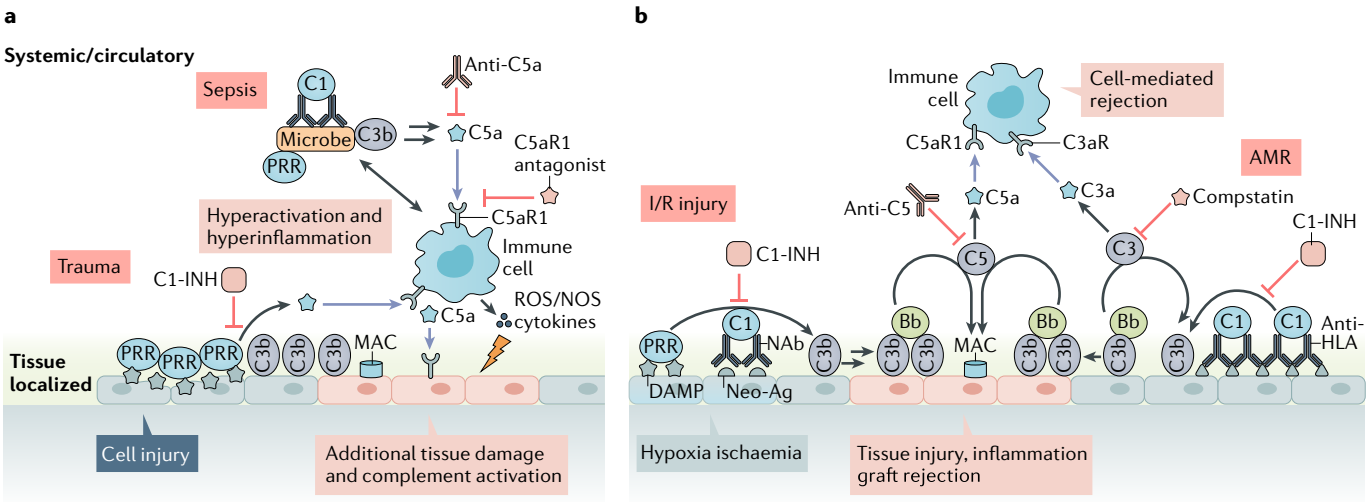


Fig. 2 | **Examples of acute or transient complement-mediated disorders with currently evaluated treatment strategies. a** | Trauma-related and sepsis-related tissue damage are examples of complement involvement during severe inflammatory response syndrome. Microbial intruders (sepsis) or cell injury (trauma) lead to massive complement activation and stimulation of immune cells (for example, via the C5a anaphylatoxin). The resulting effector molecules, such as reactive oxygen and nitrogen species (ROS and NOS, respectively), cytokines or the membrane attack complex (MAC), cause additional tissue damage and fuel a vicious hyperinflammatory cycle that may lead to multiple organ failure. Whereas prevention of pattern recognition receptor (PRR)-mediated complement initiation by C1-esterase inhibitor (C1-INH) is currently evaluated in trauma, sepsis trials largely focus on blocking the C5a–C5aR1 signalling axis. **b** | Complement is a major contributor to transplant-related complications. Ischaemia–reperfusion (I/R) injury during transplantation (but also in stroke or myocardial infarction) triggers the exposure of damage-associated molecular patterns (DAMP) and/or neoantigens (Neo-Ag) that, upon reperfusion, are sensed by the PRR complexes of the lectin pathway and natural antibodies (NAb) via the classical pathway invoking a complement response. Similarly, mismatch with human leukocyte antigen (HLA) or ABO antigens after transplantation leads to pronounced classical pathway activation and may drive antibody-mediated rejection (AMR). In addition, these processes may contribute to immune cell activation and, consequently, to cell-mediated rejection. Therapeutic intervention at the initiation stage (using C1-INH), the amplification loop (using C3 inhibitors of the compstatin family) or at terminal effector pathways (using anti-C5 mAbs) are currently being evaluated.

and the development of this drug candidate for cardiovascular and other indications was later discontinued by Celldex.

Mirococept (APT070), a cytotopic complement inhibitor encompassing the first three short consensus repeat domains of CR1 fused to a membrane-tethering peptide and a membrane-inserting myristoyl group[46], has also entered clinical development as a treatment option for I/R injury during transplantation. A multicentre phase II trial is assessing the efficacy of APT070 in preventing kidney I/R injury and reducing the incidence of DGF in cadaveric renal allografts[47] (TABLE 1).

Proof of concept for the therapeutic efficacy of targeting C3 in I/R injury has largely been provided by studies of surface-directed AP inhibitors in models of intestinal and cerebral ischaemia–reperfusion and post-ischemic stroke. Indeed, surface-directed inhibition of C3 convertase activity via chimeric recombinant constructs that combine regulatory and C3 opsonin-binding moieties has ameliorated key pathological indices in preclinical models of stroke[48,49]. C3 deficiency and site-targeted complement inhibition with either CR2-Crry (inhibiting all complement pathways) or CR2-FH (inhibiting the AP) have been found to significantly reduce infarct size and improve neurological recovery in the acute phase after stroke in a model of transient middle cerebral artery occlusion[50]. A recent preclinical study has provided further leverage for the translational potential of C3 inhibition in ischemic stroke[51] by employing a 'fusion' complement inhibitor (B4Crry) that targets all three complement pathways at the level of the C3 convertase[52]. Its inhibitory moiety, Crry, is a functional analogue of the human C3 regulators CD46 and CD55. By virtue of its single-chain variable fragment moiety (B4) that specifically recognizes a stroke-associated neoepitope in the ischemic brain, this inhibitor homes into the ischemic region, blocking C3 opsonization, and improving long-term motor and cognitive recovery after systemic delivery[52]. Overall, a series of preclinical I/R studies in various organs have provided a robust conceptual basis for developing C3-based therapeutics as new treatment options for ameliorating the early neurodegenerative consequences of ischemic and haemorrhagic stroke.

Adding further diversity to the toolbox of complement therapeutics tested in cerebral I/R injury, antibody blockade of the key LP enzyme, MASP2, has markedly improved neurological and histopathological outcomes after focal cerebral ischaemia, suggesting that LP targeting may be therapeutically beneficial in patients with ischemic stroke[53]. Of note, the inhibitory MASP2 antibody (HG4) used in these studies is a derivative of the human MASP2-targeting mAb OMS721, which has been clinically developed by Omeros for several complement-mediated diseases[54].

Table 1 | **Complement therapeutics in various stages of clinical development for complement-mediated indications**

| Target | Drug candidate/trial sponsor | Drug class/mode of administration | Mechanism of action | Stage of clinical development/trial identifier |
|---|---|---|---|---|
| ***Sepsis/multiorgan dysfunction*** | | | | |
| C1s/r, MASPs/other proteases | C1-INH (Cetor), Radboud University | Protein, IV | CP/LP inhibition, other serine proteases | Phase III (NCT01766414) |
| C5a | IFX-1/CaCP29, InflaRx | mAb, IV | Blocking binding of C5a to C5aR1 | Phase II (currently on hold) (NCT02246595) |
| ***Kidney I/R*** | | | | |
| C1s/r, MASPs/other proteases | C1-INH (Berinert), Cedars-Sinai Medical Center | Protein, IV | CP/LP inhibition, other serine proteases | Phase II (NCT02134314) |
| C3/C5 convertases | Mirococept (APT070), Medical Research Council, UK | Protein, IV | Inhibition of CP and AP C3/C5 convertases | Phase II (completed) (ISRCTN49958194) |
| ***Myocardial infarction*** | | | | |
| C3/C5 convertases | TP10/CDX-1135 (soluble complement receptor 1), Avant Immunotherapeutics | Protein, IV | Inhibition of CP and AP C3/C5 convertases | Phase II (completed 2005) (NCT00082121) |
| ***Trauma/SIRS*** | | | | |
| C1s/r, MASPs/other proteases | C1-INH (Cetor/Cinryze), Sanquin/ UMC Utrecht | Protein, IV | CP/LP inhibition, other serine proteases | Phase III (terminated) (NCT01275976) |
| ***Kidney Tx/antibody-mediated rejection*** | | | | |
| C1s/r, MASPs/other proteases | C1-INH (Berinert), Cedars-Sinai Medical Center | Protein, IV | CP/LP inhibition, other serine proteases | Phase I/II (NCT01134510) |
| | C1-INH (Cinryze), Shire | Protein, IV | CP/LP inhibition, other serine proteases | Phase III (NCT02547220) |
| C5 | Eculizumab (Soliris), Alexion | mAb, IV | Blockage of C5 activation | Phase II (NCT01567085, NCT01399593) |
| ***ABO-incompatible kidney Tx*** | | | | |
| C5 | Eculizumab (Soliris), Mayo Clinic/ Alexion | mAb, IV | Blockage of C5 activation | Phase I/II (terminated) |
| C3 | AMY-101, Amyndas | Non-PEGylated peptide, IV | Inhibition of C3 activation | Plan for phase Ib announced |
| ***Positive cross match (anti-human leukocyte antigen) kidney Tx*** | | | | |
| C5 | Eculizumab (Soliris), Alexion/Mayo Clinic | mAb, IV | Blockage of C5 activation | Phase I/II (NCT00670774) |
| ***Haemodialysis-induced inflammation*** | | | | |
| C3 | AMY-101, Amyndas | Non-PEGylated peptide, infusion into the dialysis line | Inhibition of C3 activation | Plans for phase Ib announced |
| ***Periodontal disease*** | | | | |
| C3 | AMY-101, Amyndas | Non-PEGylated peptide, local (intragingival) | Inhibition of C3 activation | Phase IIa (NCT03694444) |
| ***PNH*** | | | | |
| C5 | Eculizumab (Soliris), Alexion | mAb, IV | Blockage of C5 activation | In the clinic |
| | Ravulizumab/ALX1210 (Ultomiris), Alexion | mAb, IV/SQ extended dosing interval | Blockage of C5 activation (targets same epitope as eculizumab) | In the clinic |
| | SKY59/RO7112689, Hoffmann-La Roche | mAb, IV/SQ | Blockage of C5 activation (different C5 epitope) | Phase I/II (NCT03157635) |
| | Tesidolumab/LFG316, Novartis | mAb, IV | Blockage of C5 activation (different C5 epitope) | Phase II (NCT02534909) |
| | Pozelimab/REGN3918, Regeneron | mAb, IV/SQ | Blockage of C5 activation (different C5 epitope) | Phase I (NCT03115996) |
| | ABP959, Amgen | mAb, IV | Biosimilar of eculizumab | Phase III (NCT03818607) |
| | SB12, Samsung Bioepis | mAb, IV | Biosimilar of eculizumab | Phase I (NCT03722329) |

