Combination of Rituximab, Low-Dose Cyclophosphamide, and Prednisone for Primary Membranous Nephropathy: A Case Series With Extended Follow Up

Reza Zonozi, Karen Laliberte, Noah R. Huizenga, Jillian K. Rosenthal, Anushya Jeyabalan, A. Bernard Collins, Frank B. Cortazar, and John L. Niles

Rationale & Objective: B-cell depletion with rituximab has emerged as a first-line therapy for primary membranous nephropathy (MN). However, most patients do not achieve complete remission with rituximab monotherapy. In this case series, we report longer-term remission and relapse rates, anti-phospholipase A_2 receptor (PLA_2R) antibody levels, B-cell levels, and serious adverse events in patients with primary MN who received rituximab combined with an initial short course of low-dose oral cyclophosphamide and a course of rapidly tapered prednisone.

Study Design: Single-center retrospective case series.

Setting & Participants: 60 consecutive patients with primary MN treated with the combination of rituximab, low-dose cyclophosphamide, and prednisone at the Vasculitis and Glomerulonephritis Center at the Massachusetts General Hospital.

Findings: After treatment initiation, median follow-up was 38 (interquartile range [IQR], 25-62) months; 100% of patients achieved partial remission, defined as a urinary protein-creatinine ratio (UPCR) < 3 g/g and a 50% reduction from baseline, at a median of 3.4 months. By 2 years after treatment initiation, 83% achieved complete remission, defined as a UPCR < 0.3 g/g. The median time to complete remission was 12.4 months. Immunologic remission (defined by an anti-PLA_2R titer < 14 RU/mL) was achieved by 86% and 100% of anti-PLA_2R seropositive patients (n = 29) at 3 and 6 months, respectively, after treatment initiation. After 1 year, the median UPCR fell from 8.4 (IQR, 5.0-10.7) to 0.3 (IQR, 0.2-0.8) g/g (P < 0.001). No patient relapsed throughout the duration of B-cell depletion. Relapse occurred in 10% of patients at 2 years after the onset of B-cell reconstitution following the last rituximab dose. Over a combined follow-up time of 228 patient-years, 18 serious adverse events occurred. One death occurred unrelated to treatment or primary MN, and 1 patient progressed to kidney failure requiring kidney replacement therapy.

Limitations: Absence of a comparison group.

Conclusions: All patients with primary MN treated with combination therapy achieved partial remission and most achieved a durable complete remission with an acceptable safety profile.

Complete author and article information provided before references.

Correspondence to R. Zonozi (rzonozi@yahoo.com)

Am J Kidney Dis. 78(6):793-803. Published online June 24, 2021.

doi: 10.1053/j.ajkd.2021.04.014

Primary membranous nephropathy (MN) is a kidney-limited autoimmune disease due to pathogenic immunoglobulin G (IgG) autoantibodies targeting endogenous podocyte antigens. The known podocyte target antigens include the phospholipase A_2 receptor (PLA_2R) and thrombospondin type 1 domain-containing 7A, among others.[1-4] The formation of in situ immune complexes in the subepithelial space leads to complement-mediated podocyte injury.[5] Clinically, the nephrotic syndrome ensues with its attendant risks of venous thromboembolism, cardiovascular disease, and immunodeficiency.

Editorial, p. 774

Targeting B cells with the anti-CD20 monoclonal antibody rituximab has emerged as a first-line therapy for primary forms of membranous nephropathy that are at risk for progression to kidney failure or have persistent nephrosis despite conservative therapy. One or two courses of rituximab monotherapy has demonstrated efficacy for a proportion of patients, but only a minority of patients achieve complete remission, and greater than one-third of patients have no remission.[6]

We conducted a single-center retrospective analysis of 60 consecutive patients with primary MN undergoing a treatment protocol based on rituximab-induced continuous B-cell depletion combined with an initial short course of low-dose oral cyclophosphamide and a rapid prednisone taper. We describe outcomes for these patients after use of this combination therapy.

Methods

Study Design and Patients

This is a retrospective analysis of 60 consecutive patients from July 6, 2009, to May 22, 2019, with primary MN treated at our Vasculitis and Glomerulonephritis Center at the Massachusetts General Hospital in Boston, MA, using our

PLAIN-LANGUAGE SUMMARY

Membranous nephropathy (MN) is an autoimmune disease resulting in the nephrotic syndrome. Patients are at an increased risk for progressive kidney injury, cardiovascular disease, thromboembolic disease, and infections. B-cell depletion with rituximab has emerged as a new first-line therapy. However, only a minority of patients achieve complete remission with rituximab monotherapy. We analyzed a series of 60 patients with primary MN whom we treated with rituximab combined with an initial short course of low-dose oral cyclophosphamide and a rapidly tapered course of prednisone. All patients achieved partial remission, and the majority achieved complete remission. All patients rapidly achieved immunologic remission. The relapse rate was low, and the safety profile was acceptable.

previously published combination regimen of rituximab, cyclophosphamide, and prednisone.[7] This report includes the 15 patients described in our initial report, with extended follow-up observation, and 45 additional patients who have been subsequently treated. The study was approved by the Massachusetts General Brigham Human Research Committee. The requirement for informed consent was waived by the ethics committee due to the retrospective nature of the study.

All patients were diagnosed with biopsy-proven MN or with the presence of the nephrotic syndrome accompanied by a positive serum anti-PLA_2R antibody. All patients met at least 1 of the following criteria before starting the combination therapy: (1) persistent urinary protein-creatinine ratio (UPCR) > 4 g/g for > 6 months despite treatment with an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker; (2) declining kidney function, defined as a 30% rise in serum creatinine from baseline attributed to MN; (3) debilitating or life-threatening symptoms due to the nephrotic syndrome; (4) recurrence of a UPCR > 4 g/g following remission after a prior immunosuppressive treatment; or (5) refractory disease, defined as failure of an alternative immunosuppressive regimen to induce a partial remission after at least 6 months of therapy. The nephrotic syndrome was defined as having a UPCR > 3.5 g/g, a serum albumin value < 3 g/dL, and peripheral edema. All patients in our consecutive series had at least 1 year of follow-up evaluation at the time of data analysis, and no patients were excluded because of loss to follow up, early discontinuation of treatment, an adverse event, or death. All patients underwent routine evaluation for secondary causes of MN. Patients with secondary MN were not included in this series.

Treatment Regimen

The treatment protocol of rituximab, cyclophosphamide, and prednisone for primary MN was defined by our group's standards of clinical care. The 3-drug regimen is outlined in Box 1. Rituximab was administered as two 1,000 mg intravenous (IV) doses separated by 2 weeks. Thereafter, rituximab was administered as one 1,000 mg IV dose every 4 months for 2 years with the aim of continuous B-cell depletion. The rituximab dosing interval was based on data demonstrating peripheral B-cell reconstitution begins 16 weeks after rituximab administration in a significant subset of patients.[8] B-cell depletion was defined as total $CD19^+CD20^+$ cell count < 5 cells/μL. Peripheral B-cell reconstitution was defined as total $CD19^+CD20^+$ cell count ≥ 5 cells/μL.

