



Membranoproliferative glomerulonephritis: no longer the same disease and may need very different treatment

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ABSTRACT

Membranoproliferative glomerulonephritis (MPGN) is a pattern of glomerular injury that may be primary or secondary to infections, autoimmune diseases and haematological disorders. Primary C3G and IC-MPGN are rare and the prognosis is unfavourable.

Based on immunofluorescence findings, MPGN has been classified into complement-mediated C3 glomerulopathy (C3G) and immune complex-mediated MPGN (IC-MPGN). However, this classification leaves a number of issues unresolved. The finding of genetic and acquired complement abnormalities in both C3G and IC-MPGN indicates that they represent a heterogeneous spectrum rather than distinct diseases. An unsupervised hierarchical clustering in a cohort of patients with primary C3G and IC-MPGN identified four distinct pathogenetic patterns, characterized by specific histologic and clinical features, and genetic and acquired complement abnormalities. These results provide the groundwork for a more accurate diagnosis and the development of targeted therapies.

The drugs that are currently used, such as corticosteroids and immunosuppressants, are frequently ineffective in primary C3G and IC-MPGN. Eculizumab, an anti-C5 monoclonal antibody, has been used occasionally in single cases or small series. However, only a few patients have achieved remission. This heterogeneous response could be related to the extent of terminal complement activation, which may vary substantially from patient to patient. Several drugs that target the complement system at different levels are under investigation for C3G and IC-MPGN. However, clinical trials to test new therapeutics will be challenging and heavily influenced by the heterogeneity of these diseases. This creates the need to characterize each patient to match the specific complement abnormality with the type of intervention.

Keywords: C3 glomerulopathy, complement inactivating agents, complement system, membranoproliferative glomerulonephritis, rare diseases

INTRODUCTION

Membranoproliferative glomerulonephritis (MPGN), also known as mesangio-capillary glomerulonephritis, is a pattern of glomerular injury observed in kidney biopsies, with characteristic light microscopic changes: mesangial hypercellularity, endocapillary proliferation and duplication—double contours—of the glomerular basement membrane (GBM) [1].

Historically, MPGN was classified, on the basis of light microscopy and glomerular ultrastructure, as type I, II and III:

- (i) type I MPGN is characterized by discrete immune deposits in the mesangium and subendothelial space;
- (ii) type II MPGN is characterized by continuous, dense, ribbon-like deposits along the basement membranes of the glomeruli, tubules and Bowman's capsule; and
- (iii) type III is similar to type I MPGN, except that subepithelial as well as subendothelial deposits are noted [2].

The histopathologic finding of MPGN is one of the most challenging, since it does not refer to a specific disease entity but may instead be the result of different aetiologies.

In 2011, a classification on the basis of immunofluorescence (IF) was proposed [3, 4] that divides MPGN into (i) C3 glomerulopathy (C3G), characterized by dominant glomerular C3 deposition (at least two orders of intensity stronger than any other immune reactant) and little or no immunoglobulin (Ig) deposition; and (ii) immune complex-mediated MPGN (IC-MPGN), with significant glomerular Ig and complement deposition. Using electron microscopy (EM), C3G may be further classified as dense deposit disease (DDD, type II MPGN in the previous classification), with highly electron-dense deposits in the GBM, and C3 glomerulonephritis (C3GN), with mesangial, subendothelial, subepithelial and intramembranous deposits, but without the typical