Table 1 (cont.) | **Complement therapeutics in various stages of clinical development for complement-mediated indications**

| Target | Drug candidate/trial sponsor | Drug class/mode of administration | Mechanism of action | Stage of clinical development/trial identifier |
|---|---|---|---|---|
| ***PNH (cont.)*** | | | | |
| C5 (cont.) | Coversin/OmCI, Akari Therapeutics | Protein, SQ | Inhibition of C5 activation | Phase II/III (NCT03427060, NCT03829449) |
| | Zilucoplan/RA101495, Ra Pharmaceuticals | Peptide macrocycle, SQ | Allosteric inhibition of C5 activation | Phase III announced (NCT03078582) |
| | Cemdisiran/ALN-CC5, Alnylam | RNA interference therapeutic, SQ | Inhibition of hepatic expression of C5 | Phase II (NCT02352493) |
| C3 | APL-2, Apellis | PEGylated peptide, SQ | Inhibition of C3 activation | Phase III (NCT03500549) |
| | AMY-101, Amyndas | Non-PEGylated peptide, SQ | Inhibition of C3 activation | Plans for phase IIa announced |
| Factor B | LNP023, Novartis | Small molecule, oral | Inhibition of AP C3 convertase | Phase II (NCT03439839) |
| Factor D | ACH-4471/ACH-0144471, Achillion | Small molecule, oral | Inhibition of AP C3 convertase | Phase II (NCT03053102, NCT03472885) |
| ***Autoimmune haemolytic anaemias (CAD, wAIHA)*** | | | | |
| C1s | Sutimlimab/BIV009/ TNT009, Sanofi/Bioverativ | mAb, IV | CP inhibition/inhibition of C1s protease | Phase III (NCT03347422, NCT03347396) |
| C3 | APL-2, Apellis | PEGylated peptide, SQ | Inhibition of C3 activation | Phase II (NCT03226678) |
| ***aHUS*** | | | | |
| C5 | Eculizumab (Soliris), Alexion | mAb, IV | Blockage of C5 activation | In the clinic |
| | Ravulizumab/ALX1210 (Ultomiris), Alexion | mAb, IV extended dosing interval | Blockage of C5 activation (same C5 epitope) | Phase III (NCT03131219) |
| | Coversin/OmCI, Akari Therapeutics | Protein, SQ | Inhibition of C5 activation | Phase II/III (NCT03829449) |
| C5aR1 | Avacopan/CCX168, ChemoCentryx | Small molecule, oral | Antagonist of the C5aR1 receptor | Phase II (NCT02464891) |
| MASP2 | OMS721, Omeros | mAb, IV | LP inhibition, blockage of MASP2 activity | Phase III (NCT03205995) |
| ***Transplant-associated TMAs*** | | | | |
| C5 | Eculizumab (Soliris), Children's Hospital Medical Center, Cincinnati | mAb, IV | Blockage of C5 activation | Phase II (NCT03518203) |
| MASP2 | OMS721, Omeros | mAb, IV | LP inhibition, blockage of MASP2 activity | Phase II (basket trial: NCT02222545) |
| ***Anti-neutrophil cytoplasmic antibody-associated vasculitis*** | | | | |
| C5aR1 | Avacopan/CCX168, ChemoCentryx | Small molecule, oral | Antagonist of the C5aR1 receptor | Phase III (NCT02994927) |
| ***IgA nephropathy*** | | | | |
| MASP2 | OMS721, Omeros | mAb, IV | LP inhibition, blockage of MASP2 activity | Phase III (NCT03608033) |
| ***C3G*** | | | | |
| C3 | APL-2, Apellis | PEGylated peptide, SQ | Inhibition of C3 activation | Phase II (basket trial: NCT03453619) |
| | AMY-101, Amyndas | Non-PEGylated peptide, SQ | Inhibition of C3 activation | Plans for phase IIa announced |
| Factor B | LPN023, Novartis | Small molecule, oral | Inhibition of AP C3 convertase | Phase II (NCT03832114) |
| C5aR1 | Avacopan/CCX168, ChemoCentryx | Small molecule, oral | Antagonist of the C5aR1 receptor | Phase II (NCT03301467) |
| MASP2 | OMS721, Omeros | mAb, IV | LP inhibition, blockage of MASP2 activity | Phase II (NCT02682407) |
| Factor D | ACH-4471/ACH-0144471, Achillion | Small molecule, oral | Inhibition of AP C3 convertase | Phase II (NCT03459443) |

Table 1 (cont.) | **Complement therapeutics in various stages of clinical development for complement-mediated indications**

| Target | Drug candidate/trial sponsor | Drug class/mode of administration | Mechanism of action | Stage of clinical development/trial identifier |
|---|---|---|---|---|
| ***Ocular diseases (AMD)*** | | | | |
| C5 | Eculizumab (Soliris), Alexion | mAb, IVT | Blockage of C5 activation | Phase II in GA (NCT00935883) |
| | Tesidolumab/LFG316, Novartis | mAb, IVT | Blockage of C5 activation | Phase II in GA (NCT01527500) |
| | Zimura/avacincaptad pegol, Ophthotech Corp | RNA aptamer, IVT | Inhibition of C5 expression | Phase II in GA (NCT02686658)/ phase II in combination with Lucentis/anti-vascular endothelial growth factor in wet AMD (NCT03362190) |
| Factor D | Lampalizumab, Genentech/Roche | Fab, IVT | Blockage of AP C3 convertase formation | Phase III in GA (terminated) (NCT02247531, NCT02247479) |
| Properdin | CLG561, Alcon/Novartis | mAb, IVT | Inhibition of AP amplification | Phase II in GA (tested in combination with LFG316) (NCT02515942) |
| FB | IONIS-FB-$L_{Rx}$, Ionis Pharmaceuticals/Roche | Antisense oligonucleotide, SQ | Inhibition of hepatic FB expression | Phase II in GA (NCT03815825) |
| C3 | APL-2, Apellis | PEGylated peptide, IVT | Inhibition of C3 activation | Phase III in GA (NCT03525613) |
| ***Cancer*** | | | | |
| C5aR1 | IPH5401, Innate Pharma | mAb, IV | Blockade of C5aR1 signalling | Phase I/II: IPH5401 tested in combination with anti-programmed cell death 1 ligand 1/durvalumab in advanced solid tumours (NCT03665129) |
| DR5 | GEN1029, GenMab | HexaBody, IV | Enhancement of CDC against $DR5^+$ tumours | Phase I/II (NCT03576131) |
| ***Autoimmune neuromuscular disorders (gMG)*** | | | | |
| C5 | Eculizumab (Soliris), Alexion | mAb, IV | Blockage of C5 activation | In the clinic |
| | Ravulizumab/ALX1210 (Ultomiris), Alexion | mAb, IV extended dosing interval | Blockage of C5 activation (same epitope on C5) | Announced plans for phase I/II |
| ***Recurrent neuromyelitis optica*** | | | | |
| C5 | Eculizumab (Soliris), Alexion | mAb, IV | Blockage of C5 activation | Phase III (NCT01892345) |
| ***Inflammatory skin diseases (hidradenitis suppurativa)*** | | | | |
| C5a | IFX-1/CaCP29, InflaRx | mAb, IV | Blocking binding of C5a to its receptor C5aR1 | Phase IIb completed (NCT03487276) |

aHUS, atypical haemolytic uraemic syndrome; AMD, age-related macular degeneration; AP, alternative pathway; C1-INH, C1 esterase inhibitor; C3G, C3 glomerulopathy; CAD, cold agglutinin disease; CDC, complement-dependent cytotoxicity; CP, classical pathway; DR5, death receptor 5; Fab, antigen-binding fragment; FB, Factor B; GA, geographic atrophy; gMG, generalized myasthenia gravis; I/R, ischaemia–reperfusion; IV, intravenous; IVT, intravitreal; LP, lectin pathway; mAb, monoclonal antibody; MASPs, mannose-binding lectin-associated serine proteases; OmCI, *Ornithodoros moubata* complement inhibitor; PNH, paroxysmal nocturnal haemoglobinuria; SIRS, systemic inflammatory response syndrome; SQ, subcutaneous; TMA, thrombotic microangiopathy; Tx, transplantation; wAIHA, warm antibody autoimmune haemolytic anaemia.

### *Organ transplantation*

It is increasingly appreciated that solid organ transplantation triggers several pathogenic pathways that are tightly intertwined with various effectors of complement activation, both in the vasculature and on the allograft surface[55,56]. In addition to its cardinal role in triggering donor organ inflammatory damage via CP/LP-mediated neoepitope recognition during I/R, complement activation is also considered a major pathogenic driver in acute antibody-mediated rejection (ABMR) following allogeneic organ transplantation[32,57,58] (FIG. 2b). Moreover, its multifaceted role in driving adaptive immune stimulation and humoral responses is growingly appreciated as a factor further affecting long-term graft function[32]. Sensitization of transplant recipients by donor-specific human leukocyte antigen (HLA)-directed or ABO-directed alloantibodies marks a key initial trigger for both acute transplant rejection and for fuelling chronic cell-mediated organ injury and rejection (that is, across HLA and ABO transplantation barriers)[56,58]. Given the pervasive nature of complement's involvement in the transplant process, it is hardly surprising that pharmaceutical companies are devoting attention to targeted therapeutics for organ transplantation. Defining the optimal point for complement modulation in transplantation is still highly debated; however, effective complement modulation could likely be achieved in a narrow time window that could be vital for promoting early graft accommodation and thus prolonging the transplant's survival[32,55].

