



Developments in anti-complement therapy; from disease to clinical trial

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ABSTRACT

The complement system is well known for its role in innate immunity and in maintenance of tissue homeostasis, providing a first line of defence against infection and playing a key role in flagging apoptotic cells and debris for disposal. Unfortunately complement also contributes to pathogenesis of a number of diseases; in some cases driving pathology, and in others amplifying or exacerbating the inflammatory and damaging impact of non-complement disease triggers. The role of complement in pathogenesis of an expanding number of diseases has driven industry and academia alike to develop an impressive arsenal of anti-complement drugs which target different proteins and functions of the complement cascade. Evidence from genetic and biochemical analyses, combined with improved identification of complement biomarkers and supportive data from sophisticated animal models of disease, has driven a drug development landscape in which the indications selected for clinical trial cluster in three ‘target’ tissues: the kidney, eye and vasculature. While the disease triggers may differ, complement activation and amplification is a common feature in many diseases which affect these three tissues. An abundance of drugs are in clinical development, some show favourable progression whereas others experience significant challenges. However, these hurdles in themselves drive an ever-evolving portfolio of ‘next-generation’ drugs with improved pharmacokinetic and pharmacodynamics properties. In this review we discuss the indications which are in the drug development ‘spotlight’ and review the relevant indication validation criteria. We present current progress in clinical trials, highlighting successes and difficulties, and look forward to approval of a wide selection of drugs for use in man which give clinicians choice in mechanistic target, modality and route of delivery.

1. Introduction

Scientists have strived for decades to develop drugs to treat complement-mediated diseases. By the end of the 20th century, many anti-complement agents had shown promise *in vitro* and in animal models, but few drug candidates had progressed to man and those that did were not developed further. Among the preclinical molecules being tested at that time were antibodies against complement components which blocked function, such as anti-mouse C5 and anti-human C5 (Frei et al., 1987; Thomas et al., 1996); their function *in vitro* and efficacy in animal models of disease was readily established (De Vries et al., 2003; Huugen et al., 2007; Ravirajan et al., 2004). By the early 21st century the humanised anti-human C5 monoclonal antibody, eculizumab, was progressing through clinical development and, in 2007, it was approved by the FDA for use in the rare but devastating disease: paroxysmal nocturnal haemoglobinuria (PNH) (Brodsky et al., 2008; Hillmen et al., 2006; Rother et al., 2007). Clinical validation of anti-complement

therapy was a landmark in complement drug discovery; this breakthrough, combined with ground-breaking data emerging from genome wide association studies (GWAS), which demonstrated key roles of complement in wide-spread disease (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; Klein et al., 2005), drove a renaissance in anti-complement drug discovery. This has brought us to the current day with many new drugs progressing through late stage clinical development and numerous others in discovery or preclinical stages (Morgan and Harris, 2015; Ricklin et al., 2018).

There are a number of challenges associated with developing drugs against complement, including the sheer quantity of circulating protein, the central role of complement in fighting infection, and the identification of an appropriate target and an appropriate disease, or drug indication (Harris, 2018). In a number of diseases, complement plays a driving role in pathogenesis, whereas in others, complement is an ‘exacerbator’ of disease, inducing increased pathology initiated by a different disease trigger, thus driving inflammation and tissue damage

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(Ricklin et al., 2016). PNH is an example of the former, where a deficiency in complement regulators renders erythrocytes and other cells, such as platelets, susceptible to complement attack and subsequent lysis or activation (Hill et al., 2017; Hillmen et al., 1992). In this disease, identification of an appropriate drug target is less challenging as the pathogenic mechanism is clear; inhibition of MAC formation using eculizumab prevents intravascular haemolysis and rapidly improves quality of life for patients. The choice of target may not be so clear in other potential indications, and even in PNH, the quest for optimal target selection continues and many drug candidates are currently being evaluated. In the last decade, technological advances have accelerated genetic and biochemical analyses to the point where understanding of intricate disease mechanisms is facilitated and rationale for specific indication selection is strengthened. The number of potential targets in the complement system is expanding as the role of complement beyond lysis and in cross-talk with other biological systems, such as coagulation, is becoming clearer. Companies and academic institutes are developing drugs which target every pathway in the system and these drugs include small molecules, peptides, biologics, antibodies and DNA-based therapeutics.

Due to the cascade nature of complement and the large number of proteins involved, selecting distinct points of intervention may result in different therapeutic effects. For a detailed insight into the function of

the complement system we refer to recent reviews on the topic and only provide a brief overview of the system here (Fig. 1) (Holers, 2014; Ricklin and Lambris, 2013). In most cases, complement is triggered by foreign or altered surfaces. In the classical pathway, recognition of immune complexes (and other non-immunoglobulin moieties) by C1q activates the associated serine proteases, C1r and C1s, which cleave the plasma proteins C2 and C4 to form a C3 convertase complex (C4b2a) on the activating surface. The same result is achieved when pattern recognition proteins of the lectin pathway (mannose-binding lectin [MBL], ficolins, collectins) bind to pathogen carbohydrate patterns and induce MBL-associated serine proteases to cleave C2/C4. The generated C3 convertases activate C3, resulting in the deposition of C3b (opsonisation) and the release of C3a. Through interplay with the proteases factor B and D (FB, FD), surface-bound C3b forms additional C3 convertases. The alternative pathway is primarily responsible for the rapid amplification of opsonisation on unprotected cells and also provides a means of constant background activity. The continuous deposition of C3b and stabilisation of convertases by properdin leads to a gradual shift to the assembly of C5 convertases, which cleave C5. Whereas the released C5a is among the strongest chemoattractants and pro-inflammatory modulators, primarily via binding to C5a receptor 1 (C5aR1), the C5b fragment can induce the assembly of membrane attack complexes (MAC) that damage or induce lysis of attacked cells. To

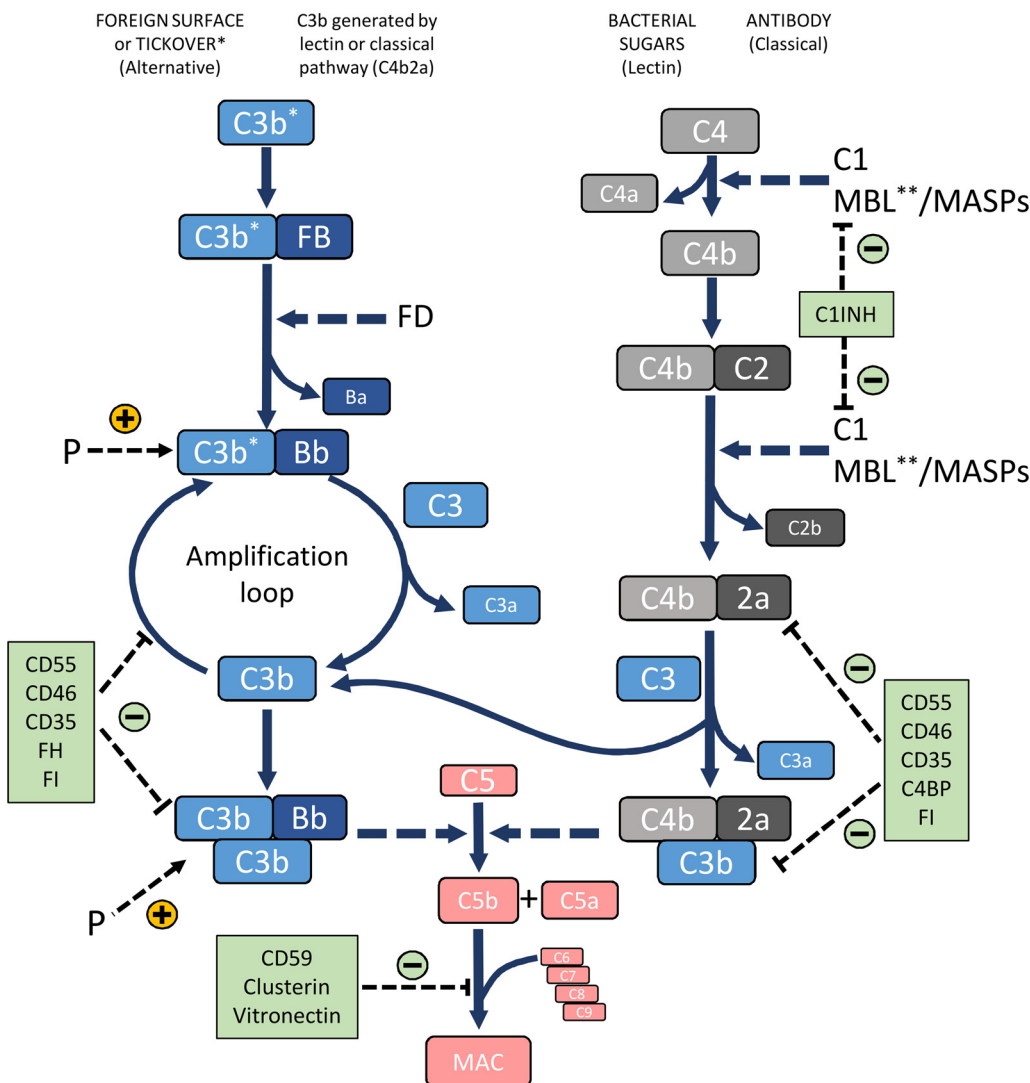


Fig. 1. Activation and control of the complement cascade. Activated C3 (C3b) deposited on a target surface can bind factor B (FB) which is activated by factor D (FD) to form the C3-cleaving enzyme (convertase) C3bBb. This enzyme cleaves further C3 to C3a and C3b, the latter decorates the surface (opsonisation) or binds the C3 convertase to form the C5 convertase, C3bBbC3b. Both the C3 and C5 convertase can be stabilised by properdin (P). Activation of the classical and lectin pathways results in covalent binding of C4b to the target surface, this binds C2 which is activated by C1r/s or the mannose-binding lectin-associated serine proteases (MASPs) to form the classical/lectin C3 convertase, C4b2a. Further cleavage of C3 to C3b results in formation of the C5 convertase, C4b2a3b. Cleavage of C5 marks the start of the lytic, or terminal, pathway. C5a is highly proinflammatory, while C5b binds C6 to initiate formation of the MAC (membrane attack complex); C7, C8 and multiple molecules of C9 bind to C5b6 to form the transmembrane pore. There are many proteins which control the cascade, either at the initiation level (C1-inhibitor [C1INH] inhibits C1r/s and the MASPs), or at the level of C3b and the convertase enzymes (including CD55, CD46, CD35, factor H [FH], factor I [FI] and C4-binding protein [C4BP]). Various proteins either inhibit C9 polymerisation and thus formation of the MAC on the membrane (CD59) or render it soluble (clusterin, vitronectin), resulting in soluble terminal complement complexes. *Tickover and contact with foreign surfaces may generate hydrolysed C3, i.e. C3(H2O), which shares structural and functional properties with C3b. **Alongside mannose-binding lectin (MBL), the lectin pathway may also be initiated by ficolins and collectins.

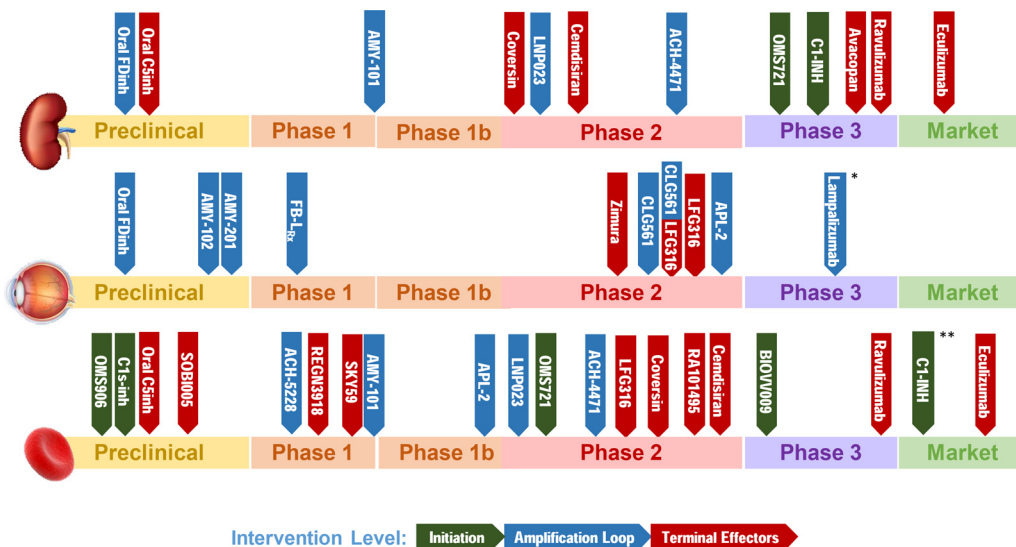


Fig. 2. The development pipeline for anti-complement drugs in the kidney, eye and vasculature. Complement drugs in preclinical and clinical stages of development are shown, and their stage of testing in each tissue is indicated. The schematic is based on company press releases and corporate presentations (as of April 2018), publications and conference proceedings. The mechanism or complement pathway for each drug is indicated by colour code: green, initiation of complement; blue, amplification loop; red, C5 and terminal pathway. * Lampalizumab has been evaluated in two phase 3 trials, both of which were recently discontinued; ** The approved indication for C1-INH is a disorder not primarily driven by complement (i.e. hereditary angioedema). Adapted from Ricklin et al. (2018).

control complement activation and the deleterious effector functions, human cells engage a number of potent regulators. For example, the initiation of the classical and lectin pathways is controlled by C1 esterase inhibitor (C1-INH). Owing to the central role of the convertases, an entire panel of regulators is involved in keeping these enzyme complexes in check. Membrane-bound (CD35, CD46, CD55) or soluble (C4b-binding protein [C4BP], factor H [FH]) inhibitors disassemble the convertases and/or enable the degradation of C3b by factor I (FI). Importantly, C3b and its cleavage fragments (iC3b, C3dg) are involved in phagocytic or adaptive immune signalling via binding to complement receptors (CR). Finally, the assembly of MAC is prevented by the membrane regulator CD59. Whereas the balance between activating and regulating processes enables an efficient immune surveillance, excessive activation or insufficient regulation can contribute to clinical complications (Rodriguez de Cordoba et al., 2012).

Complement is implicated in a wide spectrum of diseases affecting multiple organs. The current drug development landscape illustrates that the same drug is being applied to multiple diseases which affect a number of different organs (Fig. 2). The picture is dominated by diseases affecting the vasculature, the kidney and the eye. In the haematological indications, PNH, although very rare, is a very attractive indication to select as there is already clinical validation (with eculizumab) and the appropriate drug targets are understood. Diseases of the kidney are also prominent in the drug development path, in many of these pathologies there is unmet need with no prospect of long-term therapy. Aside from this, the pathogenic mechanisms have become clear in recent years and in some diseases, such as C3 glomerulopathy (C3G) and atypical haemolytic uraemic syndrome (aHUS), the disease is clearly driven by the alternative pathway of complement, thus facilitating selection of drug target. The clear genetic association between the alternative pathway and the wide-spread disease, age-related macular degeneration (AMD), has made this a favoured indication, and anti-complement drugs targeting the eye feature predominantly in company pipelines.

Treating these and other conditions with anti-complement therapy is not without its challenges, which include amount and frequency of drug dose, and selection of local (if possible) or systemic delivery. In this review, we focus on the three tissues highlighted above, the vasculature, kidney and eye, and we summarise the indications which are **currently** in clinical trial(s). We provide the rationale for indication selection and describe the progress in clinical development. Where possible, we highlight the successes and difficulties, such as dose or delivery, which provide additional insight into the challenges of drug development. Only by learning from current clinical trials in man, can we hope to develop the best ‘tool box’ of anti-complement drugs for the future.

2. The kidney

2.1. Renal indications involving complement

The kidney is highly susceptible to a large number of complement-mediated disorders, and renal diseases as target indications have been particularly attractive for drug companies. This is because in several diseases, such as aHUS and C3G, the pathogenic mechanisms are well-defined and the contribution of complement is prominent. Deposition of activated complement components is evident in most cases of renal diseases, sometimes in association with well-recognised classical triggers, such as antibodies, but frequently without any apparent cause until further diagnostic clues are scrutinised. The susceptibility of the kidney to complement-mediated disease suggests that its anatomy or function make it particularly sensitive to complement dysregulation (Fig. 3) (Ricklin et al., 2018; Thurman, 2015). The renal diseases, or indications, which have been of most interest to companies and academics developing anti-complement drugs are outlined below, together with a review of current clinical developments in that field.