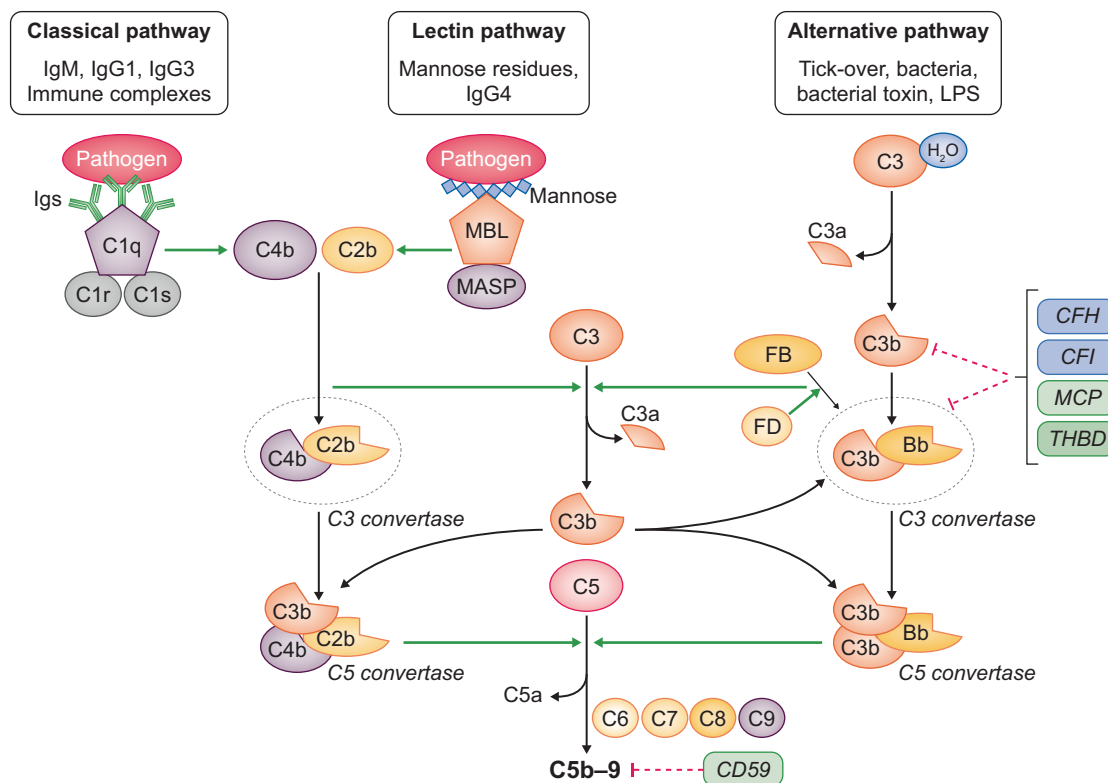


FIGURE 1: The complement cascade. Schematic overview of the complement cascade, illustrating the three activation pathways (classical, lectin and alternative) with the C3 convertase complexes of the classical/lectin and of the alternative pathway (dotted circles in the figure) and the common terminal pathway that leads to C5 cleavage and the formation of the anaphylatoxin C5a and of the membrane attack complex, composed of C5b, C6, C7, C8 and many copies of C9. The classical pathway is triggered by the binding of C1q to antibody-antigen complex. The lectin pathway is similar to the classical pathway, but is activated by the binding of MBL to mannose residues, which activates MASP proteins. At variance, the alternative pathway is continuously activated in plasma by low-grade hydrolysis of C3 (C3H₂O, tick-over). The latter binds factor B to form a C3(H₂O)B complex. Factor D (FD) cleaves factor B to form the alternative pathway initiation C3 convertase that cleaves C3 to C3b. The activation is then amplified by the covalent binding of a small amount of C3b to hydroxyl groups on cell-surface carbohydrates and proteins of target cells such as bacterial cells. This C3b binds factor B to form the amplification loop C3 convertase C3bBb. C3b also binds to the C3 convertase, forming the C5 convertase enzyme C3b2Bb. The alternative pathway is highly regulated so as to prevent non-specific damage to host cells and limit the deposition of complement on the surface of pathogens. This fine regulation occurs through a number of membrane-anchored (green) and fluid-phase (light blue) regulators. CFI, complement factor I (degrades C3b and C4b); CFH, complement factor H (acts as cofactor for factor I for C3b cleavage and favours the decay of the C3 convertase of the AP); MCP, membrane cofactor protein (binds C3b and C4b and has cofactor activity for both the classical and the alternative pathways); THBD, thrombomodulin (increases CFH cofactor activity, activates TAFI-mediated C3a and C5a inactivation); CD59 (blocks the C9 association with C5b-8 to prevent C5b-9 formation on host cells).

electron-dense deposits of DDD. The term C3G is also used to define non-specific alterations or other proliferative patterns that share C3-dominant glomerular staining [5].

The current classification is based on the assumption that C3G arises from abnormalities in the control of the alternative complement pathway, whereas IC-MPGN derives from the deposition of IC that trigger the classical complement pathway (Figure 1). Although dividing MPGN into C3G and IC-MPGN seemed to provide an advance toward an aetiology-based diagnostic approach, some pitfalls emerged that will be discussed later in this review.

