C5 Convertase Blockade in Membranoproliferative Glomerulonephritis: A Single-Arm Clinical Trial

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Rationale & Objective: Primary membranoproliferative glomerulonephritis (MPGN) is a rare glomerulopathy characterized by complement dysregulation. MPGN progresses rapidly to kidney failure when it is associated with nephrotic syndrome. We assessed the effects of C5 convertase blockade in patients with MPGN and terminal complement activation.

Study Design: Prospective off-on-off-on open-label clinical trial.

Setting & Participants: Consenting patients with immune complex–mediated MPGN (n = 6) or C3 glomerulonephritis (n = 4) with sC5b-9 (serum complement membrane attack complex) plasma levels > 1,000 ng/mL and 24-hour proteinuria with protein excretion > 3.5 g identified from the Italian Registry of MPGN and followed up at the Istituto di Ricerche Farmacologiche Mario Negri IRCCS (Bergamo, Italy) between March 4, 2014, and January 7, 2015.

Intervention: Anti-C5 monoclonal antibody eculizumab administered during 2 sequential 48-week treatment periods separated by one 12-week washout period.

Outcomes: Primary outcome was change in 24-hour proteinuria (median of 3 consecutive measurements) at 24 and 48 weeks.

Results: Median proteinuria decreased from protein excretion of 6.03 (interquartile range [IQR], 4.8-12.4) g/d at baseline to 3.74 (IQR, 3.2-4.4) g/d at 24 weeks ($P = 0.01$) and to 5.06 (IQR, 3.1-5.8) g/d ($P = 0.006$) at 48 weeks of treatment, recovered toward baseline during the washout period, and did not significantly decrease thereafter. Hypoalbuminemia, dyslipidemia, and glomerular sieving function improved during the first treatment period. 3 patients achieved partial remission of nephrotic syndrome and all had undetectable C3 nephritic factors before treatment. Mean measured glomerular filtration rate was 69.7 ± 35.2 versus 87.4 ± 55.1 and 75.8 ± 42.7 versus 76.6 ± 44.1 mL/min/1.73 m^2 at the start versus the end of the first and second treatment periods, respectively, among all 10 study participants. Unlike C3, sC5b-9 plasma levels normalized during both treatment periods and recovered toward baseline during the washout in all patients.

Limitations: Single-arm design, small sample size.

Conclusions: Eculizumab blunted terminal complement activation in all patients with immune complex–mediated MPGN or C3 glomerulonephritis and nephrotic syndrome, but persistently reduced proteinuria in just a subgroup.

Trial Registration: Registered in the EU Clinical Trials Register with study no. 2013-003826-10.

Complete author and article information (including a list of the members of the EAGLE Study Group) provided before references.

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Membranoproliferative glomerulonephritis (MPGN) affects approximately 1 to 2 per million people per year in Western countries.[1,2] Eighty percent of cases occur before the age of 25 years. Approximately 40% of cases, in particular those associated with nephritic or nephrotic syndrome, progress to kidney failure over 10 or fewer years and most patients need long-term kidney replacement therapy by the age of 25.[3] Moreover, the disease recurs in approximately 50%[4] to 90%[5] of those who receive a kidney transplant and disease recurrence is associated with high rates of graft loss.[6] Typical glomerular changes include thickening of the capillary wall and mesangial expansion with hypercellularity and increased matrix deposition that are associated with deposition of immunocomplexes and/or complement factors at the subendothelial and/or mesangial level.[7,8]

On the basis of ultrastructural findings, for decades MPGN has been categorized as type 1 (with mesangial and subendothelial deposits), type 2 (dense deposit disease [DDD]; with mesangial and intramembranous highly electron-dense deposits), and type 3 (with subendothelial, intramembranous, and subepithelial deposits). More recently, a new classification has been suggested on the basis of the different distribution of immunoglobulin and C3 glomerular deposits at immunostaining evaluation. In most cases, termed immune complex–mediated MPGN (IC-MPGN), immune complex deposits predominate over C3 deposits, whereas in the less common form of MPGN, named C3 glomerulopathy (C3G), there is a dominant C3 deposition (C3-related signal at least 2 orders of magnitude greater than any other immune reactant) and little or no immunoglobulin staining.[9] C3G is further categorized into DDD, when distinctive highly electron-dense osmiophilic deposits are found within the glomerular basement membrane, or C3 glomerulonephritis (C3GN), when predominant subendothelial and occasionally subepithelial and intramembranous deposits are observed, but without the typical electron-dense

deposits of DDD.[9] The term C3G is also used to define nonspecific alterations or other proliferative patterns sharing C3-dominant glomerular staining.[9]

This new classification is based on recent evidence that dysregulation of the complement system plays a central role in the pathogenesis of MPGN.[10] In most cases, enhanced C3 breakdown and C3 hypocomplementemia reflect predominant dysregulation of the C3 convertase of the alternative pathway,[11] in particular in the fluid phase.[12] However, in IC-MPGN, C1q binding to immunoglobulin-antigen complexes may also result in activation of the classical complement pathway.[7] Very high plasma levels of sC5b-9 (serum complement membrane attack complex) also indicate massive activation of the terminal complement pathway.[12] In these patients, increased sC5b-9 production reflects concomitant activation of the C5 convertase of the alternative pathway caused by genetic C3 and complement factor B (CFB) abnormalities[13] or by autoantibodies (nephritic factors [Nefs]), which may stabilize both C3 (C3Nef) and C5 convertases.[14,15] Mutations in complement factor H (CFH) or acquired anti-CFH autoantibodies may also result in loss of function with impaired decay of both convertases.[16]

In CFH-deficient mice showing terminal complement activation and kidney changes reminiscent of MPGN in humans, the finding that either C5 gene silencing or treatment with anti-C5 antibodies ameliorates disease outcome[17] has been taken to suggest that independently of the underlying pathology, patients with high plasma sC5b-9 levels might benefit from inhibition of the terminal complement pathway through pharmacologic C5 blockade. The introduction into clinical practice of eculizumab, a humanized anti-C5 monoclonal antibody approved for the treatment of paroxysmal nocturnal hemoglobinuria[18] and atypical hemolytic uremic syndrome,[19] offered the opportunity to explore the effect of C5 blockade, which has been reported in a small number of case reports.[20-24] However, the data are conflicting.[10,24] The heterogeneous response to eculizumab treatment could be related to the extent of terminal complement activation, which may vary substantially from patient to patient. On the basis of this consideration, we evaluated the effect of eculizumab in 10 patients with biopsy-proven MPGN and high plasma sC5b-9 levels in the context of a sequential off-on-off-on design.[25,26]

Methods

Study Participants

Eligible patients were those in a prospective cohort who had been referred to the Italian Registry of MPGN at the Aldo e Cele Daccò Clinical Research Center (CRC) for Rare Diseases of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS (Bergamo, Italy) and who had biopsy-proven MPGN with creatinine clearance > 20 mL/min/1.73 m^2, proteinuria with protein excretion persistently >3.5 g/d in adults or >40 mg/m^2/h (or 2 mg/mg protein-creatinine ratio in spot urine samples) in children, serum C3 levels < 80 mg/dL, and plasma sC5b-9 levels > 1,000 ng/mL (corresponding to the mean + 10 standard deviation [SD] of values in our healthy controls) in at least 2 consecutive measurements performed at a median of 10 weeks apart, and who had received stable conservative therapy, including angiotensin-converting-enzyme inhibitors and/or angiotensin receptor blockers, during the last 6 months. We excluded patients 75 years and older, those with evidence of secondary MPGN, severe chronic kidney histologic changes (>50% of sampled glomeruli globally sclerotic[27] and/or >50% of renal cortex with interstitial fibrosis and tubular atrophy[28]), steroid or immunosuppressive therapy during the last 6 months, any clinical condition expected to affect completion of the study or confound the study findings, inability to understand the potential risks and benefits of the study, and legal incapacity (of patients or their parents or guardians). Pregnant or lactating women or fertile women without effective contraception were also excluded.[3]

Study Approval and Participant Consent

The CRC conducted the study in cooperation with the participating centers listed in the EAGLE Study Group Organization (see Article Information) and monitored all study activities. The Agenzia Italiana del Farmaco and ethics committees of participating centers approved the study, which was done in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. The study was registered on October 28, 2013, in the EU Clinical Trials Register with the number 2013-003826-10.

