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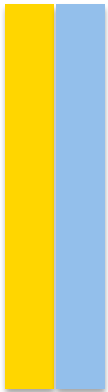

C3 Glomerulopathies: understanding the pathogenesis and treatment options

Literature Review

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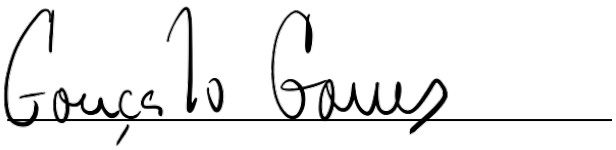

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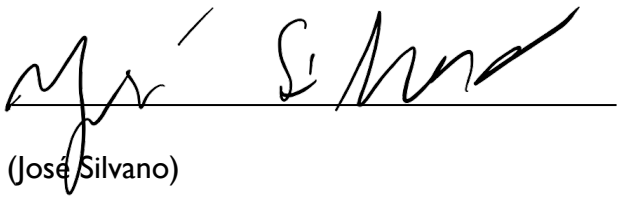

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RESUMO

Introdução: As Glomerulopatias de C3 compreendem um grupo de doenças raras mediadas pelo complemento que partilham o mesmo processo patológico. Definem-se histologicamente por uma predominância de deposição glomerular de C3, evidenciada por técnicas de imunofluorescência. O prognóstico é desfavorável, com metade dos doentes a progredir para doença renal terminal dez anos após o diagnóstico. Atualmente, o tratamento restringe-se essencialmente a uma estratégia conservadora, uma vez que ainda não foram aprovadas opções terapêuticas dirigidas a esta entidade.

Objetivos e metodologia: Esta revisão pretende promover uma compreensão atualizada relativamente à C3G, com particular foco na patogénese da doença e respetivas implicações para as opções terapêuticas em desenvolvimento. Nesse sentido, foi realizada uma revisão bibliográfica primariamente pela perquirição dos artigos pertinentes publicados na base de dados PubMed®. Ademais, foi realizada uma análise das referências citadas nos artigos selecionados, por forma a incluir publicações relevantes à revisão que pudessem não ter sido abrangidas na pesquisa inicial. Quando relevante, foram incluídos dados não revistos por pares relativos a terapêuticas de inibição do complemento em desenvolvimento, nomeadamente resultados apresentados em congressos e em comunicados de imprensa.

Discussão: Tendo em conta o papel fundamental da desregulação da via alternativa na patogénese da C3G, a inibição do eixo C3/Convertase de C3 tem sido assumida como uma estratégia terapêutica promissora para esta entidade. Nesse sentido, foram desenvolvidos vários fármacos com o intuito de inibir especificamente cada um dos fatores envolvidos neste eixo. Da mesma forma, foram desenvolvidos fármacos com o intuito de replicar os reguladores naturais da ativação do complemento e fármacos com o intuito de silenciar a produção sistémica de C3. Embora a inibição da via terminal tenha mostrado resultados insatisfatórios, a eficácia terapêutica da inibição específica da anafilatoxina C5a também tem sido investigada.

Conclusão: Os desenvolvimentos recentes na terapêutica dirigida ao complemento, inicialmente motivados pelo sucesso do eculizumab e, mais recentemente, pela aprovação do pegcetacoplan, cunham com esperança o surgimento de novas terapêuticas que possam alterar a gestão do doente com C3G. Pela complexidade e heterogeneidade da doença, será provavelmente essencial uma abordagem personalizada a cada doente.

Palavras-chave: *C3 Glomerulopathy*, *C3 Glomerulonephritis (C3GN)*, *Dense Deposit Disease (DDD)*, *Alternative complement pathway*, *Complement therapeutics*

ABSTRACT

Introduction: The C3 Glomerulopathies (C3G) comprise a group of rare complement-mediated diseases, which share the same disease process defined histopathologically by a predominance of complement C3 deposition on immunofluorescence. The prognosis is poor, resulting in progression to end-stage renal disease (ESRD) within ten years of diagnosis in approximately half of the patients. Currently, the management of C3G primarily relies on a conservative therapeutic strategy, as no disease-specific therapies have yet been approved.

Objectives and methodology: This review aims to provide a current, comprehensive understanding of C3G, with an emphasis on its pathogenesis and respective implications for emerging approaches in the rapidly evolving field of complement therapeutics. To achieve this, a literature review was performed, primarily focusing on pertinent articles published in the PubMed® database. The references cited in these articles were also examined to incorporate any significant publications that might have initially been overlooked. Additionally, non-peer-reviewed findings on emerging complement therapeutics were considered, as presented in congresses or press releases.

Discussion: Considering the pivotal role of alternative pathway (AP) dysregulation in the pathogenesis of C3G, inhibition of the C3/C3 convertase axis has swiftly risen as a promising therapeutic strategy for this condition. Thus, several drug candidates have been developed to specifically inhibit each of the factors involved in this axis, as well as to mimic natural regulators of complement activation or silence systemic expression of C3. While terminal pathway inhibition has shown limited efficacy, the efficacy of specific impairment of the anaphylatoxin C5a is also being investigated.

Conclusion: Recent developments in complement therapeutics, initially fueled by the success of eculizumab and, most recently, by the approval of pegcetacoplan, raise the very realistic hope for the emergence of new therapies that could change the course of clinical management in C3G. Considering the complexity and heterogeneity of this disease, a personalized medicine approach will likely be necessary.

Keywords: C3 Glomerulopathy, C3 Glomerulonephritis (C3GN), Dense Deposit Disease (DDD), Alternative complement pathway, Complement therapeutics

LIST OF ABBREVIATIONS

ACEi	Angiotensin-converting enzyme inhibitors
aHUS	Atypical Hemolytic Uremic Syndrome
AKI	Acute Kidney Injury
ANCA	Antineutrophil Cytoplasmic Antibody
AP	Alternative Pathway
ARB	Angiotensin Receptor Blockers
CA	Cofactor Activity
C1-INH	C1 Inhibitor
cDNA	Complementary DNA
C3G	C3 Glomerulopathy
C3GN	C3 Glomerulonephritis
C4BP	C4 Binding Protein
CA	Cofactor Activity
CL	Collectins
CP	Classical Pathway
CR	Complement Receptors
CR1	Complement Receptor 1
CVF	Cobra Venom Factor
DAA	Decay Accelerating Activity
DAF	Decay Accelerating Factor
DAMPs	Damage-Associated Molecular Patterns
DDD	Dense Deposit Disease
ESRD	End-Stage Renal Disease
Fcn	Ficolins
FDA	United States Food and Drug Administration
FH	Factor H
FHL-1	Factor H-Like 1
FHR1-5	Factor H-Related proteins
GFR	Glomerular Filtration Rate
IC-MPGN	Immune Complex-Mediated Membranoproliferative Glomerulonephritis
IgAN	IgA Nephropathy
KDIGO	Kidney Disease: Improving Global Outcomes
LP	Lectin Pathway
MAC	Membrane Attack Complex
MASP	Mannose-Associated Serine Protease
MBL	Mannose-Binding Lectin
MAD	Multiple Ascending Dose
MCP	Membrane Cofactor Protein
MMF	Mycophenolate Mofetil
NeF	Nephritic Factor
PNH	Paroxysmal Nocturnal Hemoglobinuria
PAMPs	Pathogen-Associated Molecular Patterns
PEG	Polyethylene Glycol
RISC	RNA-induced Silencing Complex

RNAi	RNA interference
SCR	Short Consensus Repeat
siRNA	Small interfering RNA
TP	Terminal Pathway
uPCR	Urine Protein-to-Creatinine Ratios
TEAEs	Treatment-Emergent Adverse Events
Vn	Vibronectin

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INTRODUCTION

C3 Glomerulopathies (C3G) are a rare group of kidney diseases that arise from the dysregulation of the alternative complement pathway (AP). Diagnosis is established by kidney histopathology, characterized by a predominance of glomerular C3 staining on immunofluorescence, at least two orders of magnitude greater than any other immune reactant[1]. Based on the specific localization patterns and characteristics of deposits within renal tissue, electron microscopy permits the distinction between two subgroups: Dense Deposit Disease (DDD) and C3 Glomerulonephritis (C3GN).

