High-Dose Rituximab and Early Remission in PLA2R1-Related Membranous Nephropathy

Barbara Seitz-Polski,[1,2,3] Karine Dahan,[4] Hanna Debiec,[5,6] Alexandra Rousseau,[7] Marine Andreani,[2] Christelle Zaghrini,[3] Michel Ticchioni,[1] Alessandra Rosenthal,[1] Sylvia Benzaken,[1] Ghislaine Bernard,[1] Gérard Lambeau,[3] Pierre Ronco,[4,5,6] and Vincent L.M. Esnault[2]

Abstract

Background and objectives Different rituximab protocols are used to treat membranous nephropathy. We compared two rituximab protocols in patients with membranous nephropathy.

Design, setting, participants, & measurements Twenty-eight participants from the NICE cohort received two infusions of 1-g rituximab at two-week intervals, whereas 27 participants from the Prospective Randomized Multicentric Open Label Study to Evaluate Rituximab Treatment for Membranous Nephropathy (GEMRITUX) cohort received two infusions of 375 mg/m^2 at one-week interval. We measured serum rituximab levels and compared remission at month six and before any treatment modification and analyzed factors associated with remission and relapses.

Results Remissions occurred in 18 (64%) versus eight (30%) from the NICE and GEMRITUX cohort (P=0.02) at month six, and in 24 (86%) versus 18 (67%) participants (P=0.12) before treatment modification. Median time to remission was 3 [interquartile range (IQR), 3–9] and 9 [IQR, 6–12] months for NICE and GEMRITUX cohorts respectively (P=0.01). Participants from the NICE cohort had higher circulating level of rituximab and lower CD19 counts (3.3 μg/L [IQR, 0.0–10.8] versus 0.0 [IQR, 0.0–0.0] $P<0.001$ and 0.0 [IQR, 0.0–2.0] versus 16.5 [IQR, 2.5–31.0] $P<0.001$) at month three, lower level of anti-PLA2R1 antibodies at month six (0.0 [IQR, 0.0–8.0] versus 8.3 [IQR, 0.0–73.5] P=0.03). In the combined study population, lower epitope spreading at diagnosis and higher rituximab levels at month three were associated with remissions at month six (13/26 (50%) versus 22/29 (76%) P=0.05 and 2.2 μg/ml [IQR, 0.0–10.9] versus 0.0 μg/ml [IQR, 0.0–0.0] $P<0.001$ respectively). All non-spreaders entered into remission whatever the protocol. Eight of the 41 participants who reached remission had relapses. Epitope spreading at diagnosis (8/8 (100%) versus 16/33 (48%) P=0.01) and incomplete depletion of anti-PLA2R1 antibodies at month six (4/8 (50%) versus 5/33 (9%) P=0.05) were associated with relapses.

Conclusions Our work suggests that higher dose rituxan protocol is more effective on depletion of B-cells and lack of epitope spreading is associated with remission of membranous nephropathy.

CJASN 14: ●●●–●●●, 2019. doi: https://doi.org/10.2215/CJN.11791018

[1]Department of Immunology, Hôpital l'Archet, Centre Hospitalier Universitaire de Nice, Université Côte d'Azur, Nice, France; [2]Department of Nephrology–Dialysis–Transplantation, Hôpital Pasteur, CHU de Nice, Université Côte d'Azur, Nice, France; [3]Institut de Pharmacologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, Université Côte d'Azur, Nice, France; [4]Department of Nephrology and Dialysis, Hôpital Tenon, Assistance Publique des Hôpitaux de Paris, Paris, France; [5]Unité Mixte de Recherche_S 1155, Institut National de la Santé et de la Recherche Médicale, Paris, France; [6]Sorbonne Université, Université Pierre et Marie Curie University Paris 06, Paris, France; and [7]Department of Clinical Pharmacology and Clinical Research, Hôpital Saint Antoine, AP-HP, Paris, France

Correspondence: Dr. Barbara Seitz-Polski, Laboratoire d'Immunologie, Hôpital l'Archet, CHU de Nice, Université Côte d'Azur, Route St-Antoine de Ginestière, 06202 Nice Cedex 3, France. Email: seitz-polski.b@chu-nice.fr

Introduction

Membranous nephropathy is defined by the presence of subepithelial immune complex deposits with alteration of the glomerular basement membrane. Most cases of membranous nephropathy are associated with antibodies against podocyte antigens such as neutral endopeptidase in the neonate, and M-type phospholipase A2 receptor (PLA2R1) or thrombospondin type-1 domain-containing 7A (THSD7A) in 70%–80% and <5% of adult patients, respectively (1–3). The pathogenic role of anti-PLA2R1 antibodies is not yet proven, but antibody titers rise during clinically active phases and decrease before clinical remission (4–7), which led to the proposal of a serology-based approach for the treatment of PLA2R1-related membranous nephropathy (8). Spontaneous remissions and ESKD may both occur in about a third of the patients (9). High titers of anti-PLA2R1 antibodies at presentation are associated with poor clinical outcome (10,11). Therefore, reducing anti-PLA2R1 antibody levels has become an important goal of therapy (12).