Prompted by the clinical success of anti-C5 therapy in haematological and renal indications, recent studies have evaluated the safety and efficacy of complement intervention in mitigating allograft injury in conditions

ranging from ABMR and recurrent post-transplant aHUS, to post-transplant recurrent C3 glomerulopathies and antiphospholipid antibody syndrome[59]. Therapeutic modulation at the level of C5 has shown promise in alleviating complications of early ABMR and reducing incidence rates of kidney graft rejection in transplant patients with circulating anti-HLA donor-specific antibodies[60,61] (TABLE 1). However, a fair proportion of graft recipients fail to respond to anti-C5 treatment, implying that a broader and more comprehensive intervention may be warranted. Two pilot studies have shown that C1-INH treatment, in combination with intravenous (IV) immunoglobulin, is well tolerated in the treatment of ABMR and that this regimen is associated with improved renal allograft function[62,63]. Moreover, Cinryze, a plasma-derived preparation of C1-INH, has advanced to a multicentre phase III trial assessing its efficacy for the treatment of ABMR in sensitized kidney transplant patients[64] (TABLE 1). It should be noted, however, that the beneficial impact of C1-INH treatment on kidney graft survival may well extend beyond its inhibitory activity in the CP or LP, summoning also its ability to intercept proteases of the coagulation and fibrinolytic/kinin pathways.

While C1-INH could potentially reduce transplant-associated CP and LP activation (for example, during I/R injury), given its broad inhibitory activity on the serine proteases C1s/r and MASPs, respectively, the amplifying capacity of the AP on the opsonized surfaces of the donor organ would remain unhindered, thus imposing an inflammatory burden on graft function and survival[32]. In this regard, C3 convertase modulation or direct C3 interception could feature as plausible strategies for improving graft accommodation in transplant recipients, given the multifaceted impact of C3 activation on cell-mediated and humoral inflammatory pathways operating locally on the graft's surface and in the vascular endothelium[65]. Endorsing the potential of C3 intervention in clinical transplantation across ABO barriers, Amyndas has already announced plans to develop its C3-targeted inhibitor AMY-101 as a treatment option for increasing graft accommodation in patients undergoing ABO-incompatible kidney transplantation[66] (TABLE 1).

### *Sepsis-associated inflammation*

Polymicrobial sepsis is a systemic inflammatory condition associated with high morbidity and mortality[67]. It evokes excessive intravascular activation of the complement system that, coupled to overwhelming cytokine signalling, contributes to systemic innate immune dysfunction and thromboinflammation that are disseminated to distal organs, culminating in multiorgan failure[68]. The pathogenic role of distinct complement effectors in sepsis-induced inflammatory damage has been demonstrated in both rodent and primate models of *Escherichia coli*-induced or polymicrobial (caecal ligation puncture) sepsis[69,70]. From a translational perspective, small-sized C3 inhibitors of the compstatin family and C5 inhibitors (for example, RA101295, Ra Pharma) have both shown therapeutic efficacy in preclinical models of sepsis, particularly in combination with CD14 blockade, an approach that abrogates proinflammatory lipopolysaccharide signalling[71,72]. Of note, complement inhibition at the level of C3 or C5 has shown promising results in primate models of sepsis by maintaining vital organ integrity and attenuating thromboinflammatory damage when delivered as an early rescue regimen[70,73].

While all these approaches have yielded promising results at a preclinical stage, they are still far from being translated into clinically meaningful therapies. Nonetheless, clinical efforts have been spearheaded by a humanized mAb targeting C5a (CaCP29/IFX-1, InflaRx), a key inflammatory effector in sepsis (FIG. 2a). This antibody therapeutic has undergone phase II trials in patients with severe sepsis or septic shock with evidence of organ dysfunction and pulmonary or abdominal infection[74], but no further plans have been announced for these indications thus far[74] (TABLE 1). Of note, clinical development of IFX-1 for another inflammatory indication, the chronic skin disorder hidradenitis suppurativa, recently led to a major setback, as this anti-C5a agent failed to meet its primary end point (that is, clinical response score) in a large multicentre phase II study involving patients with moderate to severe hidradenitis suppurativa[75] (TABLE 1). C1-INH has also been evaluated as a potential modulatory strategy for sepsis in a pilot study of human endotoxaemia closely resembling lipopolysaccharide-induced septic shock models. Although this study advanced to phase III, further plans for the clinical development of C1-INH as a treatment for septicaemia have not been announced[76] (TABLE 1). C3 interception by the peptidic C3 inhibitor Cp40 afforded early organ protection, reducing systemic thromboinflammation in non-human primates (NHPs) subjected to trauma-induced haemorrhagic shock, thereby indicating that C3 may be a tractable target for developing 'rescue' therapies for trauma-associated multiorgan failure[69]. However, the complex nature of the inflammatory circuits involved in septicaemia and sepsis or trauma-induced multiorgan dysfunction poses a significant challenge in developing targeted anti-inflammatories based exclusively on complement modulation. This challenging situation is reflected by the limited commitment of complement drug makers to pursuing clinical programmes in this disease area. From a different perspective, complement activation can be exploited as a beneficial effector mechanism for containing hospital-acquired bacterial infections and dampening systemic inflammation. In this regard, Aridis has been developing mAbs against *Pseudomonas aeruginosa* (that is, AR-101/Aerumab) with enhanced complement-fixing properties that facilitate opsonophagocytic clearance of the pathogen. AR-101 has completed phase IIa testing and shows promise as a new adjunctive anti-infective treatment for patients with severe *P. aeruginosa* pneumonia[77].

### *Haemodialysis-induced inflammation*

Haemodialysis (HD) represents the mainstay of clinical management for alleviating the complications of a failing kidney in patients with end-stage renal disease (ESRD)[78,79]. The clinical challenge in treating patients with ESRD is further accentuated by the staggering shortage of donor organs worldwide[80]. Interestingly, even modern 'biocompatible' HD filters evoke

appreciable levels of complement activation and fuel thromboinflammatory responses that can propagate inflammation to distal organs[10]. The parallel activation of multiple innate immune systems, including the contact and coagulation cascades, imposes a growing burden on patients with ESRD that causes deterioration in their quality of life over time, increasing their risk for anaemia, atherosclerosis and cardiovascular disease[10].

Several preclinical studies have supported the pathogenic involvement of all three complement pathways in HD-induced inflammation, while indicating a critical role for the AP in amplifying the complement-driven proinflammatory response elicited during HD[33,78]. Recent translational efforts in primate and human models of HD-induced inflammation have provided proof of concept for the therapeutic targeting of complement. Administration of a single dose of C1-INH in an ex vivo perfusion model of HD resulted in attenuated C3 activation and decreased levels of key proinflammatory and prothrombotic markers implicated in cardiovascular disease predisposition[81]. These findings are in line with earlier observations in a clinically relevant NHP model of HD-induced inflammation in which C3 inhibition has yielded promising results[82]. A single intravenous bolus injection of the compstatin analogue Cp40 (that is, the active component of AMY-101) completely abrogated complement activation in cynomolgus monkeys subjected to HD and led to an increase of the anti-inflammatory cytokine IL-10 during the HD session[82]. Taken together, these studies have identified a window of opportunity for developing anti-inflammatory therapies in HD that could target C3 activation on the biomaterial surface[33] (TABLE 1). It should be clarified, however, that the beneficial activity of C1-INH in HD may extend well beyond its capacity to modulate the CP and LP on the biomaterial surface because of its promiscuous inhibitory activity on other proteases of the fibrinolytic, clotting and kinin pathways[10]. Of note, the transient nature of complement inhibition during an HD session significantly lowers the risk of infectious complications, thus obviating the need for prophylactic vaccination or other antimicrobial treatments. With the production costs of peptides being lower than those of classical biologics (for example, antibodies)[83,84], and with the prospect of injecting C3-inhibitory peptides directly into the dialysis circuit for a short duration of treatment, this immunomodulatory strategy could likely offer an effective and comparatively affordable treatment for patients undergoing HD.

### *Periodontal disease*

A plethora of preclinical and translational studies are reshaping our understanding of the fine immunoregulatory role of tissue-resident microbiota in health and disease[85]. Periodontitis is a prevalent oral inflammatory disease that afflicts millions of people in the Western world[86]. It is instigated by dysbiotic microbiota that thrive on local inflammation in the gingival cavity, promoting the gradual destruction of the tooth-supporting tissues and eventually leads to bone loss[87]. The inflammophilic microbiome causes periodontal disease by subverting the innate immune response at the level of complement activation and Toll-like receptor signalling[88]. Preclinical genetic and pharmacological studies of complement inhibition in rodent and NHP models of naturally occurring and ligature-induced periodontitis have unveiled the key pathogenic role of the complement–Toll-like receptor crosstalk in driving microbial dysbiosis, periodontal inflammation and osteoclastogenic bone loss[14]. More importantly, these studies have identified potential points of therapeutic intervention such as C3, the central hub of the system, and the downstream proinflammatory C5a–C5aR1 signalling axis[14]. Local injection of the C3-targeted inhibitory peptide Cp40 in the interdental papilla of primates afflicted with periodontitis has led to clinically meaningful reduction of key inflammatory indices, attenuating the osteoclastogenic bone loss that occurs in periodontal disease[89]. Following up on preclinical safety and tolerability studies[90], the Cp40-based therapeutic AMY-101 recently received Investigational New Drug approval by the FDA for the conduct of the first clinical study to evaluate its efficacy in adults with gingivitis (ClinicalTrials.gov identifier: NCT03694444) (TABLE 1).

Taken together, these translational studies attest to the safety and efficacy of transiently applied local C3 intervention in oral inflammatory diseases (for example, periodontitis) and pave the way for a novel adjunctive, host-modulatory therapy that could benefit patients with periodontal diseases by targeting complement in the oral cavity.