Atypical haemolytic uraemic syndrome (aHUS) is a disease characterised by a classical triad of thrombocytopenia, microangiopathic haemolytic anaemia and acute kidney injury (Sethi and Fervenza, 2014). In approximately 60% of patients the root cause has been linked to genetic abnormalities in genes encoding complement activating and/or control proteins and in a further 10% of patients to autoantibodies which bind functional domains of FH (see Table 1). Other non-complement genes have been associated with aHUS, such as diacylglycerol kinase epsilon (DGKE), but their mechanistic role in pathogenesis has not yet been elucidated (Lemaire et al., 2013). In the complement-mediated form of the disease, the alternative pathway amplification loop of the complement system is usually hyperactive and the focus of attack is at the surface of endothelial cells within the kidney which leads to cell activation and dysfunction, swelling and damage (Fig. 3c) (Kavanagh and Goodship, 2010; Rodriguez de Cordoba et al., 2014, 2012). Thrombus formation within the glomeruli and capillaries of the kidney are evident in aHUS, and while it is likely that complement activation fragments such as C5a and MAC drive this process, synergistic effects of genetic variants within the coagulation system have not been ruled out (Kavanagh and Goodship, 2011). In aHUS, many patients carry one normal and one mutant form of the complement gene and frequently these single gene mutations are not completely penetrant for disease. Concurrence of multiple risk factors is commonly required for disease manifestation; these include common and rare variations in complement genes, and other factors such as pregnancy,

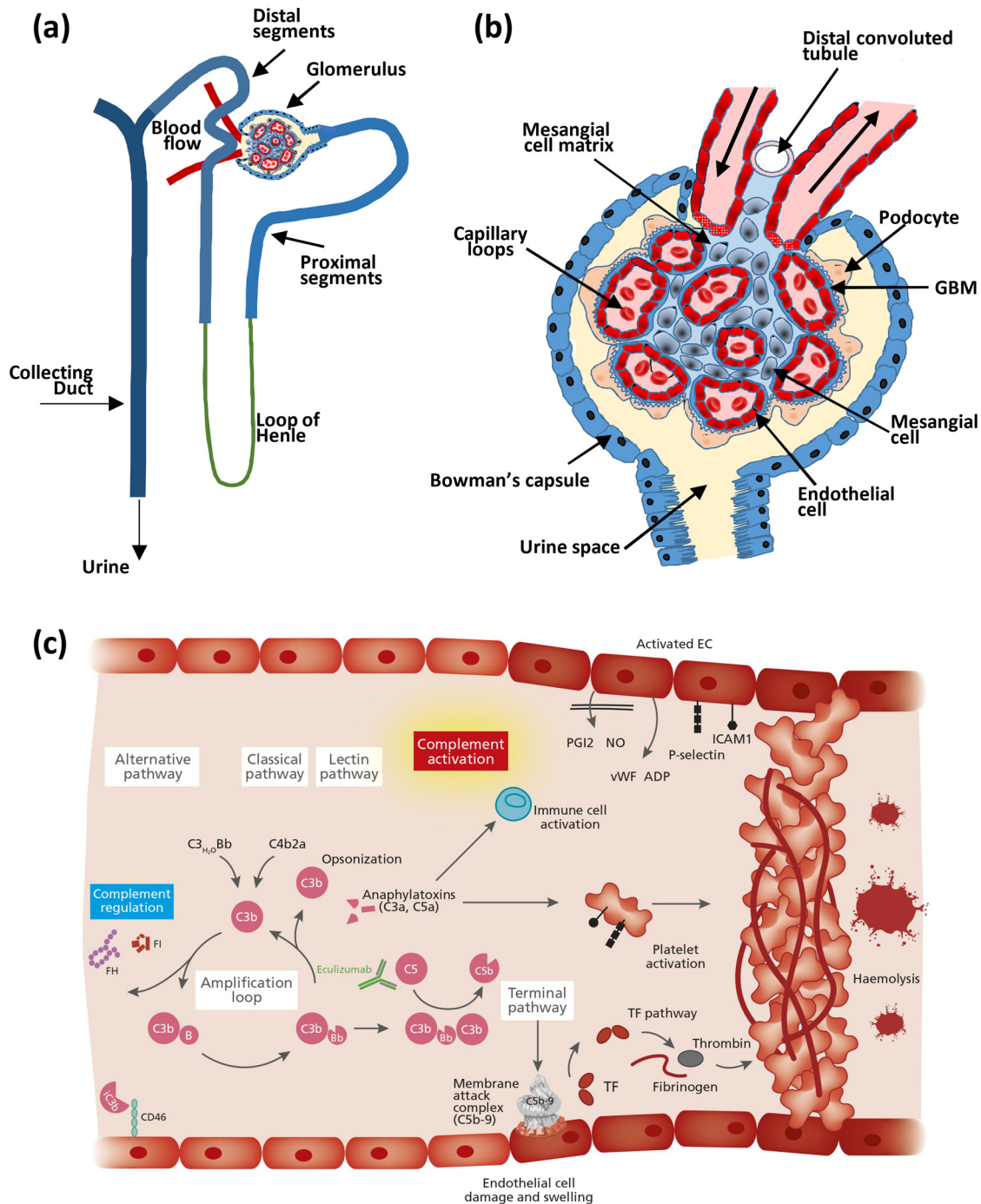


Fig. 3. Kidney structure and pathogenic mechanism in aHUS. (a) A healthy kidney has approximately 1 million nephrons, these are the microscopic and functional unit of the kidney, each comprising a renal corpuscle and tubule. (b) The renal corpuscle is comprised of a glomerulus, which holds a network of capillaries supported by mesangial cells, encapsulated within the Bowman's capsule. The Bowman's capsule serves as a collecting space for fluid filtered through the glomerulus. Blood is filtered as it passes through three layers: the capillary endothelial cells, the glomerular basement membrane and the foot processes of the podocytes, the cells which line the capsule. The glomerular basement membrane (GBM) forms at the interface between the endothelial cells and podocytes. The glomerular capillary wall determines the size and charge selective features of the glomerular barrier to macromolecules. Complement attack and/or deposition occurs at many sites within the glomerulus and tubule, including the GBM, the mesangium, around the foot processes of the podocytes, and at sub-endothelial and sub-epithelial sites. Sites of pathology in different indications are described in the main text; inappropriate complement attack impairs the ability of the kidney to filter blood and produce urine. (c) In aHUS, complement control at the surface of the endothelial cells is defective. The pathway most impacted is the alternative pathway, triggered by FB binding C3b to form the proenzyme C3bB. FH cleaves FB resulting in the convertase C3bBb which generates further C3b and ultimately the C5 convertase, C3bBbC3b. In health, control proteins such as CD46, FH and FI control the C3 and C5 convertase enzymes at the cell surface, but in aHUS these mechanisms are impacted by autoantibodies and gene mutations resulting in excessive production of the activation products, C5a and MAC. The anaphylatoxin, C5a, activates platelets and immune cells, whereas MAC activates endothelial cells and causes physical damage, resulting in endothelial swelling. Activated endothelial cells also express proinflammatory mediators and adhesion molecules. Subsequent microthrombi forming in the capillaries results in platelet consumption, vascular occlusion and mechanical haemolysis of erythrocytes. Eculizumab binds C5 and prevents cleavage by the convertase, thus preventing formation of both C5a and MAC and blocking downstream pathogenic events such as thrombus formation and endothelial cell activation and damage. NO, Nitric oxide; PGI₂, prostaglandin I₂; TF, tissue factor; vWF, von Willebrand factor. Part (c) reproduced with permission from the National Renal Complement Therapeutics Centre 2016/2017 annual report (<http://www.typicalhus.co.uk/>).

infection or use of certain drugs which may in turn impact systemic complement activity (Harris et al., 2012; Rodriguez de Cordoba et al., 2012). There are studies suggesting that the release of heme during aHUS-related hemolysis further induces an activation of the alternative pathway on endothelial cells, thereby providing a ‘secondary hit’ that exacerbates tissue damage (Frimat et al., 2013).

Eculizumab binds complement component C5, preventing its cleavage and thus blocking formation of both C5a and MAC; it was approved for aHUS by the FDA in 2011 and its use has been life-changing, relieving further attack on the kidney soon after administration and preventing progression to end-stage renal failure if used early enough in the disease (Wong et al., 2013). It is not yet clear whether C5a or MAC, or in fact both, are responsible for the pathogenic role of complement; it may therefore be interesting to see whether drugs targeting C5a-mediated signalling currently in development (see below) will significantly reduce disease progression by themselves. Meanwhile, as eculizumab prevents formation of all activation products downstream of the C5 convertase, it has a profound therapeutic impact and has quickly developed into the gold standard of aHUS treatment in many markets. Risks associated with C5 blockade include meningococcal infection which is managed with prophylactic vaccination and, in some countries, additional use of prophylactic antibiotics. Drawbacks to eculizumab include high cost that restricts drug availability in several countries, high dose (1200 mg for an adult) necessitating intravenous infusion every other week, and breakthrough symptoms when for some reason, such as an acute phase episode, complement C5 levels are increased (Peffault de Latour et al., 2015; Schutte et al., 1975). Although aHUS is rare, 0.42 per million population (UK) (Sheerin et al., 2016), and well-managed through administration of eculizumab, it remains of interest to drug companies as new and improved therapies might limit the drawbacks outlined above such as route and frequency of administration. Due to the extreme rarity of the disease, recruitment to clinical trials may be difficult in countries where eculizumab is available for treatment. Never-the-less clinical trials are ongoing and in years to come clinicians treating patients with aHUS may find that there is a selection of drugs approved and available with varying modes of action and routes of administration.

C3 glomerulopathy, C3G, is also rare and encompasses a heterogeneous group of syndromes encompassing dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) (Iatropoulos et al., 2018; Pickering et al., 2013). As with aHUS, C3G is a disease in which the complement system is dysregulated, typically within the amplification loop. Unlike in aHUS, the dysregulation usually affects fluid phase activation (Martinez-Barricarte et al., 2010). It is clear that systemic activation of complement leads to high levels of complement deposition in the kidney, particularly at the glomerular basement membrane (GBM), but whether systemic or local activation of C3 is causative is unclear. Deletion of FH in mice leads to spontaneous disease, and the normal phenotype can be restored by reinstating control of the amplification loop either systemically or locally (see Table 1). While the means of dysregulation are heterogeneous, the attraction of this indication for clinical trials is that alternative pathway dysregulation is central to the pathogenic process, and within that pathway and the amplification loop, blockade of one of many drug targets will significantly impede activation (Table 1). Due to the abundance of complement biomarkers of disease, there is potential to seek mechanistic signals in addition to clinical endpoints to confirm that the target is engaged. As with aHUS, mutations in genes encoding complement activating and control proteins are strongly associated with diseases, in addition to autoantibodies, particularly nephritic factors (NeF) (Iatropoulos et al., 2018; Servais et al., 2012). The latter typically bind neopeptides on the C3 or C5 convertase causing dysregulation of those enzymes, although antibodies to FH, FB and C3b have also been described (Paixao-Cavalcante et al., 2012; Skerka et al., 2009). While their causative nature is unclear, NeF are present in a high proportion of patients with DDD. DDD and C3GN are primarily distinguished by

histological parameters and other, more subtle, changes in complement biomarkers. Dysregulation at the level of C3 is more common in both conditions, while over-activation at the level of C5 may associate more with C3GN (Tanuma et al., 1990). Various strategies, such as immunosuppression or plasma exchange, may help patients with C3G, but to date there is no treatment to prevent end stage renal disease. There are limited case reports and trial data pertaining to the use of eculizumab in C3G, but outcomes are variable and are likely linked to the extent of C5 dysregulation in an individual patient (Zuber et al., 2012).

Immune complexes in the kidney are notably absent in aHUS and C3G, but are present in other diseases of the kidney. In **immune complex glomerulonephritis (IC-MPGN)**, immune complexes activate the classical pathway and deposits of activated C3 and immunoglobulin are found in the kidney. The disease is heterogeneous with variable presence or absence of complement gene mutations, autoantibodies and complement abnormalities in the circulation (Table 1) (Iatropoulos et al., 2018). Although considered a disease driven by immune complexes, it is intriguing that NeF are present in a high proportion of patients and mutations in alternative pathway genes have been detected. This suggests that dysregulation of the amplification loop may play a role in disease pathogenesis, perhaps ‘tipping the balance’ in an already compromised state of complement control. Other diseases linked to abnormal antibody deposition in the kidney include **IgA nephropathy**, typified by deposition of IgA in the kidney along with signs of lectin and alternative pathway activation (Table 1) (Daha and van Kooten, 2016). It is thought that abnormal glycosylation of the IgA molecule leads to recognition by anti-glycan antibodies. Mannan-binding lectin (MBL) accumulates in the glomeruli of some patients and, together with C4d deposition, correlate with worse outcome (Espinosa et al., 2014); these data together with early signs that clinical inhibition of the lectin pathway (anti-MASP2, Omeros; see below) might have therapeutic benefit in these patients, suggest that the lectin pathway plays a role in driving pathology. Genetic deficiency of the complement factor H-related proteins 1 and 3 (FHR-1, -3) associates with lowered risk of disease (Gharavi et al., 2011), thereby also implicating a contribution of the alternative pathway as the FHRs prevent localisation of the regulator FH, which controls the alternative pathway C3 and C5 convertases (Goicoechea de Jorge et al., 2013; Tortajada et al., 2013).

Lupus nephritis (LN) is a common clinical manifestation and cause of morbidity in systemic lupus erythematosus (SLE), in which immune complexes deposit in the kidney causing complement activation, renal cell damage and thrombotic microangiopathy (TMA) (Yu et al., 2017). Up to 50% of patients with SLE will have clinically evident kidney disease at presentation; during follow-up, renal involvement occurs in up to 75% of patients (Bomback et al., 2016). Complement plays an intriguing dual role in SLE pathogenesis. While deficiency of classical pathway components C1, C4 and C2 is high risk for SLE, probably due to a failure in clearance of cell debris and immune complexes (Pickering et al., 2000; Walport, 2002), complement is required to drive inflammation and tissue damage in active disease, often leading to low levels of the central components, C3 and C4, due to secondary consumption (Table 1). The exact role and mechanism of complement-mediated damage in LN is unclear. Immune deposits usually consist of anti-double-stranded DNA antibodies targeting nucleosomal antigens and antibodies binding chromatin in the mesangium and GBM; immune-histologic studies demonstrate immunoglobulin, C1q and C3 deposition. Depending on the site of immune complex formation, influx of inflammatory cells can vary, histologic analysis reveals proliferative glomerulonephritis at different sites within the glomerulus, or a membranous nephropathy-type appearance (Bomback et al., 2016). Renal manifestations are also common in the pauci-immune glomerulonephritis, **ANCA (anti-neutrophil cytoplasmic antibody)-associated vasculitis (AAV)** (Manenti et al., 2015). As AAV is pauci-immune in nature, it was originally thought that antibody and complement played little role in pathogenesis of the disease. However, availability of

improved reagents to detect complement in tissues, coupled with elegant mechanistic studies, demonstrate a clear role of the alternative pathway and C5a in driving disease. Neutrophil priming leads to increased adhesiveness of cells to walls of small blood vessels in many organs; intracellular proteins such as myeloperoxidase (MPO) become surface-localised, and autoantibodies to MPO activate complement leading to release of C5a (Jennette et al., 2013; Noone et al., 2018). This binds back to C5a receptor 1 (C5aR1) on neutrophils, further activating the cells and setting up a vicious cycle of inflammation. In AAV, levels of alternative and terminal pathway activation fragments are elevated, and renal biopsies frequently indicate activation of the alternative pathway (Table 1). Therapeutically interfering with C5a-mediated signalling might have benefit in AAV as evidenced by the positive phase 2 outcome from trial of avacopan (see below); treatment with this C5aR1 antagonist was shown to have a steroid-sparing effect (Chen et al., 2017; Jayne et al., 2017). **Membranous nephropathy (MN)** is another renal indication driven by immunoglobulins, it is commonly associated with antibodies against M-type phospholipase A2 receptor (PLA2R), often of IgG4 isotype (Beck et al., 2009). While it is clear that primary MN is associated with complement activation, the exact mechanism and role of the IgG4 antibodies have yet to be elucidated as they are typically non-complement activating. In MN, antibodies permeate the GBM and form immune complexes on podocyte membranes. Subsequent complement activation, probably sub-lytic, leads to cellular damage and thickening of the GBM. Complement deposits of activated C3, C4 and MAC are evident in the absence of C1q (distinguishing from secondary forms of MN) (Ma et al., 2013); identification of MBL in renal biopsies suggests that the lectin pathway might be responsible for complement deposition (Table 1).

Various complications of **renal transplant** have been the focus of numerous clinical trials with limited success to date. However, the potential to prolong transplant life, and the huge benefit to patients by preventing rejection, keeps this indication in the spotlight. **Ischemia/reperfusion (I/R) injury** is a common cause of acute kidney injury (AKI) following transplant resulting in delayed graft function. Complement activation associated with I/R injury tends to manifest in the tubulointerstitium as evidenced by C3 deposition in the tubular basement membrane in humans and animal models - it is likely that the alternative and terminal complement pathways play a key role in pathogenesis (McCullough et al., 2013; Thurman et al., 2005). Acute tubular necrosis due to ischemia is one of the most common causes of renal failure in man. Localisation of injury within the tubule may be a result of disruption of the apical-basolateral organization of the tubular epithelium due to ischemia, and access of proteins which are normally restricted to the apical side. It is highly likely that short-term amelioration of the I/R insult will go a long way to improving outcome following renal transplant. **Antibody-mediated rejection (AMR)** is another key factor restricting success of transplantation. Donor-specific antibodies (DSA) due to HLA/ABO mismatch can cause acute rejection within hours with massive activation of complement and tissue necrosis (Stegall et al., 2012). Effective pre-screening can minimise risk, but there is potential for anti-complement agents to prevent complement activation for a sufficient period to result in organ accommodation, particularly in cases such as ABO mismatch (Fiane et al., 1999). The role of *de novo* DSAs which develop post-transplant in chronic AMR is not clear, although emerging evidence implicates C1q and complement in driving pathology which will eventually result in loss of transplant function (Yell et al., 2015). Whether or not complement inhibition will be of benefit in chronic AMR remains to be investigated.

All of the diseases listed above have been, or are, in clinical trials for anti-complement therapy. In a number of these indications, the mechanisms of complement dysregulation and/or inappropriate activation, are well-characterised. Clearly these well-defined diseases are of interest to companies developing drugs; exploratory biomarkers are obvious and relatively simple to monitor and potentially correlate with clinical endpoints. In other indications, the mechanism might not be so

clear, but a growing body of evidence is highly suggestive of key involvement of the complement system. Some drugs in clinical development hit multiple pathways (such as C3 inhibitors), thereby having potential for those indications where the exact causative factor is unknown, but the pathogenic entity lies between a well-defined trigger and an endpoint of complement deposition or activation in the tissue. Much of the information discussed below is gleaned from company websites and conference proceedings and these are cited where practical and possible; the reader is referred to several recent reviews which highlight and discuss the extensive number of drugs which are in pre-clinical and clinical development by different companies (Morgan and Harris, 2015; Ricklin et al., 2018).

2.2. Clinical development of drugs targeting the alternative pathway amplification loop in renal disease

The amplification loop of the alternative pathway is a major driving force behind complement activation and often determines the onset of C5 convertase formation, thereby initiating subsequent C5 cleavage and terminal pathway activation. Even in diseases where pathological mechanisms are mediated by the lectin or classical pathways, blockade of the alternative pathway can frequently provide therapeutic benefit. Intervention in the amplification loop is considered an attractive proposition in drug development as the upstream lectin and classical pathways are functional and can fight infection through opsonophagocytosis; at the same time damage-driven opsonisation is reduced and, downstream, the terminal pathway is critically impacted, restricting production of the proinflammatory mediators C5a and MAC.