Careful evaluation can help identify an underlying cause in C3G or IC-MPGN cases. In their proposed algorithm for the clinical management of these two diseases, Fakhouri *et al.* [6] suggest that underlying conditions, such as infectious-related GN and plasma cell dyscrasia, should be ruled out in patients

with C3G, while autoimmune diseases, chronic infections, liver cirrhosis and sickle cell anaemia must be ruled out in IC-MPGN cases. Infectious-related GN is a common cause of acute nephritic syndrome in children that develops following an infection. Histologically, it is characterized by proliferative GN, bright glomerular C3 IF staining with or without Ig and subepithelial hump-like deposits on EM. Most patients recover within days to weeks, and the overall prognosis is good. [7] However, in a subgroup, the disease follows an atypical course, with chronic C3 consumption, persistent proteinuria, and progression to end-stage renal disease (ESRD). In many of these atypical cases, an underlying defect in the alternative pathway regulation may be detected and these are most likely examples of primary C3G in which the regulatory defect is unmasked when complement activation is triggered by infection [8].

Monoclonal gammopathies may be associated either with C3G or IC-MPGN [8] and should be ruled out, especially in adults. Both IF on frozen tissue and IF on paraffin-embedded tissue with pronase digestion [9] have been used to identify monoclonal deposits. More recently, Nasr *et al.* [10] described an IF protocol with antibodies that target combined heavy/light chain epitopes (HLC IF). Although confirmatory studies in high numbers of patients are required, HLC IF appears as a promising complementary technique to confirm or exclude the diagnosis of monoclonal gammopathy in MPGN and other glomerular diseases. When chronic infections, autoimmune diseases or paraprotein-related kidney diseases are excluded, and a clear underlying aetiology cannot be identified, C3G and IC-MPGN are considered primary or idiopathic.

Primary C3G and IC-MPGN are rare, with an estimated prevalence of 12–16 per 100 000 population in Europe [11]. The clinical picture is characterized by a variety of symptoms, ranging from mild disease with asymptomatic microscopic haematuria and/or proteinuria, to severe disease with nephritic or nephrotic syndrome (NS) and renal impairment. In general, outcome is poor. Relevant prognostic factors, reported in both adults and children, include NS at onset and a higher proportion of sclerotic glomeruli and crescents in kidney biopsies [12]. The risk of ESRD is similar for C3G and IC-MPGN (4–41% versus 9–41%) [13, 14].

Compared with other forms of GN, patients experience comparable rates of survival on dialysis and following kidney transplantation, but significantly higher rates of allograft loss due to disease recurrence (54–60% in C3G versus 43% in IC-MPGN) [14].

In patients with DDD, an association with retinal abnormalities, with the appearance of drusen-like deposits, is described. In fact, on EM, these deposits look similar in both the Bruch's membrane and the GBM. More recently, the development of drusen and other types of retinal pathologic features has also been documented in C3GN [15]. Acquired partial lipodystrophy, characterized by the loss of subcutaneous fat in the upper half of the body, may be noted in patients with C3G [16].

This review aims to explore how our current understanding in systems biology, and genetic and clinical diagnostics will enable the identification of the multifaceted mechanisms that underlie C3G and IC-MPGN, improving prognosis and the choice of targeted treatment options.

C3G AND IC-MPGN: A DISEASE SPECTRUM

The IF-based classification and related assumption that C3G is complement-mediated, while IC-MPGN is secondary to conditions that cause the glomerular deposition of IC, leaves some issues unsolved [6].

If the C3 dominant criterion were used, up to 20% of cases exhibiting DDD at EM examination would not be classified as C3G [5, 17]. In a recent report, 8 out of 48 DDD patients were mis-classified as IC-MPGN on IF [18]. In a large cohort,

including biopsies from 319 patients with primary MPGN, 60% did not meet the criteria for C3G [17].

Among patients who underwent repeat biopsies, 40% had different IF staining patterns on the initial and follow-up biopsies and 17% exhibited a shift from C3G to IC-MPGN or vice versa [17]. Some children may present at onset with a biopsy characterized by proliferative GN and an IC-MPGN pattern with co-dominant C3 and Ig staining, with a subsequent biopsy showing dominant C3 [19]. It seems likely that this relates to IC induced by infections or other triggers that initiate the disease in a patient who has an underlying alternative pathway abnormality, and that C3-dominance may become evident following classical pathway inactivation after resolution of infection or immunosuppressive therapy. Glomerular C4d immunostaining has been suggested as a marker of classical and/or lectin pathway activation and to differentiate between IC-MPGN (C4d-positive) and C3G (C4d-negative) [20]. This finding, however, has not been confirmed by subsequent studies, which showed positive glomerular C4d in over half of C3G patients, particularly in paediatric cases [19, 21].