Given the extreme rarity of this disease, precise epidemiological data is difficult to gather, particularly since the only diagnostic criterion is based on the interpretation of a renal biopsy sample. Consequently, incidence and prevalence estimates are vary between regional biopsy and referral practices. Despite these challenges, small cohort studies have produced rough estimates, suggesting an estimated incidence of 0.2–2 cases per million per year[2,3]. Although more frequently diagnosed during childhood and adolescence, the disease can also affect individuals later in life. Manifestations of C3 glomerulopathy can vary widely, ranging from asymptomatic hematuria and proteinuria to more acute presentations that show the classic signs and symptoms of glomerulonephritis, often accompanied by low serum C3 levels. Typically, the persistence of these symptoms and sustained low C3 levels prompt the need for a biopsy, leading to a formal diagnosis and thorough evaluation.

Despite carrying one of the highest risks for kidney failure of all primary glomerular diseases, there are currently no approved therapies targeting the underlying cause of C3G. Consequently, about 50% of the patients progress to end-stage renal disease (ESRD) within 10 years of the diagnosis, although cases of more rapid progression are not unusual[2,3]. Furthermore, the recurrence of the disease and subsequent allograft loss is a common complication among post-transplantation patients, a predicament faced by 50-75% of patients in this group[3,4].

Considering the multifaceted role of the complement system in human pathophysiology, precise, targeted inhibition and well-timed spatial modulation of its activity may offer novel opportunities for therapeutic drug design and efficient clinical intervention. Thus, taking into account the central role of complement dysregulation in the pathogenesis of C3 glomerulopathy, recent attention has been directed toward anti-complement agents as potential therapeutic options.

This review provides an up-to-date survey of current knowledge regarding C3G, particularly emphasizing its pathogenesis and the implications for emerging approaches

in the rapidly evolving field of complement therapeutics.

Of note, this article adheres to the most recent complement nomenclature[5]. In line with this, the larger fragments resulting from cleavage, which bind to the cell surface to propagate the cascade, are signified with a “b”. Conversely, the smaller, liberated fragments are denoted with an “a”.

METHODS

The literature search strategy employed in this review article involved querying articles published in the PubMed® database, with no temporal restrictions for inclusion. Initial article selection was based on title, followed by a review of the respective abstracts. Subsequently, the selected articles were read in their entirety. Relevant articles referenced in those initially selected were examined and included if considered pertinent. Furthermore, web searches were conducted to identify recent findings regarding new complement therapeutics presented in congresses or press releases that were not yet peer-reviewed. The Zotero software was utilized to facilitate the organization and management of bibliographic references and prevent article duplication.

THE COMPLEMENT SYSTEM

Unraveling the complexity of C3G demands a foundational comprehension of the complement system (**Figure 1**). This intricate network, comprised of more than 40 individual proteins or activation fragments that can exist either bound to surfaces or in a soluble form, plays a central role in innate and adaptive immunity. It maintains a delicate equilibrium between activation and regulation processes, allowing it to identify and target harmful microorganisms for elimination, clear immune complexes and apoptotic cells from the bloodstream, and enhance the humoral response, all while sparing healthy cells. Additionally, the complement system is intricately interconnected with numerous other pathways, contributing to a broad spectrum of responses, which range from the activation of platelets[6] and the induction of coagulation responses[7] to the release of cytokines and the modulation of T-cell responses[8], reviewed elsewhere[9,10,11]. The effectiveness of the complement system relies on its quick response time and broad range of activity, and, as a result, its sensing abilities may not always be highly specific and completely reliable, thus paving the way for complement-associated diseases. While the relationship between the complement system and disease has long been acknowledged[12], it is only in recent times that we have begun to grasp the full

extent of its complexity, significance, and impact on disease processes, mainly motivated by the anti-C5 antibody eculizumab's clinical and commercial success in treating paroxysmal nocturnal hemoglobinuria (PNH)[13].

The complement system is activated through three distinct initiation pathways: the classical, lectin, and alternative pathways. Each pathway is triggered by unique stimuli, leading to an amplification phase that transitions into the terminal phase of the complement system. The classical pathway (CP) and lectin pathway (LP) share a similar activation mechanism. The CP is triggered by detecting immune complexes or non-immunoglobulin activators, such as pathogen- or damage-associated molecular patterns (PAMPs, DAMPs), while the LP is triggered by the recognition of microbial polysaccharides on a surface through mannose-binding lectin (MBL), ficolins (Fcn), or collectins (CL). Upon detection of these triggers, C1r/C1s proteases or MASP-1/MASP-2 (mannose-associated serine proteases) are respectively activated, leading to the cleavage of C4 and C2. This results in the formation of C4b2b, the C3 convertase of the CP and LP, which cleaves available C3 to release an anaphylatoxin, C3a, and induce a conformational change in the remaining C3b, thus opsonizing the cell in proximity to the initiating surface. Notably, the formation of C3b enables the binding of the protease Factor B (FB), creating a pro-convertase (C3bB) that is quickly transformed by Factor D (FD) into an active C3 convertase (C3bBb). This convertase can cleave more C3 into C3b, creating an amplification loop for C3b deposition that can contribute up to 80% of the overall response[14,15].

Concurrently, the complement system employs a "tick-over" mechanism in its alternative pathway (AP), characterized by its indiscriminate nature that allows for an increase in baseline activity. In this process, a small fraction of C3 undergoes spontaneous hydrolysis to form a distinct conformer termed C3(H_2O), which interacts with and binds to FB to form C3(H_2O)B, further cleaved by FD to generate C3(H_2O)Bb, another C3 convertase. This process is swiftly halted in healthy human cells or the fluid phase; however, an amplification process is initiated on foreign or damaged cells. This positive feedback mechanism results in an increased deposition of C3b, which converts C3 convertases into C5 convertases once the density of C3b reaches a certain threshold.

Subsequently, these convertases cleave C5 into two components: the smaller anaphylatoxin C5a and the larger C5b, the foundational unit for assembling the membrane attack complex (MAC), also known as C5b-9. While the lytic activity of the MAC is often the most identifiable effector function, it applies only to a subset of susceptible cells. In numerous instances, the overarching response is driven by the broad receptor-mediated functions of complement opsonins, such as C3b/iC3b and, to

a lesser degree, C4b and C1q. The inflammatory impact of the anaphylatoxins, such as C3a and C5a, further supports these functions. Thus, during the activation process, the release of C3a and C5a instigates the chemoattraction and priming of various immune cells, facilitated by signaling through anaphylatoxin receptors; in parallel, the interaction of opsonins with complement receptors (CR) in these cells triggers immune responses, which include immune shuttling via CR1, the stimulation of adaptive immune responses via CR2, and phagocytosis through integrin receptors CR3 and CR4[16].

To focus complement activation on proper targets and prevent damage to the host, the system is delicately regulated by fluid-phase and surface-bound molecules, which control activation in body fluids and on various cellular and non-cellular surfaces (such as basement membranes)[17–21]. In the glomerular microenvironment, the need for rigorous complement control is emphasized by the observed presence of C3 immunoreactivity in the glomeruli in approximately 33% of kidneys obtained from clinically healthy donors and at post-mortem examinations[22,23], which may be attributed to the specific affinity of this complement component for certain glycocalyx proteins, paralleling the finding that C3 shows preferential binding to laminin rather than type IV collagen and fibronectin[24].

Fluid-phase regulators include C1 inhibitor (C1-INH), C4 binding protein (C4BP), factor H (FH) and factor H-like 1 (FHL-1), factor I (FI), anaphylatoxin inactivators (carboxypeptidase-N and carboxypeptidase-R), vibronectin (Vn, S protein) and clusterin, while most nucleated cells express a panel of four membrane-bound complement regulators that act at the convertase/opsonin level, including decay accelerating factor (DAF, CD55), membrane cofactor protein (MCP, CD46), and complement receptor 1 (CR1, CD35); or, at the MAC level, specifically CD59.