We identified reactive epitopes in the CysR, CTLD1, and CTLD7 domains of PLA2R1 (13) and found that patients with anti-CysR–restricted activity (nonspreaders) had lower proteinuria and better prognosis, with higher rate of spontaneous remission and lower rate of kidney failure progression, compared with patients with epitope spreading beyond the CysR domain (13).

Rituximab, an anti-CD20 chimeric antibody, can trigger B cell death by apoptosis (14,15), complement-mediated cytotoxicity (16), and antibody-dependent cellular cytotoxicity (17,18). Rituximab induced clinical remission in 60%–80% of patients with primary membranous nephropathy in several nonrandomized studies (19–21),

and its efficacy was established in our recent randomized, controlled clinical trial, Prospective Randomized Multicentric Open Label Study to Evaluate Rituximab Treatment for Membranous Nephropathy (GEMRITUX), using two doses of 375 mg/m^2 at 1-week intervals (22). We confirmed in this trial that PLA2R1 epitope spreading at baseline was independently associated with a lower rate of remission in this trial (23).

There are still uncertainties regarding the rituximab protocol that should be used in nephrotic patients (24). Cravedi *et al.* (25) proposed titrating rituximab to circulating CD20 cells. Fervenza *et al.* (26) showed that four doses of rituximab resulted in more effective B cell depletion. Moroni *et al.* (27) demonstrated that a single low dose of rituximab (375 mg/m^2) was poorly effective in patients with membranous nephropathy. The reason for these discrepancies may be that rituximab pharmacokinetics studies have demonstrated a large interindividual variability, related to either disease or genetic factors, which could explain differences in the clinical response (28–31). Residual serum levels of rituximab were detected in several patients up to 6–9 months after the first infusion, owing to recycling from endothelial cells *via* FcRn receptors (32). In patients with membranous nephropathy, the rituximab $t_{1/2}$ was 11.5 days, as compared with 18.0 days in patients with rheumatoid arthritis (26). In previous studies where 1-g rituximab infusions were given at 2-week intervals, Fervenza *et al.* (20,26) did not find differences in serum rituximab levels between responders and nonresponders at any time point until day 15 postdose.

The aim of this study was to compare two protocols of rituximab in two prospective cohorts of anti-PLA2R1–positive patients.

Materials and Methods

Study Design and Patient Population

Patients were enrolled after signing informed consent from the two prospective studies. The NICE cohort recruited consecutive participants with primary membranous nephropathy in the Department of Nephrology at Pasteur Hospital in Nice (Clinicaltrials.gov identifier NCT02199145), testing epitope profile at diagnosis to predict the need of immunosuppressive therapy (rituximab); only participants recruited in Nice Hospital, treated by rituximab and followed at least 1 year were included in this study. The GEMRITUX cohort is part of a French multicenter, randomized, controlled trial (Clinicaltrials.gov identifier NCT01508468) testing rituximab added to antiproteinuric therapy against antiproteinuric therapy alone (22); only participants with anti-PLA2R1 antibodies treated with rituximab were included in this study. The inclusion criteria were (*1*) biopsy-proven diagnosis; (*2*) primary membranous nephropathy defined by the absence of anti-nuclear antibodies, negative hepatitis B and C serologies, and negative cancer workup; (*3*) positive anti-PLA2R1 antibodies measured by ELISA; and (*4*) persistent nephrotic

Table 1. Baseline characteristics and outcome data in rituximab-treated participants from NICE and GEMRITUX cohorts