Within the amplification loop there are multiple targets, C3 (or C3b), FB, FD and properdin, and all have been targeted in drug development (Ricklin and Lambris, 2016). Various antibodies have been developed which bind activated C3b, possibly to lower dose compared to blocking native C3, but to the best of our knowledge, none remain in clinical development (DiLillo et al., 2006; Katschke et al., 2009). A blocking agent against properdin has also been reported by Novartis; this antibody, termed CLG561, is not in trial for renal disease. Two agents are currently in clinical trial which directly target C3 activation in renal indications. They are both based on the C3 inhibitor compstatin, which binds to native C3 and prevents its activation by C3 convertases (Ricklin and Lambris, 2008). AMY-101 is under development by Amyndas for multiple indications (<http://amyndas.com/>), which include C3G and ABO mismatch kidney transplant. AMY-101 is a latest-generation compstatin analogue with improved affinity and pharmacokinetic (PK) properties, including potential for administration via the subcutaneous (SC) route (Mastellos et al., 2015; Ricklin and Lambris, 2008). The phase 1 trial (NCT03316521) results in healthy volunteers were announced in December 2017 stating that AMY-101 could be administered SC every 48 h and maintain effective C3 blockade with a satisfactory safety profile (Amyndas Press Release, 2017). AMY-101 has been granted orphan designation for C3G and PNH by EMA and FDA and phase 1b/2 clinical trials are imminent. A second compstatin-derived drug candidate, APL-2, is based on an earlier analogue of the C3 inhibitor, which had originally been licenced to Potentia. In view of the weaker target-binding when compared to AMY-101, it has been derivatised using PEG to link two cyclic peptide moieties to provide longer half-life (<http://www.apellis.com/>). APL-2 also demonstrated satisfactory safety profile in phase 1, which largely alleviated prior fears that systemic inhibition of the complement system due to blockade of its central component, C3, puts patients at risk of severe infection; all subjects were vaccinated against *Neisseria meningitidis* types A, C, W, Y and B, *Streptococcus pneumoniae* and *Haemophilus influenzae* Type B (Hib). In total 51 subjects were enrolled in placebo-controlled studies; twenty-four subjects received APL-2 as a single dose (top dose 1440 mg) and sixteen subjects received repeated daily doses of APL-2 for a month (top dose at 270 mg/day). Alternative pathway haemolytic activity was impacted following a single dose of

1440 mg APL-2 and following multiple daily doses of 180 and 270 mg (Grossi et al., 2016). These dosing levels were required due to the high plasma concentration of the target, C3 (> 1 mg/ml), nonetheless, both studies concluded that pharmacological doses of APL-2 were safe and well tolerated and that APL-2's PK/PD profile supported daily SC administration (Apellis Press Release, 2016). The dosing regime of 180 mg or 270 mg daily (SC) is current protocol for phase 1 studies in PNH (NCT02588833). While the lead indications, PNH and AMD, are described below, APL-2 is currently being trialled in patients with a variety of biopsy and biomarker-confirmed renal diseases, including IgA nephropathy, LN, MN, C3GN and DDD (Phase 2, NCT03453619; first posted to ClinicalTrials.gov March 2018). The drug is being administered once a day for 16 weeks with a 6 month safety follow-up and a primary endpoint of change in baseline proteinuria at 16 weeks. While these trials will provide long-awaited insight into the efficacy and safety of C3 blockade, it will be interesting to see in which disease indications PEGylation proves beneficial. Whereas the addition of PEG units may confer important benefits concerning solubility and/or PK, high PEG concentrations may potentially influence the safety profile, and short-lived complement inhibitors may allow rapid restoration of complement activity in case of adverse events (Ivens et al., 2015; Risitano et al., 2014).

A sea change in anti-complement therapy is marked by the recent development of orally bioavailable anti-complement drugs. Two small molecule (SM) drugs with activity on the amplification loop have been developed by Achillion (ACH-4471; <http://www.achillion.com/>) and Novartis (LPN023; <https://www.novartis.com/>). The Novartis agent, LPN023, binds the active site of FB and thus inhibits the convertase and C3 cleavage. The phase 2a/2b dose ranging study was first posted to ClinicalTrials.gov in December 2017 (NCT03373461), marking the start of a long-awaited trial. The agent will be taken twice daily (BID) in patients with IgA nephropathy to establish clinical proof-of-concept and dose. The change in urine protein to creatinine concentration (from original baseline measurement) is being monitored as a primary endpoint; secondary outcomes probe renal function in more depth and examine drug PK and impact on the complement alternative pathway through measurement of plasma Bb and sC5b-9. As with APL-2, all patients receive triple vaccination prior to receiving the drug.

The second orally-bioavailable drug, the FD inhibitor ACH-4771, has been developed by Achillion. By blocking the catalytic site of FD, the drug prevents formation of functional alternative pathway C3 convertases. In phase 1 studies, ACH-4771 was administered twice a day at a single dose of 1200 mg, delivering almost complete inhibition of alternative-pathway activity in *ex vivo* blood samples at 24 h (Achillion Presentation, 2016). While further phase 1 studies assess PK of modified formulations (NCT03384186), ACH-4771 is being administered three times daily (TID) with an initial dose of 100 mg in phase 2 PNH studies (Achillion Press Release, 2017a) (NCT03053102). The same dose is being administered in the phase 2a proof-of-mechanism study in C3G/IC-MPGN trial (NCT03124368, initiated April 2017); initial data from two patients with C3G demonstrated that the drug significantly inhibited the alternative pathway *in vivo*, resulting in elevated intact C3 and decreased levels of the activation fragment Bb. Importantly, renal function improved (albumin creatinine ratio, ACR) after 14 days (Achillion Press Release, 2017c). A randomised, placebo-controlled phase 2 trial (NCT03369236) is now recruiting (posted ClinicalTrials.gov December 2017) with primary outcome measures of change from baseline in renal biopsy at 6 months, based on a score incorporating changes in both the activity index and C3 staining, and improved renal function. Orphan drug status for C3G was granted by the FDA in December 2017 and a 12 month open-label proof-of-concept trial has now been posted (March 2018; NCT03459443) in C3G/IC-MPGN. Achillion have also announced (December 2017) initiation of first-in-human studies of their 'next generation' FD inhibitor, ACH-5228, also administered orally and reported to have several-fold improved activity and better PK than ACH-4771 (Achillion Press Release,

2017b). This initial phase 1 trial is a randomised, placebo-controlled, single ascending dose study of ACH-5228 in healthy volunteers, approximately 28 subjects are expected to be enrolled to assess safety, tolerability and to explore PK/PD relationships (Achillion Press Release, 2017d). Novartis also report an orally-bioavailable SM inhibitor of FD with interesting properties in preclinical testing (Maibaum et al., 2016), but clinical development has not been reported. Similarly, Ra Pharma lists a peptide-based inhibitor of FD in their development strategy, this is currently in preclinical testing with C3G as potential indication (<http://rapharma.com>), though further details have yet to emerge (RaPharma Investor Presentation, 2018).

As mentioned above, a transplanted kidney can be subject to many assaults from antibody-driven complement activation to I/R injury. A phase 2 trial being conducted in the UK asks the question whether infusion of the kidney pre-transplant with a drug termed mirococept (originally APT070), which localises to endothelial membranes and inhibits the C3 and C5 convertases, will improve renal outcome (ISRCTN Registry, ISRCTN49958194) (Kassimatis et al., 2017). The primary endpoint is delayed graft function (DGF), defined as the requirement for dialysis during the first week after transplantation. The trial was registered in 2012 and has yet to publish data.

2.3. Clinical development of drugs targeting C5, C5a and MAC in renal disease

Approval for the use of eculizumab for treatment of PNH and aHUS was a landmark in complement research and anti-complement drug development (Hillmen et al., 2006). It provided critical clinical validation that complement could be effectively inhibited in man and save lives. Two phase 2 trials were conducted in aHUS in 2009 and the results were remarkable (Legendre et al., 2013). In the first trial patients with plasma-therapy resistant progressive TMA (measured by low platelet counts), impaired renal function and evidence of hemolysis were recruited and received eculizumab for 26 weeks; most continued for a long-term extension phase to 64 weeks (adults, NCT00844545; adolescents, NCT00844844). Seventeen patients were recruited, hematologic values normalised with improved platelet count and improved renal function. In the second trial 20 patients were recruited who had long duration of disease and chronic kidney damage and who had received prolonged treatment with plasma exchange or infusion but had no decrease in the platelet count of more than 25% for at least 8 weeks during PE/PI treatment (adults, NCT00838513; adolescents, NCT00844428). Twenty were treated for 26 weeks and 19 continued into the extension phase to 62 weeks. In 16 of the 20 patients, TMA was inhibited and in the remaining four a transient decrease in platelet count meant that they did not reach primary endpoint. All 20 patients came off plasma exchange/infusion and did not require dialysis. Intervention early in the disease resulted in greater improvement in glomerular filtration rate (GFR) in both trials. These remarkable data from clinical trials of eculizumab in the rare diseases, aHUS and PNH, together with the discovery in 2005 that complement also contributed significantly to the etiology of common diseases (such as AMD) (Edwards et al., 2005; Hageman et al., 2005), drove a vigorous resurgence in development of anti-complement therapeutics for multiple targets and multiple diseases (Morgan and Harris, 2015; Ricklin et al., 2018).

Many of these molecules are described above and targets lie in the activation pathways of complement, however, C5 remained, and still is, a firm favourite as a drug target and various strategies have evolved since approval of eculizumab to block C5 activity *in vivo*. One agent, ravulizumab (ALXN1210), a pH-switched version of eculizumab being developed by Alexion (www.alexion.com) (Sheridan et al., 2018), is in phase 3 trials for aHUS (NCT03131219, NCT02949128). This next-generation version of eculizumab has been engineered in the antigen-binding region to release the target, C5, at pH6. This results in C5 being released in the slightly acidic endosome where it is subsequently

targeted for degradation, whereas the antibody is recycled to the cell surface devoid of the antigen and available to bind further C5. This ‘pH switch’, together with engineering of the Fc domain, has resulted in a drug with improved PK/PD characteristics compared to the original ‘parent’ eculizumab. It is administered every 8 weeks, rather than fortnightly, with a maintenance dose of 3300 mg (in a 60–100 kg adult). This is higher than that of eculizumab in aHUS (1200 mg) although it is given less frequently. The success with eculizumab in aHUS and PNH has led to many explorations of its efficacy in other indications, including the challenge of kidney transplantation. Eculizumab has been tested in many clinical trials for DGF and I/R injury. The clinical trial databases are littered with such trials, frequently investigator-led and terminated due to poor enrolment. The definitive study of eculizumab in DGF comes from Alexion’s PROTECT DGF world-wide phase 2/3 registration trial which treated 286 patients and completed in November 2016 (NCT02145182). The primary outcome measure was incidence of DGF defined as the requirement for dialysis for any reason in the first seven days post-transplant, and this endpoint was not met. The trial results have yet to be released but these data are expected imminently. The reason for the failure of this trial is not clear, but it suggests that blockade of C5a and MAC alone are not sufficient to prevent complement-mediated damage to the kidney, or that other mechanisms beyond complement play a key role in kidney injury post-transplant. Disappointing results were obtained in Alexion’s phase 2 trial in AMR of living donor kidney transplant recipients requiring desensitization (NCT01399593); 102 patients were transplanted and received eculizumab or standard of care (SOC). The primary endpoint was treatment failure rate at 9 weeks, but the trial was terminated as statistical significance was not reached. Study limitations included potential reporting bias with many sites involved, unequal exposure to randomised prevention therapy between groups, and subsequent treatment of SOC patients with eculizumab following diagnosis of AMR. Testing of eculizumab in a small trial for treatment of AMR following renal transplantation (no desensitisation required; NCT01895127) was also terminated due to lack of efficacy. Eculizumab continues to be tested in different indications, some of which are kidney diseases, but to date no other renal disorder has shown great promise. While success in aHUS is striking, data remain insufficient to recommend eculizumab as a first-line agent for the treatment of rapidly progressive C3G (Bomback et al., 2012; Goodship et al., 2017a, Herlitz et al., 2012). Even in aHUS, there is debate whether the treatment of this condition requires continuous anti-C5 therapy or whether an intermittent treatment once symptoms occur would be sufficient (Sahutoglu et al., 2016). While such a treatment scheme would be more cost-effective, additional studies will be required to define which patients may potentially benefit from this adaptation.

One strategy to overcome high target concentration is to target at the RNA or DNA level and, indeed, Alnylam Pharmaceuticals have developed an RNAi, Cemdisiran (ALN-CC5), that is liver-targeted (through GalNAc conjugation) and blocks hepatic production of C5 (<http://www.alnylam.com/>). Initial clinical studies demonstrated that the agent effectively knocked-down circulating C5 by 98% and a single SC dose of Cemdisiran (600 mg) achieved greater than 90% C5 knock-down six months following administration (Alnylam Press Release, 2016). Whereas these inhibition levels appear to be insufficient to achieve treatment success with Cemdisiran monotherapy in PNH (see below), the conditions for such an approach may be more favourable in the case of aHUS. To this end, a phase 2 clinical trial of Cemdisiran monotherapy in aHUS was launched late 2017 (NCT03303313), in which 600 mg is administered as monthly SC injection and increase in platelet count is assessed (Alnylam Press Release, 2017). Given that C5 blockade is required extremely rapidly when an individual presents with an acute episode of aHUS, and it takes a number of days for C5 to be suppressed with RNAi therapy, it can be speculated that an additional therapeutic agent may need to be used in the initial stages of therapy to rescue renal function before C5 levels are subsequently knocked down

using Cemdisiran.

While the blockade of C5 prevents formation of both disease-causing products, C5a and MAC, the specific interference with any of these effectors individually may provide further insight into disease mechanisms and provide clinical benefits in some diseases. This is particularly true in the case of aHUS, for which it has not been resolved whether both C5a and MAC act as drivers of the condition. Avacopan is an orally bioavailable small molecule antagonist of C5aR1, developed by ChemoCentryx (Bekker et al., 2016; Jayne et al., 2017), which has progressed to multicentre phase 3 trial in AAV (NCT02994927). Its potential to induce disease remission at 26 weeks in immunosuppressed individuals and maintain remission for one year is being compared to SOC (steroids) and should read out in 2019. Company releases and conference papers report a decrease in renal inflammation and improved proteinuria in AAV patients treated with avacopan, raising hope that the agent could improve outcome in renal disease. Indeed, in 2016, ChemoCentryx reported a case of C3G treated with avacopan with positive outcome and stabilisation of renal function (Chemocentryx Press Release, 2016a). The company obtained orphan disease designation for C3G in 2017 and initiated a multicentre placebo-controlled phase 2 trial late 2017 (NCT03301467); patients with biopsy-proven disease and with elevated circulating terminal complement complex (TCC, also known as sC5b-9) will be assessed for improvement in histologic index following 6 months of twice-daily dosing (30 mg/dose) with avacopan. The focus on orphan disease encompasses further indications including a skin disease (Hidradenitis Suppurativa) and two further renal indications (Chemocentryx Corporate Presentation, 2018). A small phase 2 study (NCT02384317) in IgA nephropathy completed in 2016 and improved proteinuria was reported after 12 weeks (Chemocentryx Press Release, 2017), and a phase 2 trial in aHUS commenced in 2015 (NCT02464891). In this trial, six patients were administered avacopan twice daily (30 mg) for two weeks and blood was taken at various times during that period for analysis of *ex vivo* pro-thrombotic properties on a cultured human microvascular endothelial cell line (presented at the American Society of Nephrology (ASN) Kidney Week 2016 Annual Meeting (Chemocentryx Press Release, 2016b)). Although this study supported a role for C5a in thrombus formation and a potential role for avacopan in therapy, all patients were on peritoneal dialysis or hemodialysis, treatments which are in themselves complement-activating; definitive evidence of the *in vivo* impact of this agent in aHUS awaits further clinical trials. Recently, Akari announced that they have initiated phase 2 trials with their C5 inhibitor coversin in aHUS (Akari Press Release, 2018); this tick-derived protein binds to both C5 and leukotriene B4 and has been clinically developed for treatment of PNH (see below); further details regarding the aHUS trial are awaited.

2.4. Clinical development of drugs targeting other pathways in renal disease

The anti-MASP2 monoclonal antibody, OMS721, which blocks the lectin pathway, has also progressed to phase 3 in renal indications and has received orphan drug designation for aHUS and IgA nephropathy (Corporate presentation January 2018). In 2017, Omeros opened an open-label multi-centre phase 3 trial aiming to recruit 80 adult/adolescent patients with aHUS with increase platelet count as primary endpoint (NCT03205995). Drug loading is achieved through intravenous administration followed by SC maintenance dosing. A phase 2 trial (NCT02682407) in glomerulopathy commenced in 2016 and recruited patients with C3G, LN, MN and IgA nephropathy. Patient numbers in the IgA nephropathy arm of the open label phase 2 trial were limited, but following 12 weeks of treatment, daily steroid use was reduced or stopped altogether and proteinuria improved. Follow-up reports in August 2017 indicated that in three out of four patients, the improved renal function continued to persist post-cessation of dosing. Omeros has achieved orphan and breakthrough designation of OMS721 in IgA nephropathy and larger, randomised, double-blinded placebo-

controlled phase 3 trials are expected in 2018. A similar improvement in tapering of steroids and improved proteinuria was evident in the LN arm of the phase 2 trial. As the lectin pathway proteins, including MASP-2, have been shown to exert procoagulant activities (Dobo et al., 2016), it will be interesting to see whether in some of these indications the modulation of coagulation pathways contributed to the outcome.