Finally, abnormalities resulting in dysregulation of the alternative pathway are found in around 50% of patients with primary IC-MPGN. These include genetic abnormalities affecting complement regulators, mainly factor H (FH), or the two components of the alternative pathway C3 convertase, C3 and factor B (FB) [12, 14], and/or acquired abnormalities, including C3NeF or C5NeFs (autoantibodies that stabilize the alternative pathway C3 or C5 convertases) [22], activating anti-FB or anti-C3b antibodies [23] and inhibitory anti-FH antibodies [24, 25]. Notably, the percentages of primary C3G and IC-MPGN patients carrying genetic and/or acquired alternative pathway abnormalities were comparable [12–14]. Rearrangements affecting the genes encoding the five FH-related proteins and leading to internal duplications, deletions or hybrid genes that result in the deregulation of FH activity were described in C3G patients [26]. However, more recently, they have been associated with primary IC-MPGN as well [24].

Altogether, the above-mentioned studies underline the complexity of C3G and IC-MPGN.

Through an unsupervised cluster analysis in a cohort of 173 C3G and IC-MPGN patients, we explored whether patients could be separated into relatively homogeneous groups [13]. This approach, which places patients with many commonalities close together so that each individual cluster has greater homogeneity than the whole, has previously enabled the identification of disease subtypes in Parkinson disease, Alzheimer's disease, asthma and other conditions [27]. We included in the analysis 34 histologic, biochemical, genetic and clinical features that were available at disease onset. We identified four clusters that indicate the existence of distinct disease entities characterized by specific pathogenetic mechanisms (Figure 2). Clusters 1–2 included patients with fluid-phase alternative pathway activation at both C3 and C5 levels, highlighted by low serum C3 and high plasma levels of the soluble terminal complement complex (sC5b-9) (Figures 1 and 2), but those in

		Cluster 1	Cluster 2	Cluster 3	Cluster 4
Glomerular C3	Score	2.7	2.7	2.8	2.5
Mutations or NeFs	%	75	63	79	14
C3NeFs	%	22	15	78	/
C5NeFs	%	78	85	22	/
Serum C3	mg/dl	↓↓	↓↓	↓↓	N
Plasma sC5b-9	ng/ml	↑↑	↑↑	N/↑	N
Glomerular IgG	Score	0.4	2.0	0.5	1.0
Glomerular C1q	Score	0.3	1.6	0.3	0.6
Highly electron-dense deposits	%	7	0	73	0

Clusters 1–3: Fluid-phase complement activation

Cluster 1: Fluid-phase C3 and C5 convertase activation

Cluster 2: Fluid-phase C3 and C5 convertase activation + classical pathway activation

Cluster 3: Fluid-phase C3 convertase activation prevalent

Cluster 4: Solid-phase complement activation

FIGURE 2: Characteristics of the four clusters obtained through unsupervised cluster analysis. Clusters 1 to 3 showed evidence of fluid-phase alternative pathway activation and a high prevalence of complement gene abnormalities (mutations) and/or nephritic factors (NeFs). In clusters 1 and 2, alternative pathway activation occurs both at the C3 and C5 levels, as documented by low serum C3 and high levels of soluble C5b-9. Cluster 2 is distinguished by the fact that these patients also have signs of activation of the classical pathway (Ig and C1q staining in biopsy). In cluster 3, fluid-phase C3 convertase activity predominates over C5 convertase activity, as shown by mostly normal sC5b-9 levels. Most of these patients have high electron-dense deposits in the glomerular basement membrane. Most patients in clusters 1 and 2 carried NeFs that stabilized both the C3 and the C5 convertases (C5NeFs), whereas C3NeFs stabilizing the C3 convertase only were mainly found in cluster 3. Cluster 4 patients separated from the others since they have normal serum C3 levels but intense C3 staining in the kidney, indicating solid-phase alternative pathway activation in the kidney. N, normal value. Green rectangles highlight abnormalities in circulating blood; red rectangles highlight glomerular abnormalities.