These regulators engage at each stage of the complement system. In the initiation phase, excessive complement activation on a target and in plasma is mitigated by C1-INH by associating with C1r or C1s, instigating their release from C1q, or by blocking the analogous active sites in MASPs[25,26]. In the amplification phase, these operate in three unique ways: inhibiting the generation of the convertases; dismantling or separating the convertases, an action termed as decay-accelerating activity (DAA); or via the restricted proteolytic cleavage of C4b and/or C3b, a mechanism referred to as cofactor activity (CA), which demands a synergistic interaction between the plasma-serine protease, factor I, and a cofactor protein, such as MCP on cell membranes or FH/C4BP in the fluid-phase[27–29]. During the terminal phase, vibronectin controls the fluid-phase MAC by inhibiting its adherence to cell membranes, while CD59 suppresses the final steps of MAC assembly that deposit on self-tissue[30,31]. Furthermore, anaphylatoxins can be rendered inactive by plasma enzymes carboxypeptidase-N and carboxypeptidase-R[32,33].

As the main soluble regulator of the AP, FH is considered the most critical regulator in C3G[34–36], exerting its influence on the C3 convertase by functioning as convertase decay accelerator and as co-factor for factor I in the cleavage of C3b. Structurally, FH is a linear protein composed of 20 short consensus repeat (SCR) units, and its inhibitory effects are contingent on the ability of the initial four N-terminal SCRs to interface with C3b effectively[37,38]. The two terminal SCRs of FH on the carboxyl end are constituted by another primary binding site for C3b, which is specifically devised to bind with C3b or its by-product, C3d, when covalently bonded to a self-surface[39–42]. This binding site interacts with the glycomatrix, whose composition varies at different anatomic sites, thereby modulating the interaction potency of this complement regulator with diverse surfaces[43].

In addition, there is a growing appreciation for the splice variant of FH, FHL-1. This shorter isoform of FH, which comprises the initial seven SCRs and a unique four-amino-acid C-terminus[44,45], is characterized by distinctive cell surface specificity, thus partaking in a unique role in complement control on surfaces compared to its larger counterpart[43,46]. Analogous to FH, FHL-1 binds to C3b and exhibits both cofactor and convertase decay accelerating activities[38]. Despite its relatively smaller size (seven as opposed to twenty domains), which imposes constraints on its regulatory impact in circulation due to a decreased surface recognition capacity, the role of FHL-1 may gain considerable prominence in tissue environments owing to its advantageous penetration profile[47].

An even larger change in perception has been observed for FH-related proteins (FHRs). These proteins share specific characteristics with FH, such as surface- and opsonin-binding properties, but do not seem to possess the same complement-regulatory capacities and may present in a dimeric form[21]. While the role of FHR proteins is less characterized and, in part, remains a matter of controversy[48], their clinical significance has been unequivocally established since their discovery, with numerous reports linking anomalous FHRs to 'typical' complement-associated diseases[49–53]. While there have been indications of some forms of complement-inhibiting activity associated with FHRs, recent findings solidly endorse a counterpoint role to FH and FHL-1 for FHRs in the complement activation process. They are proposed to drive the activation of the AP directly by binding C3b and fostering the formation of the C3 convertase C3bBb, and, in parallel, they appear to augment the activation of the alternative pathway by competing with the regulators FH and FHL-1[21,48,54–59]. Additionally, FHRs may influence complement activation through interactions with other host molecules, such as by attracting pentraxins that can bind C1q and permit CP activation, or by being drawn in

by C-reactive protein to stimulate AP activation further[56,57]. These functionalities warrant further research, particularly concerning their physiological significance. Nevertheless, the correlation between increased complement activation and elevated FHR levels, or pathological, avidity gain-of-function dimerization mutants of FHR-1, FHR-2, and FHR-5 in complement-mediated diseases, such as C3G (see “Drivers of Disease” section), is compellingly indicative of a substantial role for FHR as balancing agents for the inhibitory effects induced by FH/FHL-1, thereby modulating the primary regulators of the alternative pathway.

Lessons from complement inhibition therapeutics

Recent basic, translational, and clinical research has begun to cast doubt on the traditional understanding of the TP’s initiation, specifically through the proteolytic activation of C5. The genesis of this investigative interest was the demonstration of unexpected results in clinical complement analytics and patient monitoring among patients treated with eculizumab for PNH[60,61] and aHUS[62]. These investigations uncovered a phenomenon of pharmacodynamic breakthrough, where residual C5 activity persevered even in the presence of excessive quantities of distinct C5 inhibitors[63], which bind their target with picomolar or nanomolar affinity[64]. Intensive research showed that a C5 priming event on C3b is needed for proteolytic activation of C5 by bimolecular complement convertases (C3bBb or C4b2b)[65–68], challenging the function of the conventionally accepted trimolecular convertase C3bBb3b (and by analogy, C4b2b3b)[69,70]. The research findings illustrated that only high surface densities of C3b (in the absence of convertases) can reversibly recruit C5.

It was also demonstrated that C4b shares this functionality with C3b, enabling C5 activation without C3, a phenomenon now referred to as C3 bypass activation of C5[68], posing a contradiction to a central dogma of complement biology. Although it has little physiological relevance, this finding is important for developing complement therapeutics and thus has immense translational importance. This finding offers a compelling explanation for the inconsistent outcomes observed with inhibiting TP activation across various in vitro and ex vivo assays when using compstatin[71,72], a small cyclic peptide designed to target C3 whose analogs are presently evaluated for their therapeutic potential in clinical trials for complement-mediated diseases, including C3G.

In addition, it has been demonstrated that C5 can undergo conformational activation even in the presence of stoichiometric C5 inhibitors. Specifically, it was shown that eculizumab-bound C5 can potentially become 'entrapped' on a highly concentrated

C3b surface, ultimately assuming a conformation capable of assembling cytolytic MACs. Mannes et al. postulated that eculizumab-bound C5, when interacting intensively with an unnaturally dense C3b surface, remains in a primed conformation for a prolonged period, eventually transitioning to a C5b-like conformation[68]. This transition, which resembles C3's autoactivation to C3(H_2O), has been denoted as $C5_{conf}$, representing C5's conformational activation to a C5b-like state on dense C3b surfaces.

Considering these recent findings regarding the central complement proteins C3 and C5, there appears to be unexplored potential for uncovering novel insights further upstream in the complement cascade. In one seminal study, Zhang et al. assessed the potential therapeutic benefits of mitigating FD, through elimination or inhibition, in a C3G mouse model[73]. The C3G mouse model used in this study was FH-KO, widely recognized as a disease model due to its ability to mimic the underlying causes of C3G observed in a specific patient subset characterized by FH deficiency[74]. Surprisingly, the study revealed that serum from the FH/FD double-KO mice could deposit small amounts of C3b on surfaces conducive to activating the AP sufficiently to incite complement-mediated rabbit erythrocyte hemolysis. On a mechanistic level, this can be elucidated by the persistent presence of C3(H_2O)FB, which permits FB to adopt an open, enzymatically active conformation even without proteolytic activation by FD. Essentially, this flexibility in conformational change facilitates a limited degree of AP activation confined to tissue surfaces without FD, culminating in considerable AP-induced tissue damage[73].

The novel insights unraveled in this extensively explored field can be attributed to the swift progress in complement-targeted therapeutics (further reviewed elsewhere[75]). Consequently, future seemingly scientific inconsistencies harbor the potential to offer novel mechanistic insights and refine therapeutic strategies.