Although C1 esterase inhibitor (C1-INH) has a long-standing history for the treatment of a non-complement-mediated disease (i.e. hereditary angioedema), the applicability of C1-INH as a possible complement-targeting therapeutic has gained renewed interest. C1-INH blocks C1r and C1s of the classical pathway as well as MASPs and complement-unrelated serine proteases. It is readily available on the market, with various manufacturers (Pharming, CSL Behring and Shire) providing different preparations, but has only sparsely been applied in a complement therapeutic context. CSL Behring completed two single centre, randomised phase 1b/2 trials examining the safety and efficacy of Berinert in reducing complement-dependent AMR or DGF. The first (NCT0134510) enrolled 20 patients, finding no severe adverse events or episodes of AMR during the study period (Vo et al., 2015). The second (NCT02134314) completed in March 2017, enrolling 70 patients, looking for improved kidney function after deceased donor kidney transplantation with high risk of I/R injury and DGF. While results are expected imminently, CSL Behring initiated a further double-blind, randomised-withdrawal, placebo-controlled phase 3 study (July 2017) to evaluate Berinert as add-on therapy to SOC in AMR (NCT03221842). In the meantime, Shire is recruiting patients for a phase 1 study to study efficacy of Cinryze, with or without heparin, as a donor pre-treatment strategy to decrease the incidence of DGF (NCT02435732, started 2015, last update September 2017). Of note, this presents a different strategy compared to CSL Behring, as donors of kidney grafts, rather than the recipients, are treated with Cinryze prior to transplantation, in an effort to increase graft condition by decreasing complement activity in the donor.

2.5. Biomarkers and stratification

As disease mechanisms and biological traits predisposing to disease are becoming clearer, it is increasingly possible to stratify patients for clinical trial in order to maximise chance of success; getting the ‘right drug to the right patient’ is critical for effective anti-complement therapy (Ricklin et al., 2017, 2018). Stratification for clinical trial can frequently reach beyond clinical phenotype to include other markers such as genetic variants and protein biomarkers, including complement activation fragments in plasma and in tissues (Wong et al., 2016). Plasma biomarkers can provide invaluable mechanistic insight to enable subgroups of patients to be identified. For example, elevated TCC in plasma may be an indicator that C5 is being abnormally and excessively activated; or presence of C5a, despite blockage of convertase-mediated C5 cleavage, may be an indicator that other enzymes, such as thrombin or elastase, are activating C5 (Riedemann et al., 2017). In these cases, treatment with a C5a-blocker or a C5aR1-blocker, alone or in combination, might provide additional therapeutic impact. In C3G, while dysregulation of complement at the level of C3 is evident in most cases, evidence points to a subset of patients with dysregulation at the level of C5 (Le Quintrec et al., 2015; Lebreton et al., 2017; Nester and Smith, 2016); these patients may benefit from anti-C5/C5a therapy (for example, NCT03301467). Elevated TCC and lowered levels of properdin may indicate elevated C5 convertase formation and assist identification of the correct patient subgroup (Corvillo et al., 2016).

Complement biomarkers can also provide an exquisitely sensitive signal to demonstrate on-target action of a drug enabling rapid proof that it is having an effect on disease mechanism (Ricklin et al., 2017). As a first line diagnostic, haemolytic activity of complement in blood (serum) can be assessed using a number of laboratory assays, including hemolysis (CH50, AH50) or ELISA-based systems. More informative biomarkers may read out closer to drug target, for example, it should be

possible to prevent C3 consumption in C3G patients and restore C3 levels to normal range by inhibiting the amplification loop of complement by one of many different means, whether that be inhibition of FD or FB, or blockade of C3 cleavage; the read-out should be rapid following drug loading as C3 is constantly synthesised by the liver. Similarly, blockade of C5 will prevent formation of MAC (tissues) and TCC (soluble) and plasma measurements can rapidly demonstrate that the drug has bound its target and is having pharmacological impact. Various clinical trials use complement plasma biomarkers as clinical trial endpoints (as examples NCT03373461, NCT03124368), and a growing number use histologic index with a focus on complement deposition (e.g., NCT03301467).

3. The eye

3.1. Ocular indications involving complement

The eye is a challenging organ to treat due to anatomical barriers between it and the rest of the body (Fig. 4), never-the-less, common diseases afflict the eye and rewards, both to health services and to population health, could be high for a successful therapeutic (Clark and Bishop, 2018; Mohlin et al., 2017). Complement is implicated in a number of diseases which afflict the eye (Table 1), but the disease which particularly caught the attention of the scientific community and pharmaceutical industry was **age-related macular degeneration (AMD)** (Fig. 4c). This is a common blinding disease in the developed world affecting 1.5% of individuals over 40 years old in the USA; in white women (of European-descent) aged over 80 the incidence rate even exceeds 15% (Friedman et al., 2004). Complement turns over naturally in the eye, just as in most other organs, and is controlled through the actions of the fluid phase and membrane bound regulators (Bora et al., 1993; Sohn et al., 2000). This complement turnover, and the actions of resident phagocytic cells, are critical to maintain an infection-free environment, and the level of therapeutic complement inhibition therefore needs to be carefully considered. Moreover, some changes in chronic eye disorders only progress very slowly and may be difficult to monitor, therefore often requiring lengthy trials. Despite such technical challenges, it was AMD with its strong disease link to complement and high prevalence, which brought complement and anti-complement therapy back into the limelight around 13 years ago. In AMD, small deposits comprising protein and lipid start to accumulate early in the disease process, particularly within the Bruch's membrane, the stratified sheet of extracellular matrix which separates the retinal pigment epithelium, the most posterior element of the retina, from the choroid (Mohlin et al., 2017). These deposits, termed drusen, disrupt the health of the retina by interfering with clearance of waste products and nutrient supply and are characteristic of the early form of the disease (see Fig. 4). Late stage blinding disease is termed geographic atrophy (GA or ‘dry’ AMD), characterised by death of the retinal pigment epithelium (RPE) and associated photoreceptors, or neovascular AMD, characterised by invasion of fragile blood vessels from the choroid into the retina where leaky vessels cause retinal distortion and scarring (‘wet’ AMD). Dry AMD currently lacks therapeutic options whereas wet AMD is treated with anti-VEGF (vascular endothelial growth factor) injections directly into the eye. It had been known for some time that drusen were coated with activated complement (Mullins et al., 2000) but pathogenic consequences were unclear. The discovery in 2005 of strong associations between AMD disease risk and common variations in genes encoding proteins of the complement system, particularly with a common variant in the control protein FH (Tyr402His), fuelled vigorous research in this area and drove a new era of complement drug discovery (Gold et al., 2006; Hageman et al., 2005; Haines et al., 2005; Klein et al., 2005; Ricklin and Lambris, 2007; Spencer et al., 2008). The exact role of complement in AMD pathogenesis remains unclear. A number of structure/function studies demonstrate that risk-associated variants of complement proteins produce a more active

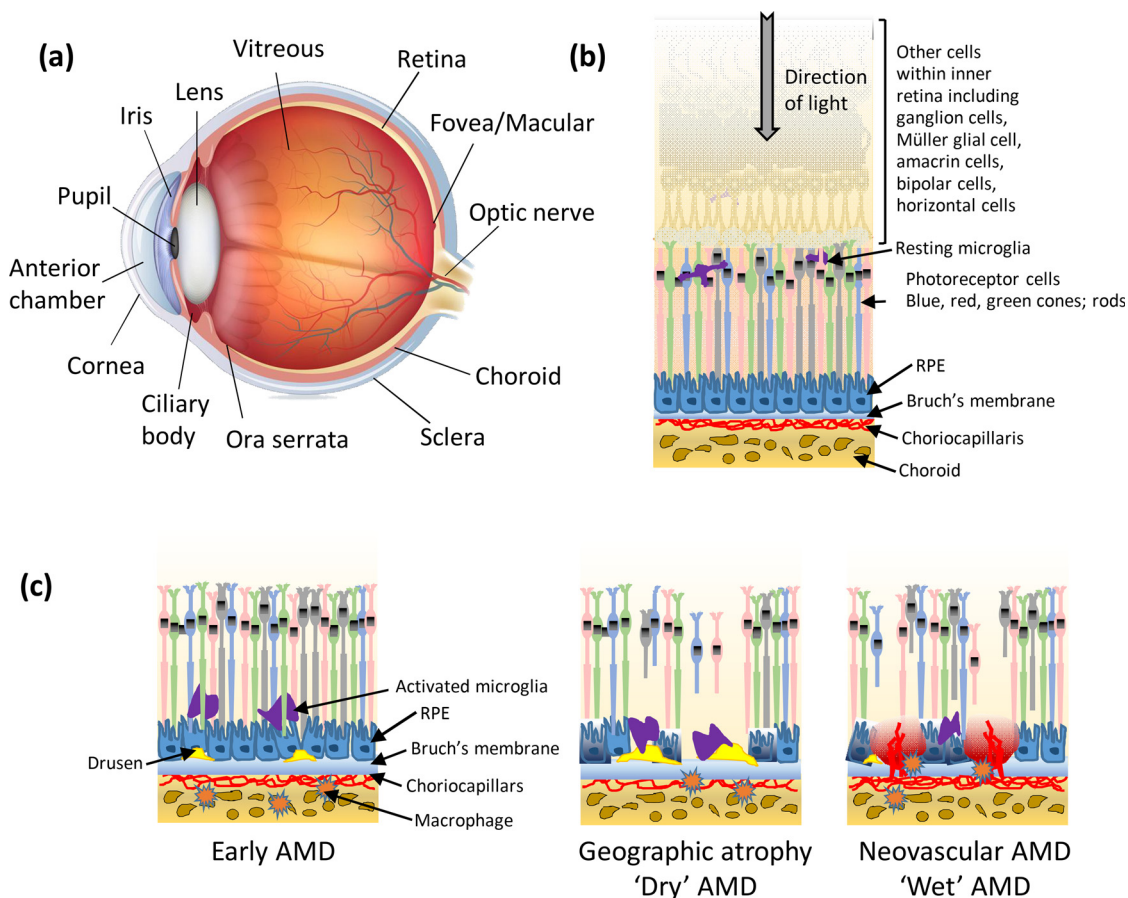


Fig. 4. Structure of the eye and pathogenic mechanisms in AMD. (a) The retina is located towards the posterior of the eye above the choroid; the macula is responsible for central vision and has the highest density of photoreceptors. (b) In health, the RPE cells, which obtain nutrients from the choroid below, support and maintain the integrity of the photoreceptor cells; the retina is patrolled by resting microglia. (c) In early AMD (left), deposits known as drusen form within the Bruch's membrane; these deposits comprise protein/lipid and cellular debris and are highly complement activating. The Bruch's membrane becomes thickened, microglia become activated and accumulate in the sub-retinal space, macrophages are evident in the choroid. In dry AMD (centre), the choroidal blood vessels atrophy and the RPE cells start to die, leaving photoreceptors without support, this results in confluent areas of atrophy and cell death. In wet AMD (right), choroidal vessels break through the Bruch's membrane and become leaky. Activated microglia and macrophage accumulate in the subretinal space, the RPE and immune cells release proinflammatory mediators and vascular endothelial growth factor (VEGF) setting up a vicious cycle of vessel growth and inflammation, resulting in cell death and retinal atrophy.

complement system, although this is not the case with FH 402His (Heurich et al., 2011; Rodriguez de Cordoba et al., 2012; Tortajada et al., 2013). Elegant structural and functional studies suggest that the 402His form of FH, or its smaller splice product FH-like 1 (FHL1) bind less well than the 402Tyr variant to structures in the eye, thus potentially providing less protection against chronic inflammation in the eye (Clark et al., 2010, 2013; Prosser et al., 2007; Weismann et al., 2011). Interestingly, these studies demonstrate preferential binding of ocular glycosaminoglycans (GAGs) to the GAG-binding domain in the central part of FH (in short consensus repeat [SCR] 7) where the 402 variant is located in FH and FHL1, rather than to the binding domain in the carboxy terminus in FH, a structural region known to be of critical importance in protecting the kidney from complement-mediated damage.

In many diseases complement acts to exacerbate rather than drive pathology, and the following disorders may fall under that umbrella. **Recessive Stargardt macular degeneration (STGD1)** is a disease which has some parallels with AMD, such as lipofuscin accumulation and complement dysregulation; it is caused by mutations in the gene for the ABCA4 (ATP binding cassette subfamily A member 4) transporter in photoreceptor outer segments; patients exhibit buildup of bisretinoid-containing lipofuscin pigments in the RPE, as there is increased oxidative stress and slow degeneration of photoreceptors. Recent literature indicates that complement is over-activated in the retina in the mouse

model of disease (*Abca4*^{-/-} mice), and that gene therapy to provide additional complement control could directly modulate complement activation and partially rescue the disease phenotype (Lenis et al., 2017). **Uveitis** is inflammation of the uveal tract and can occur at many sites within the eye; it can be localised to the anterior (iris, anterior ciliary body), intermediate (vitreous, posterior ciliary body, or ora serrata) or posterior part of the uvea (choroid, retina) and is a significant cause of blindness worldwide. The exact role of complement in uveitis is unclear, and likely complement has a role as a mediator of an inflammatory vicious cycle rather than as a driver of disease, but data from animal models indicate that complement blockade at the level of C5 may have therapeutic benefit (Copland et al., 2010). Novartis completed a phase 2 trial with LFG316 anti-C5 in non-infectious intermediate-, posterior- or panuveitis (NCT01526889) but data have yet to be reported. There are other disorders in which complement plays a role, but to the best of our knowledge anti-complement agents are not yet being tested in clinical trials; these diseases include glaucoma and diabetic retinopathy (Mohlin et al., 2017).

3.2. Clinical development of drugs targeting the amplification loop in ocular disease

Genetic associations with AMD dominate within genes encoding proteins that activate, inhibit or impact in some way on the

amplification loop of complement. It is therefore not surprising that drug discovery and development for AMD has largely focussed in this area (Fig. 2, Table 1). Two opposing strategies could be employed to downregulate complement in the eye –supply of additional regulators or blockade of activators. Presumably the ideal scenario would be one which modulates complement activation rather than blocks it altogether as blockade could also prevent efficient clearance of debris. Genentech advanced an inhibitor of FD (lampalizumab) to late stage clinical testing, but failed to meet primary endpoint in two phase 3 trials (CHROMA and SPECTRI; NCT02247479, NCT02247531; (Genentech Media Statement, 2017; Roche Media Release, 2017). Post-hoc analysis of the phase 2 trial (NCT02288559), which studied rate of growth of retinal atrophy in people with GA due to AMD, demonstrated that patients carrying a specific genetic variant in a locus close to the factor I (FI) gene, had 44% decrease in rate of progression over 18 months of treatment when compared to only 20% in the overall study population (Roche Investor Update, 2013a,b), but the functional or biological consequences of that SNP were not clear. The phase 3 trials were identically-designed, double-masked, randomised studies evaluating the efficacy and safety of a 10 mg dose of lampalizumab administered every four or six weeks by intravitreal injection, versus sham injections, in more than 1800 participants at more than 275 sites in over 20 countries (Holz et al., 2018). The primary efficacy endpoint was evaluated at one year (week 48) and was measured by fundus autofluorescence (FAF) analysis of the GA lesion in the macula. Detailed analyses demonstrate that in this large study, the FI biomarker had no effect on outcome (Holz et al., 2018). Despite the setback with lampalizumab, FD remains a target of interest in the treatment of AMD. A new generation of small molecule FD inhibitors as those from Achillion (ACH-5548), currently in phase 1 in healthy volunteers (Achillion Press Release, 2017d), and Ra Pharma (preclinical) (RaPharma Investor Presentation, 2018) may provide much-anticipated insight as to whether systemic complement inhibition may result in therapeutic benefit in AMD.

One other agent in trials for GA is APL-2 (by Apellis), this is expected to start phase 3 trials in the near future. APL-2 was administered by the intravitreal route in phase 2a studies in GA; this trial, termed FILLY, investigated outcomes from sham, monthly (15 mg) and bi-monthly (15 mg) injections of APL-2 in a total of 246 patients (Apellis Company Presentation, 2018a,b). Similarly to the lampalizumab trials, primary efficacy endpoint was change in GA lesion size from baseline to month 12; patients receiving monthly injections showed a 29% difference in lesion growth compared to sham injection with the biggest impact being evident in the second six months of treatment. Following cessation of treatment and follow-up for a further 6 months, the original rate of progression of the GA growth resumed. It was noted that in 18% of the patients receiving APL-2 monthly, there was evidence of new onset of wet AMD at the 12 month monitoring point, which was higher than in the sham-treated group. However, it is likely that many of these patients would have developed wet AMD as part of the natural disease process as 38% of patients had wet AMD in the fellow (untreated) eye at the start of the study. It is possible that the exudative events were not related to ‘fresh’ neovascularisation or were linked to subclinical lesions missed at baseline, these possibilities warrant further study. It is interesting to note that Apellis launched a small phase 1b/2 open label trial in wet AMD (NCT03465709; March 2018) with a primary outcome measurement of safety over 18 months of treatment. It is to be noted that there were three incidences of endophthalmitis reported in the FILLY trial, two were culture-positive for *Staphylococcus* and one culture-negative. Total blockade of complement within the ocular space can increase risk of infection, but despite the many complement-targeted trials in AMD to date these numbers have been small. In the case of APL-2, the risk-benefit profile at 18 months in the FILLY trial supported progression to phase 3; further study and larger phase 3 trials (expected to initiate 2018) will indicate whether these adverse events prove significant. A previous phase 1 clinical trial with the non-

PEGylated derivative (termed POT-4) was carried out by Potentia Pharmaceuticals in neovascular AMD; this completed in 2010 with a satisfactory safety profile reported in patients (NCT00473928) (Biobusiness Briefs, 2009); Potentia Pharmaceuticals was acquired by the spin out company, Apellis Pharmaceuticals, in 2014. Amyndas lists plans to initiate clinical development of next-generation compstatin analogs in AMD (www.amyndas.com/pipeline), yet no phase 1 trial has been started to date. In addition, Amyndas has a small, engineered version of FH in preclinical development for AMD (mini-FH; AMY-201). Although full-length FH has been considered as a FH therapeutic, it is challenging to purify or produce. Recombinant regulator constructs such as mini-FH may render the approach of controlling convertase activity in the eye more feasible (Schmidt et al., 2016).