cluster 2 also exhibited markers of activation of the classical pathway in the biopsy (C1q and IgG staining) (Figures 1 and 2) and the highest prevalence of NS. Patients in cluster 3 had fluid-phase alternative pathway activation, mainly at the C3 level, and highly electron-dense deposits in the GBM (Figures 1 and 2). Finally, cluster 4 was characterized by solid-phase-restricted complement activation with glomerular C3 deposits and a normal complement profile in the blood, and had the highest risk of ESRD. In this regard, clusters did better at predicting renal survival than the classification into IC-MPGN, DDD and C3GN [28]. Notably, while a large majority of DDD patients fell into cluster 3, C3GN and IC-MPGN patients were distributed among clusters, reinforcing the overlap between C3GN and IC-MPGN and the heterogeneity of the two histologic groups. Genetic and acquired complement abnormalities were highly prevalent in clusters 1–3 but rare in cluster 4 (Figure 2). Further analysis revealed that variants affecting C3 and CFB as well as C5NeFs [13, 22] were more prevalent in clusters 1 and 2, whereas cluster 3 patients had a higher prevalence of C3NeFs and of heterozygous CFH variants compared with the other clusters [13, 22].

The cluster analysis approach was subsequently validated by an independent group in another cohort of C3G and IC-MPGN patients, with similar results [29].

CURRENT TREATMENT OF C3G AND IC-MPGN

For a number of reasons, the optimal treatment for primary C3G and IC-MPGN has not been established yet.

In several studies, patients were classified as having idiopathic MPGN, while they may have been affected by secondary C3G and IC-MPGN; early studies were performed before the extensive use of angiotensin inhibitors; the classification criteria have changed over time and the small numbers of patients limited the possibility of conducting randomized clinical trials.

Common principles of the management of primary C3G and IC-MPGN are similar to those recommended for other proteinuric glomerulopathies: a low-salt diet, treatment of hypertension, reduction of proteinuria with angiotensin inhibition and treatment of dyslipidaemia. Immunosuppressive

therapy is often prescribed, although the choice of drugs and duration of treatment are based on retrospective analyses, limited case series and observational studies, rather than randomized controlled intervention trials.

Current guidelines [30] suggest that in patients with primary C3G and proteinuria >0.5 g/day despite supportive therapy, or moderate inflammation on renal biopsy or abnormal kidney function, a mycophenolate mofetil (MMF) trial with oral glucocorticoids should be attempted. The optimal duration of MMF therapy is uncertain, and up to 50% of patients relapse when treatment is stopped.

In patients with primary IC-MPGN, with a normal estimated glomerular filtration rate (eGFR) and non-nephrotic-range proteinuria, conservative treatment is preferred, since the long-term outcome is relatively benign in this setting. For patients who present with NS or elevated serum creatinine, steroids, in addition to angiotensin inhibition therapy, have been suggested [31, 32]. Several alternative regimens (calcineurin inhibitors, cyclophosphamide, intravenous boluses of methylprednisolone, plasmapheresis) have been proposed in non-responding patients, but the data on outcomes are scanty. Rituximab, a chimeric anti-CD20 antibody that depletes B-cell lymphocytes, has emerged in the last decade as a treatment alternative for patients with primary glomerular diseases, particularly idiopathic membranous nephropathy [33]. As for patients with MPGN, there are encouraging data for rituximab in patients with secondary forms and proliferative GN with monoclonal immune deposits. Results in primary IC-MPGN are scanty and the few reports in primary C3G are essentially negative [34].

Eculizumab, an anti-C5 monoclonal antibody, has been employed occasionally in single patients or small series of patients with primary C3G and IC-MPGN. The hypothesis is that by inhibiting the cleavage of C5, thereby precluding the formation of C5a and the terminal complement complex (C5b-9) (Figure 1), the drug might protect the kidneys from complement-mediated damage. Published data show that a high level of plasma sC5b-9 is potentially a marker of responsiveness [35]. Based on this consideration, we evaluated the effect of eculizumab in the context of a sequential off-on-off-on design in 10 patients (six primary IC-MPGN and four primary C3GN) with nephrotic-range proteinuria and high plasma sC5b-9 levels [36]. The finding that sC5b-9 plasma levels were fully normalized by eculizumab in all subjects, whereas proteinuria decreased in only three patients, is consistent with evidence that disease activity, at least in some patients, is only partially mediated by the activation of the terminal complement pathway. It could also be assumed that in non-responder patients, the activation of other upstream C3-convertase-dependent pathways, which cannot be blocked by eculizumab, may cause kidney damage despite the inhibition of the terminal pathway. Indeed, cluster analysis has revealed that dysregulated complement activation may occur within the circulation and/or within glomeruli and may deplete C3 or both C3 and C5 in distinct groups. The heterogeneous response to eculizumab treatment could be related to the extent of terminal complement activation, which may vary substantially from patient to patient.