Patient Characteristics	NICE Cohort (*n*=28)	GEMRITUX Cohort (*n*=27)
Baseline characteristics		
Age[a]	63 [51; 71]	51 [40; 63]
Sex ratio (F/M)	7/21	6/21
Body mass index (kg/m^2)	25.4 [23.3; 27.2]	25.4 [23.5; 28.4]
Proteinuria (g/g of creatinine)	5.9 [4.9; 7.6]	8.4 [4.4; 11.0]
Serum albumin (g/dl)	2.0 [1.5; 2.5]	2.1 [1.8; 2.6]
Dose of rituximab received (g)[b]	2 [2; 2]	1.45 [1.44; 1.46]
Serum creatinine (mg/dl)	1.2 [0.9; 1.5]	1.1 [0.9; 1.3]
BP (mm Hg)		
Systolic	121 [114; 130]	124 [110; 140]
Diastolic	82 [66; 80]	77 [64; 84]
Time from kidney biopsy to rituximab infusion	6.0 [6.0; 12.0]	8.0 [6.0; 13.0]
Anti-PLA2R1 titer (RU/ml) at first infusion	165.0 [67.0; 245.5]	102.5 [36.1; 672.5]
Spreading		
No	11 (39%)	7 (26%)
Yes	17 (61%)	20 (74%)
Characteristics at month 3		
Serum rituximab (μg/ml)[b]	3.3 [0.0; 10.8]	0.0 [0.0; 0.0]
Proteinuria (g/g of creatinine)[b]	2.6 [1.1; 5.0]	4.8 [3.2; 7.4]
Serum albumin (g/dl)	2.7 [2.0; 3.0]	2.7 [2.1; 3.1]
Anti-PLA2R1 (RU/ml) titer	0.0 [0.0; 19.0]	0.0 [0.0; 60.5]
Rate of PLA2R1 antibody depletion	16/27 (59%)[c]	14/25 (56%)
Spreading		
No	17 (61%)	13 (54%)
Yes	11 (39%)	11 (45%)
CD19 count (/mm^3)[b]	0.0 [0.0; 2.0]	16.5 [2.5; 31.0][d]

Quantitative values are medians [interquartile ranges]; qualitative values are numbers. F/M, female/male; PLA2R1, M-type phospholipase A2 receptor.
[a]$P<0.05$.
[b]$P<0.001$.
[c]One point is missing.
[d]Two points are missing.

proteinuria (*i.e.*, urinary protein-to-creatinine ratio >3.5 g/g) after maximal tolerated antiproteinuric treatment, early deterioration of kidney function, or complications of nephrotic syndrome.

Serum and morning spot urine samples were prospectively collected at the first infusion and every 3 months after the first rituximab infusions (*i.e.*, month 3, month 6). Remissions were defined according to the 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines. Complete remission was defined by a urinary protein-to-creatinine ratio <0.3 g/g, accompanied by a normal serum albumin concentration and a preserved kidney function. Partial remission was defined by urinary protein-to-creatinine ratio <3.5 g/g with >50% reduction of proteinuria, accompanied by an improvement or normalization of the serum albumin concentration and preserved kidney function. A relapse was defined by an increase of urinary protein-to-creatinine ratio >3.5 g/g after remission.

Outcome

The first aim of this study was to compare the efficacy of rituximab in term of clinical remission at 6 months and before treatment modifications in two cohorts of PLA2R1-related membranous nephropathy treated with two different regimens of rituximab.

The second aim was to identify factors associate with remission at month 6 and before treatment modifications in the two combined cohorts.

Measurement of Anti-PLA2R1 Antibodies by ELISA

Serum levels of total IgG anti-PLA2R1 antibodies were measured by the ELISA test developed by EUROIMMUN AG (Lübeck, Germany) (33). Participants were considered as anti-PLA2R1–positive when levels were >14 RU/ml.

Measurement of PLA2R1 Epitope Spreading by ELISA

CysR (Ala-26 to Lys-164), CTLD1 (Thr-223 to Asn-359), and CTLD7 (Thr-1102 to Glu-1237) PLA2R1 domains were produced in HEK293 cells and reactivity of sera of patients with membranous nephropathy toward these domains were analyzed essentially as previously described for the GEMRITUX cohort (23). Participants with only anti-CysR reactivity were defined as nonspreaders, whereas participants with additional anti-CTLD1 and/or anti-CTLD7 reactivity were defined as spreaders (*i.e.*, participants with intramolecular epitope spreading within PLA2R1).