DRIVERS OF DISEASE

The flip side of this instant, forceful, and broadly applicable defense machinery is that it may cause considerable damage when directed against host tissue. This is the case especially in the AP, as it is constitutively active at a low level and plays a pivotal role in amplifying the complement response, ultimately accounting for more than 80% of terminal pathway activation regardless of the activating pathway[14,15]. Such dysregulation of the AP in the fluid phase forms the pathological basis of C3G.

The foundational understanding of the pathophysiological process in C3G is primarily attributed to seminal studies employing mouse models, which elucidated critical aspects of the pathological mechanism. One of the earliest observations made

was that FH-deficient (Cfh-/-) mice exhibited reduced C3 plasma levels coupled with elevated plasma C3b levels, a pattern indicative of C3 convertase hyperactivation. Shortly after birth, these mice developed renal pathology similar to human C3G, including C3 glomerular deposition in the absence of immunoglobulin, and subendothelial electron-dense deposits resembling C3GN[76]. In addition, it was evidenced that the absence of FB in these FH KO mice (Cfh-/- Cfb-/-) resulted in the cessation of C3 cleavage due to the inhibition of C3bBb formation, effectively preventing the manifestation of the C3G phenotype, which further affirmed the essential role of C3 convertase formation in disease occurrence[76]. In contrast, the absence of C5 in the FH KO mice (Cfh-/- C5-/-) did not prevent the disease but notably attenuated its severity[74]. This finding aligns with the known function of C5, operating in the terminal but not the initiating pathway of the complement cascade. Remarkably, this protective effect was not seen in FH KO mice deficient for C6 (Cfh -/- C6 -/-), suggesting a significant role for C5 (specifically C5a), but not the MAC, in the development of renal lesions[74].

Data accumulated in these experimental models correlate with in vivo studies in humans. Through the use of laser microdissection and mass spectrometry, a significant accumulation of AP and TP complement components within C3G glomeruli has been documented, with C3 being the most prominent and present at concentrations 5–10 times higher than other complement components[77,78]. Furthermore, a series of in vivo investigations underscore the active and continuous nature of C3 deposition in C3G glomeruli. Research carried out by Medjeral et al. revealed the presence of C3b/iC3b/C3c or C3dg deposits in the majority of C3G patients studied[2], while an investigation involving 12 C3G cases by Sethi et al. demonstrated that glomerular C3 deposits were primarily composed of C3dg[79]. These findings substantiate the notion of active C3b deposition, as opposed to passive entrapment of intact C3 within the C3G glomeruli. In parallel, in a study examining 19 different complement biomarkers, Zhang et al. documented notably lower C3 levels in C3G patients and decreased FB levels, with an accompanying increase in FB breakdown products, Bb and Ba[80]. Thus, these results reaffirmed the significant involvement of AP C3 convertase in the pathogenesis of C3G. Intriguingly, a correlation between C3 cleavage and disease activity or severity has not yet been substantiated[2,3,81,82], implying the possible involvement of additional factors downstream of the C3 cleavage step in determining the severity of renal pathology.

Complement dysregulation in patients with C3G is frequently driven by autoantibodies to various complement proteins and complexes. The primary autoantibodies linked with C3G are denoted as nephritic factors (NeFs), a heterogeneous group of antibodies against neoepitopes mainly generated within C3 or C5 convertases.

These antibodies have the capacity to stabilize the respective molecules, extending their half-life, which leads to an associated decrease in serum levels of C3 or C5 and a corresponding elevation in the levels of their cleavage products[83]. The most frequently identified autoantibodies target C3bBb, known as C3NeFs, and are reported in 40-80% of patients[3,82,84], although there are broad interindividual differences in the nature and/or the level of C3NeFs. Crucially, a study comprising 40 C3NeFs-positive patients (13 children and 27 adults) investigated the relationship between C3 nephritic factors and disease outcome, discovering that patients' IgG's ability to stabilize C3 convertase was markedly higher in the group exhibiting rapid disease progression (defined as a decline in GFR ≥5 ml/min per year) than in the group with slow progression (defined as a decline in GFR <5 ml/min per year). The two groups did not significantly differ in age at disease onset, proteinuria, and renal function; however, the median renal survival was 30 months for patients with rapid progression versus 288 months for those with slow progression. Thus, the C3NeFs' stabilizing capacity was identified as a biomarker associated with renal outcomes in C3G[85]. Moreover, C5NeFs, which target the C5 convertase, are also prevalent, reported in 42% of patients[86]. Notably, the ability of C5NeF to stabilize C5 convertase is correlated with sC5b-9 in patients, substantiating its direct involvement in C5 convertase overactivation in C3G. In addition, sC5b-9 levels were found to correlate inversely with C3 levels, indicating that a significant portion of C5 convertase overactivation results from C3 convertase formation rather than an isolated mechanism. Autoantibodies less commonly observed include C4NeFs (directed against C4b2a) and autoantibodies against FH, FB, and C3b. Collectively, these are identified in approximately 10% of patients diagnosed with C3G[87,88]. The potential therapeutic value of identifying, neutralizing, or eliminating these antibodies is yet to be ascertained. Nonetheless, considering the success of such interventions in other autoantibody-associated glomerular diseases, it seems plausible to regard these antibodies as prospective targets for therapeutic intervention in future management strategies.

Furthermore, comprehensive genetic testing has demonstrated that approximately 25% of C3G patients carry rare variants or genomic rearrangements in disease-associated complement genes, which are either responsible for gain of function in C3 convertase components (C3 and FB) or loss of function of AP regulators such as FH and FI[3]. It is also worth noting that multiple variants in complement-related genes are not uncommon in patients diagnosed with C3 glomerulopathy. This inherent genetic complexity might elucidate the infrequent incidence of familial cases wherein the affected individual shares the same diagnosis with a first or second-degree relative. One possible explanation for this pattern can be traced to the influence of specific haplotypes, which

can contribute to the variable penetrance of C3G, possibly by modulating the circulating levels of specific complement proteins[3,82,89]. Several well-documented familial cases of C3G have been reported in recent years, many of which are linked to genomic rearrangements within the FH gene family. A significant proportion of these cases are characterized by genetic anomalies involving the rearrangement of the CFH locus, the susceptibility of which arises from its origin, which can be traced back to partial gene duplications of the FH gene, leading to the emergence of unique CFHR fusion genes[48,90,91]. Most genomic rearrangements specifically associated with C3G result in the addition of two amino-terminal SCR domains, which generates an extra dimerization domain. These abnormal FHR proteins are thought to lead to enhanced complement de-regulation at surfaces, especially in the kidney, likely because of their enhanced oligomer formation and thus enhanced avidity towards disease-relevant ligands, leading to increased glomerular C3 deposition and the manifestation of C3G[54,55,92]. The prototypical example of this process is the CFHR5 gene variant, which is endemic to Cyprus and affects approximately 1 in 6,000 persons. This variant creates an FHR5–FHR5 fusion protein in which the first two SCRs of FHR5 are duplicated, resulting in variably penetrant C3GN[50]. Of note, even with an observable genotype-phenotype correlation, clear pathological effects remain to be functionally validated, an essential step that underpins the confirmation of disease association and the elucidation of the underlying disease mechanism[93]. In addition to the aforementioned genetic abnormalities in complement genes, some disease-linked polymorphisms in CFH, C3, CFB, and MCP may influence the complement activity and susceptibility to inflammatory and infectious diseases. For instance, the CFH-H1 haplotype has been found to confer a higher risk for the development of C3G, whereas CFH-H2 has been found to confer protection[94].

CURRENT AND EMERGING THERAPIES FOR C3 GLOMERULOPATHY

At present, the management of C3G primarily relies on a conservative therapeutic strategy centered on renin-angiotensin system blockade. General immunosuppressive therapy with mycophenolate mofetil (MMF) plus steroids may be considered in moderate-to-severe cases. If patients fail to respond to these therapies, the off-label use of eculizumab is sometimes considered, although no anti-complement therapeutic has yet been approved for treating C3G. Given the current lack of specific therapeutic approaches, renal prognosis remains poor.