THERAPIES UNDER INVESTIGATION

The recent success of eculizumab for the treatment of other complement-mediated rare diseases, such as paroxysmal nocturnal haemoglobinuria (PNH) and atypical haemolytic uremic syndrome, has boosted the interest of pharmaceutical industries in the clinical development of inhibitors that target different molecules of the complement cascade [37, 38]. Several ongoing clinical trials are testing new complement inhibitor drugs in C3G and IC-MPGN (Table 1). The oral C5aR1 antagonist CCX168 (avacopan), which demonstrated a good safety profile and promising results for the treatment of ANCA vasculitis, is currently being investigated in a phase 2 double blind, placebo controlled trial in C3G patients (NCT03301467). Preliminary results presented at the 2021 ERA-EDTA meeting (Late Breaking Clinical Trials, Symposium 0.1) showed an increase of C3G histologic index activity score (primary end point) at 26 weeks in the placebo but not in the avacopan group; however, the difference did not reach statistical significance. Of note, eGFR at 26 weeks decreased versus baseline in the placebo group, whereas there was a significant improvement in the avacopan group. Proteinuria also improved in the avacopan group.

C5a is a potent anaphylatoxin that, through interacting with the C5aR1 receptor, increases vascular permeability, induces oxidative bursting and the release of pro-inflammatory cytokines in myeloid cells, and has chemotactic properties for myeloid and certain lymphoid cells [39]. Avacopan blocks the interaction of C5a with C5aR1 and potentially exerts an anti-inflammatory effect, particularly in patients such as those in clusters 1 and 2 in our study [13], with C5 convertase dysregulation and inflammatory lesions observed on biopsy. Compared with eculizumab, avacopan does not affect the formation of the terminal complement complex that plays a key role in controlling several bacterial infections, potentially having less impact on the defence against microbes.

However, terminal pathway inhibitors do not affect complement activation upstream of C5 and do not prevent the formation of C3 activation fragments and their accumulation in glomerular immune deposits of C3G and IC-MPGN patients [40, 41]. Agents that target the alternative pathway are thus being tested in C3G and IC-MPGN. This type of medication will be particularly suitable for patients in cluster 3 [13], who are characterized by prevalent alternative pathway C3 convertase activation and dysregulation (Figure 2).

In this regard, ACH-0144471 (danicopan) [42] is a small, orally active inhibitor of factor D (FD). FD is a serine protease, mainly produced by adipose tissue, that catalyses the cleavage of FB, a rate-limiting step that converts the inactive enzyme proconvertase C3bB into the active C3 convertase C3bBb of the alternative pathway (Figure 1, Table 1). By inhibiting FD, danicopan specifically inhibits alternative pathway activity. An open-label phase 2 study in PNH documented the effective inhibition of haemolysis in all participants. Two proof-of-concept phase 2 studies with this FD inhibitor, a randomized placebo-controlled phase 2 study in C3G (NCT03369236) and a single-arm phase 2 study in C3G or IC-MPGN patients (NCT03459443), have completed recruitment. The results are

Table 1. Interventional clinical trials (phase 2, 3) with complement inhibitor in patients with primary C3G and IC-MPGN