Concurrent with rituximab initiation, oral cyclophosphamide was administered at 2.5 mg/kg daily for 1 week, then 1.5 mg/kg daily for 7 weeks. The cyclophosphamide dose was adjusted for kidney function as outlined in Box 1. The cyclophosphamide dosing was not allowed to exceed 150 mg daily for the first week and 100 mg daily thereafter. The prednisone taper is also outlined in Box 1. Glomerular filtration rate (GFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[9] Minor modifications of the treatment regimen were permitted at the discretion of the treating physician.

Follow-up and Monitoring

The study follow-up period for all patients was defined as starting from initiation of combination therapy (date of

Box 1. Treatment protocol.

Rituximab
- 1,000 mg IV: days 1, 15; and months 4, 8, 12, 16, 20, 24

Cyclophosphamide[a,b]
- 2.5 mg/kg oral daily: week 1
- 1.5 mg/kg oral daily: weeks 2-8
- Stop

Prednisone
- 60 mg daily: week 1
- 40 mg daily: week 2
- 30 mg daily: week 3
- 20 mg daily: week 4
- 15 mg daily: weeks 5-8
- 12.5 mg daily: weeks 9-12
- 10 mg daily: weeks 13-16
- 7.5 mg daily: weeks 17-20
- 5 mg daily: weeks 21-24
- 2.5 mg daily: weeks 25-28
- Stop

Prophylaxis[c]

Abbreviations: eGFR, estimated glomerular filtration rate; IV, intravenous.
[a]The dose was adjusted for eGFR as follows: 10% dose reduction if eGFR was 60-90 mL/min/1.73 m^2, 25% if 45-59 mL/min/1.73 m^2, 33% if 30-44 mL/min/1.73 m^2, 40% if 15-29 mL/min/1.73 m^2, and 50% if <15 mL/min/1.73 m^2 or on dialysis.
[b]The dose of cyclophosphamide was not allowed to exceed 150 mg daily for the first week and 100 mg daily for the remaining 7 weeks.
[c]All patients received standard of care prophylaxis to *Pneumocystis* pneumonia, osteoporosis, and gastrointestinal ulceration that ended usually when prednisone finished.

the first rituximab dose) until the date of the last follow-up clinic visit during the study period. The schedule of clinic visits was typically at presentation, 1 week (first rituximab dose), 3 weeks (second rituximab dose), 2 months, 4 months, and then every 4 months during the active treatment phase. After the final rituximab dose, patients were seen every 4 to 6 months for monitoring. The following laboratory values were routinely checked at each clinic visit: complete blood count, comprehensive metabolic panel, spot urine total protein, and spot urine creatinine. A complete blood count was checked every 2 to 3 weeks while patients were receiving cyclophosphamide to monitor for leukopenia.

B-cell depletion was monitored by sending peripheral blood for flow cytometry prior to each rituximab infusion. Serum immunoglobulin levels were also checked before each rituximab infusion. Most patients were tested for autoantibodies to the M-type PLA_2R, which was performed on peripheral blood via enzyme-linked immunosorbent assay (ELISA) (Euroimmun) or from staining deposits on kidney biopsy samples for PLA_2R via immunofluorescence. ELISA findings were interpreted as follows: <14 RU/mL: negative; 14-20 RU/mL: borderline; and >20 RU/mL: positive.

Primary Outcome

The primary outcomes measured were attainment of partial remission and attainment of complete remission. Partial remission was defined as a UPCR < 3.0 g/g and a ≥50% reduction in the UPCR from baseline. Complete remission was defined as a UPCR < 0.3 g/g. All UPCRs were determined with a spot collection.

Secondary Outcomes

Secondary outcomes included the change in proteinuria, serum creatinine, serum albumin, total cholesterol, triglycerides, and serum IgG levels from baseline to 1 year after treatment initiation. Changes in serum anti-PLA_2R antibody titer and rates of relapse were also assessed. Immunologic remission was defined as a negative ELISA for serum anti-PLA_2R antibody (<14 RU/mL). Relapse was defined as a UPCR > 3 g/g after having achieved complete remission or partial remission. All relapses were reviewed to differentiate clinically insignificant fluctuations of UPCR values from clinical relapse.

Serious Adverse Events

Hospitalizations and life- or organ-threatening events are tracked in our clinic's treatment flowsheets. Details of the events were ascertained by the treating physician at each patient visit, including those involving hospitals other than our institution.

Statistical Analysis

Continuous variables are presented as median and interquartile range (IQR) or mean and standard deviation, as appropriate. Categorical variables are presented as frequencies and percentages. Baseline parameters were compared using the Wilcoxon rank-sum test or Fisher's exact test, as appropriate. Longitudinal differences in clinical parameters and anti-PLA_2R titers from baseline to various time points were analyzed with the Wilcoxon signed-rank test. Univariate logistic regression analysis was used to assess the odds ratio of achieving complete remission in seropositive patients with baseline PLA_2R titer as the independent variable.

Kaplan-Meier method was used to examine time to partial remission and time to complete remission, both starting from the date of the first rituximab infusion. Patients were censored on the date of their last follow-up visit if the primary outcome was not achieved during the study period. One competing event (death) occurred in 1 patient after attaining partial remission and before attaining complete remission. The analysis was stratified by disease status (initial presentation vs relapsing/refractory disease) and anti-PLA_2R status (associated vs not associated vs unknown). Differences between Kaplan-Meier curves were assessed using the log-rank test and Cox proportional hazards model. The Kaplan-Meier method was used to measure time to relapse. No relapse events occurred during treatment. Time 0 for relapse was measured from onset of B-cell reconstitution following the final rituximab dose, which represents treatment-free time. We considered remission to be durable if the patient had at least 2 years of relapse-free time since onset of B-cell reconstitution. All comparisons are 2-tailed, with $P < 0.05$ considered statistically significant. Analyses were carried out using STATA version 15 (StataCorp).

Results

Baseline Characteristics

Between July 6, 2009, and May 22, 2019, there were 60 consecutive patients with primary MN who were initiated on treatment with rituximab, cyclophosphamide, and prednisone (combination therapy). The median baseline UPCR was 8.4 (IQR, 5.0-10.7) g/g, and the median baseline serum creatinine and eGFR values were 1.17 (IQR, 0.93-1.61) mg/dL and 64 (IQR, 45-85) mL/min/1.73 m^2, respectively. Eighteen patients (30%) had a baseline eGFR of 30-60 mL/min/1.73 m^2, and 8 patients (13%) had a baseline eGFR < 30 mL/min/1.73 m^2. Baseline characteristics are summarized in Table 1.