Ionis Pharmaceuticals have recently announced phase 2 development (placebo-controlled, double-masked trial; NCT03446144) of their antisense agent (ligand conjugated antisense; LICA) targeting complement FB (FB-LR_x). The company aims to enrol 120 participants with GA secondary to AMD with a single SC dose every other week. Primary outcome measurement will be percent change in plasma FB, and secondary outcome measurements include safety and rate of change of the GA lesion. Phase 1 studies completed in 2017 and achieved dose-dependent reductions in FB and demonstrated a positive safety and tolerability profile (Ionis Press Release, 2017). Antisense technology provides an excellent opportunity to circumvent dosing issues associated with extremely high levels of protein drug target in the circulation and potentially provides the means to modulate complement activity rather than ablate it. Ionis have previously demonstrated that SC administration of FB antisense to cynomolgus monkeys for 13 weeks at 40 mg/kg/week resulted in dramatic reduction in FB levels in blood and, consequently, in the eye (Grossman et al., 2017); it remains to be seen whether systemic inhibition of complement can slow or halt progression of GA.

3.3. Clinical development of drugs targeting C5, C5a and MAC in ocular disease

Various agents against C5 have been tested in clinical trials for AMD. Blockade of C5 cleavage stops both C5a and MAC formation, thus preventing inflammation driven by proinflammatory C5a receptors and the NLRP3 inflammasome, known to be active in RPE cells in advanced AMD (Laudisi et al., 2013; Tseng et al., 2013). Eculizumab (described above) has been administered by intravenous infusion every other week in a dry AMD phase 2 trial (COMPLETE, NCT00935883); patients were treated for 6 months and observed for another 6 months. Thirty patients were enrolled but the rate of GA progression was the same in controls and eculizumab-treated patients at both 6 and 12 months. However, there were no drug-related adverse events and none of the eyes converted to wet AMD (Yehoshua et al., 2014). Novartis also tested their anti-C5 antibody, LFG316, in both dry and wet AMD using intravitreal administration. In the dry AMD trial (NCT01527500), LFG316 was administered monthly for 18 months in patients with geographic atrophy (GA); the trial data are pending in ClinicalTrials.gov but in a conference proceeding (Angiogenesis, Exudation and Degeneration Meeting, 2016), the trial was reported to have failed with no difference in GA lesion growth at month 12. Of note, a 12 month combination trial of LFG316 together with the anti-properdin antibody CLG561 is ongoing in GA (NCT02515942); properdin is a positive regulator of the amplification loop and supports surface-associated complement activation, particularly C5 cleavage; thus, this trial represents a double-hit on complement amplification and C5 activation. CLG561 is also being tested (in the same trial) as a monotherapy in GA with potential to inhibit formation of both the C3 and C5 convertase enzymes. A phase 2 trial of LFG316 in wet AMD completed in 2013 but data have yet to be reported (NCT01535950).

One other C5 agent, an aptamer (i.e. single strand nucleic acid ‘antibody’) termed Zimura (originally known as ARC1905;

avacincaptad pegol sodium), is being developed by Ophthotech (Ophthotech Corporate Presentation, 2018). The company has ongoing trials in dry AMD (GA) (NCT02686658; phase 2b) and wet AMD (NCT03374670; phase 2a), in both cases the drug is administered intravitreally. The GA trial is randomised, double-masked and sham-controlled and zimura is given at multiple dose levels every month for 18 months; similarly to the trials described above, the primary efficacy endpoint is mean rate of change in GA over 12 months measured by FAF at three time points. Recruitment started in 2016 with the original goal to treat ~300 patients with GA, with an interim analysis scheduled at 18 months before further large-scale enrolment. Following release of the top line data from the phase 3 lamalizumab (Genentech) and the phase 2 APL-2 (Apellis) trials, Ophthotech announced a modification to decrease patient numbers and shorten time to primary efficacy endpoint (Ophthotech Press Release, 2017); the top line data should be available in the second half of 2019. The phase 2a trial in wet AMD (NCT03362190) commenced October 2017 and should produce top line data late 2018; Zimura is being administered over 6 months in a dose ranging study as a combination therapy with anti-VEGF antibody (0.5 mg Lucentis), an interesting approach given that VEGF blockade is suggested to decrease expression of complement regulators, such as FH, in the retina (Keir et al., 2017). Ophthotech is expanding their portfolio of C5 blockade in ocular conditions by trial of Zimura in STGD1. In this double-masked sham-controlled phase 2b trial (NCT03364153), patients will be treated for 18 months; recruitment was announced in January 2018 and the top line data should read out in 2020 (Ophthotech Press Release, 2018). A similar approach is being taken for therapy in idiopathic polypoidal choroidal vasculopathy (PCV); although definitive evidence for a role of complement in PCV is so far lacking, it is a disease with clinical features very similar to neovascular AMD and association with a SNP in FH which affects function (Coscas et al., 2015; Tanaka et al., 2011). An open label phase 2a safety study (NCT03374670) started recruitment in March 2018 for combination therapy of zimura with aflibercept (Eylea), a soluble decoy fusion protein of VEGF receptor fused to human IgG1; the initial data should be available towards the end of 2019.

3.4. Biomarkers and stratification

The most prevalent disease of the eye, in which complement plays a major role, is AMD; while the strongest case can be made for anti-complement therapy in this indication, it is one of the most confounding when considering drug delivery and patient stratification. At the very least, it should be possible, and probably advisable, to stratify patients according to their key genetic risk factor, whether it be the complement genes ('chromosome 1 disease'), including the FH Tyr402His variant (rs1061170), or variants at the HTRA1/ARMS2 locus ('chromosome 10 disease') (Kanda et al., 2007; Yang et al., 2006). Data from the phase 2 trial of lamalizumab in AMD hinted that better outcome might be obtained in patients stratified for a specific complement, however, data analysis from the phase 3 trials demonstrated that outcome was not improved in patients carrying the FI biomarker (Holz et al., 2018).

Even if it is possible to define a population most likely to have a significant complement driven disease, questions remain as to the best route of administration, systemic versus local, and the best part of the cascade to inhibit, amplification loop alone, C3, or C5 and beyond. Most therapeutic approaches to date have focussed on intravitreal blockade of the alternative complement pathway or the amplification loop. It is of note that whereas the lectin and classical pathways remain active with blockade of FD or FB, drugs which target both C3 or C5 impact all major activation pathways and MAC formation. It is a matter of some debate as to whether systemic or local therapy is the best strategy. Systemic biomarkers of complement activation are present in AMD patients, although these could be a marker of an active complement system and a 'mirror' of what is happening within the eye, rather

than a consequence of it (Hecker et al., 2010; Reynolds et al., 2009; Scholl et al., 2008). A follow-up study of liver transplant recipients with AMD implicated local production of complement, rather than systemic (originating from liver) as being of greater importance (Khandhadia et al., 2013). It is clear that the individuals carrying disease-risk complement variants (a risk complotype) have increased levels of complement deposited in the choriocapillaris and the Bruch's membrane. It is also notable that most complement proteins, including FH, do not traverse the Bruch's membrane, but some, such as FHL-1, C5a and FD are able to pass through. It remains to be determined whether complement located on the choroidal side of the Bruch's membrane, the retinal side, or indeed, in both locations, contribute significantly to AMD pathogenesis but evidence to date suggests that complement dysregulation in early disease is present within the Bruch's membrane and the choriocapillaris layer (Clark and Bishop, 2018); it might be envisaged that sub-RPE or choroidal delivery might dampen complement-mediated inflammation at the relevant site.

As blockade of C3 using APL-2 in phase 2 GA trials (Apellis) demonstrated a decrease in the rate of progression of the atrophic lesion, data from the upcoming large phase 3 trial are eagerly anticipated and will be highly informative with regards to planning of future clinical trials in AMD. It is of note, however, that in this phase 2 trial, and also the MAHALO phase 2 trial with lamalizumab, treatment appeared to slow disease progression rather than stop or reverse it, and that disease progressed at the pre-treatment rate once therapy (with APL-2) ceased. Inhibition of complement in advanced AMD may be of limited benefit, and the goal must surely be to detect early disease and develop a therapeutic agent which can significantly halt progression. The cost of administering intravitreal injections into a large population with signs of early AMD would be huge and the practicalities unfeasible. Readout from clinical trials of systemic therapy, with potential to modulate complement rather than completely ablate, will be significant and informative (such as Ionis FB antisense, upcoming phase 2). Drugs which provide additional control in the retina rather than ablate activation may also provide critical insight, these could be based on the naturally occurring regulators, such as FH, FI, CD46, CD55, and CD59. One such agent 'mini FH' or AMY-201, is under development for AMD (www.amyndas.com), and a second agent, HMR59 (AAV-CAGsCD59; Hemera Biosciences), is in phase 1 open-label safety trial for delivery of the MAC inhibitor, CD59, to patients with GA, although little information is available to date (NCT03144999). Delivery to the retina via gene therapy is an attractive proposition as administration could be limited to once in a lifetime (Moore et al., 2017; Naldini, 2015; Ramo et al., 2008; Schnabolk et al., 2018).

4. The vasculature

4.1. Indications involving the circulatory system driven by complement

Despite intracellular and tissue-located complement activation gaining increasing attention (Arbore et al., 2017; Hajishengallis et al., 2017), the blood circulation still remains the major compartment for complement-mediated immune surveillance and, consequently, complement-related disorders (Ricklin et al., 2016). While complement has evolved to instantly react to foreign intruders, circulating blood cells and the vasculature of the host may fall victim to the cascade's defensive actions, either due to bystander effects or dysregulation of the activation pathways. Among those are several autoimmune diseases, some of which have been discussed above due to their major manifestation in the kidneys, and acute-phase disorders related to exposure to pathogen- or damage-associated molecular pattern as for example during sepsis, trauma or transplantation (Ricklin et al., 2016). Yet is has been one of the least prevalent complement-related diseases, **paroxysmal nocturnal haemoglobinuria (PNH)**, which has captured attention and fuelled discussions regarding complement-targeted interventions in the past years (Fig. 5, Table 1).

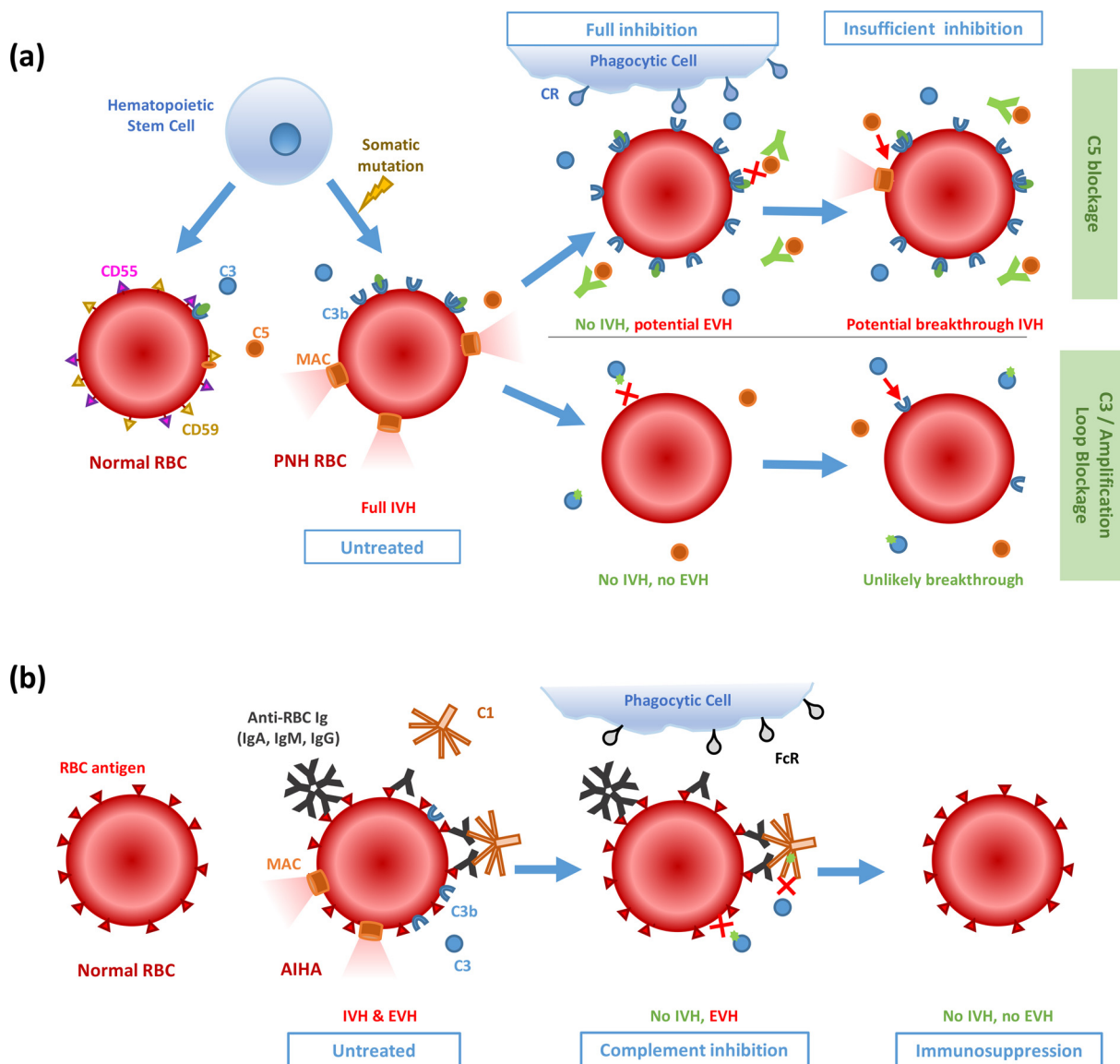


Fig. 5. Complement-mediated mechanisms of haemolysis in PNH and AIHA. (a) Red blood cells are normally protected from homologous complement attack by the GPI-anchored cell-surface regulators CD55 and CD59; whereas CD55 controls the C3 and C5 convertases, CD59 prevents MAC formation. In PNH, clones of hematopoietic stem cells, lacking ability to synthesise and attach GPI anchors, expand in the circulation. These cells do not have CD55 and CD59 on their cell surface and are thus exquisitely sensitive to direct MAC-mediated lysis (i.e., intravascular haemolysis, IVH). In patients treated with eculizumab (top right), C5 cannot be cleaved to form C5b and MAC formation is blocked; assuming all C5 is blocked by the drug, this rescues the erythrocytes from IVH and dampens activation of platelets and other immune cells. However, in the case of total blockade ('full inhibition'), activated C3 continues to accumulate on the cell surface due to the deficiency of CD55. This results in a dense array of opsonins accumulating on the membrane, potentially facilitating extravascular haemolysis (EVH) due to phagocytic uptake. Moreover, ongoing formation of C5 convertases will instantly activate residual free C5 during insufficient blockage, thereby leading to breakthrough IVH. Therapeutic blockade at the level of C3 would prevent the coating of opsonins and MAC formation, thus preventing both IVH and EVH. Since generation of C5 convertases requires a high density of C3b, small amounts of residual free C3 are less likely to induce instant IVH. (b) In AIHA, autoantibodies of the IgA, IgG and IgM isotypes recognize epitopes on erythrocytes and trigger the classical pathway of complement activation, leading to IVH. Particularly in the case of IgG, affected erythrocytes are also cleared by non-complement-mediated mechanisms through binding of the immunoglobulins to Fc receptors (FcR) on immune cells. In some cases, therapeutic complement inhibition may therefore not be fully sufficient to suppress all forms of haemolysis in AIHA. Preventing the formation of autoantibodies by means of immune suppression may be considered as an alternative approach.

In patients suffering from PNH, a variety of events can lead to an acquired somatic mutation in hematopoietic stem cells (e.g. in the *PIGA* gene) rendering them incapable of producing the glycosylphosphatidylinositol (GPI) anchor, which tethers various surface proteins to the plasma membrane (Hill et al., 2017; Takeda et al., 1993). As a consequence, PNH patients show clonal populations of blood cells with a deficiency in membrane regulators, including CD55 and CD59 (Hillmen et al., 1992). Despite their high density in blood and natural lack of one complement regulator, CD46, healthy erythrocytes are sufficiently protected from low level opsonisation, such as that which

may occur as bystander deposition during infection. PNH erythrocytes, however, have a largely reduced capacity for controlling the amplification loop (CD55) and MAC formation (CD59). Any encounter with increased complement activation leaves PNH erythrocytes highly vulnerable to (bystander) opsonisation, MAC-mediated membrane damage and, consequently, intravascular haemolysis (Hill et al., 2017). The resulting haemolytic anaemia is a defining symptom of PNH, though it is often the increased risk of developing thrombotic events, due to reasons not yet fully resolved, that impact morbidity and life expectancy (10–20 years after diagnosis) most profoundly. For decades,

frequent blood transfusions and, if possible, bone marrow transplantation had been the only treatment options available. And even though ill-controlled complement activation was linked to the pathogenesis of PNH very early (Ham and Dingle, 1939), the expected low market (the estimated incidence rate is 1–10 cases/million/year) and concerns about therapeutic interference in the complement system prevented the rapid development of a complement-targeted drug for PNH. For many patients, the treatment options profoundly improved in 2007, when Alexion received approval for eculizumab in PNH (NCT00122330). By blocking convertase-mediated activation of C5, eculizumab prevents the formation of MAC on PNH erythrocytes, thereby efficiently halting intravascular haemolysis in most patients. Even during clinical trials, it became evident that eculizumab treatment could lead to a rapid normalization of haemolytic makers such as lactate dehydrogenase (LDH) levels (Brodsky et al., 2008; Hillmen et al., 2006; Rother et al., 2007). Of equal importance, the treatment was generally well-tolerated with few adverse events, which built confidence in controlling systemic complement activity therapeutically (Hillmen et al., 2013). There were, however, reported cases of patients suffering from meningococcal infections, which led to mandatory vaccination before starting treatment. In the decade since its official availability in the clinic, the risk-benefit profile has largely been confirmed, and eculizumab has meanwhile changed the natural history of the disorder (Risitano and Marotta, 2016). Eculizumab treatment is not without drawbacks, however, and not all patients benefit fully from the treatment. Whereas some rare cases of non-responders can be pinpointed to a SNP in C5 (p.R885H) that prevents eculizumab from binding (Nishimura et al., 2014), a third of patients remain transfusion-dependent despite showing some improvement in clinical parameters (Risitano and Marotta, 2018). One of the potential reasons for this insufficient response is that eculizumab prevents MAC formation but not opsonization and amplification via the alternative pathway. This accumulation of C3 opsonins on circulating PNH erythrocytes, which is well-confirmed experimentally, may have clinical consequences (Risitano et al., 2009). Firstly, the opsonins (C3b, iC3b, C3dg) may act as ligands for immune cells and drive the clearance of PNH erythrocytes by phagocytes; such extravascular haemolysis has been demonstrated *in vitro* and *in vivo* (Fig. 5) (Lin et al., 2015; Risitano et al., 2009). Moreover, ongoing opsonization gives rise to a steady presence of C5 convertases; even small transient amounts of non- or insufficiently blocked C5 may therefore lead to formation of MAC. The resulting breakthrough haemolysis appears to be most prominent during episodes of strong complement activity in the circulation (Harder et al., 2017). Which of the above-mentioned mechanisms primarily contributes to insufficient response to eculizumab, and the resulting transfusion dependence, is not fully resolved and may differ between patients. New treatment modalities may therefore be critical to expand the number of PNH patients that fully benefit from complement-targeted therapy. Finally, and as mentioned above, the treatment cost of eculizumab is extraordinary high with annual expenses exceeding \$500,000/£340,000 per patient in some markets, and severely restricting or preventing access to the drug in many countries.