ID number	Acronym/other ID number	Targeted protein	Status	Conditions	Interventions	Phase	Number of patients	Age
NCT02093533	EAGLE 2013-003826-10	C5	Completed	C3G; IC-MPGN	Eculizumab	2	10	Up to 75 years
NCT03301467	ACCOLADE CL011_168	C5aR1	Active, not recruiting	C3G	Avacopan Placebo	2	88	12 years and older
NCT03369236	ACH471-204	Factor D	Completed	C3G; IC-MPGN	Danicopan Placebo	2	13	17–65 years
NCT03124368	ACH471-201	Factor D	Completed	C3G; IC-MPGN	Danicopan	2	6	16–65 years
NCT03459443	ACH471-205	Factor D	Completed	C3G; IC-MPGN	Danicopan	2	22	17–65 years
NCT03832114	CLNP023X2202	Factor B	Completed	C3G; post-Tx C3G recurrence	Iptacopan	2	27	18–65 years
NCT03955445	CLNP023B12001B Extension study	Factor B	Recruiting	C3G; post-Tx C3G recurrence	Iptacopan	2	27	18 years and older
NCT04817618	APPEAR-C3G CLNP023B12301	Factor B	Recruiting	C3G	Iptacopan Placebo	3	68	18–60 years
NCT03453619	APL2-201	C3	Active, not recruiting	C3G	Pegcetacoplan	2	21	18 years and older
NCT04572854	NOBLE APL2-C3G-204	C3	Recruiting	Post-Tx C3G or IC-MPGN recurrence	Pegcetacoplan	2	12	18 years and older

Retrieved from ClinicalTrials.gov database (26 October 2021) <https://clinicaltrials.gov/ct2/home>.

C5aR1, C5a receptor; C3G, C3 glomerulopathy; IC-MPGN, immune complex-mediated membranoproliferative glomerulonephritis; Tx, transplant.

not yet publicly available, but very recently the company stated that they would halt development of the drug for C3G and IC-MPGN, citing that the phase 2 study data showed a suboptimal clinical response, due to an insufficient pharmacokinetic and pharmacodynamic response and incomplete inhibition of the alternative pathway (<https://medcitynews.com/2020/07/alexion-drops-kidney-disease-program-for-drug-that-was-part-of-930m-achillion-buyout-last-year/>). The company also stated that it had a more potent FD inhibitor, ALXN2050, which is currently undergoing a phase 2 study in PNH (NCT04170023). ALXN2050 could also be assessed in C3G and IC-MPGN.

In vitro and *in vivo* studies indicated that a very high degree of FD inhibition (likely more than 95%) needs to be achieved to efficiently block the alternative pathway of complement. Indeed, in experiments where serum from FD deficient mice was mixed with serum from wild-type mice, 2.5% of normal FD levels fully restored alternative pathway activity. This finding was confirmed in humans. Experiments in which human sera, immunodepleted of FD, were mixed with normal human sera revealed that 1.8% of normal FD levels induced 50% activation of the alternative pathway [43]. *In vivo*, the transplantation of wild-type adipose precursors into lipodystrophic mice that lacked adipose tissue and had virtually no FD resulted in 1–5% of wild-type circulating FD levels, which however supported as much as 30% of normal alternative pathway activity [43]. These findings are of particular relevance for C3G and IC-MPGN patients, who may require an even higher degree of FD inhibition, since they suffer from hyperactivity of the alternative pathway due to C3 convertase dysregulation.

In addition, circulating levels of FD are dependent on kidney function, since FD is filtered through the glomerulus and catabolized in the proximal renal tubule [44]. An inverse correlation between plasma FD levels and creatinine clearance has been reported in patients with various renal diseases [45]. Preliminary results from the two clinical trials of danicopan in C3G and IC-MPGN confirmed an inverse correlation between FD levels and renal function, so that patients with renal impairment had higher than normal FD levels [46], which represents another hurdle for efficient alternative pathway inhibition in these conditions. A phase 1 clinical study has been planned (NCT04623710) involving healthy subjects and three cohorts of patients with severe, moderate or mild impairment of renal function, respectively, to determine the effect of renal dysfunction on the pharmacokinetics and pharmacodynamics of the new anti-FD inhibitor ALXN2050.

Altogether, the above evidence indicates that the response to FD inhibition may vary greatly in C3G and IC-MPGN patients, depending on the degree of alternative pathway dysregulation and on FD levels. Specific *in vitro* and *ex vivo* assays of alternative pathway C3 convertase activity [47] should be performed in patients before and during treatment with FD inhibitors to establish whether they may benefit from the drug and, if so, to establish the effective dose.

Another alternative pathway inhibitory drug is LNP023 (iptacopan, Table 1), a small, orally active molecule that binds to FB. It does not prevent the formation of the C3 convertase, but it specifically inhibits C3 convertase enzymatic activity, blocking the conversion of C3 to C3b (Figure 1) [48]. *In vitro*, iptacopan inhibited complement activation in sera from C3G patients and inhibited the activity of

the C3 convertase stabilized by C3NeFs isolated from C3G sera [48].