Combining available biopsy and serological data, the primary MN was PLA_2R-associated in 44 patients (73%), non–PLA_2R-associated in 7 (12%), and unknown in 9 (15%). Baseline serum autoantibodies to PLA_2R were present in 29 patients of the 49 patients with available data. A kidney biopsy was performed in 56 patients, and the diagnosis was made serologically in the remaining patients. Colocalization of IgG and PLA_2R staining on deposits seen on immunofluorescence was

Table 1. Baseline Characteristics

Variable	Value
No. of patients	60
Age, y	59 [52-66]
Female sex	23 (38%)
Systolic BP, mm Hg	141 [124-150]
Diastolic BP, mm Hg	80 [70-86]
Serum creatinine, mg/dL	1.17 [0.93-1.61]
eGFR, mL/min/1.73 m^2	64 [45-85]
eGFR category	
>60 mL/min/1.73 m^2	34 (57%)
30-60 mL/min/1.73 m^2	18 (30%)
<30 mL/min/1.73 m^2	8 (13%)
UPCR, g/g	8.4 [5.0-10.7]
UPCR category	
<4 g/g	9 (15%)
4-8 g/g	17 (28%)
>8 g/g	34 (57%)
Nephrotic syndrome[a]	50 (83%)
Albumin, g/dL	2.7 [2.3-3.1]
Total cholesterol, g/dL	312 [248-389]
Triglycerides, mg/dL	188 [145-270]
Receiving ACEI or ARB	56 (93%)
Receiving statin	45 (75%)
Treatment indication	
Failure of ACEI or ARB[b]	30 (50%)
Decline of kidney function	17 (28%)
Debilitating symptoms from nephrotic syndrome	25 (42%)
Relapsing disease	19 (32%)
Refractory disease	9 (15%)
PLA_2R-associated disease	
Yes	44 (73%)
No	7 (12%)
Unknown	9 (15%)
Anti-PLA_2R antibody titer, RU/mL	160 [61-244]

Data are presented as median [interquartile range] and n (%). Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; eGFR, estimated glomerular filtration rate; PLA_2R, phospholipase A_2 receptor; UPCR, urinary protein-creatinine ratio.
[a]Nephrotic syndrome was defined as UPCR > 3.5 g/g, serum albumin < 3 g/dL, and peripheral edema.
[b]Failure of ACEI or ARB was defined as persistent UPCR > 4 g/g despite at least 6 months of therapy.

present in 22 kidney biopsies of the 25 samples that were tested.

Thirty-three (55%) patients received the combination therapy as an initial therapy for de novo disease. The remainder of patients (n = 27) received the combination therapy as a second-line therapy for relapsing or refractory disease after being referred from outside physicians who had previously attempted immunosuppressive treatment of the patient's primary MN. Sixteen patients (59%) out of 27 who received combination therapy as a second-line therapy and 12 patients (36%) out of 33 who received combination therapy as an initial therapy met more than 1 treatment indication as outlined in Methods. The most common treatment indication for patients receiving combination therapy as the initial therapy was failure of 6 months of an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker to result in a reduction of UPCR to < 4 g/g. The most common indication for patients receiving combination therapy as a second-line therapy was disease relapse (UPCR > 4 g/g). Twenty-seven patients had received prior immunosuppressive treatment. Six patients (22%) out of these 27 had received at least 2 different immunosuppressive treatment regimens before receiving the combination therapy. For patients treated with combination therapy for disease relapse, the median immunosuppression-free interval was 10 (IQR, 0-84) months. In all but 2 cases, the UPCR at initiation of combination therapy was greater than the UPCR 6 months prior (median difference: 5.9 [IQR, 2.9-8.0] g/g).

Primary Outcome

The median follow-up time was 38 (IQR, 25-62) months. 60 patients (100%) achieved partial remission during the study period. The median time to partial remission was 3.4 (IQR 1.0-7.2) months (Fig 1A). There was no difference in the time to partial remission when stratified by anti-PLA_2R status (log rank P = 0.3) (Fig 1B), nor in patients receiving combination therapy as initial treatment versus patients receiving combination therapy as second-line therapy (hazard ratio, 1.5 [95% CI, 0.9-2.6]; P = 0.1).

Of the 60 patients treated with the combination therapy, 49 patients achieved complete remission during the study period. By Kaplan-Meier analysis, the complete remission rate at 1, 2, and 3 years after treatment initiation was 45.0% (95% CI, 33.5%-58.4%), 82.7% (95% CI, 71.2%-91.5%), and 87.6% (95% CI, 76.6%-95.1%), respectively (Fig 1C). It took a median of 12.4 (IQR, 8.7-20.6) months to achieve complete remission. All 49 patients who achieved complete remission had a subsequent confirmatory UPCR < 0.3 g/g and normalization of serum albumin. There was no difference in the time to complete remission when stratified by anti-PLA_2R status (log rank P = 0.9) (Fig 1D), nor in patients receiving the combination therapy as their initial treatment versus patients receiving the combination therapy as a second-line therapy (hazard ratio, 1.0 [95% CI, 0.6-1.8]; P = 0.9).

The 11 out of 60 patients who failed to attain complete remission during the study period are outlined in Table S1. Despite not achieving complete proteinuric remission, all of the seropositive patients achieved immunologic remission. Multiple patients had pre-existing kidney damage and/or severely decreased kidney function at baseline, which highlights the possibility that their ongoing low-grade proteinuria reflects residual structural disruption from scar rather than active disease. This was exemplified in a patient who underwent a repeat kidney biopsy 20 months after initiating treatment with combination therapy to evaluate persistent low-grade proteinuria (Fig 2). The repeat biopsy demonstrated glomerular scarring and histologic remission of immune deposits.