Current Standard of Care for C3 Glomerulopathy

An ideal treatment strategy employing existing therapeutics for C3G has yet to be conclusively determined. Nonetheless, the Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group has proposed guidelines based on expert consensus and accumulated clinical experience from numerous clinical case series[95].

According to expert opinion, primarily drawn from retrospective studies and data extrapolated from non-correlated proliferative glomerulonephritides, clinical decisions regarding initial therapy are based upon moderate-to-marked proliferation on biopsy and an arbitrary proteinuria threshold of 2 g/day, splitting patients into two groups: mild disease and moderate-to-severe disease[96]. For patients categorized as having mild disease, health-promoting measures are offered along with the administration of anti-proteinuric drugs such as angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB). In severe disease, immunosuppressive therapy with MMF plus steroids should be contemplated. The theoretical advantage of this therapeutic approach lies in its ability to impede T-cell and B-cell proliferation, thus attenuating cell-mediated immune responses and autoantibody formation[97]. However, immunosuppressive treatment efficacy varies significantly[2,3,98–102].

For patients with progressive disease that is unresponsive to the aforementioned therapies, eculizumab can be considered as an off-label treatment option[95]. Eculizumab is a monoclonal antibody that binds with high affinity to C5, preventing its cleavage and thereby inhibiting the formation of C5a and the terminal complement complex (C5b-9) without affecting upstream C3 convertase activity. Despite demonstrating clinical potential in managing PNH and aHUS, prototypical disorders of complement dysregulation[13,103], eculizumab has not yet garnered adequate evidence supporting its use as a therapeutic agent even for rapidly progressing C3G. No randomized clinical trials have been undertaken, and the data primarily stems from two retrospective studies[104,105] in conjunction with two prospective non-controlled trials[106,107]. Each trial followed a treatment protocol of four weekly 900mg dosages, followed by a biweekly 1200mg dosage, guided by prior experience of eculizumab use in aHUS.

The first prospective trial encompassed six participants, each exhibiting proteinuria >1 g daily and/or AKI upon enrolment. After 12 months of eculizumab intervention, three patients met the study-defined criteria for renal response, following reduction of sCr levels (from 1.8 to 1.4 mg/dL) in two of them, and urinary protein/creatinine ratio in the other one (5.8 to 1.8 g/g). Additionally, another patient exhibited histopathological signs of improvement despite stable clinical parameters,

thus culminating in a total response rate of 67%. Given that eculizumab normalized the soluble C5b-9 levels in all patients who initially exhibited elevated levels, the authors inferred that an elevated soluble C5b-9 level could potentially serve as a valuable marker of response to this therapeutic agent[106]. However, more recent studies have challenged this idea. The most comprehensive case series to date, which involved 26 C3G patients undergoing eculizumab treatment (including 13 children/adolescents), presented a global clinical response in 6 patients (23%), a partial clinical response in another 6 (23%), and no response in the remaining 14 (54%). Those who responded to treatment demonstrated lower eGFR, more rapidly progressive disease, and increased extracapillary proliferation on kidney biopsy. At the same time, features of AP activation did not differ between the subgroups[104]. Furthermore, in a prospective single-arm clinical trial involving 10 patients with heavy proteinuria (>3.5 g/24h) and strong terminal complement activation (sC5b9 > 1,000 ng/mL), only 3 (30%) achieved a significant reduction in 24-hour proteinuria, even though sC5b-9 plasma levels promptly and entirely normalized in all patients[107]. In the most recent retrospective study, 11 patients underwent eculizumab treatment with a median follow-up period of 68 months. By the end of the treatment, eight patients (57.1%) exhibited a negative outcome, six patients (42.9%) had a stable outcome, and none showed an improvement. The study only identified young age and shorter durations of treatment initiation as valuable predictors of response[105].

Overall, these findings are consistent with the understanding that eculizumab primarily targets a single aspect of C3G — glomerular inflammation — and may have little to no effect on the primary driver of the disease, C3 complement dysregulation. This observation aligns with data from animal models of C3G, which indicate that liberation of the anaphylatoxin C5a has a pathophysiological role in C3G, whereas cytolysis by MAC formation has not[74]. Furthermore, these studies highlight the necessity for additional research to delineate clinical parameters to identify patients who might derive at least some benefit from anti-C5 therapy.

Emerging Therapies: Focus on Anti-Complement Strategies

The complement cascade, comprised of over 40 effector and regulatory proteins, presents a myriad of intervention points for the inhibition of specific steps which may be more advantageous in certain pathological conditions than solely targeting the TP. Even though eculizumab has shown limited effectiveness in the treatment of C3G, it is essential to acknowledge that its clinical and commercial success in other complement-

mediated disorders has ignited renewed interest in complement inhibition as a therapeutic strategy, motivating numerous large and small pharmaceutical firms to launch development programs with a diverse range of targets.

Taking into account the limited efficacy of anti-C5 therapy, coupled with the present understanding that C3 convertase overactivation is a central pathophysiological driver of C3G, inhibition of the C3/C3 convertase axis has swiftly risen as a promising therapeutic strategy for this condition, by targeting one of the three components of AP convertase - C3, FB, or FD. A notable deviation from this approach is avacopan, which blocks the signaling of anaphylatoxin C5a. In addition to these stoichiometric inhibitors, which adhere to their activated targets in a 1:1 ratio and maintain inhibition as long as they remain bound, a couple of non-stoichiometric inhibitors are also under investigation in clinical trials for their potential use as complement inhibitors in C3G. These include TP10, also known as soluble complement receptor 1 (sCR1), and ARO-C3, an RNA interference (RNAi) approach aimed at silencing the expression of C3. The unique appeal of non-stoichiometric inhibitors lies in their potentiated inhibitory mode, whereby a single inhibitor molecule has the capacity to inhibit multiple target proteins. **Table 1** provides a comprehensive overview of clinical trials currently investigating novel complement inhibitors for the treatment of C3G.

Moreover, the recent approval of pegcetacoplan for patients with PNH[108,109] represents a seminal event in the evolution of complement therapeutics that could potentially transform the clinical management trajectory of numerous complement-mediated diseases, particularly those where anti-C5 therapy has demonstrated mixed or insufficient responses. Until recently, clinical experience with complement inhibition was limited to a singular mechanistic strategy. Therefore, this recent shift is a crucial turning point, as it not only validates the effectiveness of compstatin technology in a clinical context but also alleviates safety concerns related to sustained C3 inhibition.

Anti-C3 Therapies: The Role of Compstatin

Compstatins comprise a family of structurally related cyclic peptides which exhibit selective binding affinity to primate and human native C3 and its bioactive fragments C3b and C3c[110]. Their primary mechanism of action involves hindering the access of C3 to the preformed convertases and interrupting the formation of new convertases[111,112], which stands in contrast to the strategies adopted by natural regulators, which either destabilize the C3 convertase or accelerate the degradation of the enzyme complex. Importantly, compstatins do not inhibit the hydrolysis of C3 into its active form, C3(H_2O)

(i.e., tick-over activation), nor do they obstruct the convertase-independent cleavage of C3 by certain proteases, such as thrombin, which might occur under specific disease states. Consequently, compstatin and its analogs efficaciously regulate the primary pathways of complement activation and mitigate amplification of the response whilst preserving residual (upstream) complement activity.

This family of complement C3 inhibitors was identified in 1996 by phage display screening, resulting in a 13 amino acid-long, disulfide-bridged peptide[113]. Since then, compstatin analogs with increased inhibitory activity, enhanced target residence and favorable pharmacokinetic profiles for systemic administration have been developed. In the case of C3G, clinical trials are being developed for Pegcetacoplan (second-generation) and AMY-101 (third-generation).