An open-label, non-randomized phase 2 study on the efficacy, safety and tolerability of iptacopan in patients with C3G on the native kidney or after transplant has been carried out (NCT03832114) and the long-term extension study is ongoing (NCT03955445).

At the 2021 ERA-EDTA meeting, preliminary results from 12 adult patients with biopsy-proven native C3G who received iptacopan for 12 weeks were presented. Iptacopan inhibited AP activity, and plasma C3 levels recovered with complete normalization in five of seven tested patients at 12 weeks [49]. Most importantly, urinary protein excretion fell by 49% at 12 weeks and renal function stabilized. The treatment was well tolerated, with no treatment-emergent severe adverse events. A multicentre, randomized, double-blind, placebo-controlled phase 3 study on the efficacy and safety of iptacopan in C3G is ongoing (NCT04817618).

Drugs that target C3 are also under clinical development. Specifically, APL-2 (pegcetacoplan), a synthetic cyclic peptide conjugated to a polyethylene glycol polymer, binds specifically to C3 and inhibits C3 activation from all three pathways (Table 1). In addition, APL-2 binds to C3b and prevents the activity of the C3 and C5 convertases (Figure 1).

This drug could be particularly suitable for patients with the characteristics found in cluster 2 (Figure 2) [13], in whom alternative pathway C3 and C5 convertase dysregulation is combined with the activation of the classical pathway.

The safety and efficacy of pegcetacoplan has been investigated in a phase 2 open-label study (NCT03453619) in patients with different glomerulopathies, including C3G. Preliminary results from C3G patients were presented at the 2020 American Society of Nephrology meeting [50]. Of eight recruited patients, two non-compliant patients were excluded from the analysis. The other six had an increase in serum C3 and a decrease in plasma sC5b-9 levels, indicating that pegcetacoplan was able to modulate complement hyperactivity in C3G, at both the C3 and C5 level. During treatment, there was a trend toward a reduction in proteinuria (mean reduction of 24-h urinary proteins at day 84: 50%) and an increase in serum albumin. A phase 2, open-label randomized study is ongoing to evaluate the safety and efficacy of pegcetacoplan in the post-transplant recurrence of C3G or IC-MPGN (NCT04572854).

The risks of new anti-complement agents remain to be quantified and it should be taken into account that drugs that target complement upstream C5, either by blocking the alternative pathway or by more broadly inhibiting C3, may have greater effects on reducing the patient's defence against bacterial infections. Anti-meningococcal, anti-pneumococcal and anti-*Haemophilus influenzae* vaccinations should be administered at least 2 weeks before the start of treatment. Antibiotic prophylaxis may be considered for the overall period of anti-complement treatment.

CONCLUSIONS

In summary, primary C3G and IC-MPGN represent a disease spectrum that is heterogeneous in terms of pathophysiology

and clinical course. The current classification is based only on histologic data and does not consider aetiology, and we believe this is suboptimal. Several drugs targeting the complement system at various levels, which is the core of disease pathogenesis, are under investigation in clinical trials for C3G and IC-MPGN.

However, clinical trials to test new therapeutics are difficult to carry out due to the rarity of the disease. In addition, because each drug may act only in specific subgroups of patients, its effect in the overall C3G and IC-MPGN population will likely be diluted. The choice of drug should be tailored to each patient's individual characteristics, including clinical, histologic and biochemical parameters, and genetic and acquired complement abnormalities.

The unsupervised cluster analysis has shown promising results in terms of sorting C3G and IC-MPGN patients into relatively homogeneous groups, but further studies are required to provide additional parameters needed for a more precise cluster stratification, to better predict responses to specific complement inhibition therapies. Specific *in vitro* and *ex vivo* tests are needed to verify the potential responsiveness of each patient to a given complement inhibitory drug and evaluate the dose associated with the maximal effect. A computational tool with an easy-to-handle interface that would enable the assignment of patients to a cluster using a limited set of genetic, laboratory and clinical data is required to enable the transition from clinical research to diagnostic development. A European consortium coordinated by our group is working on the DECODE project, financed by the 2020 ERAPERMED call (<http://www.frrb.it/it/progetti-finanziati-decode>), with the aim of filling the above knowledge gaps to create a precise classification of C3G and IC-MPGN patients that will be essential for the success of clinical trials.

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CONFLICT OF INTEREST STATEMENT

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