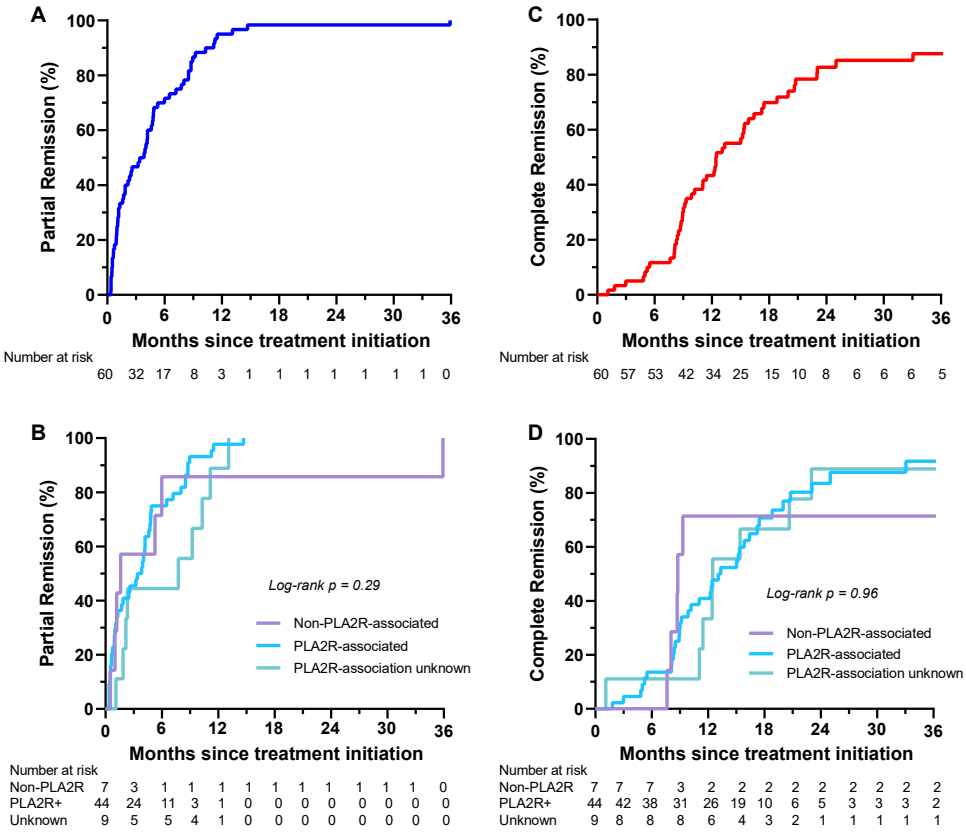

Figure 1. Kaplan-Meier curves for partial and complete remission. Kaplan-Meier curves for the overall group and when stratified by PLA_2R status are shown for partial remission (A and B) and complete remission (C and D). Abbreviation: PLA_2R, phospholipase A_2 receptor.

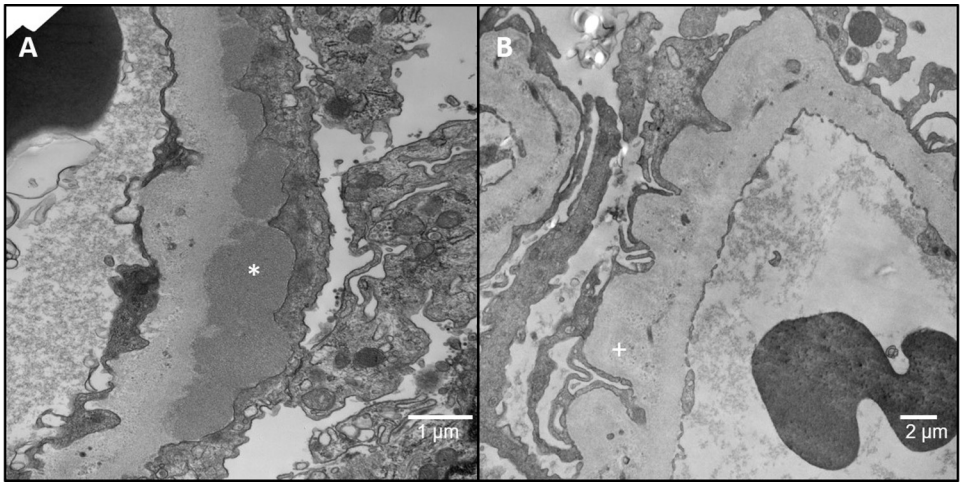

Figure 2. Kidney biopsy before and after treatment with combination therapy in one of the 11 patients (patient 6 from Table S1) who did not achieve complete proteinuric remission. (A) Amorphous electron-dense deposits (white asterisk) in the subepithelial location of the GBM before treatment. (B) The GBM 20 months after initiating treatment, no longer demonstrating electron-dense deposits. There are irregular electron-lucent zones where deposits formerly occupied (Ehrenreich-Churg stage IV resorptive changes), marked by the white plus sign (+). Both A and B depict glomerular scarring. This patient has residual ultrastructural disruption likely accounting for persistent low-grade proteinuria despite histologic remission of immune deposits. The immunofluorescence on repeat biopsy demonstrated negative staining for IgG and only irregular staining for C3 along the capillary wall (not pictured). Abbreviations: GBM, glomerular basement membrane; IgG, immunoglobulin G.