Whereas PNH represents the rare case of blood- and vasculature-targeted complement disorders without a strong involvement of immunoglobulins, many autoimmune diseases cause antibody-induced complement activation with adverse clinical consequences. For example, **autoimmune haemolytic anaemias (AIHA)** define a class of blood disorders mediated by the recognition of erythrocytes by autoantibodies, which leads to activation of the classical pathway with subsequent opsonization, amplification and MAC-mediated lysis. AIHA is an acquired disorder, either idiopathic or secondary to other immune and nonimmune diseases or treatments, and highly heterogeneous with various serological forms and clinical presentations (Liebman and Weitz, 2017). AIHA types have been termed ‘warm’ or ‘cold’ (also referred to as **cold agglutinin disease; CAGD**) based on the relative temperature at which the autoantibodies bind erythrocytes. AIHA can be IgA, IgG and/or IgM mediated, involving the complement system

depending on the isotype. AIHA is diagnosed based on a positive direct antiglobulin test (DAT) for either immunoglobulins or C3d, in combination with haemolytic anaemia. Although DAT tests can be negative for immunoglobulins, especially in the case of IgM, complement deposition on erythrocytes can be detected as a footprint for autoantibodies (Meulenbroek et al., 2015a,b). AIHA’s are typically treated by targeting the autoantibody production, through immunosuppression or rituximab (anti-CD20), but these treatments require some time to take effect (Barcellini, 2015). Therapeutic interference of complement activation serves as a more immediate treatment to cover this time window, as well as a therapeutic option during acute severe exacerbations of the disease. Furthermore, complement inhibition might be beneficial during transfusions, as freshly transfused erythrocytes are prone to targeting by autoantibodies and destruction by subsequent complement activation. Similarly to PNH, intravascular haemolysis is the main symptom of AIHA.

In other **autoimmune and antibody-mediated diseases**, the effect of complement activation is focused on the vasculature and/or peripheral tissue. Antibody-mediated nephritides, such as LN, IgA nephropathy, and AAV have been mentioned above. Other examples include antiphospholipid syndrome, bullous pemphigoid, and multiple sclerosis, and we refer to specialised literature for additional insight. **Myasthenia gravis (MG)** has recently gained particular attention after becoming the third approved indication for eculizumab treatment. In MG, autoantibodies target the neuromuscular junctions, resulting in muscle weakness and fatigue. MG is categorised as either pure ocular MG, affecting the eye musculature and resulting in ptosis and/or diplopia (~20%), or generalised MG, affecting multiple muscles throughout the body (~80%) (Hehir and Silvestri, 2018). The majority of MG-related autoantibodies target the acetylcholine receptor (AChR), which can cause increased AChR endocytosis. Importantly, the binding of autoantibodies may also activate complement via the classical pathway, and complement was implicated in MG pathology as early as the late 1970’s (Sahashi et al., 1978). The role of complement has been demonstrated in various animal models (Morgan et al., 2006), and complement blockade protected against development of experimentally acquired MG (Biesecker and Gomez, 1989; Hepburn et al., 2008, 2007; Piddlesden et al., 1996; Zhou et al., 2007). These observations led to several clinical trials exploring the treatment of generalised MG using complement inhibitors (discussed below). It is likely that even more autoimmune disorders will move into the spotlight in the coming years, especially since autoimmunity is not always recognised as being part of complex pathologies.

Whereas aHUS is the most prominent example of a complement-driven TMA, it is still not fully resolved which other forms of **thrombotic microangiopathies** may involve complement (Meri, 2013; Vieira-Martins et al., 2016). For example, a contribution of complement in thrombotic thrombocytopenic purpura (TTP) or Shiga toxin-producing *E. coli*-induced HUS remains debated. The strongest body of evidence is accumulating in the case of haematopoietic stem cell transplantation (HSCT)-associated TMA (Jodele et al., 2016a). There, it was shown that patients with proteinuria and elevated sC5b-9 had very poor survival (< 20%) compared to patients with normal levels of these markers (Jodele et al., 2014). Further studies revealed associations to several alterations in complement genes or FH autoantibodies (Jodele et al., 2016b), and treatment of high-risk patients with eculizumab showed promising results (see below). The current hypothesis suggests that HSCT-TMA is not driven by complement, but that endothelial injury due to transplantation-related stress such as chemotherapy, radiation, surgery or infection triggers complement activation; in patients with genetic susceptibility, this insult cannot be sufficiently controlled, thereby leading to clinical complications and poor survival (Jodele et al., 2016a). Finally, antibody-mediated complement activation is also a hallmark of solid organ **transplantation-related complications**, including hyperacute rejection due to HLA and/or ABO incompatibility, and in **acute-phase disorders** involving the sudden exposure of pathogen- and/or damage-related molecular patterns such as during sepsis or trauma.

4.2. Clinical development of complement-targeting drugs in haematological/vascular diseases

In view of the complement-centred disease mechanism, the confirmed therapeutic impact of complement inhibition, and the commercial success of eculizumab in the PNH market, it is not surprising that many pharmaceutical companies set the initial focus of their development programs on this indication (see below) (Morgan and Harris, 2015; Ricklin et al., 2018; Risitano and Marotta, 2018). However, such an approach is not without risks and has to be considered with care. PNH is an ultra-rare disease and the patient population remains low, which impacts both recruitment options and market size. Despite the limitations discussed above, the existing therapeutic option, i.e. eculizumab and its likely successor ravulizumab, are working well for many patients and it may be challenging to demonstrate superiority or, at least, non-inferiority to convince authorities in some cases. Finally, more than a decade of successful use of eculizumab in PNH has left the drug firmly established in the clinic and among patient groups, it may require greater efforts for new players to gain a foothold in the market. From a clinical and scientific perspective, however, it would be very much anticipated to have at least one additional complement-targeted drug available for the treatment of PNH, both to lower cost and improve treatment access to hitherto excluded markets, and to achieve a better understanding of optimal intervention points.

Almost 20 complement-targeted drug trials for PNH employing several clinical candidates are currently listed in ClinicalTrials.gov as having active/ongoing status, and many more preclinical lead compounds are in development for this indication; hence, there is certainly no shortage in potential options to expand the arsenal of PNH therapeutics (Risitano and Marotta, 2018). Many of the candidates act on the amplification loop or on C5 activation and have often also been considered for renal and/or ocular diseases as discussed above; these will only be briefly covered in this section, particularly concerning their effect in PNH trials. Candidate drugs acting at the level of C5 will likely have a comparable mode of action to eculizumab, although they may differ in binding site, dose and PK profile. For example, Alexion's next-generation antibody ravulizumab requires less frequent dosing, largely due to FcRn-mediated antibody recycling (see above) (Sheridan et al., 2018). The company recently reported topline results from a phase 3 study in PNH (NCT02946463), which showed non-inferiority (though not superiority) to eculizumab with a comparable safety profile (Alexion Press Release, 2018b). Intravenous infusion of ravulizumab to 125 treatment-naïve patients (single loading dose and weight-based maintenance dose of 3000–3600 mg every 8 weeks) showed similar or slightly better outcome on primary endpoints, i.e. transfusion avoidance and normalization of LDH levels, with fewer events of breakthrough haemolysis when compared to the eculizumab arm. This announcement was subsequently followed with the news that the eculizumab/ravulizumab switch study in PNH (NCT03056040) was also considered successful (Alexion Press Release, 2018a). Another C5 antibody (SKY59/RO7112689) with pH-dependent FcRn recycling for improved PK properties has been developed by Roche and Chugai and is currently in phase 1/2 trials (NCT03157635). SKY59 binds a distinct epitope from eculizumab and is thus active in patients carrying the p.R885H mutation (Fukuzawa et al., 2017). Initial reports from the first part of the study in healthy volunteers suggest a potential benefit due to the high subcutaneous bioavailability (~90%) and long half-life (~25 days), which may lower the treatment burden; it will be interesting to see how these properties translate to dose in PNH patients (Roeth et al., 2017). Other agents targeting C5 are in the clinic, such as the anti-C5 antibody LFG316 from Novartis (phase 2 PNH trial; NCT02534909), and a Regeneron agent, REGN3918 (phase 1 in healthy volunteers; NCT03115996), however, no result statements appear to have been released for these two antibodies. Finally, several companies are gearing up to produce biosimilar versions of eculizumab itself in anticipation of the patent protection running out (Ricklin et al., 2018; Risitano and Marotta, 2018).

While antibodies present the dominant species in the field of C5 inhibitors, several companies have developed other means of interference at this level for PNH. For example, coversin (Akari) is currently developed as a once-daily subcutaneous treatment option for PNH patients. After the 8-patient, single-arm phase 2 trial (NCT02591862) with daily subcutaneous dosing achieved its primary endpoint, defined as a reduction in LDH to ≤ 1.8 times the upper limit of normal (ULN) at day 28, Akari announced the initiation of phase 3 trials in 2018 (Akari Press Release, 2018). As it binds to a different epitope than eculizumab, coversin was demonstrated to be effective in a patient showing resistance to eculizumab (Akari Press Release, 2016), and phase 2 trials in patients carrying the p.R885H polymorphisms have been initiated (NCT03427060). A version of coversin with improved PK profile is in preclinical development (Kuhn et al., 2016). Another protein-based C5 inhibitor, SOBI005 from Swedish Biovitrum, fuses a C5-binding domain with a human IgG1 Fc to improve PK properties; it is currently in preclinical stages (www.sobi.com/en/Our-Focus-Areas/Research-and-Development). Whereas the above-mentioned approaches involve antibodies or small proteins, the strategy employed by Ra Pharma is based on a macrocyclic peptide. RA101495 binds to a site on C5 that is distinct from eculizumab and dosing levels make it suitable for patient self-administration (RaPharma Investor Presentation, 2018). The peptide has recently been evaluated in two separate 12-week phase 2 dose-finding trials in PNH, one involving eculizumab-treated and treatment-naïve patients (NCT03078582) and one focusing on patients with insufficient response to eculizumab (NCT03030183). Reported data show a rapid and sustained reduction of LDH levels and near-complete inhibition of haemolysis at a daily SC dose of 0.3 mg/kg. No major adverse effects or cases of infection were observed, and SC self-administration was reviewed favourably. Out of 21 patients, 16 have progressed to the long-term extension trial (NCT03225287) (Rapharma Press Release, 2018). As discussed above, Cemdisiran follows a different approach that prevents hepatic production of C5 rather than inhibiting its activity. Whereas monotherapy with this RNAi may hold promise in aHUS, treatment with Cemdisiran alone did not seem to be sufficient in PNH patients. Despite a high sustained degree of C5 blockade (> 90%), LDH levels remained > 1.5 x ULN, indicating that residual levels of C5, e.g. deriving from extrahepatic sources, were sufficient to cause intravascular haemolysis. Alnylam therefore adopted an approach that combines ALN-CC5 administration with reduced frequency of eculizumab dosing; interim phase 1 data indicate that a reduced dose of 600 or 900 mg eculizumab every 4 weeks (rather than 900 mg every 2 weeks) in combination with ALN-CC5 decreased LDH to below 1.5 x ULN, this could potentially represent a significant cost-saving (Alnylam Press Release, 2016). However, the pricing of Cemdisiran and the advent of ravulizumab will need to be considered in a final evaluation of the approach. It is clearly evident that most of the current approaches aim to lower the treatment burden for PNH patients, both by extending dosing intervals or simplifying administration routes. None of the current C5-targeted approaches appears to be suitable for oral administration, although Ra Pharma indicates the pre-clinical development of an oral C5 inhibitor.

For a chronic disease such as PNH, oral drug administration would be highly beneficial. When compared to C5-directed therapeutics, some of the strategies targeting the amplification loop appear to be closer to that goal. Achillion's clinical candidate, the small FD inhibitor ACH-4471, is currently evaluated in several phase 2 trials. These include an initial three-month trial of ACH-4471 monotherapy (NCT03053102) followed by a long-term extension trial (NCT03181633) in treatment-naïve PNH patients. In a separate 6-month study, ACH-4471 is added to eculizumab therapy in patients that show insufficient response to anti-C5 treatment itself; oral FD inhibitor doses vary from 100 to 200 mg daily, potentially higher than its dose in aHUS (NCT03472885). Other FD inhibitors with potential for oral dosing are being developed by Novartis and Ra Pharma, but no clinical programs appear to be initiated to date. In contrast to Novartis' FD inhibitor (Maibaum et al., 2016), their small molecule FB inhibitor LNP023 has meanwhile entered phase 2 trials for PNH and IgA nephropathy. In the PNH study

(NCT03439839), patients with insufficient response to eculizumab will receive a daily oral dose of LNP023 for 13 weeks in addition to ongoing anti-C5 therapy. Whereas the FD and FB inhibitors will specifically target the alternative pathway, compstatin analogues will exert a slightly broader activity by protecting C3 from convertase-mediated cleavage (Mastellos et al., 2015). The PEGylated APL-2 is being evaluated in two phase 1b PNH trials, one as monotherapy in treatment-naïve patients (NCT02588833) and one as add-on therapy in eculizumab-treated patients showing insufficient response (NCT02264639). Both studies involve SC administration with daily dosing ranging from 180 to 360 mg, although 270 mg/day appears to be the standard dosing. In the add-on study, the four enrolled patients had normalised LDH values and remained transfusion-independent one year into treatment; patients with previous high-dose eculizumab could reduce dosing to normal levels (Apellis Press Release, 2017b). Apellis earlier reported interim results from the monotherapy study, which led to a rapid normalization of LDH values to 1.1x ULN with no signs of breakthrough haemolysis in the three enrolled patients. No severe adverse events had been reported in the two studies (Apellis Press Release, 2017a). Based on the current results, Apellis plans to initiate extension trials and move directly to phase 3 later in 2018.

With phase 1 trials in healthy volunteers completed, Amyndas also announced plans for phase 2 trials in PNH and other indications with their non-PEGylated next-generation compstatin analogue AMY-101 (Amyndas Press Release, 2017). Preclinical PK/PD studies in non-human primates and using patient PNH erythrocytes indicated high efficacy of this approach (Risitano et al., 2014). Given the theoretical benefits of compounds targeting C3 or the amplification loop on opsonization and breakthrough haemolysis, it is exciting to see some of these approaches moving forward in clinical pipelines.

It has to be noted that, even though PNH has gained much attention over the past few years and has developed into the frontline indication for complement therapeutics, the disease should not be considered representative for most complement-mediated disorders. More importantly, treatment schemes developed for PNH may not, and should not, be directly translated across indications but adjusted to the pathological mechanism and resulting requirements. PNH is rather unique insofar that the disease is fuelled by the constant presence of highly vulnerable cells in the circulation, therefore strongly benefiting from continuous complement inhibition. Any interruption of the treatment will likely lead to a recurrence of the symptoms, potentially even a pharmacological rebound effect due to the presence of pre-opsonised erythrocytes. The strong involvement of the amplification loop is another aspect that has to be considered. As mentioned above, under C5 blockade the formation of C5 convertases is not prevented; any C5 that escapes binding by the C5 inhibitor may be immediately turned into C5b and initiate the formation of MAC. Current PNH therapy therefore requires constant and complete inhibition and is vulnerable to breakthrough events. This susceptibility has been shown *in vitro* and observed under clinical conditions, as for example during the clinical trial with Cemdisiran as a monotherapy (see above). The requirement for constant and complete inhibition does not necessarily apply in other conditions, however. In many cases, it is an imbalance of complement activation and regulation, sometimes undetected over years, that defines the disease (see above). Even partial inhibition of the activation or terminal pathways may thus be sufficient to restore homeostasis and resolve inflammation. As a consequence, either a titration/adjustment of required dose ranges or intermittent treatment with careful monitoring of disease recurrence could be considered. This would reduce the cost and treatment burden, and further lower the risk of infection. As indicated before, the choice of target level or combination of treatment options may further influence the outcome and minimise breakthrough events.