We obtained written informed consent from all patients or their parents or guardians. Patients who were included received a conjugated tetravalent meningococcal vaccine against serotypes A, C, Y, and W135 and a monovalent vaccine against serotype B at least 2 weeks before the first eculizumab infusion.

Study Design

This pilot, exploratory, phase 2, single-arm, prospective-cohort, open-label study was organized in two 48-week treatment periods with eculizumab, divided by a 12-week washout period in the context of an off-on-off-on design.[25,26]

All baseline clinical and laboratory measurements (Tables 1 and 2) were performed centrally at the CRC. Three consecutive 24-hour urine collections (Item S1) were submitted to measure protein, albumin, sodium, urea, and phosphate excretion. The median of the 3 measurements was recorded. When proteinuria at the screening evaluation and the median of the 3 measurements at baseline were both >3.5 g/d, the patient was eligible for study participation. Screening and baseline measurements were performed at a median of 10 weeks apart. Plasma sC5b-9, serum C3 and C4, and routine laboratory parameters were measured in the morning after overnight fasting.

Table 1. Urinary Protein Excretion in Individual Patients During the Two Treatment Periods With Eculizumab and the Washout Period and Comparisons of Mean and Median Values at Each Visit to Baseline

	Urinary Protein Excretion, g/24 h											
Pt No., Diagnosis, C3NeF	Screening	Wk 0a	Wk 1a	Wk 12a	Wk 24a	Wk 36a	Wk 48a	Wk 0b	Wk 12b	Wk 24b	Wk 36b	Wk 48b
001-01, IC-MPGN, Pos	4.1	3.83	2.96	4.78	4.06	2.23	3.09	5.83	4.22	3.40	3.77	3.81
001-03, C3GN, Pos	5.6	14.82	14.80	5.36	4.40	5.42	5.38	8.57	7.80	6.44	7.39	8.86
001-04, IC-MPGN, Neg	4.42	6.19	3.85	4.90	3.43	3.25	5.58	13.65	5.46	8.23	9.93	16.24
001-05, IC-MPGN, Neg	3.84	4.95	1.68	2.25	1.12	1.21	0.92	3.98	2.38	2.07	0.62	1.80
001-06, C3GN, Pos	7.58	13.72	11.86	8.02	7.85	5.64	8.54	9.49	6.83	13.41	—	—
001-07, IC-MPGN, Pos	10	12.41	12.66	5.96	5.04	5.51	8.03	8.22	7.88	8.10	6.43	8.43
003-01, IC-MPGN, Neg	4.83	6.00	4.93	3.63	3.17	5.05	5.75	10.67	7.24	7.25	6.89	7.79
004-01, C3GN, Neg	3.98	6.06	5.17	4.87	3.36	3.10	3.94	4.04	2.05	1.85	2.18	1.32
005-01, C3GN, Pos	4.72	4.23	4.00	4.29	4.35	3.53	4.73	5.42	5.75	5.51	10.13	8.14
006-01, IC-MPGN, Neg	3.9	4.84	3.61	2.41	2.77	3.34	2.13	4.26	1.89	5.67	5.61	2.12
Mean ± SD	5.30 ± 2.00	7.71 ± 4.21	6.55 ± 4.68	4.65 ± 1.69	3.95 ± 1.74	3.83 ± 1.52	4.81 ± 2.41	7.41 ± 3.26	5.15 ± 2.38	6.19 ± 3.42	5.88 ± 3.24	6.50 ± 4.80
P (paired *t* test)[a]	0.03	—	0.009	0.01	0.006	0.004	0.01	0.8	0.03	0.2	0.5	0.8
Median [IQR]	4.57 [4.0-5.6]	6.03 [4.8-12.4]	4.47 [3.6-11.9]	4.83 [3.6-5.4]	3.74 [3.2-4.4]	3.44 [3.1-5.4]	5.06 [3.1-5.8]	7.02 [4.3-9.5]	5.60 [2.4-7.2]	6.06 [3.4-8.1]	6.43 [3.8-7.4]	7.79 [2.1-8.4]
P (signed rank)[a]	0.01	—	0.01	0.01	0.01	0.002	0.006	0.8	0.05	0.4	0.5	0.5

Note: The 2 eculizumab treatment periods are weeks 0a to 48a and weeks 0b to 48b. The washout period is week 48a-0b. Week 0a was equivalent to baseline and 0b was equivalent to the end of the washout period.
Abbreviations: C3GN, C3 glomerulonephritis; C3Nef, C3 nephritic factor; IC-MPGN, immune complex–mediated membranoproliferative glomerulonephritis; IQR, interquartile range; Neg, negative; Pos, positive; pt, patient; SD, standard deviation.
[a]Comparison to baseline.

Table 2. Baseline Characteristics of Study Patients, Overall and According to Histologic Diagnosis (C3GN or IC-MPGN)

	Overall (N = 10)	C3GN (n = 4)	IC-MPGN (n = 6)
Demographic and Clinical Characteristics			
Age, y	20.0 ± 6.9	21.7 ± 8.6	18.8 ± 6.1
Male sex	6	2	4
Weight, kg	59.1 ± 15	62.3 ± 18.2	56.9 ± 13.1
BMI, kg/m^2	21.5 ± 3.3	23.3 ± 3.1	20.3 ± 3.0
Systolic BP, mm Hg	120.8 ± 14	127.9 ± 11.2	116.1 ± 13.7
Diastolic BP, mm Hg	74.8 ± 13	83.9 ± 9.8	68.8 ± 11.3
Pulse rate, beats/min	71.4 ± 10.7	71.8 ± 11.4	71.2 ± 11.3
Laboratory Parameters			
sC5b-9, ng/mL	2,420 [1,915-3,330]	3,069 [2,534-5,731]	2,107 [1,693-2,542]
C3, mg/dL	14.5 [10.3-24.4]	17.1 [14.6-22.1]	10.6 [9.8-21.0]
Detectable Nefs	5	3	2
Serum creatinine, mg/dL	1.21 ± 1.0	1.58 ± 1.1	0.97 ± 0.9
Serum albumin, g/dL	2.4 ± 0.5	2.1 ± 0.7	2.6 ± 0.3
Serum proteins, g/dL	4.6 ± 0.8	4.2 ± 1.0	4.9 ± 0.5
Total cholesterol, mg/dL	228.4 ± 30	236.3 ± 34.2	223.2 ± 28.4
HDL cholesterol, mg/dL	47.5 ± 13	50.0 ± 11.5	45.8 ± 14.6
LDL cholesterol, mg/dL	148.6 ± 39	145.0 ± 50.6	151.0 ± 33.8
Triglycerides, mg/dL	103.0 [77.0-231.0]	160.5 [67.5-233.5]	103.0 [77.0-145.0]
Blood glucose, mg/dL	88.3 ± 10	88.8± 4.8	88.0 ± 14.0
Hemoglobin, g/dL	11.3 ± 1.7	10.4 ± 0.6	11.8 ± 1.9
Serum calcium, mg/dL	8.3 ± 0.4	8.1 ± 0.4	8.5 ± 0.3
Serum phosphate, mg/dL	5.5 ± 0.7	5.2 ± 0.7	5.6 ± 0.7
Serum potassium, mEq/L	4.7 ± 0.7	4.8 ± 1.1	4.7 ± 0.5
Kidney Function Parameters			
mGFR, mL/min/1.73 m^2[a]	69.7 ± 35.2	49.7 ± 25.7	85.7 ± 35.6
eGFR, mL/min/1.73 m^2[b]	136.8 ± 94.8	83.3 ± 62.3	172.6 ± 100.1
Urinary protein, g/24 h	6.03 [4.8-12.4]	9.9 [5.6-14.0]	5.5 [4.8-6.2]
Urinary albumin, μg/min	3,199 [2,302-5,660]	4,625 [2,218-6,408]	2,854 [2,302-3,334]
Urinary sodium, mEq/24 h	107.4 [93-171]	107.4 [94.4-217.6]	125.6 [67.6-171.5]
Albumin fractional clearance	237.7 [117-580]	653.5 [356.9-919.3]	116.5 [85.7-237.7]
IgG fractional clearance	42.3 [22.6-195.8]	292.4 [110.8-412.2]	22.6 [12.9-42.3]
Antihypertensive Agents			
Diuretics	9	4	5
Calcium channel blockers	6	3	3
β-Blockers	3	1	2
ACE inhibitors or ARBs	10	4	6
Lipid-Lowering Agents			
Any	7	3	4
Statins	7	3	4
Omega-3 fatty acid	1	1	0
Ezetimibe	1	0	1

