Item S1. EAGLE Supplementary Methods

Patients

Fourteen of the 205 patients with biopsy-proven MPGN referred to the Italian MPGN Registry at April 2014 had a secondary form of the disease, 17 were on peritoneal or extracorporeal dialysis, 33 had received a kidney transplant and 40 had C3 serum levels in normal range. Of the remaining 101 patients, 84 had proteinuria <3.5 g/24 hours (n=15), sC5b-9 plasma levels <1000 ng/ml (n=14) or both (n=55). Of the 17 potentially eligible patients, six were found to have proteinuria <3.5 g/24 hours and two serum sC5B9 levels <1000 ng/ml at baseline evaluation. Thus, nine of the patients identified from the Registry were eligible for study participation. One additional patient (from the United Kingdom) who had spontaneously referred to the CRC was found to fulfill all the selection criteria. Thus, ten patients entered the study. All of them completed the first 48-week treatment period and the washout period. One patient progressed to ESKD during the second 48-week treatment period. Thus nine patients completed the study.

Measurements

Blood for centralized measurement of sC5B9 plasma levels was collected on EDTA and centrifuged at 2000xg for 20 min at 4°C. Plasma was stored at - 20°C (till six months after the sampling) or at – 80°C. sC5b-9 levels were assessed by enzyme-linked immunoassay commercially available from Quidel (MicroVue SC5b-9 Plus). C3 and C4 levels were measured in sera obtained by blood collected without anticoagulant and centrifuged after 30 min clotting at room temperature. Other laboratory parameters were assessed by standard techniques. Urinary albumin concentration was measured by nephelometry.

The GFR was centrally determined at the laboratory of the CRC by using the iohexol plasma clearance technique and results were adjusted for body surface area. On the morning of renal function evaluation, 5 ml of iohexol solution (Omnipaque 300, GE Healthcare, Milan, Italy) were injected intravenously over 2 minutes. Venous blood was sampled at different times and iohexol plasma levels were measured by high-performance liquid chromatography. The clearance of iohexol was calculated according to a one-compartment model (CL_1) by the formula: CL_1=Dose/AUC, where AUC is the area under the plasma concentration-time curve. Plasma clearances were corrected by using the formula $CL = (0.990778 \times CL_1) - (0.001218 \times CL_1^2)$, and GFR values were normalized to 1.73 m^2 of body surface area (BSA). Mean intra-individual coefficient of variation and reproducibility index of repeated measurements in subjects with GFR ranging from near-terminal (pre-dialysis) to normal or even higher than normal (hyperfiltration) values, were 5.59% and 6.28%, respectively.

Genetic analysis

Targeted Next-generation sequencing for the genetic screening of all exons and flanking regions of *CFH, CD46*, *CFI*, *CFB*, *C3* and *THBD* genes was performed by highly multiplex PCR using the Ion AmpliSeq™ Library Kit 2.0 followed by template preparation and sequencing on an Ion PGM Sequencer as previously described.Variants fulfilling the following criteria were considered as mutations: 1. variants previously identified in patients with complement disorders and with functional assays supporting

variant pathogenicity; 2. variants with allele frequency ≤0.001 in any subpopulation of the ExAC database and CADD score ≥10.

Urine collections

Three consecutive 24-hour urine collections were performed during three consecutive days, thus no time elapsed between collections. Patients were asked to start collecting the urine in the morning and complete the collection in the morning of the following day for the three consecutive days. The last collection was completed the morning of the visit. They collected the urines in three containers (one container for each 24-hour collection) and at the time of the visit the three containers were collected by the investigators and proteinuria was measured in urine sampled from each container. The median of the three measurements in the three collections was recorded for statistical analyses.

Safety and Exploratory parameters

Safety parameters, including routine clinical and laboratory parameters, were also evaluated at reference centers every week during the eculizumab Induction Phase and every four weeks during the 44-week Eculizumab Maintenance Phase. Additional evaluations were allowed whenever deemed clinically appropriate, in particular for safety reasons. Additional plasma, serum and urine samples were collected and stored for exploratory analyses to be planned after study end on the basis of study results to investigate potential mechanisms mediating the effect of eculizumab treatment on different considered outcomes (Details Table. Biomarkers).

Eculizumab administration protocol for underage patients according to body weight

Body Weight	**Induction Phase**	**Maintenance Phase**
≥40 kg	900 mg weekly x 4	1200 mg at week 5; then 1200 mg every 2 weeks
30 - <40 kg	600 mg weekly x 2	900 mg at week 3; then 900 mg every 2 weeks
20 - <30 kg	600 mg weekly x 2	600 mg at week 3; then 600 mg every 2 weeks
10 - <20 kg	600 mg weekly x 1	300 mg at week 2; then 300 mg every 2 weeks
5 - <10 kg	300 mg Weekly x 1	300 mg at week 2; then 300 mg every 3 weeks

Table S1: GFR changes over time (ml/min/1.73m^2 per month) during the whole follow up period, the first and second treatment period and the wash-out period in the study group as a whole (Overall) and in the three patients who achieved remission and in the six patients who did not. In one patient the GFR was not measured by exhogenous iohexol because of reported history of allergy to contrast agents.