Pegcetacoplan (also known as APL-2) is a compstatin analog bridged by a PEG moiety to improve plasma residence, thereby enhancing its half-life. An in vitro study conducted within a hemolysis model utilizing C3b-recovered sheep erythrocytes and purified complement proteins, supplemented with IgG positive for C3NeF or C5NeF, demonstrated that Pegcetacoplan (at a concentration of 25 μg/mL) effectively prevents the formation of both alternative pathway C3 and C5 convertases. Furthermore, it inhibited the activity of preformed convertases and concurrently attenuated the prolonged convertase activity mediated by C3NeF and C5NeF[114]. A 48-week, open-label Phase II trial (DISCOVERY; NCT03453619) further revealed Pegcetacoplan's potential as a C3G treatment in a multi-center evaluation involving five patients. The trial aimed to assess preliminary efficacy and safety, with patients receiving daily subcutaneous infusions of 360 mg Pegcetacoplan, which was later adjusted to 1080 mg per infusion twice weekly from Week 24. The primary endpoint was the change in proteinuria from baseline to Week 48, measured via 24-hour urine protein-to-creatinine ratios (uPCR), with additional evaluations of serum C3, albumin, and creatinine levels, alongside safety parameters. The study concluded with a significant 73.3% reduction in proteinuria (3.48 mg/mL at baseline to 0.93 mg/mL at week 48), along with increased serum albumin and C3 levels and stable serum creatinine. Notably, no severe adverse events were reported, nor did any treatment-emergent adverse events (TEAEs) result in discontinuation. Thus, these findings support the contention that Pegcetacoplan reduces proteinuria while preserving renal function and maintaining a solid safety profile[115]. A phase III trial (VALIANT; NCT05067127) is currently in progress. Simultaneously, this trial is being expanded to provide more comprehensive data on its long-term safety and efficacy (NCT0580953).

AMY-101 is a third-generation non-PEGylated compstatin analog based on Cp40,

which has entered clinical development as a complement inhibitor for patients with C3G. It has shown largely improved activity, target residence, and pharmacokinetic properties. Compared to the original compstatin, it offers significant improvement in binding affinity to human C3 (6,000-fold), enhanced inhibitory potency, and an extended in vivo half-life (~12 hours after a single intravenous injection)[116,117]. The underpinnings of AMY-101's potential clinical impact have been substantiated through in vitro investigations involving Cp40 using sera from C3G patients[118]. In this study, Cp40 demonstrated its ability to halt abnormal C3 turnover consistently across all tested C3G patient sera. Parallelly, it was observed that Cp40 prevented proteolytic activity when introduced after C3 convertase formation. An additional experiment involved patient-derived C3NeF/C4NeF hemolytic assays, where the inclusion of Cp40 effectively prevented the cleavage activity of NeF-stabilized convertases. Transitioning from the in vitro realm to human trials, AMY-101 was subjected to a Phase 1 clinical study in 2017 to assess the drug's safety, tolerability, pharmacokinetics, and pharmacodynamics (NCT03316521)[119]. Involving 50 healthy male volunteers as study subjects, the trial's results demonstrated a favorable safety profile for AMY-101, as it was well tolerated and did not result in significant adverse events. At the time of this review's composition, no ongoing clinical trials evaluate the use of AMY-101 in the context of C3G treatment.

Anti-FD and Anti-FB Therapies: A Targeted Approach

Unlike the total inhibition of the amplification loop achieved by C3 inhibition, FD or FB inhibition only partially restricts the classical or lectin pathway activities due to the absence of C3b amplification by the alternative pathway, thereby offering a more targeted approach. To date, clinical trials pertaining to C3G have utilized Danicopan (also known as ACH-4471, ACH-0144471, or ALXN2040) and BCX9930 for FD inhibition, while FB inhibition has been pursued with the use of Iptacopan/ LNP023. Furthermore, a novel Phase I study has been registered exploring the use of NM8074, a monoclonal antibody that selectively binds to Bb (NCT05647811).

Before delving into the current landscape of anti-FD therapeutics, it is crucial to understand the evolving perspective on the role of FD. Until recently, FD was thought to be the rate-limiting protease of the AP, a viewpoint grounded in the observation of low plasma concentrations and investigations using reconstituted FD deficient plasma[120,121]. However, more recent insights suggest that under conditions marked by increased AP activity, such as C3G, extremely low levels of FD – approximately 1% of plasma levels – are sufficient to activate it, particularly if the C3 convertase is stabilized. This notion

implies that to achieve a comprehensive shutdown of the AP, over 90% enzyme inhibition may be necessary[122]. Additionally, the recent recognition that FB can undergo conformational activation in the absence of FD[73] (see "Lessons from complement inhibition therapeutics" section) can explain why FD inhibition might not succeed in completely preventing C3b kidney deposition in C3G patients.

Danicopan (also known as ACH-4471, ACH-0144471 or ALXN2040) was the pioneering oral, clinical-stage inhibitor of FD. In initial results from a Phase 1 study, Danicopan exhibited rapid and complete inhibition of AP activity for at least 16 hours following a single oral dose of 1200 mg. However, complications arose when multiple doses at and above 500 mg twice daily were linked to elevations in liver enzymes in some subjects, thereby limiting the maximum dosage for future clinical trials to 200 mg[123]. Subsequent Phase 2 trials, which targeted patients with C3G or various types of immune complex-mediated membranoproliferative glomerulonephritides (IC-MPGN) (NCT03369236 and NCT03124368), demonstrated suboptimal bioavailability and consequently did not meet key efficacy endpoints. This occured as plasma C_{trough} values did not reach the projected concentrations required to maintain ≥90% AP inhibition derived from Phase 1 studies[124], which aligns with current knowledge of C3G and IC-MPGN, as even minute breakthrough AP activation - quickly amplified via the AP amplification loop - can uphold C3 convertase upregulation, C3b deposition, and consequent disease progression. As a result, the development of Danicopan for the treatment of C3G has since been discontinued. At present, a second oral FD inhibitor molecule, ACH-0145228, based on the same chemical scaffold, is under development.

More recently, another clinical-stage FD inhibitor, BCX9930, was discovered. Initial evaluations of the compound revealed an encouraging safety and tolerability profile, as in separate single ascending dose (SAD) and multiple ascending dose (MAD) cohorts, with dosages varying from 10 to 1200 mg and 50 to 400 mg every 12 hours, respectively, 108 healthy subjects were found to generally tolerate the drug well across all evaluated doses, without any serious adverse events or dose-related safety signals[125]. Furthermore, a Phase 1 open-label study employing ex vivo activated serum from both healthy individuals and C3G patients demonstrated rapid and sustained suppression of AP activity after the administration of a single oral 600 mg dose of BCX9930[125]. Within just an hour, maximal suppression (median ≥ 98% relative to pre-dose levels) was achieved in both healthy subjects and subjects with C3G, with a median 97% inhibition persisting 24 hours post-dose. Following these initial evaluations, BCX9930 was advanced to Phase 2 clinical trials at a dosage of 500 mg twice daily, in a basket trial encompassing C3G, IgAN and primary membranous nephropathy (NCT05162066).

However, these trials were placed under a transient partial clinical hold due to observed increases in serum creatinine levels, potentially indicative of kidney-related adverse effects. Following these developments, the company explored the efficacy of a reduced dose of 400 mg twice daily. Nonetheless, shortly after this, the development of BCX9930 was discontinued. Following this, the company that was advancing BCX9930 has shifted its focus towards the development of a similar compound, BCX10013.