Note: Data presented as mean ± standard deviation, median [interquartile range], or count.
Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BP, blood pressure; C3GN, C3 glomerulonephritis; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IC-MPGN, immune complex–mediated membranoproliferative glomerulonephritis; IgG, immunoglobulin G; LDL, low-density lipoprotein; mGFR, measured glomerular filtration rate; Nef, nephritic factor; sC5b-9, serum complement membrane attack complex.
[a]Iohexol plasma clearance.
[b]Chronic Kidney Disease Epidemiology Collaboration (aged ≥ 18 years) or Schwartz (aged 1-17 years) equations.

Glomerular filtration rate (GFR) was directly measured using the iohexol plasma clearance technique[29] and estimated using the serum creatinine–based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in patients 18 years and older[30] and the Schwartz formula in younger patients.[31] Immunoglobulin G (IgG) and albumin fractional clearances were calculated using standard formulas.

Then patients were transferred to the Unit of Nephrology of the Azienda Socio Sanitaria Territoriale Papa Giovanni XXIII of Bergamo, where they received the first intravenous infusion of eculizumab (Soliris; Alexion Pharmaceuticals) under the supervision of an intensivist. Adults and underage patients who weighed ≥40 kg received 900 mg of eculizumab every week for 4 weeks (induction period), 1,200 mg at week 5, and then

1,200 mg every 14 ± 2 days (maintenance period), up to completion of 48 weeks of treatment. The dosing regimen of the drug in children who weighed < 40 kg is shown in Item S1. A second identical 48-week treatment course was started after the 12-week eculizumab withdrawal (washout period).

The parameters evaluated at baseline, including GFR and albumin and IgG fractional clearances, were re-assessed centrally at weeks 1, 24, and 48 of the first treatment period; 12 weeks after treatment withdrawal; and at weeks 24 and 48 of the second treatment period. The same parameters, with the exception of GFR and fractional clearances, were evaluated at each reference center at weeks 12 and 36 of both treatment periods. At each study visit, plasma sC5b-9 and serum C3 and C4 measurements were performed centrally. No systematic changes in diet or concomitant medications were allowed during the study.

Outcomes

The primary efficacy outcome was 24-hour proteinuria at 24 and 48 weeks of the first treatment period. Secondary outcomes were GFR, albumin and IgG fractional clearances, and progression to complete (proteinuria with protein excretion < 0.3 g/d) or partial (proteinuria with protein excretion < 3.5 g/d with >50% reduction from baseline) remission of nephrotic syndrome (for further details, see the study protocol and amendments at [3] and in Supplementary File 2). Plasma sC5b-9 was measured to monitor terminal complement pathway activity, along with C3 and C4 serum levels.

C3Nef Assay

C3Nef activity was determined by purifying IgG from plasma and assessing the ability to stabilize preformed C3 convertase bound to sheep erythrocytes.[32] Complement-mediated hemolysis was induced through the addition of rat serum and measured using optical density at 410 nm. The cutoff for positive C3NeF was 10% lysis in the presence of 200 μg of IgG (mean + 2 SD of lysis with IgG from healthy individuals).

Sample Size Estimation and Statistical Analyses

This was a pilot exploratory study in a very rare disease and sample size was determined on the basis of the expected number of potentially available patients during a predefined recruitment period of approximately 1 year, rather than on the basis of a power calculation. Data were analyzed by F.P., and analyses were supervised by A. Perna.

Continuous outcome variables were evaluated using paired t test, Wilcoxon signed rank test, repeated-measures analysis of variance, or linear mixed-effect models, which included predefined baseline covariates, as appropriate. McNemar test, χ^2 test, or Fisher exact test were used for categorical variables. Baseline characteristics were presented as numbers and percentages, mean and SD, or median and interquartile range. Primary efficacy comparisons, that is, 24-hour proteinuria at 24 and 48 weeks of the first treatment period as compared to baseline, were conducted at the 2.5% level of significance to take account of multiple testing using Bonferroni adjustment. Normality for continuous variables was assessed by means of the Q-Q plot and Shapiro-Wilk test. All *P* values were 2 sided.

Results

Study Participants

From March 4, 2014, to January 7, 2015, ten patients (6 males) were included from 6 centers (Fig 1; EAGLE Study Organization). Six patients had IC-MPGN and 4 had C3GN. One patient with C3GN had a heterozygous mutation in the C3 gene, a guanine to cytosine substitution in the coding sequence predicted to lead to an aspartate to histidine substitution at amino acid 1,625 (chr19:6678010G>C (p.D1625H)). A second patient with C3GN had a homozygous mutation in *CFH*, an adenine to guanine substitution in the coding sequence predicted to lead to an arginine to glycine substitution at amino acid 78 (chr1:196642281A>G (p.R78G)). C3Nefs were observed in 5 patients: 2 with IC-MPGN and 3 with C3GN (Table 1). Five patients were children or adolescents. Baseline characteristics of patients considered as a whole or with IC-MPGN and C3GN considered separately are shown in Table 2.

Primary Outcome

Proteinuria reduction at weeks 24 and 48 as compared to baseline achieved statistical significance (Fig 2; Tables 1 and 3).

Secondary Outcomes

Proteinuria decreased significantly also at weeks 1, 12, and 36 of the first treatment period, then increased sharply toward baseline values during the washout period. This trend of increasing proteinuria stopped and reverted during the second treatment period (Fig 2; Tables 1 and 3). Changes in albuminuria and albumin and IgG fractional clearances paralleled the change in proteinuria (Table 3). Consistently, serum albumin and total protein levels increased, whereas total cholesterol level, low-density lipoprotein cholesterol level, and low-density lipoprotein to high-density lipoprotein cholesterol ratio decreased significantly during the first treatment period (Fig 2; Table 3). These variables recovered toward baseline during the washout period and never differed from baseline during the second treatment period.