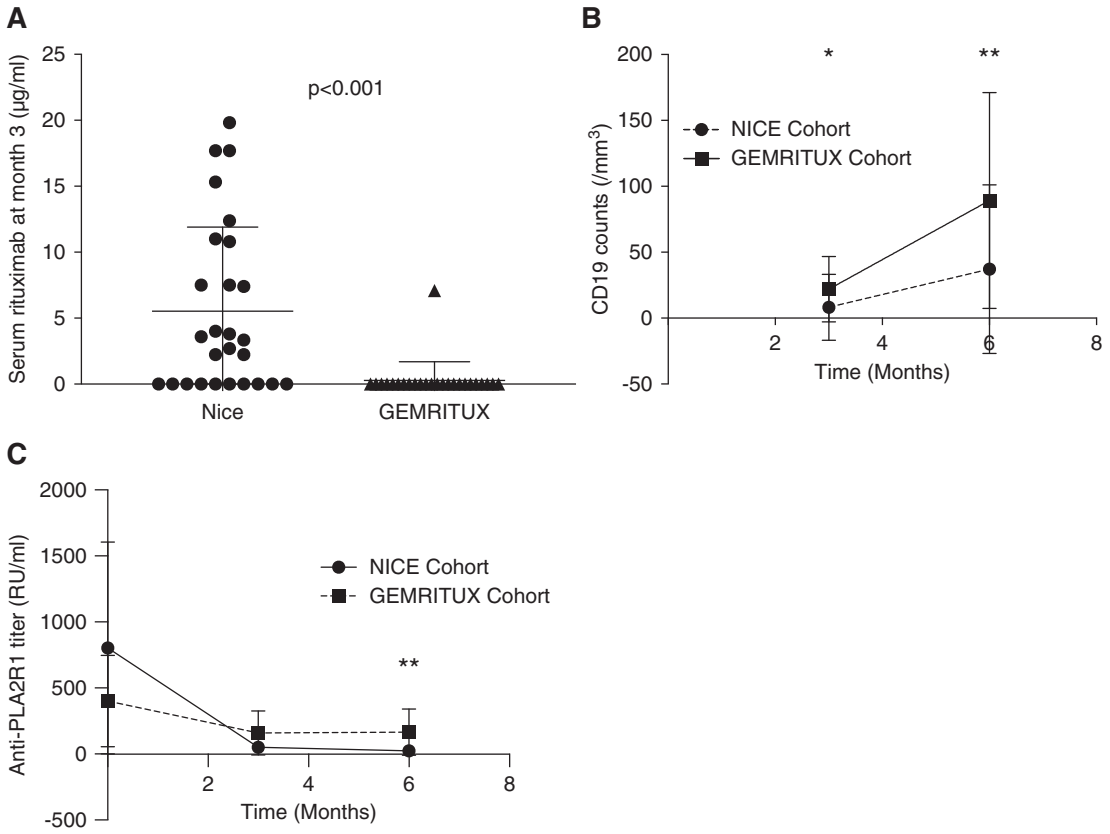


Figure 1. | **Temporal changes of circulating intermediate markers of rituximab effect in the NICE and GEMRITUX cohorts.** (A) Comparison of serum residual rituximab level at month 3 between the NICE (n=28, two infusions of 1 g at 2-week intervals) and GEMRITUX (n=27, two infusions of 375 mg/m^2 at 1-week intervals) cohorts. Note variability of residual rituximab concentrations and lower concentrations in the GEMRITUX participants (P<0.001). Threshold of detection was 2 μg/ml. (B) Evolution of CD19 count at month 3 and month 6 according to the regimens received. *CD19 count was lower in NICE cohort at month 3 (P>0.001). **CD19 count was lower at month 6 in NICE cohort (P=0.03). (C) Evolution of anti-PLA2R1 titer at baseline, month 3 and month 6 according to the regimens received. **Anti-PLA2R1 titer was lower at month 6 in NICE cohort (P>0.001).

Table 2. Outcomes of rituximab-treated participants in the NICE and GEMRITUX cohorts

Patients Characteristics	NICE Cohort (*n*=28)	GEMRITUX Cohort (*n*=27)	*P* Values
Characteristics at month 6			
Proteinuria (g/g of creatinine)	2.0 [0.7; 3.2]	3.7 [1.8; 6.5]	0.001
Serum albumin (g/dl)	3.2 [2.8; 3.5]	2.9 [2.4; 3.4]	0.24
Anti-PLA2R1 titer (RU/ml)	0.0 [0.0; 8.0]	8.3 [0.0; 73.5]	0.03
Rate of PLA2R1 antibody depletion	21/27(78%)	13/26 (50%)	0.05
Spreading			
No	25 (93%)	17 (71%)	0.07
Yes	2 (7%)	7 (29%)	
CD19 count[a]	5.0 [1.8; 48.5]	63.0 [37.0; 115.0][a]	<0.001
Remission (KDIGO guidelines)	18/28 (64%)	8/27 (30%)	0.02
Complete remission[b]	5/28 (18%)	0/27 (0%)	0.05
Characteristics at last follow-up			
Remission before any treatment modification	24/28 (86%)	18/27 (67%)	0.12
Median time to remission (mo)	3 [3; 9]	9 [6; 12]	0.01
Complete remission	9/28 (32%)	6/27 (22%)	0.55
SAEs related to rituximab	1/28 (0.03%)	1/27 (0.04%)	1