Currently in clinical development, Iptacopan (also known as LNP023) is a potent and selective FB inhibitor. This molecule emerged from optimizing an initial compound identified through a screening of a chemically diverse collection using a proteolytic assay that employed a cobra venom factor (CVF):Bb complex as a stable surrogate of the C3 convertase[126]. In preclinical studies, it has been observed that abnormal C3 cleavage in C3G patient sera can be effectively prevented through FB inhibition, which appears to require lower concentrations compared to FD inhibitors[127]. This enhanced efficacy may stem from the fact that in C3G serum, the stabilization of C3 convertase is primarily responsible for maintaining AP activity rather than its production. Notably, while FD inhibitors restrict only the formation of convertase, FB inhibition has the capacity to also interfere with already assembled convertases, given that the Bb fragment of FB forms the enzymatic foundation of the C3 convertase. The efficacy of Iptacopan was examined in a Phase 2 trial in C3G patients (NCT03832114), in which 27 patients followed a 12-week treatment with Iptacopan, resulting in a significant 45% reduction in proteinuria (measured via uPCR), sustained normalization of plasma C3 levels, significant stabilization of eGFR in native kidneys of C3G patients and a significant reduction in C3 deposit scores on renal biopsy in patients with recurrent C3G after transplantation. During the treatment period, Iptacopan showed a favorable safety and tolerability profile in both cohorts[128]. Lastly, for the seven patients who entered the extension study, their eGFR remained stable until 25 weeks (NCT03955445)[128]. Iptacopan is currently in Phase 3 clinical trials for C3G (APPEAR-C3G; NCT04817618).

Anti-C5a Therapies: Therapeutic Impairment of a Single Effector Arm

Considering the animal study findings which highlight the pathophysiological role of anaphylatoxin C5a in C3G, while MAC formation appears to be non-pathogenic (see “Drivers of Disease” section) and acknowledging the limited efficacy of anti-C5 therapy in C3G patients (see “Current Standard of Care for C3 Glomerulopathy” section), it is logical to pursue the development of inhibitors that specifically target the C5a-C5aR1 signaling axis.

Avacopan (also known as CCX168) is a low molecular weight C5aR1 antagonist. It holds the potential to inhibit C3a, C4a, and C5a, thereby manifesting a potent anti-inflammatory action. Lately, this molecule has demonstrated an encouraging safety profile along with promising signs of efficacy in patients with ANCA-vasculitis[129]. A Phase 2 randomized, double-blind, placebo-controlled trial for C3G has recently been conducted using Avacopan (ACCOLADE, NCT03301467)[130]. In this study, 57 patients were randomized in a 1:1 ratio to either receive a twice-daily dosage of 30 mg avacopan (n=28) or a placebo (n=29) over a span of 26 weeks. Following this period, consenting patients received avacopan for an additional 26 weeks. In this study, the effectiveness of avacopan was assessed using the C3G Histologic Index (C3-HI), a tool designed to measure renal disease based on renal biopsy. The primary endpoint of this trial was the C3HI Disease Activity Score, an indicator of acute glomerular inflammation. After the initial 26 weeks, avacopan treatment resulted in a non-significant 2% improvement in the primary endpoint C3G-HI disease activity, contrasted with a notable 38% deterioration observed in the placebo group. The secondary endpoint of this trial was the C3-HI Disease Chronicity Score, which specifically assessed the progression of fibrosis. Remarkably, avacopan showed a significantly lower rise in patients' disease chronicity score compared to placebo. The mean percent change from baseline was 31.7% in the avacopan group (N=26) versus 57.5% in the placebo group (N=26), reflecting a score change of 0.8 versus 1.6, respectively. Consequently, these outcomes suggest that avacopan may demonstrate a favorable effect in attenuating the progression of C3G. The importance of this study's findings is underscored by a 2021 publication that validated the use of the C3-HI and established the Disease Chronicity Score as an independent predictor of kidney failure[131].

Non-Stoichiometric Inhibitors: The Potential of TP10 (sCR1) and ARO-C3

Contrary to the previously mentioned inhibitors, which exhibit a 1:1 binding ratio with their respective targets, non-stoichiometric inhibitors possess the ability to inhibit multiple target proteins with just a single inhibitor molecule. Within the field of complement inhibition pertinent to C3G, the only non-stoichiometric inhibitors that have undergone or are currently undergoing investigation include the protein TP10, also known as soluble complement receptor 1 (sCR1), and the RNA interference (RNAi) method implemented through ARO-C3, which is designed to repress C3 expression.

TP10 (also known as sCR1) constitutes the soluble variant of the natural convertase-oriented complement inhibitor CR1, which is a membrane-bound complement receptor.

CR1 functions as an essential regulator of complement activation, concurrently demonstrating decay-accelerating activity through competitive displacement of Factor Bb and C2a catalytic fragments from convertases[132,133], and acting as a cofactor in Factor I-mediated fragmentation of C3b to iC3b, as well as in the subsequent cleavage of iC3b into C3dg and C3c[134,135]. Acknowledging CR1's multifaceted roles and implications in disease manifestation, therapeutic strategies based on CR1 are being developed to manage C3 deposition on tissues and regulate complement activation in the fluid-phase. Among these potential treatments is TP10, which comprises the soluble full-length extracellular domain of human CR1. The development of TP10 involved the synthesis of complementary DNA (cDNA) encoding the entire extracellular domain of CR1, employing molecular biology techniques, while preserving all the biological functions of its parental molecule[136–138]. In vitro, TP10 proved its efficacy in preventing C3 convertase activity in a hemolytic assay using sera from patients with DDD, restoring AP control in a dose-dependent manner and preventing hemolysis even when DDD sera contained C3NeFs[139]. This effectiveness was substantiated in animal models using FH-KO mice transgenic for human CR1, in which the utilization of TP10 led to the normalization of serum C3 concentrations, a significant reduction in new C3 deposition, and the clearance of old C3 as evidenced by the marked decrease in C3c staining[139]. Considering these findings, the United States Food and Drugs Administration (FDA) authorized a compassionate clinical trial in a pediatric patient with ESRD attributable to C3G. In this patient, a seven-dose TP10 regimen led to transient normalization of circulating levels of C3 and C5b-9, with no recorded adverse effects or detected immunogenicity associated with sCR1 administration[139]. However, a phase 1 trial designed to study TP10 in a broader collective of C3G patients (NCT02302755) was withdrawn due to recruitment challenges[140]. In this study, only a single patient was enrolled, who showed an initial, but not sustained, clinical improvement. At present, CSL040, a truncated soluble variant of CR1, holds promise as a therapeutic candidate in the management of complement-mediated disorders[141].

Furthermore, the applicability of a RNAi strategy in patients with C3G is being studied using ARO-C3, with the objective of either abolishing or significantly diminishing systemic C3 expression in the liver. This is accomplished by delivering small interfering RNA (siRNA) into the cytoplasm of the target cell type, which is subsequently incorporated into the RNA-induced silencing complex (RISC). The siRNA serves as a template, aligning the RISC with target messenger RNA sequences, thereby facilitating their ensuing degradation[142]. The precedence set by RNAi-mediated silencing of C5 is noteworthy. Cemdisiran (also known as ALN-CC5) is an RNAi therapeutic targeting C5

expression in the liver, and it is currently being evaluated for its potential in treating diseases driven by the AP of complement activation. In healthy individuals, the administration of cemdisiran was shown to be safe and well tolerated, achieving up to 99% reduction in C5 levels and decreasing serum hemolytic activity by as much as 61%. Notwithstanding, when administered to patients with PNH (n=6), it was observed that despite a maximum C5 knockdown of approximately 98% and a mean maximum classical pathway inhibition of approximately 94%, the target of lactate dehydrogenase levels less than 1.5 times the upper limit of normal was not met[143]. This outcome could potentially be attributed to the local production of extra-hepatic C5, the requirement of greater than 99% C5 inhibition for achieving a therapeutic effect, or inadequate treatment or dosing of cemdisiran. Such findings have instigated the investigation into multi-modal combination strategies (for instance, RNAi coupled with monoclonal antibodies), where the target is addressed on both transcriptional and translational fronts. Recent interim outcomes from a Phase 1/2a study assessing the safety, tolerability, pharmacokinetics, and/or pharmacodynamics of ARO-C3 in healthy volunteers (NCT05083364) have revealed an average reduction of 88% in serum C3 and a mean reduction of 91% in AH50 at the highest tested dose, while demonstrating favorable safety and tolerability profiles[144]. In light of documented local C3 synthesis in various tissues, including kidneys[145], and the potential need for near-complete alternative pathway inhibition to elicit a therapeutic effect in C3G patients, the effectiveness of this approach within this context awaits further evaluation.