Mean and standard error of the mean (SEM) GFR slope changed significantly from the first to the second treatment period (+1.33 [SEM, 0.64] vs +0.06 [SEM, 0.44] mL/min/1.73 m^2 per month; $P = 0.04$). Despite the positive trend during both treatment periods, the global slope during the entire study period was slightly negative (−0.09 [SEM, 0.23] mL/min/1.73 m^2 per month) because of a

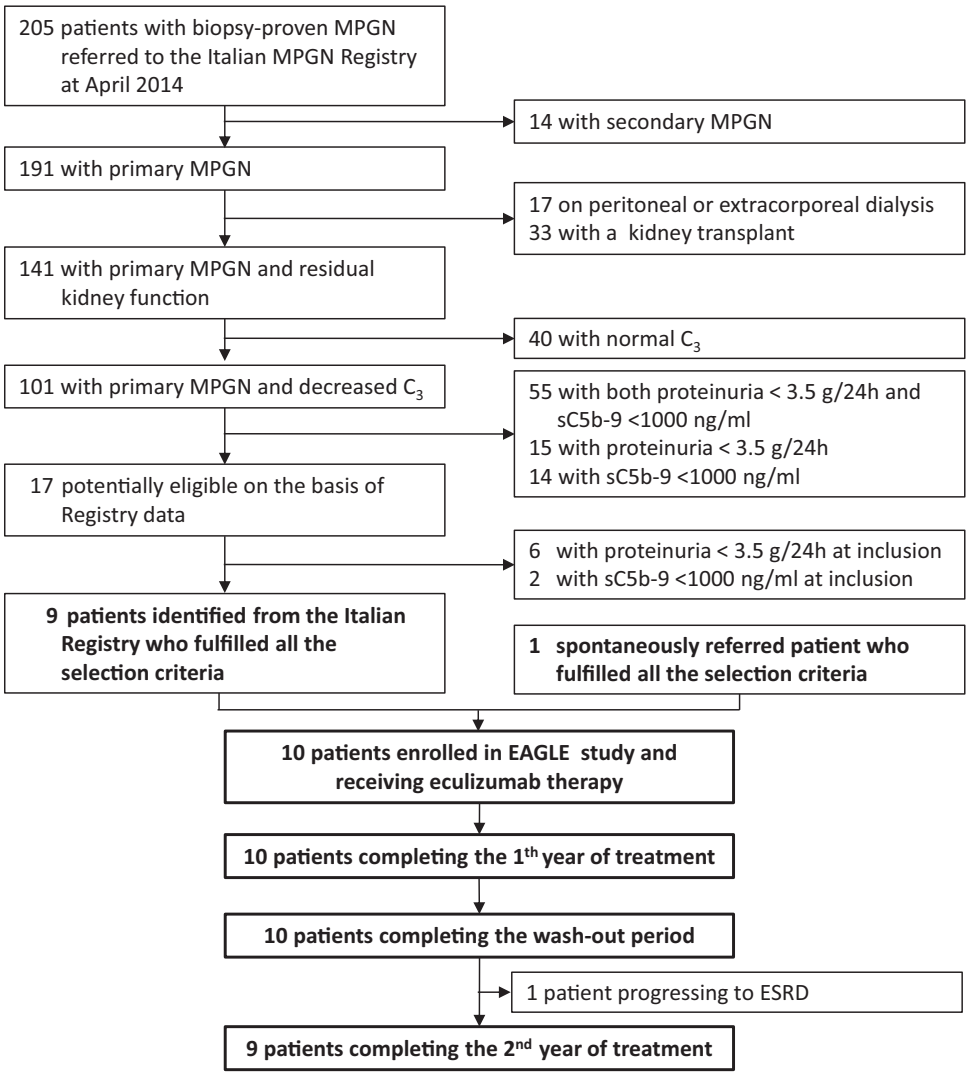

Figure 1. Study flow chart. Abbreviations: MPGN, membranoproliferative glomerulonephritis; sC5b-9, serum complement membrane attack complex.

sharp and significant ($P = 0.003$) GFR reduction during the washout period (Table S1). Estimated GFR (eGFR) showed a similar trend but widely overestimated measured GFR (Tables 2 and 3; Fig 2). In 60 simultaneous GFR estimations and measurements, GFR overestimation negatively correlated with serum albumin levels ($P = 0.02$; $r = -0.3049$). Thus, lower serum albumin levels were associated with larger GFR overestimation.

One patient progressed to end-stage renal disease (ESRD) during the second treatment period.

Body weight, body mass index, and blood pressure, as well as 24-hour urinary sodium, urea, and phosphate excretion, were relatively stable throughout the study.

Complement Inhibition

Plasma sC5b-9 levels promptly and persistently normalized during the first 48-week treatment period, recovered toward baseline values at the end of the washout period, and again normalized during the second 48-week treatment period up to study end (Fig 2; Table 3). C4 serum levels were stable and always in the normal range, whereas C3 serum levels were persistently reduced throughout the entire study period, but transiently and significantly increased during the washout period (Table 3; Fig 2). Changes in C3 levels during the washout correlated ($\rho = 0.6527$) with changes in proteinuria during the second treatment period, although this finding was of borderline statistical significance ($P = 0.05$).

Subgroup Analyses

Two patients achieved partial remission of nephrotic syndrome at completion of both treatment periods and 1 additional patient achieved the end point at completion of the second treatment period (Table 1). Baseline characteristics and changes in proteinuria, GFRs, sC5b-9 plasma levels, and C3 serum levels during the entire study period