## Targeting complement in chronic pathologies

Complement drug discovery has been propelled by studies in two archetypal complement-mediated disorders of the vascular and renal spectrum, PNH and aHUS, respectively[23,91]. The well-established pathogenic contribution of complement dysregulation to these disorders has facilitated drug development by offering researchers and drug makers clearly defined therapeutic targets corroborated by a wealth of preclinical and mechanistic studies (FIG. 3d,e). In this regard, the clinical success of the first complement-targeting drug, eculizumab, in patients with PNH and aHUS has spawned a new generation of complement therapeutics that are being benchmarked in clinical trials against the standard anti-complement therapy[18].

Undeniably, targeting the terminal complement pathway and its effectors (C5a and MAC) is a strategy that has retained the lion's share in the drug development pipelines of biopharmaceutical companies, with several anti-C5 agents currently in late-stage clinical development[92]. The projected drug market share claimed by such new therapeutics and, more importantly, the clinical necessity of developing additional anti-C5 agents for indications that already benefit from the approved therapy are matters of ongoing discussions. The limited proportion of patients who are refractory to the current treatment[93], along with the launch of Ultomiris (Alexion), the long-acting version of eculizumab[29,30], further stir this debate, posing more questions regarding the market viability of additional anti-C5 therapeutics. Of note, several companies (for example, Samsung Bioepis or Amgen) are now developing biosimilars of eculizumab that could gain access to the market, expanding the spectrum of anti-C5 therapeutics

Biosimilars
Biomedical products, such as a therapeutic antibody, that share a high degree of structural and functional similarity with a product that is already clinically approved. Similar to small-molecule generic drugs, biosimilars are typically introduced once the patent protecting the original medicinal product expires.

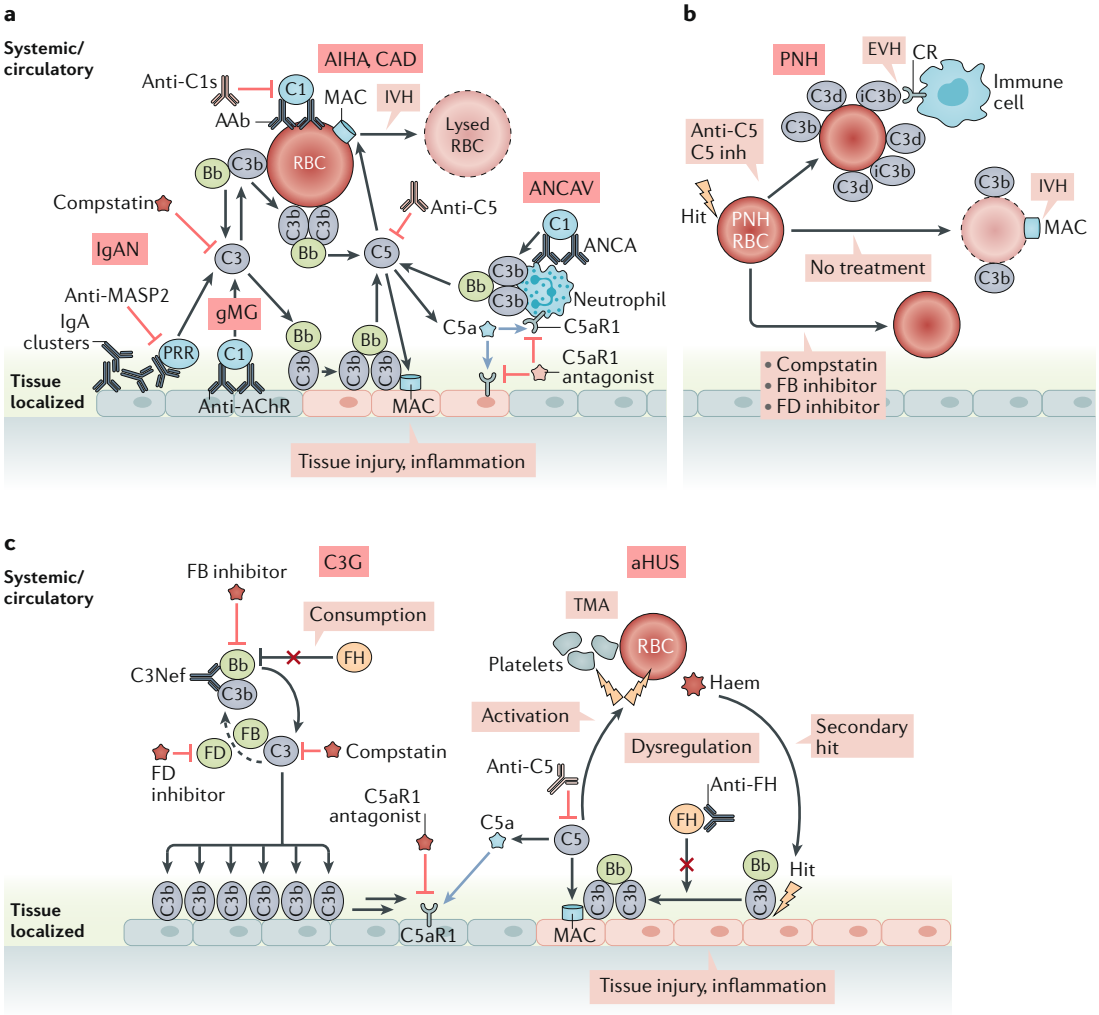


Fig. 3 | **Examples of chronic complement-mediated disorders with currently evaluated treatment strategies.** **a** | Some autoimmune conditions show a pronounced complement involvement. In autoimmune haemolytic anaemia (AHIA) and cold agglutinin disease (CAD), recognition of red blood cells (RBCs) by autoantibodies (AAbs) activates the classical pathway (CP). Upon opsonization, formation of C3 and C5 convertases causes intravascular haemolysis (IVH). Clusters of erroneously glycosylated IgA can trigger the lectin pathway in IgA nephropathy (IgAN), whereas CP activation by anti-acetylcholine receptor (AChR) antibodies contributes to some forms of generalized myasthenia gravis (gMG). Finally, anti-neutrophil cytoplasmic antibodies (ANCA) may trigger the CP to generate C5a, which stimulates neutrophils and exacerbates the development of ANCA-associated vasculitis (ANCAV). All these conditions may contribute to localized tissue injury and inflammatory complications. Current trials focus on preventing lectin pathway initiation in IgAN (using anti-mannose-binding lectin-associated serine protease 2 (MASP2)), controlling CP activation and complement amplification in AIHA and CAD (using anti-C1s monoclonal antibodies (mAbs) or C3 inhibitors of the compstatin family) or C5aR1-mediated signalling in ANCAV (using C5aR1 antagonists). The anti-C5 mAb eculizumab is approved for the treatment of gMG. **b** | In paroxysmal nocturnal haemoglobinuria (PNH), the lack of glycosylphosphatidylinositol-anchored complement regulators on clonal populations of RBC predisposes to complement-mediated IVH. Whereas C5-targeted therapies effectively prevent IVH, they do not stop ongoing opsonization. This may potentially enable extravascular haemolysis (EVH), an unwanted effect that is expected to not occur with C3-targeted or convertase-targeted entities. Currently, compstatin analogues and small molecule Factor B (FB) and Factor D (FD) inhibitors are being evaluated for this purpose. **c** | Both C3 glomerulopathy (C3G) and atypical haemolytic syndrome (aHUS) are rare complement-mediated kidney disorders with diverse causes that are typically based on complement dysregulation. In C3G, the stabilization of soluble C3 convertases by C3 nephritic factor (C3Nef) often leads to C3 consumption with massive deposition of C3 fragments. Therapeutic options under consideration focus on preventing C3 convertase formation (FD inhibitors) or convertase-mediated C3 cleavage (compstatin and FB inhibitors). In aHUS, complement activation is initiated by certain hits; dysregulation by polymorphic surface regulators, the prevention of Factor H (FH) surface recognition by autoantibodies or the presence of gain-of-function components leads to activation of C5 with formation of C5a and membrane-attack complex (MAC) that cause tissue damage, inflammation and thrombotic microangiopathy (TMA), which by itself (for example, by release of haem from RBC) can act as a secondary hit for aHUS progression. Alongside the approved eculizumab, other C5-targeted entities and C5aR1 antagonists are evaluated for the treatment of aHUS. CR, complement receptor; PRR, pattern recognition receptor.

Breakthrough haemolysis
The transient increase of markers of intravascular haemolysis (that is, elevated lactate dehydrogenase levels and decreased haemoglobin) in patients receiving treatment designed to abrogate intravascular haemolysis. Typically, breakthrough haemolysis is attributed either to pharmacokinetic or pharmacodynamic issues.

available to patients currently reliant on anti-C5 therapy[94]. Moreover, a new generation of C3-targeted therapeutics is gaining clinical traction for their ability to afford broader therapeutic coverage of clinical manifestations (for example, extravascular haemolysis) that potentially limit patient responses in disorders such as PNH[95].

With the advent of high-throughput genomic analyses, new biodiagnostic algorithms and the development of more sophisticated tools for dissecting disease mechanisms, complement drug discovery is being expanded into new disease areas that span almost the entire clinical spectrum of inflammatory pathologies, including ocular indications, neurodegenerative diseases of the central and peripheral nervous system, cancer, rare thrombotic microangiopathies, glomerulopathies, and autoimmune disorders with vascular manifestations[15,96–99] (an overview of translational studies for complement modulation in cancer and neurodegenerative diseases is provided in BOX 1). While complement modulation in cancer and neurodegenerative diseases may reveal new therapeutic modalities in the near future, these emerging indications are still in their infancy in terms of clinical translation of complement inhibitors. Therefore, this review focuses primarily on archetypal clinical disorders and disease areas with well-documented complement involvement, and on drug candidates already advancing through early or late-stage clinical development.