CONCLUSIONS AND FUTURE DIRECTIONS

C3G is an ultra-rare renal disease typically associated with poor prognosis. In the absence of specific treatment options, approximately half of the patients diagnosed with C3G develop ESRD within a decade.

Recognizing the fundamental role of complement dysregulation in the pathogenesis of C3G, significant attention has been directed toward complement inhibitor agents as potential therapeutic strategies. Notably, eculizumab's clinical and commercial success played a pivotal role in fueling interest in complement therapeutics among clinicians, pharmaceutical industries, and patients. Consequently, there is substantial anticipation surrounding the potential benefits of complement modulation in treating various kidney diseases, including C3G.

Currently, several drug candidates are approaching the final stages of development, heralding the potential introduction of these novel therapies as feasible

treatment alternatives for C3G in the near future. Within this promising context, the recent approval of pegcetacoplan for patients with PNH stands as a significant breakthrough. As the first-in-class C3 inhibitor, pegcetacoplan establishes a new precedent for the application of C3-targeted therapeutics across a broad spectrum of diseases driven by C3 dysregulation, such as C3G.

However, akin to the complement system itself, inhibiting this host defense pathway poses unique and substantial challenges. As evidenced by our experiences with eculizumab, the advent of new inhibitors of the complement system may yield unexpected findings in clinical complement analytics and patient monitoring, findings that our current understanding may not fully comprehend. These scientific puzzles are crucial to unravel, as they hold the potential to reveal unknown aspects of the complement cascade. Moreover, considering the inherent complexity of C3G, it is improbable that a “one-size-fits-all' solution will be suitable. As such, patient stratification will become an indispensable tool for tailoring the most effective complement-specific therapy. This tailored approach will need to factor in various elements such as clinical presentations, histologic findings, biochemical markers, and genetic predispositions.

More than a century after the discovery of the complement system, we stand on the precipice of groundbreaking developments in the field of complement-mediated diseases. With its unique challenges and complexities, C3G may well be the next beneficiary of these scientific advances.

APPENDIX

Table I - Clinical trials investigating complement inhibitors in C3G listed with any statuses except with status "unknown".

Stoichiometric inhibitors				
Complement target	**Complement inhibitor**	**Clinical phase**	**Reported statuses (04/2023)**	**ClinicalTrials.gov identifiers (date first posted)**
C3	Pegcetacoplan (APL-2)	Phase 2	Active, not recruiting	NCT03453619 (2018)
		Phase 2	Recruiting	NCT04572854 (2020)
		Phase 3	Recruiting	NCT05067127 (2021)
		Phase 3	Not yet recruiting	NCT05809531 (2023)
	AMY-101(Cp40)	Phase 1	Completed	NCT03316521 (2017)
FD	Danicopan (ACH-4471, ACH-0144471, ALXN2040)	Phase 2	Completed	NCT03124368 (2017)
		Phase 2	Completed	NCT03369236 (2017)
		Phase 2	Terminated	NCT03459443 (2018)
	BCX9930	Phase 2	Terminated	NCT05162066 (2021)
FB	Iptacopan (LNP023)	Phase 2	Completed	NCT03832114 (2019)
		Phase 2	Recruiting	NCT03955445 (2019)
		Phase 3	Recruiting	NCT04817618 (2021)
FBb	NM8074	Phase 1/2	Not yet recruiting	NCT05647811 (2022)
C5a	Avacopan	Phase 2	Completed	NCT03301467 (2017)
Non-stoichiometric inhibitors				
Complement inhibitor		**Clinical phase**	**Reported statuses (04/2023)**	**ClinicalTrials.gov identifiers (date first posted)**
TP10 (sCR1)		Phase 1	Withdrawn	NCT02302755 (2014)
ARO-C3		Phase 1	Recruiting	NCT05083364 (2021)

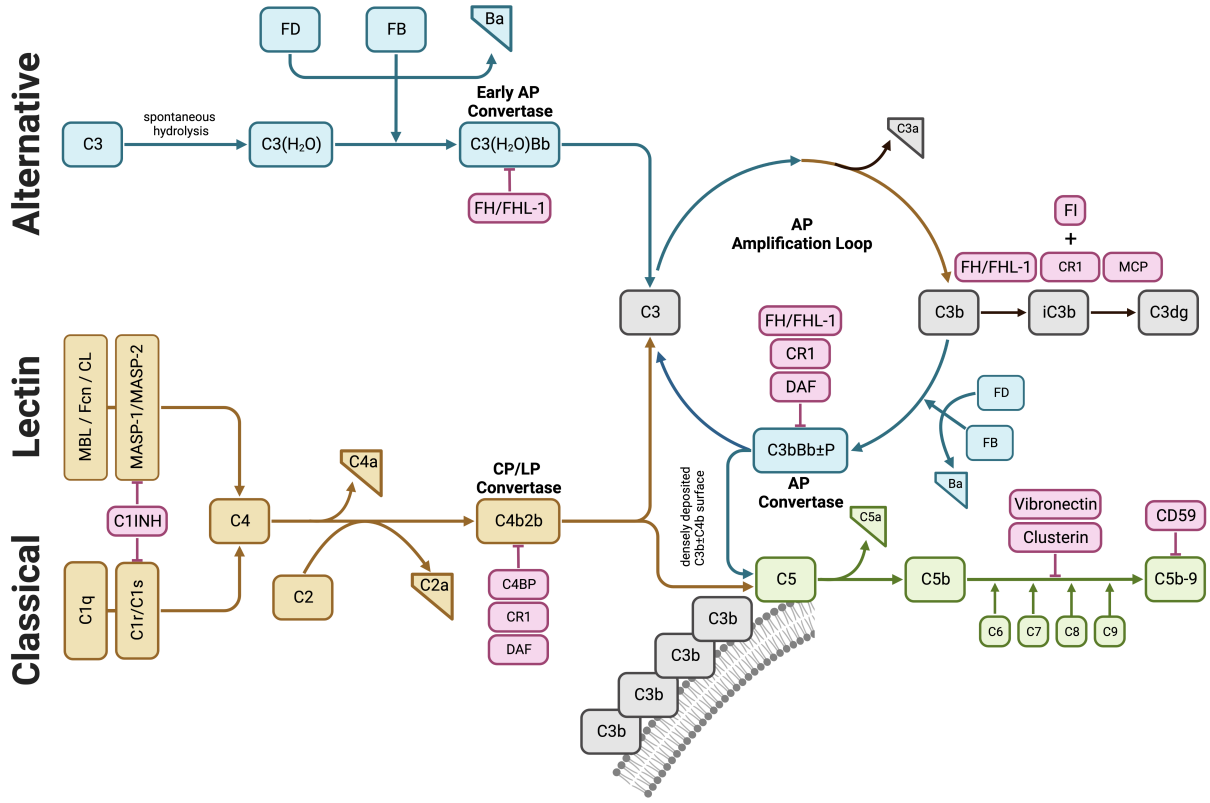

Figure 1- The complement system. The complement system is activated through three distinct pathways: classical, lectin, and alternative. Each pathway is initiated by specific stimuli, leading to the formation of C3 convertases. These convertases cleave C3 into anaphylatoxin C3a and C3b, the latter of which opsonizes cells near the initiating surface. Simultaneously, an amplification loop for C3 cleavage is created, resulting in augmented C3a generation and C3b deposition. Once a certain surface C3b density is reached, C5 is primed for cleavage by these convertases into anaphylatoxin C5a and C5b, with the latter assembling the membrane attack complex (MAC or C5b-9). Meticulous regulation of this system via fluid-phase and surface-bound molecules across all phases is crucial to avoid host damage. Key regulators in C3 Glomerulopathy (C3G) are Factor H (FH), the primary fluid-phase regulator of the alternative pathway (AP), and its splice variant, FHL-1. These regulators interact with the C3 convertase, acting as decay accelerators and as co-factors for Factor I in C3b cleavage. This figure was created using BioRender.com.

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