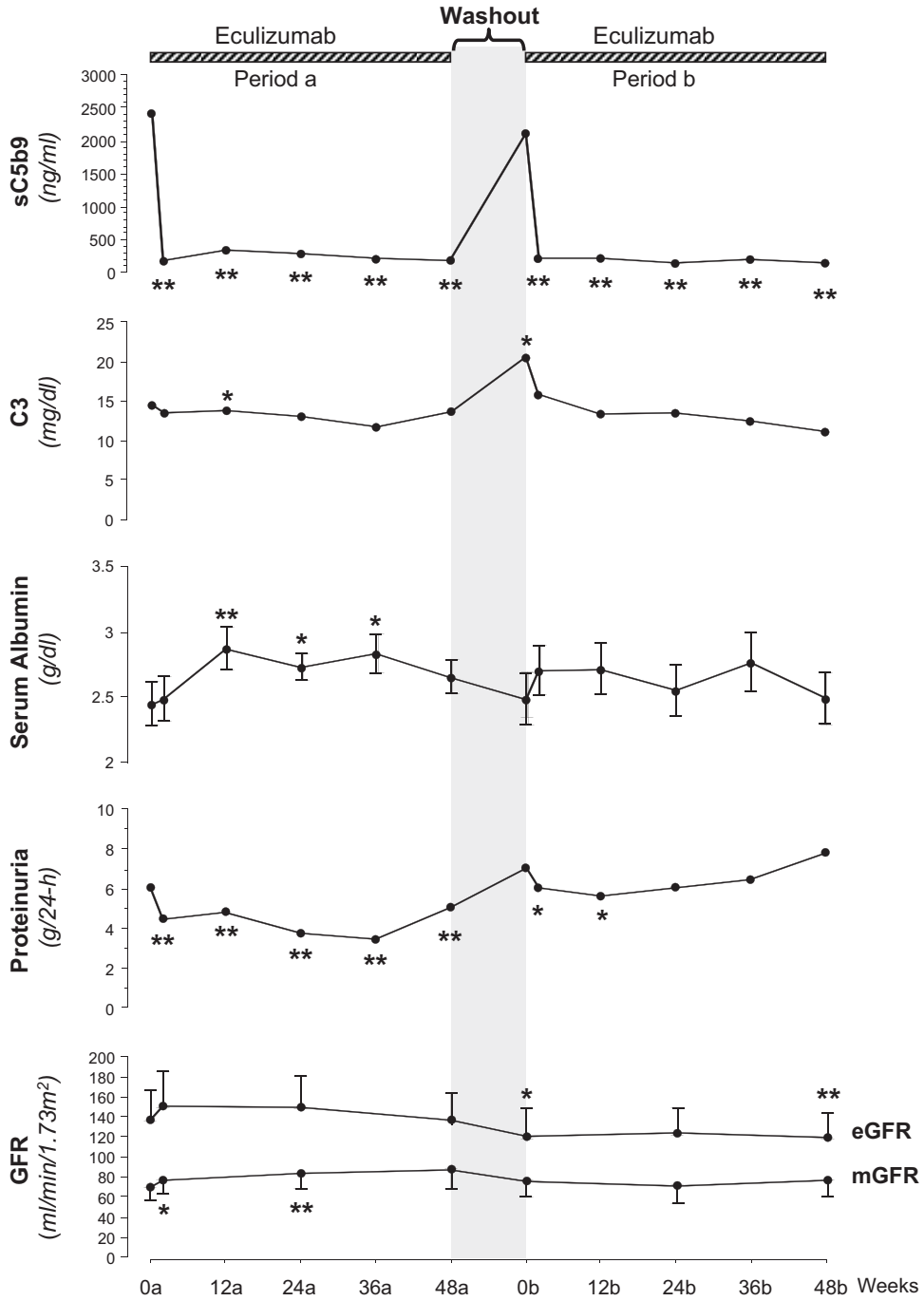

Figure 2. Changes over time in (first panel from the top) sC5b-9 (serum complement membrane attack complex) plasma levels; (second panel) C3 serum levels, (third panel) serum albumin concentration, (fourth panel) 24-hour proteinuria, and (fifth panel) measured (mGFR) and estimated (eGFR) glomerular filtration rate (GFR) during the first (period a) and the second 48-week treatment period (period b), which were separated by a 12-week washout period. Median plasma sC5b-9 levels were extremely elevated at inclusion, promptly and persistently normalized during the first treatment period, recovered toward baseline values at the end of the recovery period, and again normalized during the second treatment period. C3 median serum levels were persistently below normal range during both treatment periods, but transiently and significantly increased during the washout period as compared to baseline. Median proteinuria and mean (± SEM) serum albumin concentrations showed opposite trends (to decrease and to increase, respectively) during the first treatment period as compared to baseline. Then proteinuria increased toward baseline values at the end of the recovery period, whereas serum albumin levels only partially recovered toward baseline. Again, proteinuria significantly decreased during the initial 12 weeks of the second treatment period and was relatively stable thereafter. Serum albumin levels did not change appreciably. Mean (± SEM) measured GFR transiently increased during the first treatment period, recovered to baseline after the washout period and slightly declined thereafter. Mean (± SEM) eGFR showed a similar trend but largely overestimated true GFR throughout the whole study period. $^{*}P < 0.05$, $^{**}P < 0.01$ all versus baseline.

Table 3. Clinical and Laboratory Parameters During the Two Treatment Periods With Eculizumab (Weeks 0a-48a and 0b-48b) and the Washout Period (Week 48a to Week 0b)

	Wk 0a	Wk 1a	Wk 12a	Wk 24a	Wk 36a	Wk 48a	Wk 0b	Wk 12b	Wk 24b	Wk 36b	Wk 48b
Complement Markers											
sC5b-9, ng/mL	2,420 [1,915-3,330]	177 [114-207][a]	345 [264-396][a]	289 [225-377][a]	206 [132-418][a]	188 [147-262][a]	2,118 [1,723-2,635]	221 [161-238][a]	142 [119-252][a]	203 [139-312][a]	149 [128-194][a]
C3, mg/dL	14.5 [10.3-24.4]	13.5 [11.9-18.3]	13.8 [9.8-15.5][b]	13.1 [8.8-18.7]	11.7 [9.8-15.1]	13.8 [11.3-15.4]	20.6 [11.7-31.2][b]	13.4 [11.9-22.3]	13.5 [12.0-16.9]	12.5 [11.2-19.1]	11.1 [9.6-16.0]
C4, mg/dL	15.0 [10.3-29.4]	19.6 [12.5-26.5][b]	15.2 [14.2-28.6]	19.8 [14.6-26.4]	17.5 [12.1-23.2]	17.0 [12.9-23.2]	18.5 [14.3-26.4]	19.0 [15.0-27.8]	18.4 [13.6-27.5]	18.5 [12.8-27.1]	15.8 [11.3-17.9]
Clinical Parameters											
Body weight, kg	59.1 ± 15	58.3 ± 14	58.2 ± 12	58.1 ± 12	60.7 ± 11	59.6 ± 11	63.0 ± 10	60.8 ± 10	61.2 ± 10	60.9 ± 9	61.1 ± 9
BMI, kg/m^2	21.5 ± 3.3	21.2 ± 3.1	21.2 ± 2.7	20.9 ± 2.8	21.5 ± 2.7	21.3 ± 2.8	22.1 ± 2.8	21.5 ± 2.7	21.6 ± 2.5	21.5 ± 2.5	21.8 ± 2.6
Systolic BP, mm Hg	120.8 ± 14	115.8 ± 13[b]	116.8 ± 17	115.9 ± 16	114.3 ± 12[b]	118.4 ± 13	125.0 ± 16	119.2 ± 13	118.6 ± 11	120.2 ± 24	115.2 ± 14
Diastolic BP, mm Hg	74.8 ± 13	73.7 ± 12	73.7 ± 14	72.4 ± 10	70.6 ± 9	74.5 ± 15	77.5 ± 17	73.4 ± 12	75.4 ± 9	76.9 ± 16	71.4 ± 9
Laboratory Values											
Total chol, mg/dL	228.4 ± 30	231.9 ± 56	204.0 ± 33[b]	184.0 ± 43[a]	183.4 ± 27[b]	215.1 ± 83	241.7 ± 51	238.2 ± 59	221.2 ± 63	212.4 ± 65	214.3 ± 66
LDL chol, mg/dL	148.6 ± 39	148.0 ± 61	121.1 ± 38[b]	111.9 ± 50[a]	102.4 ± 31[b]	135.6 ± 76	146.7 ± 48	151.3 ± 59	140.7 ± 52	134.6 ± 52	134.4 ± 58
HDL chol, mg/dL	47.5 ± 13	55.2 ± 34	54.5 ± 20[b]	49.0 ± 13	53.3 ± 23	48.4 ± 16	48.3 ± 19	51.1 ± 24	42.8 ± 16	48.1 ± 26	48.7 ± 21
LDL:HDL ratio	3.45 ± 1.6	3.36 ± 1.7	2.56 ± 1.1[b]	2.53 ± 1.4[b]	2.40 ± 1.4[b]	3.26 ± 2.4	3.91 ± 2.3	3.76 ± 2.6	3.81 ± 2.0	3.29 ± 1.5	3.35 ± 2.0
TG, mg/dL[c]	103 [77-231]	107 [89-153]	112 [101-156]	113 [52-142]	118 [51-154]	109 [58-158]	113 [69-158]	114 [91-215]	118 [80-156]	119 [85-181]	85 [59-145]
Blood glucose, mg/dL	88.3 ± 10	74.4 ± 10 [a]	79.6 ± 7	93.5 ± 17	82.1 ± 6.8	87.7 ± 7.9	90.6 ± 7.2	79.8 ± 9.7	86.5 ± 9.1	78.0 ± 11.3	86.6 ± 6.4
Hb, g/dL	11.3 ± 1.7	11.3 ± 2.1	12.0 ± 1.6[b]	11.3 ± 1.9	12.1 ± 1.9[a]	11.7 ± 2.1	11.3 ± 1.8	11.9 ± 1.8[b]	11.6 ± 1.6	12.3 ± 1.8[a]	11.5 ± 1.7
[Ca], mg/dL[c]	8.34 ± 0.4	9.54 ± 3.7	7.94 ± 2.0	8.52 ± 0.3	7.77 ± 2.1	8.52 ± 0.3	8.26 ± 0.4	7.56 ± 2.0	8.31 ± 0.4	7.61 ± 2.0	8.28 ± 0.4
[PO_4^-], mg/dL[c]	5.46 ± 0.7	5.52 ± 0.5	4.65 ± 1.2	4.84 ± 0.5[b]	4.42 ± 1.2[b]	5.05 ± 0.7[b]	5.43 ± 0.9	4.23 ± 1.2[b]	5.08 ± 0.8	4.26 ± 1.2[a]	4.92 ± 0.6
[K], mEq/L[c]	4.71 ± 0.7	4.73 ± 0.5	4.86 ± 0.7	4.51 ± 0.4	4.63 ± 0.4	4.46 ± 0.3	4.83 ± 0.5	4.76 ± 0.6	4.50 ± 0.6	4.60 ± 0.5	4.56 ± 0.8
Protein, g/dL[c]	4.64 ± 0.8	4.90 ± 0.8	5.33 ± 0.5[a]	5.18 ± 0.4[b]	5.22 ± 0.6[b]	4.92 ± 0.6	4.64 ± 0.9	5.13 ± 0.7[b]	4.93 ± 0.9	5.20 ± 1.0	4.71 ± 0.9
Albumin, g/dL[c]	2.44 ± 0.5	2.47 ± 0.5	2.87 ± 0.5[a]	2.72 ± 0.3[b]	2.83 ± 0.5[b]	2.65 ± 0.4	2.48 ± 0.6	2.71 ± 0.6	2.55 ± 0.6	2.76 ± 0.7	2.48 ± 0.6