Selecting the ideal point of intervention is particularly important in the case of haemolytic diseases other than PNH and in autoimmune disorders. A phase 2 study with eculizumab in CAgD was conducted some time ago (2011–2015; NCT01303952), and a case report of a patient receiving 900 mg of eculizumab every other week described a

positive outcome for haemolysis and transfusion requirement (Röth et al., 2015), however, no subsequent studies appear to have been performed. As mentioned above, blockade at the level of C5 would not prevent upstream events such as antibody recognition or opsonization. Importantly, the major and well-defined involvement of a distinct activation pathway in antibody-induced disorders opens the door for rather selective modes of intervention with the potential to interfere much earlier in pathological events. Indeed, Bioverativ, which has been acquired by Sanofi in March 2018, is developing a humanised antibody (BIVV009, originally termed TNT003/TNT009 developed by True North) that blocks the classical pathway enzyme C1s (Shi et al., 2014). After receiving orphan designation for CAgD in 2017 and showing improvement of haemolytic events in a phase 1b study (Panicker et al., 2013), BIVV009 is currently being evaluated in two parallel phase 3 clinical trials (NCT03347396, NCT03347422) (Bioverativ Press Release, 2018). Similarly, off-label use of C1-INH (discussed above) is being evaluated in autoimmune anaemias in open label studies, including one case-report where prophylactic administration of C1-INH was beneficial in an IgM-mediated AIHA patient (Wouters et al., 2013; Wouters and Zeerleder, 2015). As with the use of C1-INH in kidney transplants (discussed above), investigations focused on increasing the survival of the transfused erythrocytes in the patient; haemoglobin and lactate dehydrogenase levels and C3 deposition on erythrocytes (determined by DAT) were used as markers. Rather than aiming at the initiation stage, inhibition of C3 activation represents another approach to prevent opsonisation and haemolysis. For example, in an *ex vivo* AIHA model, the C3 inhibitor Cp40 (developed as AMY-101 by Amyndas) was shown to prevent C3b deposition and intravascular haemolysis while also reducing extravascular clearance of opsonised erythrocytes (Baas et al., 2017). APL-2, discussed above, has also meanwhile entered phase 2 clinical trials for the treatment of AIHA, including CAgD (NCT03226678).

At least in one autoimmune disease, however, C5-targeted therapy has shown significant benefit. With MG gaining attention as a complement-mediated disorder, therapeutic studies have focussed on anti-AChR positive generalised MG. Alexion initiated a pilot phase 2 clinical trial in August 2008, looking into the applicability of eculizumab in generalised MG (NCT00727194). The study was terminated early but enrolled 14 patients, and results indicated eculizumab treatment decreased MG severity (Howard et al., 2017). This was followed-up with a randomised, double-blind, placebo-controlled, multicentre phase 3 clinical trial including 125 patients with refractory generalised MG with confirmed anti-AChR autoantibodies (NCT01997229). A similar treatment regimen as for aHUS was used. The study finalised in February 2016 and results were published recently (Howard et al., 2017). Results were positive overall, although no significant difference in the Myasthenia Gravis Activities of Daily Living Profile (MG-ADL) score (primary endpoint) was observed between eculizumab and placebo treated patients. However, secondary endpoints including other outcome or quality of life measures did indicate a beneficial effect of eculizumab. In view of the unmet clinical need, eculizumab was approved by the FDA for treating patients with generalised MG in October 2017 (Alexion Press Release, 2017). Ra Pharma has recently (October 2017) initiated a randomised, double-blind, placebo-controlled, multicentre phase 2 trial to evaluate its compound RA101495 in generalised MG (NCT03315130). The study is designed to enrol 36 patients and encompasses three arms, comparing SC injections of either 0.1 mg/kg or 0.3 mg/kg RA101495 with placebo treatment. Similarly to the eculizumab phase 2 trial, primary outcome is change from baseline in quantitative MG score, which is a standardised quantitative strength scoring system. Results are to be expected in 2019.

Finally, the clinical availability of eculizumab has allowed for an evaluation of the drug in forms of TMA other than aHUS. Whereas eculizumab was used in case studies of treatment-refractory TTP and in STEC-HUS during an outbreak in Germany, the impact of the treatment remains debated and requires larger, controlled studies to confirm a

potential benefit. In HSCT-associated TMA, earlier studies indicated that high-risk patients showing elevated levels of sC5b-9 (see above) could be treated with eculizumab. Even though they could not be vaccinated against meningitis due to immunosuppression, appropriate antimicrobial prophylaxis was provided (Jodele et al., 2016c). In an investigator-led study in 2016, 18 high-risk HSCT-TMA patients were treated with eculizumab and showed a profoundly improved survival rate (56%) when compared to high-risk patients from a separate, observational study who did not receive targeted therapy (9%) (Jodele et al., 2016d). More recently, a case study of an eculizumab-resistant patient with HSCT-TMA showed that treatment with coversin had beneficial effects, even though the patient could not be rescued due to limited supplies of the drug candidate (Goodship et al., 2017b). Based on these results, it will be interesting to see whether complement-targeted therapy will develop into a standard treatment for high-risk patients suffering from HSCT-TMA-related complications.

4.3. Biomarkers and stratification

In contrast to aHUS, C3G and AMD, which are largely driven by imbalances between the activating and regulating capacities of several complement components, the diversity and/or impact of genetic alterations is typically less pronounced in the haematological and vascular disorders. In PNH, the genetically-induced lack of GPI anchor synthesis is a common denominator, and the resulting absence of the GPI-anchored complement regulators CD59 and CD55 on clonal populations of blood cells is used as a diagnostic marker (Hill et al., 2017). Moreover, there are additional genetic alterations that have been described as influencing the success of eculizumab treatment in PNH patients. As mentioned above, occurrence of the p.R855H polymorphism leads to complete abrogation of eculizumab binding to C5 due to changes in the epitope (Nishimura et al., 2014). Whereas this alteration defines non-responders, a polymorphism in the gene for complement receptor 1 (CR1, CD35) has been associated with risk of remaining transfusion-dependent. In a study encompassing 72 PNH patients, poorly responding patients represented less than 20% of all the H/H CR1 homozygotes, 33% of all the H/L heterozygotes and 68% of all the L/L CR1 homozygotes (Rondelli et al., 2014). The molecular details of this correlation are not yet fully resolved as CR1 is involved in many biological processes such as immune adherence, the clearance of immune complexes and the degradation of C3-based opsonins (Verschoor et al., 2016). With the exception of rare cases such as one of the coversin studies, genetic variations and biomarkers are not typically utilised for patient stratification in PNH trials.

Similarly, few efforts appear to have been applied to select specific patient populations in trials related to AIHA/CAGD. This may change based on the insight that cases predominantly involving IgM appear to be more complement-driven than those dominant in IgG, which seem to have a stronger contribution of Fc γ R-mediated clearance (Meulenbroek et al., 2015a,b).

In MG, the phase 3 trials, which led to the approval of eculizumab for this indication, only enrolled patients with anti-AChR antibody-positive generalised MG who had previously failed immunosuppressive treatment and continued to suffer from significant unresolved disease symptoms (Alexion Press Release, 2017). This is representative of less than 10% of all MG patients, although it includes those with the highest unmet clinical need; it will be interesting to see whether the patient base will be expanded now that the drug is available for this indication.

5. Conclusion and outlook

During the past three decades, and particularly since the introduction of eculizumab as the first complement-specific inhibitor into the clinic more than ten years ago, complement-targeted drug discovery has seen a remarkable metamorphosis from a “high risk – small market” status with little interest by major companies to a thriving field

featuring numerous clinical candidates and high commercial interest (Morgan and Harris, 2015; Ricklin et al., 2018). The realisation that some of the potential risks associated with the general approach, in particular the susceptibility to infection, can be mitigated to a substantial degree by the means of careful target selection, dosing schemes, tissue-localised application, targeting and/or the involvement of antimicrobial strategies, built an important base for a newly gained confidence in the therapeutic strategy. In contrast to many other target areas, therapeutic complement inhibition is not restricted to a specific indication but may be viewed as a platform approach that will be of great value for the treatment of a broad range of inflammatory and degenerative diseases. While bearing great potential, this wide scope of disorders which involve complement also generates new challenges for companies entering the field. They face the question whether to strive for better therapeutic alternatives in established indications (i.e. PNH, aHUS) or explore new niches, in which the involvement of complement is not as well defined. As emphasised in this review, complement-targeted drug development has so far been focused on three major indication areas, i.e. renal, ocular, and haemolytic diseases. They are all related to cells and organs that are in constant close contact with complement and/or feature ill-protected, and therefore highly susceptible, structures such as the basement membranes of the eyes and the kidneys. The early commercial and therapeutic success with eculizumab in PNH may have been misleading as the approach may not easily be translated into other indications. Importantly, however, PNH is not necessarily representative of most complement-related disorders (see above). This has emerged as a cautionary tale in the case of AMD, in which, despite strong genetic disease association, the road to clinically available complement drugs has faced several setbacks, most recently the failure of lampalizumab. The complex anatomy and limited accessibility of the eye may well require an even more detailed understanding of tissue-localised disease mechanisms and tailored PK adjustments to achieve the necessary efficacy. Yet despite the setbacks, AMD remains a promising indication for complement drugs, and results from the next stages of clinical trials with C3 inhibitors are eagerly awaited. Owing to the observation that systemic imbalances in the complement system, caused by genetic alterations or autoimmune reactions, often manifest in the kidneys, renal diseases have quickly moved into the spotlight of complement-targeted drug development (Ricklin et al., 2018). Current clinical trials indeed indicate beneficial effects of therapeutic complement inhibition not only in aHUS but also in C3G, IgA nephropathy and lupus nephritis. Whereas the small patient population in these mostly orphan diseases may render the design of statistically well-powered trials challenging, there appears to be a trend towards “bucket trials” in which different indications are evaluated at the same time. Such trials have not only been initiated for kidney diseases and TMAs but also for haematological disorders such as CAGD and other AIHA's. Another emerging trend, at least in established indications such as PNH, is the comparison of monotherapy involving a new candidate with add-on therapies, in which the drug is administered alongside eculizumab (e.g. in the case of cemdisiran). To what degree latter approaches may contribute to higher response rates and/or a reduction of total treatment cost remains to be explored. Independent of the indication, the availability of diagnostic tools and disease markers for patient stratification and therapeutic monitoring will be critical for advancing the field (Ricklin et al., 2017).

Whereas this review puts a strong emphasis on the three major therapeutic areas in which complement-targeted drugs are currently explored, i.e. diseases of the kidneys, eyes, and the vasculature, several “frontier areas” with high potential for future approaches have been identified. These include neurological and neurodegenerative disorders such as amyotrophic lateral sclerosis, Guillain-Barré syndrome, multiple sclerosis, or Alzheimer's disease (Brennan et al., 2016; Morgan, 2015), conditions related to mucosal inflammation such as periodontal disease (Hajishengallis et al., 2016), and autoimmune disorders such as MG or antiphospholipid syndrome (Thurman et al., 2017). Moreover,

Table 1

Evidence for a role of the complement system in disease pathogenesis. A multitude of data implicates complement in a number of diseases involving the kidney, eye or the vasculature. The genetic, pharmacological and biomarker data are described in brief here along with relevant data from animal models. This Table does not represent an exhaustive list of all relevant citations, rather it provides reference to seminal citations and key reviews to enable the reader to research further. Clinical trials are cited where they provide new, unpublished insight into disease mechanism and therapeutic inhibition in a particular indication (current clinical trials are discussed in main text). Indications which are not currently in clinical trials are not cited here, these include glaucoma, diabetic retinopathy, diabetic nephropathy and others.

Evidence that complement plays an important role in disease pathogenesis; KIDNEY	
Atypical Haemolytic Uraemic Syndrome (aHUS) Genetic	<ul style="list-style-type: none"> ● Mutations in complement genes are found in ~60% of patients. ● Risk genotype is associated with disease including common variants in FH, FHRs and CD46.
Pharmacological	<ul style="list-style-type: none"> ● Blockade of C5 using eculizumab is approved for use in aHUS (FDA, 2011) and has revolutionised outcome and quality of life for aHUS patients. Other agents which also block C5 demonstrate equivalent therapeutic benefit in aHUS patients. ● Other agents progressing through clinical development also show favourable outcome; C5 blockade dominates the therapeutic approach although some companies are targeting the lectin pathway or components downstream of C5, such as C5a(R). ● Presenting patients frequently demonstrate elevated levels of activation fragments such as Bb and TCC. A corresponding decrease in components (such as C3) may be evident in some patients. ● C5b-9 staining is frequently evident on renal biopsy; C3 deposition may be detectable on endothelial cells. ● Autoantibodies (commonly to FH) are detectable in plasma in ~10% of patients. ● Mice expressing FH which lacks the membrane-targeting carboxy-terminal domains develop aHUS-like disease; genetic deficiency of FB or C5 rescues the phenotype. ● Mice expressing a gain-of-function C3 molecule (which was first detected in patients with aHUS) develop aHUS-like disease. ● Subtotal deficiency of FH in mice, in combination with renal injury, result in C5-dependent TMA resembling aHUS.
Biomarker	<ul style="list-style-type: none"> ● Presenting patients frequently demonstrate elevated levels of activation fragments such as Bb and TCC. A corresponding decrease in components (such as C3) may be evident in some patients. ● C5b-9 staining is frequently evident on renal biopsy; C3 deposition may be detectable on endothelial cells. ● Autoantibodies (commonly to FH) are detectable in plasma in ~10% of patients. ● Mice expressing FH which lacks the membrane-targeting carboxy-terminal domains develop aHUS-like disease; genetic deficiency of FB or C5 rescues the phenotype. ● Mice expressing a gain-of-function C3 molecule (which was first detected in patients with aHUS) develop aHUS-like disease. ● Subtotal deficiency of FH in mice, in combination with renal injury, result in C5-dependent TMA resembling aHUS.
Animal models	<ul style="list-style-type: none"> ● Mice expressing FH which lacks the membrane-targeting carboxy-terminal domains develop aHUS-like disease; genetic deficiency of FB or C5 rescues the phenotype. ● Mice expressing a gain-of-function C3 molecule (which was first detected in patients with aHUS) develop aHUS-like disease. ● Subtotal deficiency of FH in mice, in combination with renal injury, result in C5-dependent TMA resembling aHUS.
C3 Glomerulopathy Genetic	<ul style="list-style-type: none"> ● Mutations may be present in complement genes of the amplification loop (C3, FH) ● Risk genotype is associated with disease including common variants in FH and C3. C3F variant of C3 is associated with nephritic factors. ● Limited data from clinical trials indicate that blockade of FD decreases C3 consumption and improves proteinuria in patients. ● Blockade of C5 may show benefit in a portion of patients. ● C3 levels are usually low due to consumption; activation fragments are high, particularly of iC3b/C3dg. Factor B may also be consumed resulting in elevated Bb. ● Low properdin and high TCC may indicate dysregulation at the level of C5. ● Autoantibodies are common, particularly nephritic factors which dysregulate the C3/C5 convertase enzymes. ● C3 is heavily deposited in the kidney glomeruli in the absence of immunoglobulin. MAC may also be evident.
Pharmacological	<ul style="list-style-type: none"> ● Limited data from clinical trials indicate that blockade of FD decreases C3 consumption and improves proteinuria in patients. ● Blockade of C5 may show benefit in a portion of patients. ● C3 levels are usually low due to consumption; activation fragments are high, particularly of iC3b/C3dg. Factor B may also be consumed resulting in elevated Bb. ● Low properdin and high TCC may indicate dysregulation at the level of C5. ● Autoantibodies are common, particularly nephritic factors which dysregulate the C3/C5 convertase enzymes. ● C3 is heavily deposited in the kidney glomeruli in the absence of immunoglobulin. MAC may also be evident.
Biomarker	<ul style="list-style-type: none"> ● Limited data from clinical trials indicate that blockade of FD decreases C3 consumption and improves proteinuria in patients. ● Blockade of C5 may show benefit in a portion of patients. ● C3 levels are usually low due to consumption; activation fragments are high, particularly of iC3b/C3dg. Factor B may also be consumed resulting in elevated Bb. ● Low properdin and high TCC may indicate dysregulation at the level of C5. ● Autoantibodies are common, particularly nephritic factors which dysregulate the C3/C5 convertase enzymes. ● C3 is heavily deposited in the kidney glomeruli in the absence of immunoglobulin. MAC may also be evident.