### *Haematological disorders*

The field of complement-mediated haematological diseases is justifiably dominated by PNH and the clinical success of anti-C5 therapy (eculizumab)[20,100]. PNH is driven by the spontaneous intravascular haemolysis of erythrocytes that lack the glycosylphosphatidylinositol-anchored complement regulators CD55 and CD59 and therefore succumb to autologous complement attack and MAC-mediated intravascular haemolysis[95] (FIG. 2d). The approval of eculizumab drastically changed the landscape of PNH by introducing the first aetiological therapy for these patients[20,101]. Despite this progress, unmet clinical needs have emerged, including the genetically driven refractory phenotype to anti-C5 in certain patients, the residual haemolysis of C3-opsonized PNH cells in extravascular compartments, and the pharmacokinetic/pharmacodynamic (PK/PD) breakthrough haemolysis observed in certain cases that evoke strong complement activation (that is, acute infections), irrespective of drug dosing levels[93,102,103]. Moreover, the excessive costs of current anti-C5 therapy (typically, some US$500,000 per year and patient, depending on the market)[104] cannot be overlooked, since they impose a sizeable economic burden on health-care systems and limit drug access in developing countries[105] (an overview of regulatory and economic aspects pertaining to complement drug development is provided in BOX 2). Altogether, these clinico-economic challenges have rekindled the interest of drug companies in developing biosimilars of eculizumab, alternative anti-C5 therapeutics, or drug candidates targeting upstream components of the AP or the central protein of the complement cascade, C3 (REF.[18]).

Addressing the challenges of patient compliance and drug plasma residence, Alexion has recently launched Soliris' successor, Ultomiris (ravulizumab), a long-acting version of eculizumab that differs only in four amino acids but exhibits longer plasma residence thanks to neonatal Fc receptor-dependent antibody recycling[106]. This antibody engineering allows for an extended IV dosing interval of 8 weeks, considerably longer than that of eculizumab[29,30,107]. After two multicentre phase III studies established the non-inferiority of ravulizumab to eculizumab in both patients with clinically stable PNH during previous eculizumab therapy and in complement inhibitor-naive patients[29,30], Ultomiris gained FDA approval for PNH in December 2018. An approval in other markets and for other indications, including aHUS and gMG, is expected to follow soon.

Since details about the expected treatment costs are scarce, it remains to be seen whether ravulizumab will alleviate the burden on the health-care system, in addition to the burden for patients regarding treatment frequency.

The arsenal of clinically developed anti-C5 agents for PNH features several mAbs that target a different epitope of C5 than does eculizumab, thus projecting clinical benefit even for those patients bearing the therapy-refractory p.R885H C5 mutation[93]. SKY59/RO7112689, a mAb developed by Roche and Chugai, displays a favourable PK profile for chronic application by exploiting a pH-dependent mechanism of neonatal Fc receptor-mediated antibody recycling[108]. Currently, SKY59 is being evaluated in phase I/II trials as a treatment option for PNH, with initial reports of high subcutaneous bioavailability and prolonged plasma residence in healthy volunteers predicting a promising clinical development path (NCT03157635). Other C5-targeting antibodies in clinical development for PNH include tesidolumab (LFG316, Novartis)[109] and pozelimab (REGN3918, Regeneron), which are registered in phase II and I studies, respectively[110] (TABLE 1).

Box 2 | **Reconciling complement-targeted orphan drug costs**

With most of the complement-targeted indications belonging to the rare-disease spectrum, complement drug candidates are poised to benefit from the orphan drug legislation that offers incentives for expedited development and protected market exclusivity[252]. Thus far, the clinical approval of a single orphan medicinal product for complement indications (eculizumab) and the recent launch of its long-acting successor (ravulizumab) have moulded a state of distorted market competition that unavoidably fosters a monopolizing culture at multiple levels[253]. Market domination by a single drug has spawned spiralling drug costs that impose a huge burden on health-care systems and patients alike. With the socioeconomic and ethical implications becoming increasingly conspicuous[254,255], drug regulatory bodies as well as medical and patient communities are now faced with urgent decision-making to reshape orphan drug development in complement indications and beyond. Clearly, incentives for orphan medicinal product development should continue, securing a reasonable margin of profit for the pharmaceutical industry, while fostering drug discovery by academic groups could also downsize research and development costs through public funding[256]. New guidelines should be adopted in order to reconcile market needs with optimal drug efficacy and ensure drug access to all patients in need of therapy. Orphan drug pricing should optimally cover the cost of research and development plus production and reflect the value of the drug in longitudinally improving a patient's quality of life. The long-awaited entry of new complement drugs into the market with projected lower production costs than biologics[83] (for example, small-molecule inhibitors and peptide-based therapeutics)[257], the imminent launch of biosimilars and the anticipated reduction in drug prices as the market expands with more approved indications should all offer a viable track towards sustainable drug market growth and optimal, affordable and disease-tailored patient management.

Besides larger biologics, the diversified toolkit of C5-targeting agents comprises smaller proteins and peptides with the capacity to block C5 cleavage. Specifically, coversin (Akari Therapeutics), a recombinant C5/leukotriene B4-targeted inhibitor derived from the soft tick *Ornithodoros moubata*, is currently being developed for PNH as a subcutaneous treatment option[111]. After achieving its primary end point — lactate dehydrogenase reduction — in a phase Ib trial in patients with PNH, coversin is now advancing to phase II trials, offering another treatment option with increased patient compliance (due to subcutaneous (SQ) dosing) that can also circumvent the genetic resistance to eculizumab[112] (TABLE 1). Constrained peptides are also gaining momentum and entering late-stage clinical development[113]. Zilucoplan (RA101495, Ra Pharma) is a macrocyclic peptide targeting C5 in a region distinct from that targeted by eculizumab[114]. This C5 inhibitor has entered clinical development for PNH as an SQ formulation and shown promising results in two phase Ib/II trials in treatment-naive and eculizumab-treated patients[115,116]; it has now advanced to an extension phase II study in patients with PNH (TABLE 1). From a different mechanistic perspective, Alnylam is developing an RNAi therapeutic (cemdisiran/ALN-CC5) that targets the hepatic expression of C5 at the mRNA level[117]. Whereas monotherapy with cemdisiran may hold promise in other indications (that is, aHUS), this strategy has not yielded optimal results in PNH, and Alnylam has redirected its programme to combine cemdisiran dosing with concomitant eculizumab treatment, albeit at a lower dosage (TABLE 1).

Clearly, all the above-mentioned approaches are capitalizing on the success of anti-C5 therapy, without significant deviation from a mechanistic standpoint. Their rationale resides in the extension of dosing intervals and in increasing patient compliance through self-administration of the drug.

Therapeutic strategies that target the AP amplification loop or the C3 protein itself offer a robust mechanistic basis for inhibiting complement in PNH, with potentially broader therapeutic coverage by blocking both intravascular and extravascular haemolysis[92]. Considerable therapeutic insight in this direction has been gained from a family of cyclic peptides targeting C3, the compstatins (for updated reviews on the design and preclinical development of compstatins, please see REFS[118,119]). Compstatins bind to a shallow pocket formed by the macroglobulin ring of the β chain of human and NHP C3 and selectively block convertase-mediated cleavage of C3 independent of the initiation pathway[120]. In fact, C3-targeted inhibition by these small peptidic inhibitors has shown clinical promise as a broader therapeutic approach by preventing intravascular MAC-mediated haemolysis and also abrogating the residual haemolysis that can result from extravascular phagocytosis of C3-opsonized PNH cells exposed to anti-C5 agents[121].

These mechanistic attributes have propelled the clinical development of two compstatin-based drug leads, that is, the PEGylated therapeutic APL-2 (Apellis) and the non-PEGylated AMY-101/Cp40 (REF.[118]) (Amyndas). APL-2 has been derivatized from POT-4, corresponding to the second-generation compstatin analogue 4(1MeW)[118], through conjugation of two peptide moieties to an extended PEG linker[122]. Both the 4(1MeW) analogue and AMY-101/Cp40 have been developed by purely academic efforts at the University of Pennsylvania and subsequently licensed to Apellis and Amyndas, respectively. APL-2 is currently in phase II studies as an add-on therapy to eculizumab[123] and as a monotherapy in treatment-naive patients with PNH[124] (TABLE 1). After reporting successful weaning of patients from eculizumab and switching to APL-2 monotherapy with favourable haematological results, Apellis has announced the enrolment of patients in a phase III study in which the C3 inhibitor's efficacy will be benchmarked against eculizumab[125]. It has also registered a multicentre extension study to evaluate the long-term safety and efficacy of APL-2 in PNH[126]. Amyndas has completed phase I studies in human volunteers, with good safety and tolerability profiles for their non-PEGylated third-generation compstatin analogue AMY-101 and has announced plans for phase II studies in PNH and other complement-mediated indications[127] (TABLE 1). AMY-101 is based on the Cp40 compstatin analogue, which has shown excellent PK/PD profiles in NHP, sustained inhibitory potency and broad therapeutic efficacy in blocking both intravascular and extravascular haemolysis of PNH erythrocytes[121]. Of note, the design of Cp40 analogues with targeted mini-PEGylation in the N-terminus or the addition of charged amino acids at the C-terminus has led to derivatives with higher solubility and improved PK profiles, thus broadening the spectrum of administration routes and likely reducing the dosing frequency of these peptidic drugs in chronic regimens[128].

Efforts to develop complement therapeutics amenable to chronic administration have culminated in two orally available, small-sized inhibitors that target the AP amplification loop at different steps[18]. To this end, both Novartis and Achillion are advancing two small-molecule inhibitors to late-stage clinical development that block the serine proteases Factor B (FB) and FD, respectively[129]. The FB inhibitor LNP023 (Novartis) blocks the active site of FB and the Bb fragment and has entered phase II trials for PNH[130,131] (TABLE 1). Meanwhile, Achillion is dosing patients with PNH with its orally available FD inhibitor, ACH-4471, which blocks the catalytic site of FD, preventing AP C3 convertase formation[132]. ACH-4471 is being evaluated in two phase II trials, both as a monotherapy in treatment-naive patients with PNH and as an add-on to eculizumab in patients with suboptimal response to anti-C5 therapy[133] (TABLE 1). Achillion has also announced plans to advance its next-generation FD inhibitors ACH-5228 and ACH-5548 to phase I trials, aiming for higher AP inhibition along with a reduced dosing frequency[134]. Of note, a series of compstatin derivatives with improved PK properties and appreciable levels of oral bioavailability in NHP are now in preclinical development[135]. The further development of oral formulations for enhancing the bioavailability of these peptidic C3 inhibitors is expected to largely improve complement therapeutic intervention, especially in those indications that rely on chronic treatment (for example, PNH).