(Continued)

Table 3 (Cont'd). Clinical and Laboratory Parameters During the Two Treatment Periods With Eculizumab (Weeks 0a-48a and 0b-48b) and the Washout Period (Week 48a to Week 0b)

	Wk 0a	Wk 1a	Wk 12a	Wk 24a	Wk 36a	Wk 48a	Wk 0b	Wk 12b	Wk 24b	Wk 36b	Wk 48b
Kidney Function											
mGFR, mL/min/1.73 m^2	69.7 ± 35.2	76.5 ± 38.2[b]	—	83.3 ± 43.9[a]	—	87.4 ± 55.1	75.8 ± 42.7	—	71.2 ± 48.6	—	76.6 ± 44.1
eGFR, mL/min/1.73 m^2	136.8 ± 94.8	150.6 ± 113.2	—	149.4 ± 101.3	—	136.9 ± 87.1	120.0 ± 90.9[b]	—	123.5 ± 84.0	—	119.3 ± 75.5[a]
Proteinuria, g/24 h	6.03 [4.8-12.4]	4.47 [3.6-11.9][a]	4.83 [3.6-5.4][a]	3.74 [3.2-4.4][a]	3.43 [3.1-5.4][a]	5.06 [3.1-5.8][a]	7.02 [4.3-9.5]	5.60 [2.4-7.2][b]	6.06 [3.4-8.1]	6.43 [3.8-7.4]	7.79 [2.1-8.4]
Albuminuria, µg/min	3,199 [2,302-5,660]	2,609 [1,710-5,005]	2,961 [1,993-3,771]	1,920 [1,386-2,020][b]	1,921 [1,407-2,333]	2,685 [1,707-3,773]	2,839 [1,891-4,491]	1,513 [916-3,081]	3,391 [1,903-3,969]	3,112 [2,029-5,684]	3,461 [998-3,921]
Natriuresis, mEq/24 h	107 [93-171]	156 [137-167]	152 [113-167]	138 [124-162]	126 [110-158]	140 [109-164]	163 [132-173]	117 [101-142]	156 [96-197]	126 [115-165]	119 [99-152]
Phosphaturia, mg/24 h	[407-663]	651 [447-709]	535 [489-597]	607 [550-736]	579 [450-659]	508 [428-776]	608 [539-625]	553 [420-678]	544 [479-691]	561 [470-570]	570 [505-683]
Urinary urea, g/24 h	18.2 [13.6-22.2]	19.1 [16.2-22.0]	16.6 [14.0-18.5]	17.3 [15.1-22.9]	17.1 [11.5-20.5]	15.4 [14.0-20.5]	16.5 [16.2-17.3]	17.4 [14.7-19.4]	15.8 [14.2-19.2]	15.7 [15.6-17.6]	15.2 [14.4-17.1
Albumin fractional clearance, ×10^{-5} mL/min	238 [117-580]	126 [76-432][a]	—	85 [60-140][a]	—	171 [25 to 264][b]	243 [93 to 436]	—	252 [61 to 449]	—	352 [42 to 685]
IgG fractional clearance, ×10^{-5} mL/min	42.3 [22.6-195.8]	24.7 [13.4-144.5][a]	—	14.6 [12.7-28.4][a]	—	32.0 [4.9-68.1][b]	50.3 [16.9-148.5]	—	59.2 [12.4-137.7]	—	121.6 [6.4-320.6]

Note: Data are mean ± standard deviation or median [interquartile range].
Abbreviations: BMI, body mass index; BP, blood pressure; [Ca], calcium concentration; chol, cholesterol; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HDL, high-density lipoprotein; IgG, immunoglobulin G; [K], potassium concentration; LDL, low-density lipoprotein; mGFR, measured glomerular filtration rate; [PO_4^-], phosphate concentration; sC5b-9, serum complement membrane attack complex; TG, triglycerides.
[a]Paired t or signed rank test versus baseline (0a): $P < 0.01$;
[b]Paired t or signed rank test versus baseline (0a): $P < 0.05$;
[c]serum value.