Periods	***Overall*** *(n=9)*	***Remission YES*** *(n=3)*	***Remission NO*** *(n=6)*
First Treatment	1.33 ± 0.64	2.44 ± 1.66	0.78 ± 0.49
Wash Out	-5.67 ± 2.01*	-6.25 ± 5.57	-5.38 ± 1.87*
Second Treatment	0.06 ± 0.44*	0.36 ± 1.37	-0.09 ± 0.27
Whole Follow up	-0.09 ± 0.23	0.51 ± 0.56	-0.39 ± 0.08

Data are means and std. errors. * $p<0.05$ vs the First Treatment Period

Table S2: Baseline characteristics of the three study patients (identification numbers: 001-05, 004-01 and 006-01) achieving partial remission during follow up and the seven patients who did not achieve this endpoint.

	Partial Remission *(n=3)*	No Partial Remission *(n=7)*
Demography and clinical characteristics		
Age *(years)*	21.1 ± 10.7	19.5 ± 5.7
Gender *(M/F)*	2/1	4/3
Weight *(Kg)*	45.7 ± 8.1	64.8 ± 13.1
BMI *(Kg/m^2)*	19.9 ± 3.7	22.2 ± 3.2
Systolic Blood Pressure *(mmHg)*	116.7 ± 20.1	122.6 ± 11.3
Diastolic Blood Pressure *(mmHg)*	69.0 ± 19.0	77.3 ± 10.1
Pulse Rate *(bpm)*	78.1 ± 4.4	68.6 ± 11.5
Laboratory Parameters		
Sc5b9 *(ng/ml)*	2542 [1916 to 8130]	2298 [1693 to 3331]
C3 *(mg/dL)*	10.9 [9.3 to 19.6]	14.6 [10.5 to 29.6]
Detectable Nefritic factor	0	5
Serum Creatinine *(mg/dL)*	0.87 ± 0.8	1.36 ± 1.1
Serum Albumin *(g/dL)*	2.6 ± 0.2	2.4 ± 0.6
Serum Proteins *(g/dL)*	4.8 ± 0.4	4.6 ± 0.9
Total Cholesterol *(mg/dL)*	212.3 ± 24.4	235.3 ± 30.7
HDL Cholesterol *(mg/dL)*	48.3 ± 22.4	47.1 ± 9.1
LDL Cholesterol *(mg/dL)*	136.0 ± 25.0	154.0 ± 43.8
Triglycerides *(mg/dL)*	99.0 [77.0 to 231.0]	107.0 [49.0 to 236.0]
Blood Glucose *(mg/dL)*	86.0± 5.6	89.3 ± 12.7
Hemoglobin *(g/dL)*	12.2 ± 2.3	10.8 ± 1.3
Serum Calcium*(mg/dL)*	8.5 ± 0.4	8.3 ± 0.4
Serum Phosphate*(mg/dL)*	5.9 ± 1.0	5.3 ± 0.5
Serum Potassium*(mEq/dL)*	4.9 ± 0.7	4.6 ± 0.8
Kidney function parameters		
Measured GFR (*mL/min/1.73m^2)**	74.9 ± 41.8	67.1 ± 35.5
Estimated GFR (*mL/min/1.73m^2)°*	179.5 ± 123.3	118.5 ± 84.3
Urinary Protein *(g/24h)*	4.95 [4.8 to 6.1]	6.19 [4.2 to 13.7]
	5.28 ± 0.7	8.74± 4.7
Urinary Albumin *(µg/min)*	2508 [2302 to 3198]	3334 [1981 to 6051]
	2669 ± 469	4032 ± 2137
Urinary Sodium *(mEq/24h)*	118.4 [93.7 to 171.5]	96.4 [67.6 to 224.4]
	127.9 ± 39.7	143.7 ± 96.6
Albumin Fractional Clearance	116.5 [85.7 to 580.1]	317.2 [133.8 to 727.0]
	260.7 ± 277.0	446.5 ± 401.3
IgG Fractional Clearance	22.6 [10.3 to 195.8]	79.5 [25.7 to 389.0]
	76.2 ± 103.8	170.4 ± 191.4

^ One patient borderline detectable * iohexol plasma clearance; ° CKD-EPI /(≥18 years) or Schwartz (1-17 years) equations. Data are means ± SD, median [IQR] or counts.