Whereas PNH represents an archetypal disease for testing AP therapeutics, other haemolytic conditions with

prominent complement involvement require tailored approaches that better align with their distinct pathophysiology. For example, autoimmune haemolytic anaemias (AIHAs) such as cold agglutinin disease (CAD) or warm antibody AIHA are fuelled by autoantibodies that trigger CP activation on erythrocytes (FIG. 2c). This attribute has propelled the clinical development of CP-targeted inhibitors for these indications. After receiving orphan drug designation for CAD in 2017, sutimlimab (BIV009/TNT009, Sanofi/Bioverativ), a humanized mAb targeting the C1s protease of the CP, entered late-stage clinical development, being evaluated as an IV infusion in two separate phase III trials in patients with CAD[136,137] (TABLE 1). Further expanding the spectrum of therapeutics for AIHAs, Apellis has also instigated a phase II trial (PLAUDIT) to assess the biological efficacy of its C3 inhibitor, APL-2, in patients with CAD or warm antibody AIHA[138] (TABLE 1). While the advent of clinical-stage drug candidates targeting various enzymes of the complement cascade (that is, C1s, FB and FD) marks an important milestone in complement drug discovery, the potential lack of therapeutic coverage due to residual enzymatic activity in cases of strong complement activation or insufficient dosing (for example, a missed dose in a daily oral administration schedule) should not be ruled out and may warrant further investigations.

The spectrum of haemolytic conditions fuelled by complement is continuously expanding, with new indications entering the clinical development spotlight[95]. Sickle cell disease (SCD), a genetically driven haemolytic disorder, has long been associated with an overactive AP but, until recently, has been neglected from a translational standpoint[139]. AP activation is likely triggered by increased exposure of certain membrane phospholipids (for example, phosphatidylserine) on SCD erythrocytes and further fuelled by the release of haem from lysed erythrocytes[139,140]. Moreover, the contribution of the AP to vaso-occlusive episodes (that is, SCD crises) and delayed haemolytic transfusion reactions in patients with SCD has been proposed[141]. In this regard, the translational value of AP modulation in SCD has recently been demonstrated in a study showing that both Factor H (FH) and smaller FH constructs can block the adhesion of sickled red blood cells to the inflamed vascular endothelium of patients with SCD[142]. Future investigations will have to determine the clinical potential of such modulatory strategies in genetic disorders such as SCD. Of note, haem-induced AP activation is now recognized as an exacerbating factor that leads to bystander haemolysis in both SCD and malaria-induced anaemia[143]. In this regard, C3 inhibition by the compstatin analogue Cp40 can abrogate haem-induced complement activation and C3 fragment deposition on erythrocytes, pointing to the therapeutic potential of C3-based inhibitors for ameliorating malaria-induced anaemia in patients infected with *Plasmodium*[144]. While direct C3 inhibition or C3 convertase modulation might be viable approaches to protect the endothelium from complement damage during intravascular haemolysis, targeting surface 'modifiers', such a P-selectin, that are upregulated in response to haem exposure, might point to an alternative therapeutic approach for quenching complement-mediated endothelial injury. In this respect, P-selectin was shown to act as a scaffold for C3 deposition on endothelial surfaces, and antibody blockade of its interaction with C3 resulted in attenuated complement deposition and reduced endothelial damage in haemolytic mice[145]. These data strongly suggest that blocking tissue-specific docking sites for C3 fragment deposition might have additive or stand-alone therapeutic potential in diseases such as SCD.

### *Chronic renal disorders*

Given its unique anatomical and functional blueprint, the kidney represents one of the organs most susceptible to complement-mediated inflammatory damage[1,146]. Disorders that predominantly affect the glomerular function, leading to acute or chronic kidney injury, have therefore been in the crosshairs of pharmaceutical companies developing complement therapeutics[18]. Under steady-state conditions, the glomerular endothelium is protected from autologous complement activation by the glycocalyx, an overlying layer of proteoglycans and glycolipids that sequester fluid-phase regulators (for example, FH), thus preventing C3 opsonization and AP amplification[146]. This structural adaptation, combined with the endothelial expression of surface-bound regulators of the C3/C5 convertases, maintains renal homeostasis. In renal disorders involving high complement turnover, this regulatory shield is compromised by genetic alterations and acquired defects (that is, rare or common gene variants in complement activators or regulators, or autoantibodies)[147,148] that collectively render the glomeruli prone to complement damage.

***Atypical haemolytic uraemic syndrome.*** aHUS is a rare renal disorder characterized by a clinical triad of thrombocytopenia, microangiopathic haemolytic anaemia and acute kidney injury[146,149]. This thrombotic microangiopathy (TMA) is tightly linked to AP dysregulation since more than 60% of patients carry genetic alterations in complement activators or regulators of the AP (for example, FH, membrane cofactor protein/CD46, Factor I, C3, FB and thrombomodulin)[150]. Notably, ~10% of patients present with autoantibodies against various domains of FH, the main fluid-phase AP regulator[148]. Complement-driven pathophysiology in aHUS culminates in persistent, uncontrolled AP amplification on the renal endothelium, which propagates endothelial thromboinflammation and can progress to end-stage kidney failure[146]. The release of haem during mechanical haemolysis has been implicated as a secondary hit that exacerbates complement-mediated tissue damage in aHUS[140,151] (FIG. 2e). Other risk factors, including pregnancy, drug use or infections, are thought to increase penetrance of the 'at-risk' phenotype among carriers of complement gene mutations[152].

Eculizumab was approved by the FDA as a treatment for aHUS in 2011, following the completion of two multicentre phase III trials in patients with aHUS[153,154]. Although clinical experience with eculizumab has raised confidence in this approach, its longitudinal use in aHUS has revealed limitations that warrant further investigation regarding the duration of treatment, recurrence of disease upon drug discontinuation and coverage

of pathogenic processes upstream of C5. Following up on eculizumab's success, ravulizumab (Ultomiris) is currently being evaluated in two phase III trials as an IV formulation for both treatment-naive and inhibitor-exposed patients with aHUS[155,156]. According to the company's recent press release, ravulizumab has achieved its primary end point in the phase III trial in treatment-naive patients with aHUS. In addition, plans have been announced for a phase III study, in which ravulizumab will be delivered subcutaneously once per week in patients with PNH and aHUS[157]. While these developments mark a clear progress for anti-C5 therapy in the aHUS drug space, challenges remain, including the need for chronic drug administration in kidney graft recipients at high risk of recurrence due to FH, Factor I or C3 gene variants[129]. Therefore, alternative therapeutics are being developed to circumvent the bottleneck of long-term IV dosing[18]. In this regard, avacopan (CCX168, ChemoCentryx), an orally administered C5aR1 antagonist that targets the C5a–C5aR1 inflammatory axis without disrupting the terminal pathway's lytic activity, is currently being evaluated in a proof-of-efficacy phase II trial in patients with aHUS[158].

From a different standpoint, blockade of the LP through MASP2 inhibition is also being pursued as a treatment option for aHUS. In fact, Omeros has advanced its MASP2-targeting antibody OMS721 into a multicentre phase III study in patients with aHUS[159]. Remarkably, however, this strategy has not been supported by publicly available preclinical or mechanistic data that could justify a projected therapeutic benefit over the standard anti-C5 therapy.

***Other TMAs.*** Capitalizing on the successful use of eculizumab in aHUS, complement intervention is now being considered in other TMAs, including haematopoietic stem cell (HSC) transplant-associated TMA (TA-TMA); pregnancy-related HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome; and infection-related or medication-related TMAs[99,160]. Although strong genetic linkage to complement dysregulation has not yet been discerned in most cases, emerging observations and clinical data support a pathogenic involvement of a dysregulated AP and overactive terminal pathway in exacerbating TMA pathology[99]. Consistently, increased levels of activated C3 fragments and terminal effectors (for example, sC5b-9) have been reported in patients undergoing HSC transplantation who develop TA-TMA[161,162]. Encouraging results in attenuating TA-TMA-associated organ injury have been obtained with eculizumab in phase II studies, but clinicians are still debating how to use this drug in a clinically safe, effective and cost-efficient way[163]. Recently, proof of principle for the efficacy of alternative C5 inhibitors in TA-TMA was provided in a paediatric patient with TA-TMA, carrying the therapy-refractory p.R885H C5 mutation, who showed signs of transient clinical improvement after subcutaneous delivery of coversin, the tick-derived C5/leukotriene B4 inhibitor (Akari)[164]. Current clinical-stage efforts in TA-TMA include the anti-MASP2 antibody OMS721, which is being evaluated in a phase II 'basket-type' trial involving patients with a spectrum of TMAs and in an extension study for compassionate use in patients with TMA benefiting from treatment[165,166].

Identifying recipients of HSC transplants at high risk for severe transplant complications before the clinical onset of disease will enable targeted interventions using either agents already available or complement therapeutics targeting C3 or the AP amplification loop that are currently in the clinical development pipeline.

***C3 glomerulopathies.*** The term C3 glomerulopathy (C3G) defines a heterogeneous spectrum of rare kidney diseases characterized by AP dysregulation both in the fluid phase and in the renal endothelium[98,167]. Uncontrolled AP activation results in prominent C3 fragment deposition in kidney biopsy samples[168]. The two major subgroups of C3G, namely dense deposit disease (DDD) and C3 glomerulonephritis (C3GN), have overlapping clinical and pathological features but also distinct morphoanatomical changes discernible by electron microscopy[167,168].

Comprehensive genetic screening has demonstrated that ~25% of patients with C3G carry rare variants in complement-related genes[169,170]. These variants include the complement genes *C3* and *FB*, which form the AP convertase complexes C3bBb (C3 convertase) and C3bBb3b (C5 convertase), the regulatory genes *CFH* and *CFI*, or complement FH-related 5 (*CFHR5*), a FH-related protein that may act as an enhancer of AP activation[98,171]. Unlike aHUS, C3G remains a challenging renal condition in terms of accurate diagnosis, biomarkers, patient stratification and aetiological treatment options[98]. To date, patient management remains palliative, with anti-hypertensive drugs, plasmapheresis or immunosuppression as the only available options[172].