Quantitative values are medians [interquartile ranges]; qualitative values are numbers. PLA2R1, M-type phospholipase A2 receptor; KIDGO, Kidney Disease: Improving Global Outcomes; SAEs, serious adverse events.
[a]Three points are missing.

Measurement of Rituximab by ELISA

Serum levels of rituximab were measured by ELISA according to the manufacturer's instructions (LISA-TRACKER Duo Rituximab; Theradiag, Croissy Beaubourg, France). This assay measures only free rituximab. The limit of detection for rituximab defined by the manufacturer was 2 μg/ml, with an intrarun variability of 8% and interrun variability of 10%.

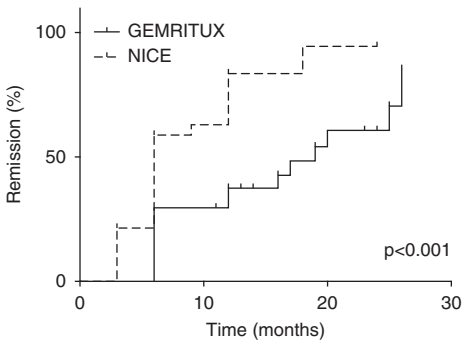
Statistical Analyses

Categorical variables were expressed as frequencies and compared using Fisher exact test. Continuous variables were expressed as medians and interquartile ranges and compared using Wilcoxon–Mann–Whitney test or Kruskall–Wallis test for multilevel variables. Correlation between residual serum rituximab level and CD19 counts were assessed by Spearman rank correlation coefficient. Adjusted analysis was performed using logistic regression. Survival curves were calculated using Kaplan–Meier estimates for survival distribution. A categorical variable combining treatment and spreading was built to investigate the effect of the interaction between treatment and spreading on the response. All tests were two-sided and a *P* value <0.05 indicated statistical significance. Analyses were performed using SAS software v.9.3 (SAS Institute, Cary, NC).

Results

Comparison of the NICE and GEMRITUX Cohorts at Baseline

The NICE cohort included 28 consecutive PLA2R1-positive participants who received two 1-g infusions of rituximab at 2-week intervals after 6 months of symptomatic treatment, or earlier in the case of acute complications according to the KDIGO guidelines. Seven participants were treated after <6 months of symptomatic treatment: two presented with thromboembolic complications at diagnosis and five participants developed progressive kidney failure. The GEMRITUX cohort included 27 PLA2R1-positive participants from the French GEMRITUX clinical trial (22) who were given two 375 mg/m^2 infusions of rituximab at 1-week intervals at least 6 months after kidney biopsy. Participants' baseline characteristics were similar, apart from an older age in the NICE cohort (63 [interquartile range (IQR), 51–71] versus 51 [IQR, 40–63] years; *P*=0.03). Proteinuria tended to be lower in the NICE cohort, although this did not reach statistical significance (5.9 [IQR, 4.9–7.6] versus 8.4 [IQR, 4.4–11.0]; *P*=0.13), (Table 1). All other baseline characteristics were similar, including anti-PLA2R1 antibody titers (NICE: 165.0 RU/ml [IQR, 67.0–245.5]; GEMRITUX: 102.5 RU/ml [IQR, 36.1–672.5]; *P*=0.65) and epitope spreading as defined by anti-CysR reactivity with additional anti-CTLD1 and/or anti-CTLD7 activities in addition to anti-CysR reactivity (61% epitope spreading in NICE versus 74% in GEMRITUX; *P*=0.39) (Table 1).



	M0	M6	M12	M18	M24	M30
NICE	28	12	6	4	4	4
GEMRITUX	27	19	17	15	13	11

Figure 2. | Time from initiation of rituximab to remission of membranous nephropathy in the NICE and GEMRITUX cohorts. Note that NICE the log-rank *P* value for remission was *P*<0.001. Numbers in the table are number at risk.