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Table 1 (continued)

Evidence that complement plays an important role in disease pathogenesis; KIDNEY	
Animal models	<ul style="list-style-type: none"> ● Complete deficiency of FH results in a C3G like disease with evidence of dense deposits ● Partial deficiency in FH (20% subtotal levels) results in C3G-like disease. ● Deficiency of FH combined with deficiency of properdin exacerbates disease, likely due to changes in the dynamics of fluid phase versus surface dysregulation of the alternative pathway. ● A natural model of C3G existed in the Norwegian Yorkshire pig strain which lacked functional FH.
Immune complex glomerulonephritis (IC MPGN)	
Genetic	<ul style="list-style-type: none"> ● Pathogenic variants are present in some patients (~16%)
Pharmacological	<ul style="list-style-type: none"> ● Acchillon has initiated a clinical trial (FD inhibition) in IC-MPGN but data are yet to be reported.
Biomarker	<ul style="list-style-type: none"> ● C3 levels may be low and TCC high; patients may show signs of C4 consumption. ● C3 is deposited in the glomeruli alongside immunoglobulin and sometimes C1q.
Animal models	<ul style="list-style-type: none"> ● Nephritic factors are present in a number of patients (~40-50%). ● To date no animal model of IC-MPGN appears to be reported.
IgA nephropathy	
Genetic	<ul style="list-style-type: none"> ● Deletion of FHR-1 and -3 is protective. ● Rare genetic variants in FHRs are associated with susceptibility
Pharmacological	<ul style="list-style-type: none"> ● Limited clinical trial data indicate that both MASP2 inhibition and blockade of C5aR1 can improve proteinuria and reduce use of steroids (ongoing trial with FB blockade is initiated by Novartis but not yet reported). ● Case reports describe use of eculizumab but outcomes are variable, may be beneficial in early inflammatory disease.
Biomarker	<ul style="list-style-type: none"> ● IgA, MBL, C3 fragments, properdin and activated FB have been detected in glomerular tissue ● Deposition of C4d associates with poor prognosis
Animal models	<ul style="list-style-type: none"> ● Complement activation fragments are elevated in plasma ● Challenging in rodents due to the different structure of antibodies; IgA is almost entirely polymeric in mice and lacks hinge region glycans ● A spontaneous model (ddY) mouse has been described although complement inhibition in this model has not been reported
Lupus nephritis (LN, as symptom of systemic lupus erythematosus, SLE)	
Genetic	<ul style="list-style-type: none"> ● Deficiency of classical pathway components C1, C4 and C2 are high risk factors for SLE ● C3 and FB variants associate with severity of disease and outcome. ● Deletion of FHR-1 and -3 is associated with risk. ● There are case reports of eculizumab in LN, might be beneficial in setting of TMA and inflammatory C5-driven disease. ● C3 and C4 levels may be low in LN indicating consumption, genetic screening will indicate whether C4 is impacted due to presence of null alleles. ● Complement activation fragments are elevated in plasma (including iC3b/ C3dg, Bb and TCC). ● Complement is deposited in kidney glomeruli (C3 fragments, Bb and MAC). ● Anti-C1q antibodies are commonly associated with renal involvement in SLE.
Pharmacological	
Biomarker	

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Cook and Pickering, 2015; Goicoechea de Jorge et al., 2011; Hegazy et al., 2002; Høgasen et al., 1995; Nichols et al., 2015; Pickering and Cook, 2008; Vermon et al., 2016; Yang et al., 2018

Iatropoulos et al., 2018, 2016; Servais et al., 2012 NCT03124368

Cook and Pickering, 2015; Marinozzi et al., 2017b, Masani et al., 2014; Servais et al., 2012; Sethi et al., 2012

Gharavi et al., 2011; Zhai et al., 2016

NCT02682407 NCT02384317

Herzog et al., 2017; Ring et al., 2015; Rosenblad et al., 2014

Endo et al., 1998; Maeng et al., 2013; McCoy et al., 1974; Rautenberg et al., 1987; Zwirner et al., 1997

Imai et al., 1985; Okazaki et al., 2012; Scivittaro et al., 1993; Tomino, 2008

Lewis and Botto, 2006; Pickering et al., 2000; Rambašek et al., 1987 Zhao et al., 2011

Coppo et al., 2015; Pickering et al., 2015

Flierman and Daha, 2007; Sinico et al., 2005; Walport, 2002; Wang et al., 1996

Table 1 (continued)

Evidence that complement plays an important role in disease pathogenesis; KIDNEY	
Animal models	<ul style="list-style-type: none"> ● Two spontaneous models in mice: MRL/lpr and NZB/W F1. ● Backcross of <i>C₃^{b/-}</i> or <i>C₃^{d/-}</i> mice onto MRL/lpr background ameliorated renal disease. ● Anti-complement therapy in MRL/lpr model ameliorated disease (soluble recombinant Crry, CR2-Crry or Crry-Ig; activation pathways). ● FB ASO modulated disease in MRL/lpr and NZB/W F1 models. ● Blockade of C5 ameliorated disease in NZB/W F1 mice.
Alkinson et al., 2008; Bao et al., 2002, 2003; Elliott et al., 2004; Grossman et al., 2016; Wang et al., 1996; Watanabe et al., 2000	
ANCA (anti-neutrophil cytoplasmic antibody)-associated vasculitis (AAV)	
Genetic	<ul style="list-style-type: none"> ● AAV is considered an autoimmune disease. No (complement) genetic susceptibility appears to have been revealed
Pharmacological	<ul style="list-style-type: none"> ● C5a is considered a driver of disease; clinical trial evidence supports blockade of the receptor, C5aR1. ● Case reports described the use of eculizumab in AAV.
Biomarker	<ul style="list-style-type: none"> ● Plasma C3 may be low in some patients; activation fragments C3a, C5a, TCC and Bb are often elevated. ● MAC and C3 fragments are frequently detected in renal biopsies. ● Factor B /Bb, properdin and C4d have variably been detected in glomeruli and small blood vessels.
Animal models	<ul style="list-style-type: none"> ● In mice, administration of murine anti-myeloperoxidase (MPO) IgG induces pauci-immune necrotizing crescentic glomerulonephritis. C5 and FB deficiency are protective; C5 blockade using antibody is also therapeutic.
Huugen et al., 2007; Schreiber et al., 2009; Xiao et al., 2007	
Membranous nephropathy (MN)	
Genetic	<ul style="list-style-type: none"> ● No confirmed links to specific complement.
Pharmacological	<ul style="list-style-type: none"> ● Clinical trials are ongoing, blockade at level of C3 or MASP2.
Biomarker	<ul style="list-style-type: none"> ● Evidence of lectin and/or alternative pathway activity in glomerulus.
Animal models	<ul style="list-style-type: none"> ● Heymann nephritis (rat) -an experimental model of MN, can be passive or active; MN results from binding of circulating antibodies to antigens within the podocyte membrane. ● Cationic BSA model (rabbit, mice, rat); injection of repeated doses of cationised bovine serum albumin (BSA) results in localisation within the glomerulus followed by a subepithelial deposition of Ig and C3.
NCT02682407 NCT02682407 Bally et al., 2016; Lhotta et al., 1999; Segawa et al., 2010; Val-Bernal et al., 2011 Border et al., 1982; Borza et al., 2013; Heymann et al., 1959; Van Damme et al., 1978	
Transplant	
Genetic	<ul style="list-style-type: none"> ● No significant association of complement genes with renal transplant outcome
Pharmacological	<ul style="list-style-type: none"> ● Clinical trial to prevent renal I/R injury using Microcept is ongoing. ● Limited success using eculizumab in renal transplant clinical trial for I/R or AMR.
Biomarker	<ul style="list-style-type: none"> ● Case reports of eculizumab in ABO mismatch transplantation. ● Limited clinical trial data support blockade of C1 for AMR. ● In I/R injury, activated C3 and MAC are detected along the tubular basement membrane. ● Donor-specific antibodies (DSA) correlate with poor outcome in transplant. C4d is frequently detected in renal tissue although this does not always correlate with presence of DSA. Literature suggests that deposition of C4d may associate with poor outcome in long-term renal function.
Ermini et al., 2016 Barnett et al., 2013; Kassimatis et al., 2017; Stewart et al., 2012; West-Thielke et al., 2018 NCT02145182, NCT01399595 NCT01895127 NCT0134510 NCT02134314 Vo et al., 2015 Harrigan et al., 2009; Loupy et al., 2011; Thurman et al., 2005; Worthington et al., 2007	

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Table 1 (continued)

Evidence that complement plays an important role in disease pathogenesis; KIDNEY	
Animal models	<p>Farrar et al., 2016, 2006; Fiane et al., 1999; Pratt et al., 2002; Thurman et al., 2003; Zhou et al., 2000</p> <ul style="list-style-type: none"> Deficiency of FB (Cβ/-) is protective in murine renal I/R injury, with decreased tubular injury and neutrophil infiltration. Mice that are deficient in C3, C5, or C6 are protected from renal I/R injury. Intra-renal local C3 (donor organ) contributes primarily to I/R injury. Collectin-11 is expressed in renal tissue following ischaemic stress and activates the lectin pathway at the ischaemic tubule surface; in the murine model, deficiency of collectin-11 in the donor kidney is protective for tubular damage. C3 inhibition prolongs survival of pig hearts perfused with human blood.
Evidence that complement plays an important role in disease pathogenesis; EYE	
Age-Related Macular Degeneration (AMD)	<p>Edwards et al., 2005; Fagemess et al., 2009; Gold et al., 2006; Hageman et al., 2005, 2006; Haines et al., 2005; Hughes et al., 2006; Klein et al., 2005; Maller et al., 2006, 2007; Raychaudhuri et al., 2011; Seddon et al., 2013; van de Ven et al., 2013; Yates et al., 2007; Yu et al., 2014</p>
Genetic	<ul style="list-style-type: none"> Common risk variants in FH, C2/FB, FI, C3, FHR-1/-3. Rare risk variants in FI, FH, C3, C9
Pharmacological	<p>Genentech advanced an inhibitor of FD (Lampalizumab) to late stage clinical testing, but failed to meet primary endpoint in two phase 3 trials (SPECTRI and CHROMA). In the phase 2 trial, MAHALO, rate of progression of geographic atrophy area was reduced (increased efficacy with CFI biomarker).</p> <ul style="list-style-type: none"> APL2 FILLY phase 2 trial (C3 inhibitor) showed reduction in rate of GA progression. Other drugs targeting amplification loop or C5 are in development but data are not yet released: Novartis, LFG316 monotherapy and LFG316/CLG561 combination therapy (NCT01527500, NCT01535950, NCT02515942); Ophthotech, Zimura, (NCT02686658). Systemic inhibition of C5 using eculizumab (NCT00935883; COMPLETE trial) had no impact on rate of GA progression. Some complement activation fragments, such as C3a, C3d, C5a, TCC, Ba and Bb, are elevated in AMD. Factor D levels are reported as increased. Activation fragments are elevated in vitreous humour. Low levels of FI are associated with risk. Complement deposits are identified in drusen, the choroid, macular, Bruch's membrane, choriocapillaris and choriocapillaris intercapillary septa.
Biomarker	<p>Anderson et al., 2010; Hecker et al., 2010; Kavanagh et al., 2015; Keenan et al., 2015; Mullins et al., 2000, 2014; Reynolds et al., 2009; Schick et al., 2017; Scholl et al., 2008</p>

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Table 1 (continued)

Evidence that complement plays an important role in disease pathogenesis; EYE	
Animal models	<ul style="list-style-type: none"> ● Aged <i>Cfh</i>^{-/-} mice have impaired visual acuity and rod response with thinning of the Bruch's membrane, disorganisation of photoreceptors and reduction in retinal blood supply. Aged <i>Cfh</i>^{+/-} mice on high fat diet develop sub-retinal deposits and RPE damage. ● Transgenic mice expressing FH with human domains SCR6-8 (either His402 or Tyr402) develop subretinal drusen-like deposits, infiltration of inflammatory cells and sub-RPE C3 deposition. There was no association with H402Y genotype. ● Mice injected with C3-expressing adenovirus exhibited signs of retinal degeneration, damage and complement deposition. ● Various models of AMD exist which are not linked to manipulation of complement genes, refer to (Pennesi et al., 2012). Some of these models, such as <i>Cc12</i>^{-/-} <i>Cx3cr1</i>^{-/-} mice the CEP-BSA immunisation model, and Ceruloplasmin/hephaestin^{-/-} mice, have increased deposition of complement in the retina. ● Laser-induced CNV, a trauma-induced model of wet AMD, responds to anti-complement therapy; deficiency of FB is protective and lack of CD5 exacerbates pathology and deficiency of C3aR and C5aR is protective. ● Some colonies of rhesus and cynomolgus macaques naturally develop AMD as they age; drusen deposits are coated with complement. Similar to humans, but unlike rodents, monkey eyes have a macula.
Recessive Stargardt macular degeneration (STGD1)	
Genetic	<ul style="list-style-type: none"> ● No confirmed links to specific complotype.
Pharmacological	<ul style="list-style-type: none"> ● Clinical trial with C5-blockade is ongoing, results are not yet available.
Biomarker	
Animal models	<ul style="list-style-type: none"> ● Complement biomarkers have not been explored in human disease. ● In the <i>Abcc4</i>^{-/-} mouse model for recessive Stargardt disease, higher levels of complement-activation products on RPE cells are observed compared to WT mice; expression of complement regulatory proteins was lower. ● Overexpression of Crry (complement receptor 1-like protein γ), reduces complement attack on the RPE and rescues both bisretinoid accumulation and photoreceptor degeneration.
Uveitis	
Genetic	<ul style="list-style-type: none"> ● No confirmed links to specific complotype.
Pharmacological	<ul style="list-style-type: none"> ● Novartis have tested anti-C5 LFG316 in active non-infectious intermediate-, posterior-or panuveitis, but results have yet to be reported. ● Increased levels of C3a, Bb, C4a, C5a in aqueous humour.
Biomarker	
Animal models	<ul style="list-style-type: none"> ● Complement is activated in experimental rat autoimmune anterior uveitis (EAAU) and disease is ameliorated by anti-complement therapy. Blockade of iC3b/CR3 interaction is beneficial. ● Genetic deficiency of C3 and production of a soluble complement inhibitor targeted to the CNS and eye are protective against experimental autoimmune uveoretinitis (EAU). ● Systemic C5 blockade using anti-C5 mAb reduces disease score and retinal damage in murine experimental autoimmune uveoretinitis (EAU).

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Table 1 (continued)

Evidence that complement plays an important role in disease pathogenesis; VASCULATURE

Paroxysmal Nocturnal Haemoglobinuria (PNH)

Genetic

- Patients feature clonal populations in blood cells, including erythrocytes, that express no or reduced levels of the complement regulators CD55 and CD59. This change is linked to acquired somatic mutations in genes involved in the biosynthesis of the GPI anchor, in particular the *PIG-A* gene. It is well established that the lack of the complement regulators, CD55 and CD59, renders PNH erythrocytes highly susceptible to complement-mediated lysis.
- PNH was the first approved indication for therapeutic blockade of C5 using eculizumab (FDA, 2007) and has profoundly changed the treatment and natural history of the disease.
- Some patients under eculizumab show no or insufficient response due to polymorphisms, extravascular haemolysis and/or pharmacological breakthrough.
- Various drug candidates, predominantly acting at the level of C5 activation or the amplification loop, have shown positive results. They were used either as a monotherapy or as add-on therapy to eculizumab.
- The absence of CD59 (or CD55) on the surface of erythrocyte populations is commonly used to confirm PNH.
- A polymorphism in C5 (p.R885H) is associated with therapeutic resistance to eculizumab.
- Polymorphisms in CD35 (CR1) have been discussed as indicator of disease progression.
- There are no commonly used animal models of the disease.
- Experimental assays are commonly performed using erythrocytes from PNH patients or by sensitizing normal erythrocytes using a combination of anti-CD55 and 2-aminoethylisothiouromium bromide (AET).

Hill et al., 2017; Hillmen et al., 1992; Takeda et al., 1993

Pharmacological

- Brodsky et al., 2008; Hillmen et al., 2006; Nishimura et al., 2014; Rother et al., 2007 NCT00122330 Akari Press Release, 2016, 2018, Alexion Press Release, 2018a,b,
- Raphama Press Release, 2016; Apellis Press Release, 2017a,b, NCT03157635, NCT02534909, NCT03115996, NCT03427060, NCT03078582, NCT03030183, NCT03225287, NCT03053102, NCT03181633, NCT03472885, NCT03439839, NCT02588833, NCT02264639
- Hill et al., 2017; Hillmen et al., 1992; Nishimura et al., 2014; Rondelli et al., 2014

Biomarker

Animal models

Ezzell et al., 1991; Fridkis-Hareli et al., 2011; Risitano et al., 2014

Autoimmune Haemolytic Anaemias (including Cold Agglutinin Disease)

Genetic

- AIHA describes a broad range of diseases with a considerable diversity of underlying triggers and mechanisms. The genetic component is often not well defined.
- Inhibition at the level of C5 has been evaluated but does not appear to be actively pursued.
- Drug candidates acting on C1s and/or other serine proteases involved in complement initiation are currently in clinical trials.
- The presence of immunoglobulins, in particular IgG and IgM, on erythrocyte surfaces is a hallmark of AIHA. The binding of autoantibodies to targets on erythrocyte surfaces triggers the classical pathway.
- Detection of IgM has been associated with a more dominant involvement of complement-mediated mechanisms.
- Similar to PNH, *in vitro* assays using patient blood are most commonly used to assess aspects of the disease
- Some rodent models of AIHA are being established but are not yet commonly used.
- MG is an autoimmune disease, of which the genetic compound is not well defined
- Anti-C5 blockade with eculizumab is efficacious in AChR-positive MG.

Pharmacological

Röth et al., 2015; Wouters and Zeerleder, 2015 NCT03347396, NCT03347422, NCT03226678

Biomarker

Meulenbroek et al., 2015a,b

Animal models

Shi et al., 2014

Myasthenia Gravis (MG)

Genetic

- MG is an autoimmune disease, of which the genetic compound is not well defined
- Anti-C5 blockade with eculizumab is efficacious in AChR-positive MG.

Pharmacological

(continued on next page)

Table 1 (continued)

Evidence that complement plays an important role in disease pathogenesis; VASCULATURE	
Biomarker	<ul style="list-style-type: none"> • Other drug candidates which bind C5 are being tested in clinical trials, data are not yet released. • Autoantibodies which target the neuro-muscular junction trigger complement resulting in deposition of C3 and MAC.
Animal models	<ul style="list-style-type: none"> • Passive and active models of MG (experimental autoimmune myasthenia gravis, EAMG) in rats and mice resemble the human disease; antibody activates complement at the neuromuscular junction. • Blockade of complement at the level of C5 or C6 ameliorates disease; administration of soluble CR1 which controls the convertase enzymes ameliorates disease. • Deficiency of C3, C4, C5 or C6 protects against EAMG.

Howard et al., 2017
NCT01997229, NCT03315130
Engel et al., 1977; Nakano and Engel, 1993; Sahashi et al., 1980
Biesecker and Gomez, 1989; Chamberlain-Banoub et al., 2006;
Christadoss, 1988; Engel et al., 1979; Hepburn et al., 2008, 2007;
Morgan et al., 2006; Piddlesden et al., 1996; Sahashi et al., 1978;
Soltys et al., 2009; Tuzum et al., 2003; Zhou et al., 2007

there is a renewed interest in tackling more complex, acute-phase disorders with strong complement involvement, in particular inflammatory complications of transplantation, haemodialysis, trauma or sepsis (Huber-Lang et al., 2016; Huber-Lang et al., 2018; Reis et al., 2015). Even in fields such as oncology, in which complement has traditionally been viewed as being important to keep progression in check, the focused inhibition of complement components may be considered alongside targeting of cancer cells with complement-activating antibodies (Reis et al., 2018). There is great hope that with the growing number of candidates in late-stage clinical trials, the arsenal of complement drugs acting at various point of intervention in the complement network that are available to clinicians will finally be expanding.

Disclosures

CLH was an employee of GlaxoSmithKline 2013–2016 and has received consultancy income from Roche, GSK, Gyroscope Therapeutics, Gemini Therapeutics and AdmiRx; all funds are donated to the University for research. DR is a co-inventor of patents and patent applications describing complement inhibitors, and has received speaker/consultancy honoraria from Roche, Novartis and Alexion. DK has received honoraria for consultancy work from Alexion Pharmaceuticals, and is a director of and scientific advisor to Gyroscope Therapeutics. RP is a co-inventor of a patent describing potentiating anti-FH antibodies and uses thereof .

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