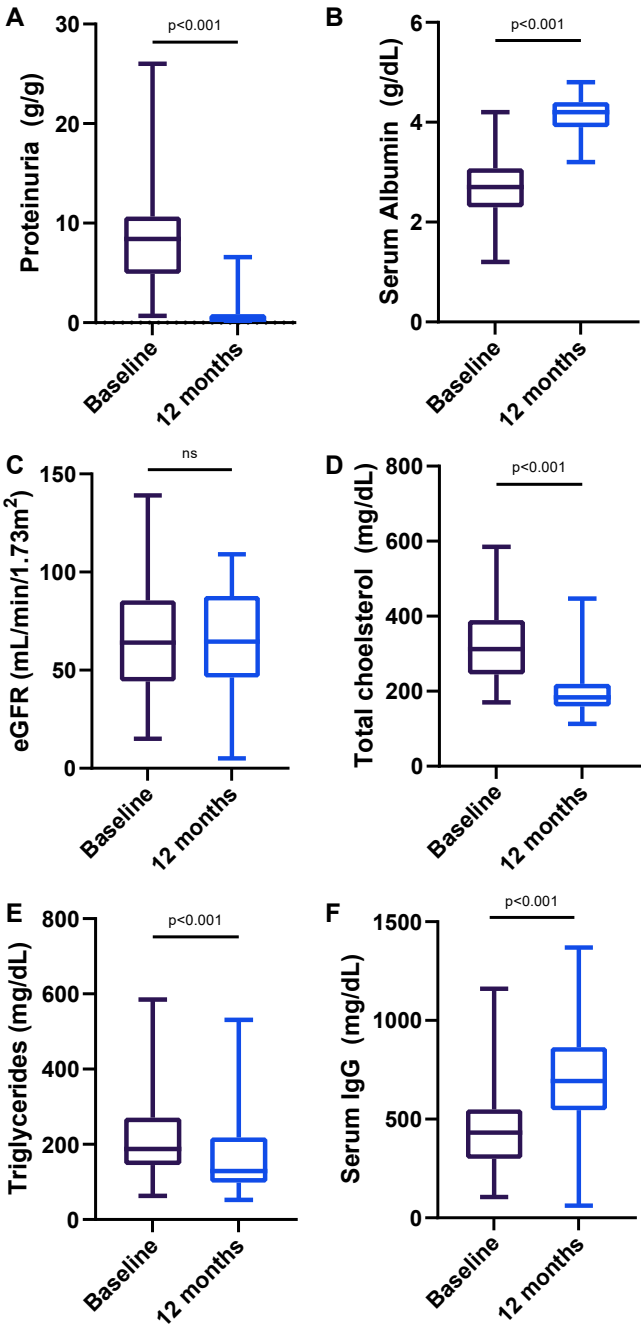

Figure 3. Early disease response at 12 months. Change in (A) proteinuria, (B) serum albumin, (C) eGFR, (D) total cholesterol, (E) triglycerides, and (F) serum IgG levels from baseline to 12 months. All box and whisker plots represent median (IQR) and the minimum-maximum range measurements. Longitudinal differences from baseline to 12 months were analyzed with the Wilcoxon signed-rank test. All differences were statistically significant except for the change in eGFR (C). The corresponding values for the median (IQR) of each parameter are listed in Table S2. The distributions of the differences in values between baseline and 12 months are depicted in Figure S1. Abbreviations: eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; IQR, interquartile range.

Secondary Outcomes

Nephrotic Syndrome Parameters

The response to treatment with combination therapy at 12 months is shown in Figure 3 and Table S2. The UPCR fell from a median of 8.4 (IQR, 5.0-10.7) g/g to 0.3 (0.2-0.8) g/g ($P < 0.001$). Overall, there was no change in serum creatinine and eGFR at 1 year. There was a significant rise in serum albumin and a significant decline in total cholesterol and triglycerides. Despite treatment with rituximab and cyclophosphamide, there was a significant rise in IgG levels (432 [IQR, 302-546] to 693 [IQR, 559-863] mg/dL; $P < 0.001$), which may be attributed to a reduction in urinary losses. The distributions of differences between baseline and 12 months are depicted in Figure S1.

Serum Anti-PLA_2R Titers

At 1, 3, and 6 months after initiating combination therapy, 64%, 86%, and 100% of patients who were anti-PLA_2R seropositive at baseline (n = 29) achieved immunologic remission, respectively. The median titer at baseline and 1, 3, 6, 9, 12, 18, and 24 months of treatment was 160.0 (IQR, 61.4-243.5), 6.3 (IQR, 0-61.0), 0.0 (0.0-6.9), 0.0 (IQR, 0.0-5.1), 0.0 (IQR, 0.0-2.0), 0.0 (IQR, 0.0-1.7), 0.0 (IQR, 0.0-0.0), and 0.0 (IQR, 0.0-0.0) RU/mL, respectively. All values were significantly different from baseline ($P < 0.001$) (Fig 4A and B).

The distributions of the differences from baseline are depicted in Figure S2. The proportion of patients who were PLA_2R seropositive at baseline who subsequently achieved complete clinical remission at 12, 18, and 24 months of follow-up was 34% (10/29), 50% (11/22), and 74% (14/19), respectively (Fig S3). In a univariate logistic regression model, the baseline anti-PLA_2R level was not associated with an increased odds of achieving complete remission in seropositive patients receiving combination therapy (odds ratio per 100 RU/mL, 0.94 [95% CI, 0.74-1.18]).

B Cells

Continuous B-cell depletion was achieved with 59 out of 60 patients during the first year of treatment and 100% of patients during the second year of treatment (Fig 5). The one patient who did not maintain continuous B-cell depletion during the first year had B-cell return before the 4-, 8-, and 12-month infusions. By study closure, the 24-month treatment protocol of scheduled rituximab infusions was finished in 47 patients, 37 of which subsequently reconstituted their circulating B cells during the study period. In these 37 patients, the median treatment-free follow-up time since the first recorded B-cell return was 18.4 (IQR, 9.9-37.3) months.

Relapse

No patient relapsed while receiving treatment—that is, throughout the duration of B-cell depletion. All relapses

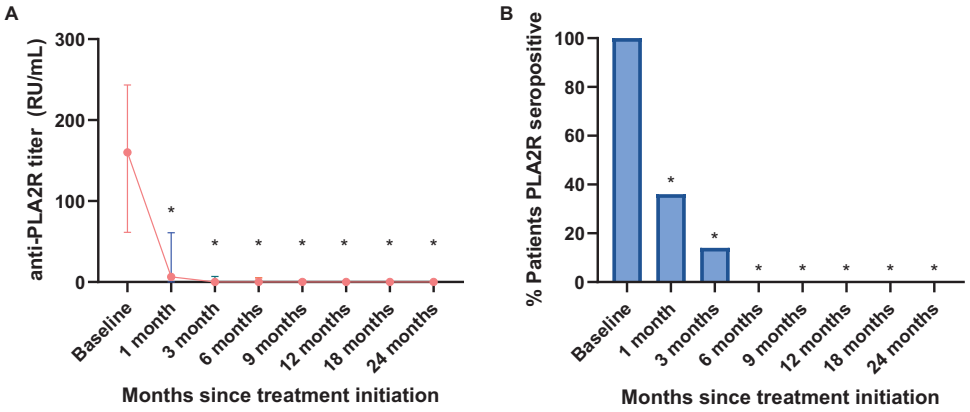

Figure 4. Anti-PLA_2R titers with treatment. (A) There is a significant decline in titer of anti-PLA_2R antibodies over time compared to baseline (time 0 versus 1, 3, 6, 9, 12, 18, and 24 months, all $P < 0.01$, represented by asterisk, as assessed by Wilcoxon signed-rank test). The dot represents median and the line represents the IQR. (B) Among patients who had detectable circulating anti-PLA_2R levels, the proportion who remained seropositive (defined by ≥ 14 RU/mL) over time. Asterisk represents a statistically significant change in proportion compared to baseline, as assessed using 95% confidence intervals of the mean. After baseline, data on anti-PLA_2R levels were available on 14, 14, 21, 11, 20, 23, and 17 patients at 1, 3, 6, 9, 12, 18, and 24 months, respectively. The distributions of the differences are depicted in Figure S2. ELISA reference ranges: negative, <14 RU/mL; borderline, 14-20 RU/mL; positive, > 20.0 RU/mL. Abbreviations: ELISA, enzyme-linked immunosorbent assay; IQR, interquartile range; PLA_2R, phospholipase A_2 receptor; RU, relative units.