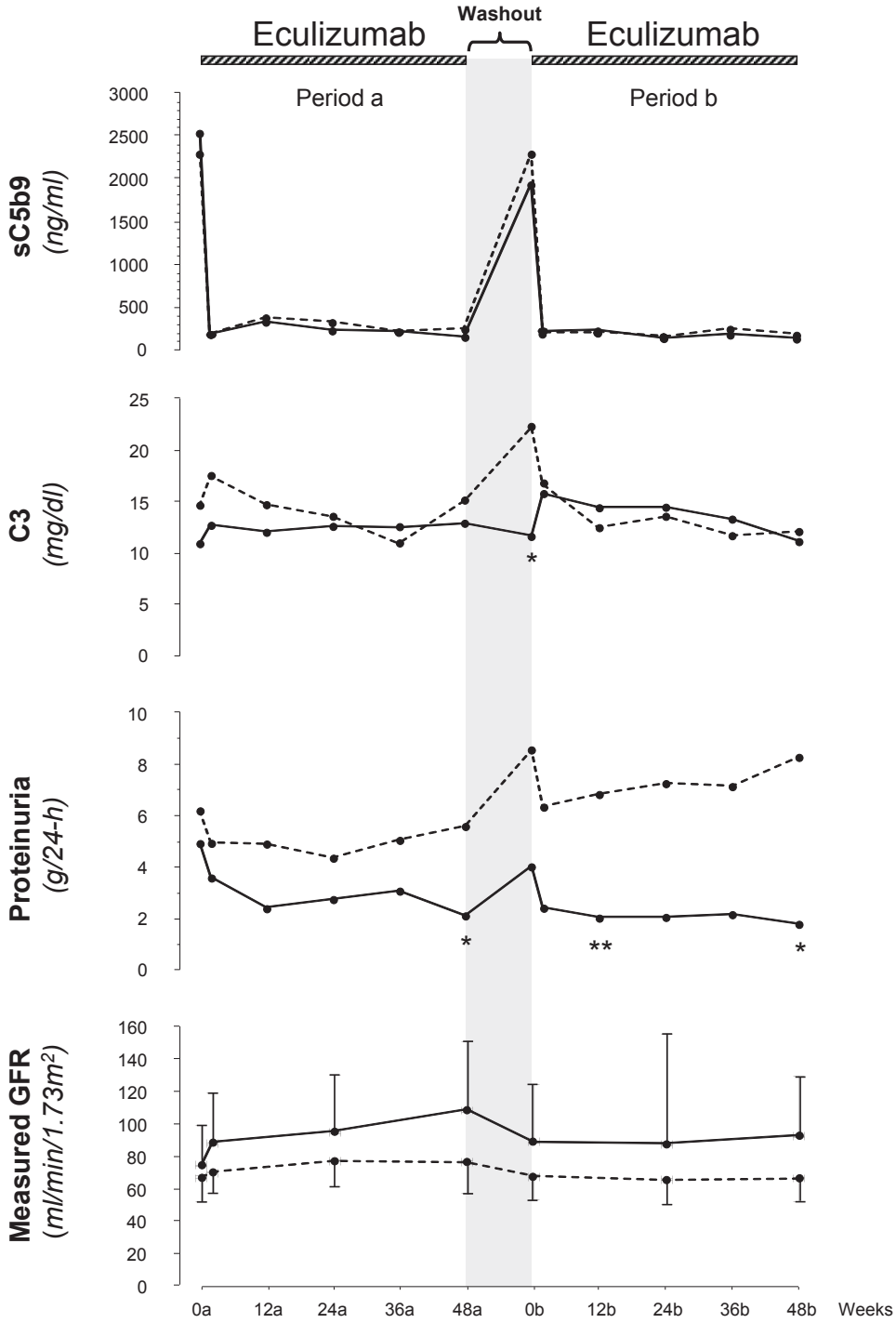

Figure 3. Changes over time in (first panel from the top) sC5b-9 (serum complement membrane attack complex) plasma levels, (second panel) C3 serum levels, (third panel) 24-hour urinary protein excretion, and (fourth panel) measured glomerular filtration rate (mGFR) during the first (period a) and the second 48-week eculizumab treatment period (period b), which were separated by a 12-week washout period, in 3 patients achieving partial remission at study end (continuous line) and 7 patients who did not achieve this end point (dashed line). In both groups, sC5b-9 median plasma levels were extremely elevated at inclusion, promptly and persistently normalized during the first treatment period, recovered toward baseline values at the end of the recovery period and again normalized during the second treatment period. In both groups, C3 median serum levels were persistently below normal range during both treatment periods. However, during the washout period, C3 levels did not change appreciably in patients who achieved partial remission and sharply increased in those who did not achieve this end point. Changes in C3 levels were significantly different between groups as compared to baseline. Median proteinuria showed an opposite trend: decreasing in patients achieving partial remission and increasing in those who did not achieve the end point. In both groups, proteinuria increased during the washout period. In both groups, (mean ± SEM) mGFR was relatively stable throughout the entire study period with a transient reduction during the washout period. *$P < 0.05$, **$P < 0.01$ between groups as compared to baseline (analysis of covariance).

in the 3 patients who achieved the end point and the 7 who did not are shown in Table S2 and Figure 3. In the 3 individuals who achieved partial remission, eculizumab therapy led to a significant reduction in 24-hour proteinuria at completion of the first treatment period as compared to baseline. Then proteinuria increased toward baseline during the washout period, after eculizumab treatment withdrawal. During the second treatment period, eculizumab reversed the trend of proteinuria increase. Proteinuria reduction remained significant at 12 weeks and at completion of the second treatment period as compared to baseline.

In 5 patients, complement-mediated hemolysis induced by 200 μg of IgG sampled from patient's plasma was >10%, and in 5, it was <10%. These patients were categorized as C3Nef negative and C3Nef positive, respectively. All 3 patients achieving partial remission were C3Nef negative, whereas 5 of the 7 who did not achieve this end point were C3Nef positive ($P = 0.2$). Mean GFR slope during the entire study period was positive in patients achieving partial remission and negative in those who did not achieve this end point (Table S1). In patients who did not achieve remission, there was a sharp increase in C3 levels during the washout period that predicted worsening of proteinuria during the second treatment period (Fig 3).

Safety

All patients completed the planned infusions. The patient who progressed to ESRD stopped eculizumab treatment at the first dialysis session. During 8 of 69 (11.6%) eculizumab infusions, there were acute reactions that recovered spontaneously and without sequelae. Acute chest pain and nausea ensued in 1 patient during the first infusion. The patient did not require hospitalization and the event was considered nonserious. Headache with blurred vision and transient temporal hemianopsia ensued in the same patient at week 20 of the first treatment period. The patient was hospitalized for 1 day and the event was categorized as serious. Five other episodes of headache, 1 with blurred vision, and 1 case of hypotension were observed during eculizumab infusion. All events were nonserious. During the washout period, 1 patient was hospitalized because of pneumococcal pneumonia associated with transient worsening of kidney function. The event was considered serious and treatment related. He recovered fully with antimicrobial therapy.

During the washout period, proteinuria increased in all patients. According to predefined protocol guidelines, resumption of treatment with eculizumab was accelerated in 3 patients because of severe worsening of proteinuria and GFR. They received the first eculizumab dose of the second treatment period after 2 (rather than 3) months of washout. In 2 of these patients, proteinuria and GFR promptly recovered to the values preceding treatment withdrawal. In the third, proteinuria and GFR did not recover fully. This patient subsequently progressed to ESRD over approximately 6 months.

Discussion

In 3 of the 10 patients with IC-MPGN or C3GN, nephrotic-range proteinuria, and strong activation of the terminal complement pathway, eculizumab therapy achieved a significant reduction in 24-hour proteinuria at completion of the first treatment period as compared to baseline. Among the 3 responders, proteinuria increased toward baseline after eculizumab treatment withdrawal during the washout period. Eculizumab administered during the second treatment period reversed the increase in proteinuria in the 3 responders, in whom proteinuria reduction was significant at 12 weeks and at completion of the second treatment period (study end) as compared to baseline. Two patients achieved partial remission of nephrotic syndrome at the end of both treatment periods despite a relapse in proteinuria during the washout. One additional patient achieved the end point at the end of the second treatment period. In the remaining 7 patients, eculizumab had no significant effect on proteinuria throughout the entire study period. One of these patients progressed to ESRD during the second treatment period.

Hypoalbuminemia and dyslipidemia ameliorated transiently during the first treatment period. However, these effects waned after treatment withdrawal. Body weight, body mass index, blood pressure, and 24-hour urinary sodium, phosphate, and urea excretion, as well as concomitant treatment with renin-angiotensin system inhibitors or statins, did not change significantly throughout the study. Thus, study findings were unlikely to be related to changes in diet or concomitant treatments. Of note, the finding that both albumin and IgG fractional clearances decreased significantly during the first treatment period and recovered toward baseline after eculizumab treatment withdrawal is consistent with improved selectivity of the glomerular barrier to plasma macromolecules.[33]

Unlike proteinuria, sC5b-9 plasma levels normalized promptly and fully during the first eculizumab treatment period, recovered toward baseline after eculizumab treatment withdrawal, and again promptly and persistently normalized during the second treatment period. C4 levels were in the normal range throughout the entire study period, whereas C3 serum levels were persistently reduced, with a transient increase during the washout period. We speculate that the transient increase in C3 levels during eculizumab treatment discontinuation could reflect either increased C3 synthesis by circulating leukocytes in response to inflammation[34] or reduced C3 consumption. The finding that proteinuria decreased in only 3 patients whereas sC5b-9 plasma levels were fully normalized by eculizumab in the entire study group is consistent with evidence that disease activity is only partially mediated by activation of the terminal complement pathway. Moreover, these data can be taken to suggest that other upstream

possibly C3-dependent pathways may play a central role in the pathogenesis of proteinuria in this context. The activation of these pathways might explain also the escape of some patients from the antiproteinuric effect of eculizumab despite persistent inhibition of sC5b-9 production, at least in the circulation.