Serum Rituximab Levels, CD19 Counts, and Anti-PLA2R1 Depletion

Rituximab serum levels were measured at month 3 in all participants. Residual levels were detected in 18 of the 28 NICE participants at month 3, but in only one of the 27 GEMRITUX participants at month 3. The median residual rituximab level at month 3 was higher with the NICE regimen (3.3 μg/ml [IQR, 0.0–10.8]) compared with the GEMRITUX regimen (0.0 μg/ml [IQR, 0.0–0.0]; $P<0.001$) (Figure 1A, Table 1). CD19 counts were lower at month 3 (0.0 [IQR, 0.0–2.0] in the NICE cohort versus 16.5 [IQR, 2.5–31.0] in the GEMRITUX cohort; $P<0.001$), and month 6 (5.0 [IQR, 1.8–48.5] versus 63.0 [IQR, 37.0–115.0]; $P<0.001$) (Figure 1B, Tables 1 and 2). Furthermore, immunologic remission defined by anti-PLA2R1 depletion at month 6 was more frequent with the NICE protocol (78% versus 50%; $P=0.05$) and anti-PLA2R1 titer was lower at month 6 in NICE cohort (0.0 [IQR, 0.0–8.0] versus 8.3 [IQR, 0.0–73.5]; $P=0.03$) (Figure 1C, Table 2).

Outcome

Outcome was analyzed at month 6 and before treatment modification.

At month 6, complete or partial remission was obtained at month 6 in 18 of the 28 (64%) NICE participants, but in only eight of the 27 (30%) GEMRITUX participants ($P=0.02$), (Table 1). Complete remissions at month 6 were only observed in the NICE cohort ($n=5$; $P=0.05$). Fourteen of the 18 NICE participants who achieved remission at month 6 had a residual rituximab concentration >2 μg/ml.

After a median follow-up of 15 [IQR, 11–19] (NICE cohort) and 24 [IQR, 22–25] (GEMRITUX cohort) months before any therapeutic modification, remissions occurred in 24 out of 28 (86%) NICE participants and 18 out of 27 (67%) GEMRITUX participants ($P=0.12$). Complete remission occurred in nine out of 28 (32%) and six out of 27 (22%) participants of the NICE and GEMRITUX cohorts, respectively. Median time to remission was 3 months [IQR, 3–9] in the NICE cohort versus 9 months [IQR, 6–12] in the GEMRITUX cohort ($P=0.01$) (Table 2), and Kaplan–Meier analysis demonstrates faster time to remission using the NICE protocol ($P<0.001$; Figure 2).

Factors associated with Remission

Clinical remissions at month 6 were associated with lower rate of epitope spreading at diagnosis (13/26 (50%) versus 22/29 (76%); $P=0.05$), and higher serum rituximab levels at month 3 (2.2 μg/ml [IQR, 0.0–10.9] versus 0.0 μg/ml [IQR, 0.0–0.0]; $P<0.001$) (Figure 3A, Table 2). Residual serum rituximab levels at month 3 were inversely correlated with