Given the prominent involvement of complement in disease pathogenesis, C3G is another chronic disorder attracting the attention of complement drug makers. The off-label use of eculizumab in patients with DDD and C3GN has yielded mixed responses thus far, without uniformly countering key pathological markers (that is, proteinuria), which has prompted the evaluation of alternative therapeutics[173]. Whereas broader intervention at the level of C3 or the overactive AP seems mechanistically more justified, targeted C5aR1 inhibition using avacopan has shown early clinical promise in C3G, with this orally available drug candidate being slated for phase II trials[174]. Of note, avacopan has advanced to late-stage clinical development for another renal disease of the autoimmune spectrum, anti-neutrophil cytoplasmic antibody-associated vasculitis[175]. In a phase II study, avacopan proved effective in replacing high-dose glucocorticoids for treating vasculitis[176]. Moreover, Omeros' anti-MASP2 mAb is also being evaluated in a basket-type phase II trial of C3G, among other nephropathies[177]. Advancing treatment options at the level of AP activation, Achillion has registered its oral FD inhibitor, ACH-4471, in a phase II trial to assess its biological efficacy in restoring C3 levels in patients with C3G[178].

C3-targeted interception has emerged as a promising therapeutic intervention given its projected broader impact on disease processes underpinning C3G[179]. In this respect, both the European Medicines Agency and

FDA have endorsed the clinical potential of C3-based inhibitors for C3G by granting orphan drug status to two candidate drugs of the compstatin family, AMY-101 and APL-2 (REFS[180,181]). The PEGylated APL-2 is being evaluated in a phase II basket trial as a daily SQ infusion in glomerulopathies, including C3G[182]. Plans for clinical trials in C3G have also been announced for AMY-101, a non-PEGylated subcutaneously administered C3 inhibitor that has recently completed phase I trials[183]. Finally, the orally available FB inhibitor LPN023 is also in early clinical development for C3G. Given the complexity of the underlying disease, it is possible that no single treatment will be universally applicable to patients with C3G. However, through stringent patient stratification and biomarker profiling, clinical trials may soon pave the way for new complement-based therapies in C3G.

The clinical promise of complement therapeutics in the renal space is further reflected by progress in IgA nephropathy (IgAN), a multifactorial immune-mediated kidney disorder linked to complement dysregulation[184] (FIG. 2c). Currently, there is no approved aetiological therapy for IgAN. Following up on promising phase II results and a breakthrough therapy designation for IgAN from the FDA, Omeros is advancing its lead LP inhibitor, OMS721, to a phase III trial that will evaluate its efficacy in ameliorating kidney damage (proteinuria) in patients with IgAN[185].

### *Ocular inflammatory disorders*

In many ways, the ocular disease space has galvanized complement drug discovery in the post-eculizumab era[18]. The eye is an immune-privileged organ with anatomical barriers that pose certain challenges in terms of local drug administration, bioavailability and sustained activity[186]. Complement has long been known to contribute to ocular tissue homeostasis in conjunction with innate immune sentinels such as resident microglia and other phagocytes[15]. However, more than a decade ago, it was thrust into the spotlight of pharmaceutical research when a common gene variation in the complement regulator FH, a p.Y402H polymorphism, became associated with increased risk for age-related macular degeneration (AMD)[187–189]. AMD is a chronic inflammatory disease of the retina that represents a leading cause of irreversible vision loss in the industrialized world[15]. In its early stages, it manifests as dense protein-rich and lipid-rich deposits, termed drusen, that accumulate in Bruch's membrane, a specialized extracellular matrix-rich layer that separates the retinal epithelium from the underlying choroid[15]. AMD progresses from its early form, marked by drusen deposition and subretinal inflammation, into two advanced forms of AMD. In the advanced 'dry' form (geographic atrophy, GA) choroidal vessels deteriorate, causing widespread retinal pigmented epithelial cell death and photoreceptor loss. The second advanced form, termed 'wet' (neovascular) AMD, features predominantly leaky blood vessels protruding into the sub-retinal space, vascular endothelial growth factor (VEGF)-driven neovascularization and further inflammatory damage, leading to retinal atrophy and vision loss[15,186]. Eyes with GA can also develop wet AMD and vice versa. Dry AMD/GA still lacks effective treatment, while the mainstay of treatment for wet AMD is the intravitreal injection of anti-VEGF agents[15].

Whereas the role of complement in AMD pathogenesis is not fully elucidated, genome-wide association studies have identified a series of common or rare risk variants in complement genes that enhance the predisposition to AMD, likely through an overactive AP or loss of AP regulatory control in the sub-retinal space[190]. Encouraged by these observations, Genentech/Roche coordinated a series of clinical trials in GA with lampalizumab, a humanized IgG Fab fragment directed against FD, the rate-limiting protease of the AP. Lampalizumab binds to an FD exosite, blocking substrate access and activation of the AP C3 proconvertase (C3bB)[191]. Although lampalizumab reduced GA lesion size in the MAHALO phase II trial, with greater drug benefit shown in a subgroup of *CFI* risk-allele carriers[192], it failed to achieve its primary end point (reduction in GA lesions) in two large multicentre phase III trials in patients with GA[27] (TABLE 1). Whereas the lampalizumab case has constituted a setback in complement drug discovery for AMD, it has also raised awareness about comprehensive patient stratification in trials, drug bioavailability and delivery into ocular tissues and the potential contribution of FD bypass mechanisms that may become operative in such pathologies, thus skewing drug efficacy[193]. Furthermore, targeting serine proteases that amplify complement responses (that is, FD and FB) may entail a risk of reducing the enzyme's activity to an extent that a residual, minimal amount of enzyme will still suffice to fuel AP activation, thereby causing pharmacodynamic breakthrough and exacerbation of the disease. These potential issues surrounding lampalizumab's trials in AMD remain relevant to other indications and need further investigation to resolve any ambiguities.

While FD targeting in ocular indications remains within the scope of certain companies[129], the drug industry is devoting more attention to C3 inhibition as a promising new platform for treating AMD. Earlier translational efforts with the compstatin analogue POT-4 (Potentia/Alcon)[194] had shown initial efficacy in NHP models of early AMD[195], but failed to recapitulate this response in a phase I study in wet AMD, likely because of insufficient dosing[194]. To compensate for the lower activity of POT-4, APL-2 has incorporated two moieties of this peptide via a bridging PEG linker[122]. A safety and efficacy phase II trial of APL-2 in patients with GA (FILLY) has recently been completed, showing reductions in GA lesion size independently of genetic variants that could modulate disease progression[196]. This PEGylated C3 therapeutic has now been advanced to two multicentre phase III studies in patients with GA on a monthly and every 2 months intravitreal dosing scheme[197] (TABLE 1). Whereas PEGylation may have afforded a longer half-life to APL-2 compared with that of its predecessor APL-1/POT-4, this approach potentially carries a risk of fuelling adverse events related to PEG accumulation (for example, tissue vacuolation)[198] or induction of choroidal neovascularization (conversion to wet AMD) after repeated subretinal injections[199]. While other PEGylated antiangiogenic agents have been effectively used in patients with wet AMD (for example, Macugen, pegaptanib sodium),

the relative PEG 'burden' of a drug, dictated by differences in dosing amounts, should be taken into account in each case. Furthermore, the possibility that C3 inhibition in the eye might favour a local M1 to M2 macrophage conversion, thereby promoting neovascularization as part of the tissue repair process, cannot be ruled out[200,201]. Although the presence of a small percentage of wet AMD conversions in the FILLY trial could be attributed to a subclinical course of disease undetectable at the baseline, concerns about neovascular AMD conversion in APL-2-treated subjects warrant further investigation.

Non-PEGylated third-generation and fourth-generation compstatins[128,202] (developed by Amyndas) with improved target affinity and PK profiles for chronic administration may confer benefits regarding dosing frequency and PEG-related effects[66]. The prolonged residence of a novel C3 inhibitor of undisclosed structure, termed AMY-106 (Amyndas) in ocular tissues at C3-saturating levels, extending over 3 months after a single intravitreal injection in NHP, indicates its clinical potential for the treatment of ocular indications associated with C3 dysregulation (for example, AMD)[66]. Of note, prolonged exposure of patients to C3 inhibitors (for example, APL-2 dosing in patients with PNH or GA) has not evoked any safety concerns thus far, further endorsing the clinical potential of this targeting strategy both systemically and locally.

AMD remains a fertile backdrop for testing new complement therapeutics spanning the entire spectrum of targets[129]. IONIS-FB-$L_{Rx}$ (Ionis/Roche), an antisense oligonucleotide targeting hepatic expression of FB, has completed phase I studies and is scheduled for a phase II study in patients with GA[203] (TABLE 1). Systemic delivery of IONIS-FB-$L_{Rx}$ has achieved FB reduction in both blood and ocular tissues of NHP, indicating its potential for modulating AMD progression[204]. However, in view of the local production of AP components in ocular tissues (that is, FB)[205] and the controversy surrounding the relative contribution of systemic versus local complement stores to retinal degeneration[206], it remains to be seen whether this elegant AP modulatory strategy will show efficacy in human AMD trials. Despite the failure of eculizumab to show clinical benefit in a phase II AMD trial[207], the ocular drug space is considering alternative anti-C5 agents such as the antibody LFG316 (Novartis)[208] and the C5-targeting aptamer (single-stranded RNA) Zimura/avacincaptad pegol (Ophthotech)[209]. Combinations of these anti-C5 agents with other therapeutics targeting the AP (anti-properdin, GLC561) or VEGF (Lucentis, anti-VEGF) are also in clinical evaluation in patients with AMD, with trial results eagerly awaited (further details on these trials can be found in REF.[129]) (TABLE 1).