(n = 4) occurred after onset of peripheral B-cell reconstitution following the last scheduled rituximab infusion. The 4 patients relapsed at 55.0, 27.8, 24.2, and 12.4 months after B-cell reconstitution, which occurred over a combined treatment-free follow-up time of 83.4 patient-years. By Kaplan-Meier analysis, the relapse rate was 10% by 2 years after B-cell reconstitution (Fig 6). At the time of relapse, B cells had accounted for 16.7% (246 cells/μL), 13.9% (242 cells/μL), 10.5% (226 cells/μL), and 18.7% (323 cells/μL) of the lymphocyte pool,

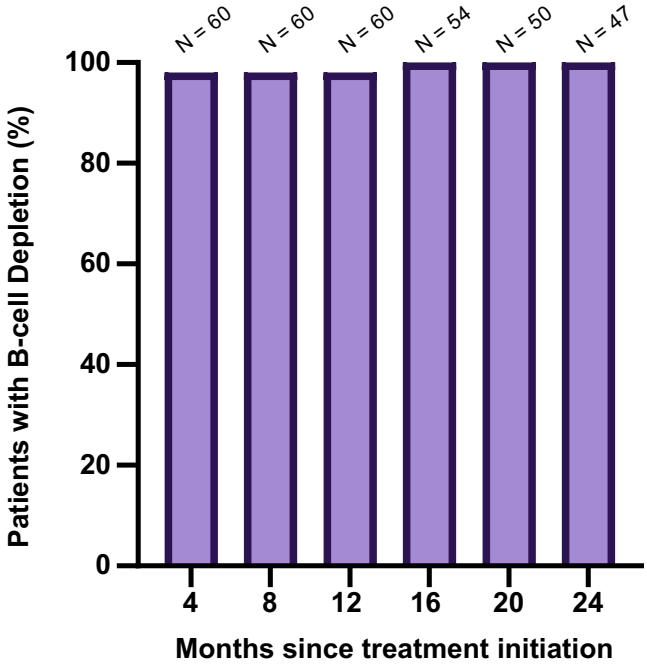

Figure 5. B-cell depletion during treatment. Bars demonstrate percentage of patients with undetectable $CD19^+CD20^+$ lymphocytes. One patient had full B-cell return before each infusion in the first year of treatment. The remaining patients maintained continuous B-cell depletion.

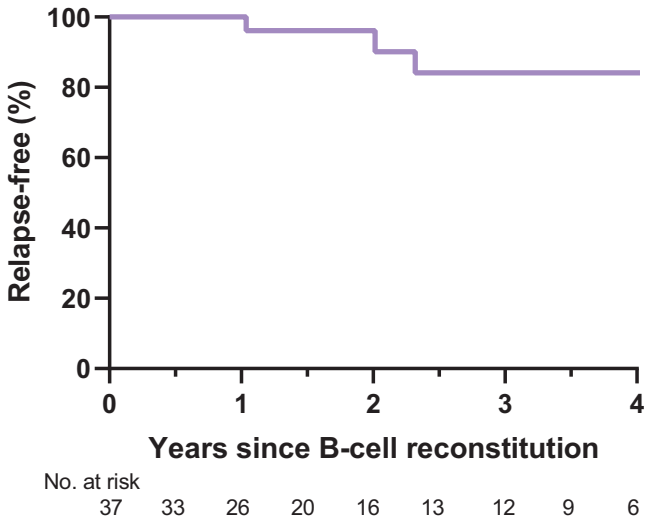

Figure 6. Kaplan-Meier curve for relapse-free survival. Relapse was defined by a urinary protein-creatinine ratio > 3.0 g/g after having achieved complete or partial remission. No relapses occurred during treatment (ie, during the period of B-cell depletion). Time to relapse was measured beginning with the onset of B-cell reconstitution following the final rituximab infusion, which represents treatment-free time.

respectively. All 4 patients had PLA_2R-associated disease, and all but 1 relapse was preceded by a significant rise in their serum anti-PLA_2R antibody titer.

After relapse, 1 patient received rituximab using the same dosing protocol without additional induction agents, while the other 3 were restarted on full combination therapy. At the last follow-up visit, 2 patients are in partial remission (achieved at 0.4 and 4.6 months) and 2 patients are in complete remission (achieved at 5.4 and 6.3 months). Finally, no additional relapses were observed when the definition of relapse was lowered to a UPCR > 1 g/g after achieving complete remission.

Serious Adverse Events

Serious adverse events (SAEs) are listed in Table 2. Over a combined follow-up time of 228 patient-years, 18 SAEs were identified. Hospitalization occurred in 17 out of 18 SAEs. The most common SAE was late-onset neutropenia from rituximab (n = 5). One patient progressed to kidney failure that required kidney replacement therapy; the patient's eGFR at treatment initiation was 13 mL/min/1.73 m^2. One death occurred from end-stage heart failure, which was unrelated to the treatment or nephrotic syndrome, and it occurred after partial remission and before complete remission. No patient developed malignancy during the study period.

Discussion

In this retrospective, single-center analysis of 60 consecutive patients with primary MN, we examined outcomes after a treatment regimen consisting of rituximab administered over a 2-year period, combined with an initial short course of low-dose oral cyclophosphamide and a rapid prednisone taper (combination therapy). First, we found all patients achieved partial remission. Second, we found most patients (83%) achieved complete remission by 2 years. To our knowledge, these are the highest rates of remission reported to date. Third, we observed a rapid depletion of circulating anti-PLA_2R antibodies in seropositive patients—specifically, all patients achieved immunologic remission by 6 months after starting the combination therapy. Fourth, we observed a durable response, as all patients remained relapse-free throughout the duration of B-cell depletion and 90% of patients remained relapse-free at 2 years after onset of B-cell reconstitution following the final rituximab dose. Finally, we found an acceptable safety profile.

Table 2. Serious Adverse Events

Adverse Event	No. of Events
Late-onset neutropenia	5
Viral upper respiratory infection	3
Community acquired pneumonia	2
Congestive heart failure (fatal)	1
Septic arthritis	1
Retropharyngeal infection	1
Clostridium difficile colitis	1
Acute diverticulitis	1
Gastrointestinal bleed	1
Noninfectious diarrhea	1
Acute liver injury	1

We report 100% of patients with primary MN receiving combination therapy achieved partial remission, and 83% of patients achieved complete remission at 2 years. Our findings compare favorably with those of prior studies assessing B-cell depletion therapy in primary MN. In the MENTOR trial, partial remission and complete remission were attained by 60% and 35% of patients, respectively, in the rituximab arm at 2 years.[6] Rituximab was dosed as two 1,000-mg IV infusions, given 2 weeks apart, and repeated once at 6 months if there was a partial clinical response, defined as a reduction in proteinuria by at least 25%, regardless of B-cell count. Notably, most patients had B-cell return at 6 and 12 months. In the GEMRITUX trial, which used 2 weekly rituximab IV doses of 375 mg/m^2, 65% and 19% of patients eventually reached partial remission and complete remission, respectively, in the postrandomized control trial observation period.[10] Similarly, most patients had detectable circulating B cells at 6 months. By contrast, nearly all patients in our series had undetectable circulating B cells throughout the entire treatment phase. Our fixed-schedule rituximab dosing strategy aims to maintain undetectable circulating B cells. The rationale for continuous depletion of B cells is to suppress autoreactive B cells from repopulating the lymphocyte pool, which may contribute to ongoing immunologic activity and limit the clinical response.[11]