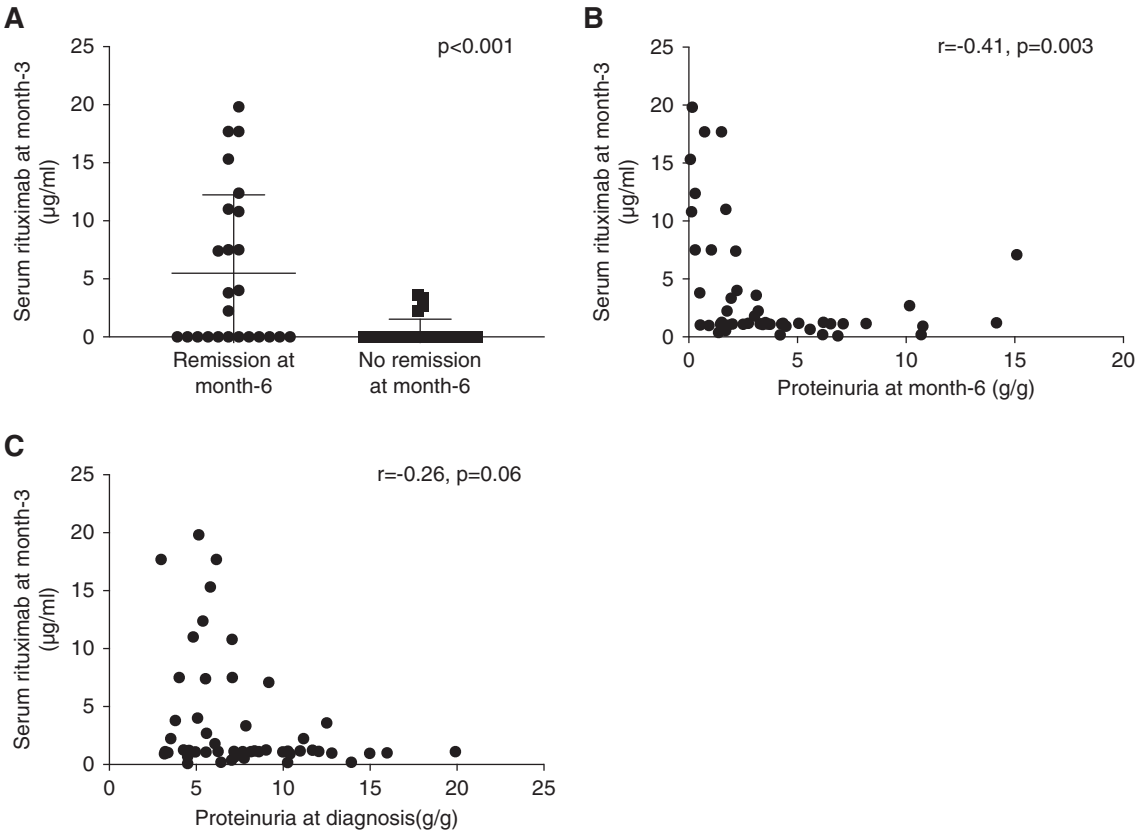


Figure 3. | **Intermediate outcomes according to serum rituximab concentration at 3 months.** (A) Comparison of serum rituximab concentrations according to remission ($n=26$) or absence of remission ($n=29$) at month 6 in the pooled NICE and GEMRITUX cohorts. Note higher concentration of residual rituximab concentrations at month 3 in participants who achieved remission ($P<0.001$). (B) Correlation of serum rituximab concentrations at month 3 and proteinuria at month 6 in the pooled NICE and GEMRITUX cohorts ($r=-0.41$; $P=0.003$). (C) Correlation of serum rituximab concentrations at month 3 and proteinuria at baseline in the pooled NICE and GEMRITUX cohorts ($r=0.26$; $P=0.06$).

proteinuria at month 3 and month 6 (r=0.42; P=0.003 and r=−0.41; P=0.003, respectively; Figure 3B), and tended to correlate with proteinuria at baseline (r=−0.26; P=0.06; Figure 3C) and with CD19 counts at month 3 and month 6 (r=−0.32; P=0.03 and r=−0.41; P=0.008, respectively). The clinical remission rate at month 6 was different according to the rituximab protocol combined with epitope spreading at baseline (P=0.003) (Figure 4A, Table 3). In adjusted analysis, remission rates were analyzed according to age, anti-PLA2R1 titers, epitope spreading at diagnosis, and the rituximab protocol. Epitope spreading at diagnosis and the rituximab protocol (1 g at the day of rituximab infusion and two weeks after rituximab infusion versus 375 mg/m^2 at the day of rituximab infusion and seven days after rituximab infusion) were independent risk factors for remission at month 6 (odds ratio, 4.34; 95% confidence interval, 1.07 to 17.5; P=0.04 and odds ratio, 5.08; 95% confidence interval, 1.3 to 19.6; P=0.02, respectively) (Table 4).

Before treatment modification, clinical remission was associated with lower anti-PLA2R1 titers and absence of epitope spreading at baseline (101.5 RU/ml [IQR, 35.9–212.3] versus 508.0 [IQR, 144.9–1620.0]; P=0.003 and 18 out of 42 (43%) versus zero out of 13 (0%); P<0.001, respectively) (Supplemental Table 1), higher residual rituximab levels at month 3 (0.0 μg/ml [IQR, 0.0–5.7] versus 0.0 μg/ml [IQR, 0.0–0.0]; P=0.02) (Supplemental Table 1). The clinical remission rate at last follow-up was different according to the rituximab protocol combined with epitope spreading at baseline (P=0.01) (Figure 4B, Supplemental Table 1). All participants with anti-CysR–restricted activity at baseline entered into remission. Because each site measured remission at last follow-up at different time points, we performed a time-to-event analysis of remission according to the two different protocols for determining epitope spreading at diagnosis (CysR profile versus CysRC1 versus CysRC1C7). Remissions occurred earlier in participants treated by the NICE protocol (rituximab 1 g the day of rituximab infusion and two weeks after rituximab infusion; P<0.001) (Figure 2) or in participants with anti-CysR–restricted activity (P=0.01; Supplemental Figure 1A), with no difference according to proteinuria at diagnosis (P=0.64; Supplemental Figure 1B) or anti-PLA2R1 titers (P=0.26; Supplemental Figure 1C).