Why eculizumab achieved a partial remission of nephrotic syndrome that was sustained up to study end in 3 patients, whereas no significant change in proteinuria was observed in the remaining 7 patients, remains elusive. Within the obvious limitation of comparisons between very small groups of patients, baseline characteristics of both subgroups did not appear to differ appreciably. However, all 3 patients achieving remission were C3Nef negative, whereas 5 of the 7 who did not achieve this end point were C3Nef positive. In addition, during the washout period, C3 levels decreased or did not change in the 3 patients who achieved remission whereas C3 level increase was observed only in the 7 patients who did not achieve this outcome. Thus, detectable C3Nefs appeared to be a risk factor for C3 level increase during the washout period and poor response to eculizumab during the second treatment period, whereas C3Nef-negative patients appeared to be to some extent protected from both events. Plasma SC5b-9 levels were extremely high at inclusion and were fully and persistently normalized by eculizumab treatment in all patients. Thus, the intensity of terminal complement pathway activation and efficiency of its inhibition in the circulation do not appear to explain different outcomes in different patients.

Immunostaining studies evaluating sC5b-9 deposition could probably help assess the pathogenic role of terminal complement activation (and consequent amplification of the C5a-mediated inflammatory process) at the tissue level and whether and to what extent different treatment effects on sC5b-9 deposition may account for the heterogeneous antiproteinuric effect of eculizumab that we observed in our study patients. These studies could also address whether activation of other upstream C3-convertase–dependent pathways that cannot be blocked by eculizumab may sustain kidney damage despite inhibition of the terminal complement pathway. Conceivably, these events could be amplified by C3Nefs because the stabilizing effect of these autoantibodies on the alternative pathway C3 convertase[35] would favor the deposition of extremely high amounts of C3b and its fragments in the kidney. A different pattern of histologic involvement could also predict treatment effect, as suggested by a recent retrospective analysis showing that in a large case series of patients with C3G, the benefit of eculizumab was most evident in those with significant extracapillary proliferation in the kidney biopsy specimen.[10,24]

However, independently of these considerations, when considering study participants as a whole, GFR was relatively stable for at least 2 years. This finding is robust because it was based on direct GFR measurements, rather than on less reliable serum creatinine–based GFR estimation equations.[36] Thus, at least in the time frame of the study, our patients appeared to be at least in part protected against the accelerated GFR loss that characterizes patients with MPGN and nephrotic syndrome.[37]

An ancillary finding of our present study was the wide difference between measured GFR and eGFR, in adults as well as in children. eGFR exceeded measured GFR by a wide margin (almost 2-fold in several cases), a finding that must be taken into consideration when eGFR values are considered to assess GFR and response to treatment in this context.[36] These data are consistent with previous evidence that serum creatinine–based GFR estimation equations greatly overestimate GFR measured using the inulin renal clearance technique in patients with hypoalbuminemia.[38] In 21 patients with serum albumin concentrations < 25.8 g/L, eGFR exceeded measured GFR by almost 60%, whereas in 21 patients with higher serum albumin levels, the overestimation was negligible.

Consistently, in our present study, we found that GFR overestimation inversely correlated with serum albumin levels and larger overestimation was observed in patients with more severe hypoalbuminemia. GFR overestimation is explained by reduced serum creatinine levels (because of increased urinary excretion) in patients with hypoalbuminemia as compared with those with normal serum albumin levels.[38] However, whether enhanced excretion is mediated by reduced tubular reabsorption, enhanced tubular secretion, or both remains elusive. Independent of involved mechanisms, in patients with nephrotic syndrome, as well as in other clinical settings,[39,40] eGFR cannot be considered as a reliable marker of kidney function and an estimate within the normal range should not be regarded as evidence of a normal GFR.[38]

Treatment was generally tolerated well. Acute but transient reactions were observed during 8 of the 69 eculizumab infusions. One event required hospitalization and was considered serious. Notably, 1 case of pneumococcal pneumonia was observed during the washout period and was most likely facilitated by terminal complement inhibition. This finding may suggest that antipneumococcal vaccination may be indicated in patients exposed to eculizumab therapy.

This was a single-arm study; however, this is usual for phase 2 proof-of-concept studies, particularly in rare diseases when it is impractical to include a placebo arm. Because of the exploratory nature of the study, all significant results require further studies that are adequately powered to confirm the findings.

The sample size was relatively small, which again reflected the rarity of the clinical condition under evaluation. Moreover, we focused on a rare subset of high-risk patients who were selected on the basis of heavy proteinuria and strong terminal complement activation. We used a sequential nonrandomized design, validated in particular in the context of rare diseases,[25,26] which allowed us to perform within-patient comparisons and obtain statistically meaningful results even without a

placebo-control arm. However, to our knowledge, this was the largest and longest trial of eculizumab treatment in MPGN reported to date. No patient with DDD happened to enter the study. This was not intentional but is most likely explained because this disease entity is seldom associated with strong activation of the terminal complement pathway.[12] There was no intention to perform any comparative analysis between patients with IC-MPGN and C3GN because response to eculizumab therapy was expected to be associated with the extent of terminal complement activation rather than MPGN type per se.[41] In our study, we focused exclusively on MPGN, which is one possible histologic pattern of C3G. Thus, whether study results can be generalized to all patients with C3G is uncertain because only 4 patients with C3GN and only those with MPGN were included. Moreover, unlike previous reports that included patients with heterogeneous characteristics (which may be likely to introduce statistical noise in data analyses), we selected a homogeneous population of patients expected to benefit the most from eculizumab therapy. Despite this approach, the results were to some extent disappointing, and treatment-induced persistent reduction in proteinuria could be observed in only 3 of the 10 patients included in the study. Whether screening for circulating C3Nefs might help identify patients who might benefit from eculizumab therapy in the long term (even despite transient treatment interruptions) may merit further investigation.

GFR was measured directly, and proteinuria, the primary efficacy variable, was evaluated centrally in 3 consecutive 24-hour urine collections to reduce variation due to random data fluctuations and imprecise urine collections. These methodological precautions, along with the sequential design, increased the statistical power of study analyses. All patients were on stable angiotensin-converting-enzyme inhibitor or angiotensin receptor blocker therapy and did not receive immunosuppression for at least 6 months before inclusion and throughout the entire observation period. Thus, the study findings were unlikely to have been confounded by the effect of concomitant medications.

Finally, despite the highly labor-intensive design and relatively demanding treatment that required repeated hospitalizations for intravenous eculizumab infusions, the study had a full retention rate of enrolled participants and good adherence to the study interventions.

In conclusion, in patients with IC-MPGN or C3GN and persistent nephrotic syndrome, response to eculizumab therapy differed widely in different cases despite similar activation of the terminal complement pathway. Detection of circulating C3Nefs and increasing C3 levels during treatment washout appeared to be associated with poor long-term response to eculizumab treatment, whereas C3NeF-negative patients with persistently reduced C3 levels appeared to benefit from eculizumab therapy even in the long-term. Conceivably, pathology studies evaluating the effect of eculizumab on the deposition of C5b-9, C3, and other complement components in renal tissue will help identify patients who might at least in part benefit from persistent C5 blockade.

Supplementary Material

Supplementary File 1 (PDF)

Item S1: Supplementary methods.

Table S1: GFR changes over time, overall and in the 3 patients who achieved remission vs the 6 who did not.

Table S2: Baseline characteristics of the 3 patients achieving partial remission during follow-up and the 7 patients who did not achieve this end point.

Supplementary File 2 (PDF)

EAGLE Protocol & Amendments.

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Data Sharing: Upon request the authors will provide access to individual participant data, which will be made available to study participants, further patients not enrolled in the present study, patients' associations, and researchers whose proposals meet methodologically sound research criteria to be discussed and shared with the study authors. Data may be requested up to 24 months after study publication. Requests for access to the study data can be submitted via e-mail to Dr Annalisa Perna (annalisa.perna@marionegri.it), head of the Laboratory of Biostatistics of the Department of Renal Medicine of the Istituto di Ricerche Farmacologiche Mario Negri IRCCS.

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