The speed and magnitude of decline in anti-PLA_2R levels predicts achievement of clinical remission.[12] Our treatment strategy demonstrates a precipitous depletion of circulating anti-PLA_2R antibodies. We observed 86% and 100% of patients to have achieved immunologic remission at 3 and 6 months after initiating combination therapy, respectively, which is consistent with prior studies evaluating cyclophosphamide-based regimens. In the STARMEN trial, the rate of immunologic remission at 3 and 6 months in the cyclophosphamide-glucocorticoid arm was 77% and 92%, respectively.[13] Another report found nearly all patients treated with cyclophosphamide achieved immunologic remission at 6 months, independent of the baseline anti-PLA_2R level.[14] By contrast, rituximab monotherapy demonstrated significantly lower rates of immunologic remission, particularly in the setting of a high baseline anti-PLA_2R level (greater than 152 RU/mL).[14] In the MENTOR and GEMRITUX trials, rituximab monotherapy led to immunologic remission in approximately half of patients at 6 months.[6,10] In aggregate, these findings suggest that anti-PLA_2R antibody levels will decline at a faster rate in patients receiving cyclophosphamide and glucocorticoids. These drugs directly attenuate autoantibody-secreting cells (ie, plasma cells and

plasmablasts), which do not express the cell surface marker CD20 and thereby are not immediately impacted by rituximab.[15]

We did not observe relapses during the active treatment phase (ie, during the period of B-cell depletion). We observed 10% of patients to have sustained a relapse at 2 years after onset of B-cell reconstitution following the last scheduled rituximab infusion. The earliest relapse was 12.4 months after B-cell reconstitution, which was 4.4 years after initiating therapy. By comparison, in patients receiving an alkylating agent-based regimen, the relapse rate approached 30% by 2.5 years.[16] Moreover, in calcineurin inhibitor–based regimens, almost half of patients relapse after drug withdrawal.[17] On the other hand, compared with rituximab-based regimens, our results are similar.[6,18-20] For example, in the MENTOR trial, 5.1% of patients in the rituximab arm experienced a relapse at 2 years from starting rituximab.[6]

While we observed favorable remission and relapse rates with our combination therapy, the potential risks of the therapy warrant further discussion. A major limitation to cyclophosphamide and glucocorticoid use in primary MN is the associated toxicity. A study primarily evaluating safety outcomes found higher rates of adverse events in patients receiving cyclophosphamide and glucocorticoids compared with rituximab monotherapy in primary MN.[21] Furthermore, another study estimated a 3-fold higher incidence rate of malignancy in patients with primary MN treated with cyclophosphamide compared with those treated without cyclophosphamide.[22] Notably, these studies evaluated a cyclophosphamide regimen involving oral dosing at 1.5 mg/kg daily typically for 12 months, which represents a cumulative dose of approximately 38 g in a 70 kg individual. The regimen also included about 5 months of treatment with high-dose glucocorticoids. For comparison, the standard cytotoxic-based regimen endorsed by current KDIGO guidelines (the "Ponticelli regimen") uses a cumulative dose of cyclophosphamide of 15.75 g over 3 months and a cumulative dose of prednisone-equivalence of approximately 14.77 g over 3 months in a 70-kg individual with normal kidney function.[23] By contrast, our treatment protocol in the present study for the same patient would use a cumulative cyclophosphamide dose of 5.95 g over a 2-month duration. This is below the dosing range generally associated with gonadal toxicity, bladder toxicity, and malignancies, none of which we observed in our series.[24-26] Moreover, our protocol for the same patient exposes the individual to a total dose of 2.63 g of prednisone and to high doses (≥20 mg daily) for less than 5 weeks.

Furthermore, with regard to overall safety, we observed 7.9 SAEs per 100 patient-years. For reference, in the MENTOR trial, 10 and 17 SAEs per 100 patient-years were observed in the rituximab arm and cyclosporine arm, respectively.[6] In the STARMEN trial, no significant difference was found in the rate of SAEs between the patients in the cyclophosphamide-glucocorticoid arm compared with the patients in the tacrolimus-rituximab arm.[13] The toxicity of treatment needs to be weighed against the risks of not achieving remission with less effective treatment. We partly attribute the acceptable safety outcomes with our combination therapy to early disease control, which reduces the infectious, cardiovascular, and thromboembolic complications of uncontrolled nephrosis. However, the most common SAE we observed in our series was late-onset neutropenia from rituximab.[27] Furthermore, although the combination regimen appeared well tolerated during the observation period, we cannot exclude the possibility that treatment-related complications will later develop. Therefore, future studies should evaluate longer-term effects of this treatment combination, ideally comparing them to a control group. Lastly, there is the inherent uncertainty of a retrospective study for detecting outside adverse events that may not have been noted during our patient visits.

Our protocol involves fixed-schedule dosing of rituximab for 2 years after which any further dosing is individually tailored to serologic or clinical relapse. For seropositive patients, an alternative approach of individually tailoring rituximab to serologic response from the outset of treatment requires future studies. Factors such as cost, safety, and patient convenience need to be studied in the context of the consequences of incomplete remission, relapse, and cumulative exposure to immunosuppression. Furthermore, the 3 specific immunosuppressive drugs in our protocol target different levels of the immune system implicated in the pathogenesis of membranous nephropathy; our data do not discern whether the same results would have been obtained without any one aspect of the protocol.

Our study has several strengths and weaknesses. The greatest strengths are the large sample size of patients with primary MN, the long duration of follow-up time, and the inclusion of patients with severe kidney disease. The main weaknesses are inherent to data collection in retrospective studies, data derived from a single center, and absence of a comparison group. Finally, all patients in our series exhibited risk factors for progressive disease, thus making it unlikely that our results were confounded appreciably by spontaneous remission.

In conclusion, we propose the combination of rituximab, low-dose cyclophosphamide, and prednisone as a multipronged approach in the treatment of patients with primary MN who are at increased risk for progression to kidney failure or have persistent nephrosis despite conservative therapy. Further studies directly comparing this combination therapy to established treatments are needed.

Supplementary Material

Supplementary File (PDF)

Figure S1: Distribution of differences of clinical parameters between baseline and 12 months.

Figure S2: Distribution of differences of PLA_2R titers from baseline to 6, 12, and 24 months.

Figure S3: Complete clinical remission in patients seropositive for PLA_2R at baseline.