## Challenges and new directions

### *Safety*

Given the key role of complement in pathogen clearance, safety has long been debated as a potential bottleneck for advancing complement therapeutics to the clinic. However, the clinical record of eculizumab has largely dissolved these concerns, showing that prophylactic vaccination against certain encapsulated bacteria (for example, *Neisseria* spp., *Haemophilus* spp. or *Streptococcus* spp.) can effectively protect patients undergoing chronic treatment[101]. The advent of C3-based therapeutics in chronic indications such as PNH has reignited this discussion, with hitherto largely hypothetical arguments[91]. Notably, primary C3 deficiencies in humans have only been associated with increased susceptibility to opportunistic infections during childhood, while this concern is largely mitigated during adulthood because of a fully matured adaptive immune compartment[210]. More importantly, insights from ongoing clinical trials applying C3-based inhibitors with prophylactic vaccination regimens have reconciled these concerns with actual clinical data. The safety and tolerability of the C3-inhibitor APL-2 in ongoing phase II/III trials in patients with PNH dosed for an extended time window and the recently reported safety of AMY-101/Cp40 in NHP under prolonged treatment have significantly negated the risk originally feared with this therapeutic approach, opening avenues for exploring such inhibitors, particularly as local treatments (for example, in AMD) or when applied to acute-phase indications (for example, haemodialysis treatment)[125,211]. The increased opsonophagocytic killing of meningococci in vaccinated individuals treated with AP inhibitors, when compared to anti-C5 agents, further supports the low infectious risk of upstream (AP) inhibition and adds more options for tailoring anti-complement therapies[212].

### *Towards personalized complement therapies*

***Patient stratification.*** The basal complement activity of an individual is largely dictated by subtle genetic variations (that is, common single nucleotide polymorphisms) in a spectrum of complement-activating or regulatory genes that define the individual's 'complotype'[213]. The impact of the complotype on clinical responses to complement therapeutics is being increasingly appreciated and has gradually 'impregnated' the design of clinical trials testing novel inhibitors (for example, in the case of the MAHALO phase II trial of lampalizumab in dry AMD/GA)[192]. However, the failure of lampalizumab to recapitulate its efficacy in two phase III trials[27] indicates that stringent stratification of patients into subgroups according to specific gene signatures may not always reflect the best course and that more comprehensive algorithms encompassing a larger set of risk-associated variants may be worth considering. Nevertheless, refined patient stratification remains an important determinant that can help reveal new indications with higher chances of benefiting from complement intervention. An illustrative example of this approach is the recent clinical approval of eculizumab as a new treatment for refractory gMG following successful phase III trials in a subgroup of patients with MG testing positive for anti-acetylcholine receptor autoantibodies[24]. Adding another layer of complexity to complement analysis, and making clinical trial design in the era of complement therapeutics even more challenging, recent studies have revealed subtle age-specific and gender-specific differences in complement protein levels and pathway-specific functional activities in the healthy population. For instance, women have a propensity

to display lower AP activity than men, which is partly attributed to significantly lower plasma levels of C3 and properdin[214]. These emerging observations resonate well with earlier studies in inbred mouse strains pointing to discrete gender-driven differences in terminal pathway activity[215]. Importantly, gender-biased complement responses should be taken into account when attempting to interpret clinical trial results (for example, effect of TP10 in patients undergoing cardiopulmonary bypass)[45], as they may have important implications in discerning the therapeutic efficacy of complement inhibitors directed at discrete components of the cascade.

***Biomarkers and diagnostics.*** Disorders with complex pathophysiological traits or multipronged complement involvement in their pathogenesis (such as C3G or complement-mediated TMAs) will benefit most from refined diagnostic criteria for patient inclusion in clinical trials[171,216,217]. Diagnostic algorithms weighing the relative contribution of distinct complement pathways to pathology (CP, LP, AP or terminal pathway) are imperative in that they may better inform patient selection and clinical trial design for testing disease-tailored complement therapeutics. The mixed patient response observed with eculizumab in the DDD/C3GN trials further attests to this necessity[173].

Undeniably, advancing novel therapies into clinical trials is tightly intertwined with a need for robust and more reliable diagnostic platforms that will standardize universally applied biomarkers for patient diagnosis and staging[217]. Indeed, more focus should be placed on monitoring dynamic changes in complement biomarkers and on ratios of activated versus total protein, rather than absolute protein levels in fluids or tissues, since the latter are merely snapshots that can misguide treatment by causing clinicians to overlook underlying pathogenic mechanisms[217,218]. The varied plasma half-lives of complement activation fragments (C3b, iC3b, C3dg and C3a/C5a) and their differential clearance rates from tissues should be taken into account when ongoing disease activity is measured by immunoassays that quantitate specific activation fragments or complexes (for example, C5b-9)[218,219]. In this direction, the advent of high-dimensional multi-omics technologies, such as single-cell RNA sequencing (RNAseq) and mass cytometry, coupled with the availability of powerful genome editing tools such as the CRISPR–Cas9 system, are expected to leverage complement diagnostics for identifying new therapeutic targets and informing complement intervention in new and unexplored indications[220,221]. Profiling complement gene expression at single-cell resolution (for example, via RNAseq) is expected to shed more light on the source of complement (that is, cell types or cell 'states'), the susceptibility of various cell types to complement damage and the relative abundance of complement proteins in specific tissue compartments during disease progression (for example, in ocular diseases). These high-end technologies can inform the design of more tailored interventions and the selection of appropriate dosing routes for indications, such as AMD, for which the source of activated complement (choroidal versus subretinal/retinal pigmented epithelial) may dictate the efficacy of complement interception[15].

### *New structural insights*

Structural advances in understanding complement mechanisms at the atomic level can point the way to new targets and treatment modalities. For instance, the recent elucidation of the structural basis for the formation of the initiating complexes of the CP, such as C1-IgG1 or C1-IgM[222–225], and the hexameric arrangement of IgG-antigen complexes on surfaces[226], have not only provided important insights into the mode of interaction between the main recognition unit of C1, C1q and the C1s/r serine proteases, but also revealed how intact C1 recognizes various CP activators (for example, IgG or IgM-containing immune complexes). This knowledge could leverage the design of tailored CP inhibitors for diseases driven by IgM neoantigen recognition (for example, ischaemias). Altogether, these structural studies have not only indicated a conserved mechanism of complement activation through the CP and LP[225] but have also enabled the design of more effective antibody therapeutics, termed 'HexaBodies', which elicit potent complement-dependent cytotoxic responses against certain haematological tumours (for example, anti-CD20, anti-CD38)[227]. Extending the spectrum of targeted indications, GenMab has recently registered its HexaBody GEN1029 in first-in-human phase I/II trials in patients with solid cancers[228]. For many years, the structure of C5aR1/CD88, a key signalling effector involved in a range of inflammatory or autoimmune pathologies and already targeted by clinical-stage therapeutics such as Avacopan/CCX118, remained elusive[229]. The recent report of the crystal structures of human C5aR1 in complex with peptidic and non-peptidic antagonists has revealed unique structural features underlying C5aR1's signalling activity[230]. These structural insights may enable the design of a new class of orthosteric or allosteric C5aR1 inhibitors that could be amenable to clinical development, providing promising drug leads that can modulate the proinflammatory C5a–C5aR1 axis.

## Outlook

Undeniably, complement drug discovery has witnessed important developments at both the discovery and clinical/translational level in recent years. Successful clinical intervention using anti-C5 therapy has not only reaffirmed confidence in complement therapeutic modulation but also propelled the development of a new generation of complement therapeutics. More than 20 therapeutic agents targeting distinct components and effector pathways of the complement cascade are now in clinical development for various indications. Clearly, the complement cascade features multiple points of therapeutic intervention that have their own merits or limitations, depending on the clinical indication and underlying pathophysiology[1,129]. The complexity of pathogenic drivers and diversity of inhibitors advancing to late-stage clinical development illustrate precisely the notion that there is no 'magic bullet' that can cater for all indications. The recent registration of basket

HexaBodies
Engineered therapeutic antibodies with strong complement-mediated cytotoxic potential due to their increased propensity to form hexameric clusters on target surfaces such as cancer cells.

or umbrella-type trials for the simultaneous evaluation of complement inhibitors in multiple conditions offers a valuable handle for screening the initial efficacy of a drug lead across several pathologies grouped under a common underlying mechanism of complement dysregulation (for example, kidney glomerulopathies or AIHAs)[138,182]. The growing appreciation that complement operates not only intravascularly but also in intracellular compartments of both lymphoid and non-lymphoid cells, contributing to homeostatic or cell-activating processes (for example, T helper 1 cell polarization of $CD4^+$ T cells), raises awareness about possible implications for complement drug design[231,232]. Although more studies are warranted to establish causality between intracellular complement targeting and human pathology, this is undoubtedly a new field with the potential to drive new therapeutic concepts.

Emerging cases of insufficient response to anti-C5 therapy and the appreciation that multiple triggers and complex genetic traits may convolute a patient's basal complement activity in yet-poorly defined ways, point to the need for more comprehensive patient stratification and robust monitoring during anti-complement treatment. Critical and unrestricted evaluation of large data sets from ongoing clinical trials will aid decision-making concerning the best treatment option for each indication[233]. In this regard, streamlining complement diagnostics by embracing new high-throughput, single-cell resolution methodologies (for example, single-cell RNAseq) and applying standardized complement assays is imperative in this new era of complement therapeutics. Consolidating trial design with refined diagnostic algorithms, disease-tailored stratification criteria and unbiased large data handling will enable more reliable evaluation of clinical outcomes in ongoing and future trials with complement inhibitors/modulators.

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**Competing interests**
J.D.L. is the founder of Amyndas Pharmaceuticals, which is developing complement inhibitors for therapeutic purposes. J.D.L. and D.R. are inventors of patents or patent applications that describe the use of complement inhibitors for therapeutic purposes, some of which are developed by Amyndas Pharmaceuticals. J.D.L. is also the inventor of the compstatin technology licensed to Apellis Pharmaceuticals (that is, 4(1MeW)7W/POT-4/APL-1 and PEGylated derivatives). D.C.M. declares no competing interests.

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