Article Information

Authors' Full Names and Academic Degrees: Reza Zonozi, MD, Karen Laliberte, RN, Noah R. Huizenga, BA, Jillian K. Rosenthal, MSN, Anushya Jeyabalan, MD, A. Bernard Collins, BS, Frank B. Cortazar, MD, and John L. Niles, MD.

Authors' Affiliations: Vasculitis and Glomerulonephritis Center, Division of Nephrology (RZ, KL, NRH, JKR, AJ, JLN), and Department of Pathology (ABC), Massachusetts General Hospital, Boston, Massachusetts; Harvard Medical School, Harvard University, Boston, Massachusetts (RZ, AJ, ABC, JLN); New York Nephrology Vasculitis and Glomerular Center, Albany, New York (FBC).

Address for Correspondence: Reza Zonozi, MD, Vasculitis and Glomerulonephritis Center, Division of Nephrology, Massachusetts General Hospital, 101 Merrimac St, Boston, MA 02114. Email: rzonozi@yahoo.com

Authors' Contributions: Research idea and study design: RZ, FBC, JLN; data acquisition: RZ, KL, NRH, JKR, AJ, ABC, FBC, JLN; data analysis/interpretation: RZ, FBC, JLN; statistical analysis: RZ; supervision: JLN. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The institutions had no role in study design; collection, analysis, and interpretation of data, writing the report, and the decision to submit the report for publication.

Financial Disclosure: The authors declare that they have no relevant financial interests.

Acknowledgements: The authors wish to thank the clinic personnel dedicated to the care of the patients at the MGH Vasculitis and Glomerulonephritis Clinic.

Peer Review: Received September 25, 2020. Evaluated by 2 external peer reviewers, with direct editorial input from a Statistics/Methods Editor, the Pathology Editor, an Associate Editor, and the Editor-in-Chief. Accepted in revised form April 18, 2021.

References

1. Beck LH Jr, Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*. 2009;361(1):11-21.
2. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med*. 2014;371(24):2277-2287.
3. Sethi S, Debiec H, Madden B, et al. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. *Kidney Int*. 2020;97(1):163-174.
4. Sethi S, Debiec H, Madden B, et al. Semaphorin 3B–associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. *Kidney Int*. 2020;98(5):1253-1264.
5. Cybulsky AV, Quigg RJ, Salant DJ. Experimental membranous nephropathy redux. *Am J Physiol Renal Physiol*. 2005;289(4):F660-F671.
6. Fervenza FC, Appel GB, Barbour SJ, et al. Rituximab or cyclosporine in the treatment of membranous nephropathy. *N Engl J Med*. 2019;381(1):36-46.
7. Cortazar FB, Leaf DE, Owens CT, Laliberte K, Pendergraft WF, Niles JL. Combination therapy with rituximab, low-dose cyclophosphamide, and prednisone for idiopathic membranous nephropathy: a case series. *BMC Nephrol*. 2017;18(1):44.
8. Breedveld F, Agarwal S, Yin M, et al. Rituximab pharmacokinetics in patients with rheumatoid arthritis: B-cell levels do not correlate with clinical response. *J Clin Pharmacol*. 2007;47(9):1119-1128.
9. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.
10. Dahan K, Debiec H, Plaisier E, et al. Rituximab for severe membranous nephropathy: a 6-month trial with extended follow-up. *J Am Soc Nephrol*. 2017;28(1):348-358.
11. Chamberlain N, Massad C, Oe T, Cantaert T, Herold KC, Meffre E. Rituximab does not reset defective early B cell tolerance checkpoints. *J Clin Invest*. 2016;126(1):282-287.
12. Ruggenenti P, Debiec H, Ruggiero B, et al. Anti-phospholipase A2 receptor antibody titer predicts post-rituximab outcome of membranous nephropathy. *J Am Soc Nephrol*. 2015;26(10):2545-2558.
13. Fernández-Juárez G, Rojas-Rivera J, van de Logt A-E, et al. The STARMEN trial indicates that alternating treatment with corticosteroids and cyclophosphamide is superior to sequential treatment with tacrolimus and rituximab in primary membranous nephropathy. *Kidney Int*. 2021;99(4):986-998.
14. Van de Logt A-E, Dahan K, Rousseau A, et al. Immunological remission in PLA2R-antibody–associated membranous nephropathy: cyclophosphamide versus rituximab. *Kidney Int*. 2018;93(4):1016-1017.
15. Oleinika K, Mauri C, Salama AD. Effector and regulatory B cells in immune-mediated kidney disease. *Nat Rev Nephrol*. 2019;15(1):11-26.
16. Ponticelli C, Altieri P, Scolari F, et al. A randomized study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 1998;9(3):444-450.
17. Cattran DC, Appel GB, Hebert LA, et al. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int*. 2001;59(4):1484-1490.
18. Fervenza FC, Abraham RS, Erickson SB, et al. Rituximab therapy in idiopathic membranous nephropathy: a 2-year study. *Clin J Am Soc Nephrol*. 2010;5(12):2188-2198.
19. Fervenza FC, Cosio FG, Erickson S, et al. Rituximab treatment of idiopathic membranous nephropathy. *Kidney Int*. 2008;73(1):117-125.
20. Ruggenenti P, Cravedi P, Chianca A, et al. Rituximab in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2012;23(8):1416-1425.
21. Van den Brand JA, Ruggenenti P, Chianca A, et al. Safety of rituximab compared with steroids and cyclophosphamide for idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2017;28(9):2729-2737.
22. Van den Brand JA, van Dijk PR, Hofstra JM, Wetzels JF. Cancer risk after cyclophosphamide treatment in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol*. 2014;9(6):1066-1073.
23. Cattran DC, Feehally J, Cook HT, et al. Kidney disease: improving global outcomes (KDIGO) glomerulonephritis work group. KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int Suppl*. 2012;2(2):139-274.
24. Koyama H, Wada T, Nishizawa Y, et al. Cyclophosphamide-induced ovarian failure and its therapeutic significance in patients with breast cancer. *Cancer*. 1977;39(4):1403-1409.

25. Yilmaz N, Emmungil H, Gucenmez S, et al. Incidence of cyclophosphamide-induced urotoxicity and protective effect of mesna in rheumatic diseases. *J Rheumatol*. 2015;42(9):1661-1666.
26. Talar-Williams C, Hijazi YM, Walther MM, et al. Cyclophosphamide-induced cystitis and bladder cancer in patients with Wegener granulomatosis. *Ann Intern Med*. 1996;124(5):477-484.
27. Zonozi R, Wallace ZS, Laliberte K, et al. Incidence, clinical features, and outcomes of late-onset neutropenia from rituximab for autoimmune disease. *Arthritis Rheumatol*. 2021;73(2):347-354.