We found a strong association between epitope spreading and anti-PLA2R1 titer (Supplemental Figure 2A). The clinical remission rate at month 6 was different according to the rituximab protocol combined with anti-PLA2R1 titer at baseline (P=0.02) (low titer was defined as <70 RU/ml) and tended to be different at last follow-up (P=0.07) (Supplemental Figures 3 and 4 A and B). We established receiver operating characteristic curve (area under the curve =0.68 [IQR, 0.53–0.83]; P=0.03) to define a threshold of anti-PLA2R1 titer associated with epitope spreading: a titer >321 RU/ml was associated with spreading, with a sensitivity of 32.4% [IQR, 19.6%–48.5%] and a specificity of 94.4% [IQR, 74.2%–99.7%] (Supplemental Figure 2, B–D).

Relapses

During follow-up, 41 participants achieved partial or complete remission and eight participants relapsed (Supplemental Table 2), with a median time to relapse of 12 months [IQR, 7–17]. There was no significant difference between participants with (n=8) or without relapse (n=33) for age,

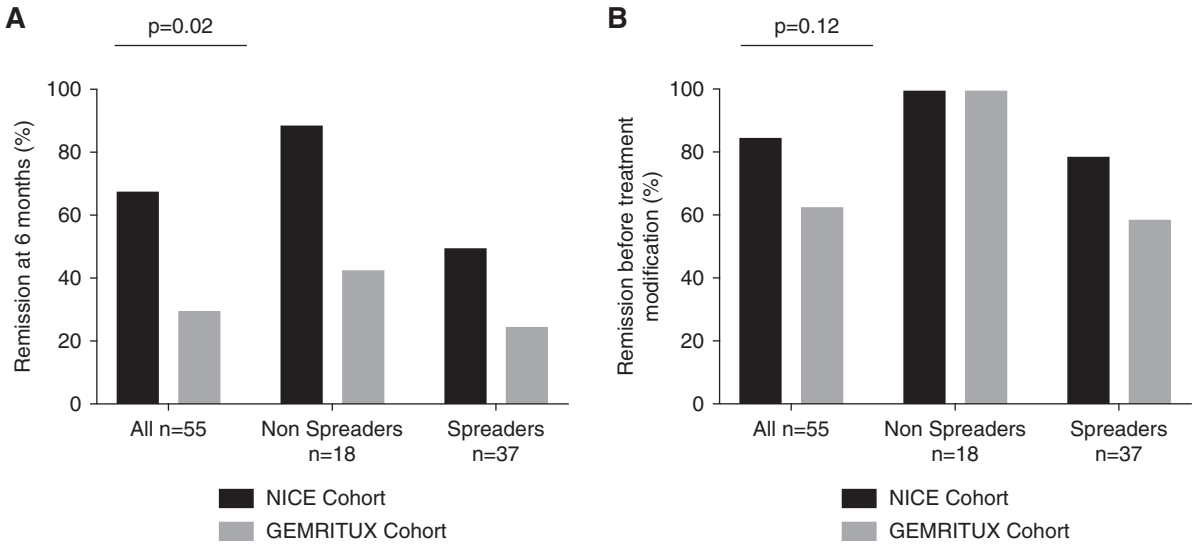


Figure 4. | Remission of membranous nephropathy in the NICE and GEMRITUX cohorts according to epitope spreading at baseline. (A) Remission at month 6 according to epitope spreading at baseline and rituximab protocol received. A categorical variable combining treatment and spreading was built to investigate the impact of the interaction between treatment and spreading on the response (P=0.003; comparing the null hypothesis that there are no differences among groups). Note that the lowest remission rate is observed in participants with epitope spreading treated by GEMRITUX protocol. (B) Remission at last follow-up according to epitope spreading at baseline and rituximab protocol received. A categorical variable combining treatment and spreading was built to investigate the effect of the interaction between treatment and spreading on the response (P=0.01; comparing the null hypothesis that there are no differences among groups). Note that all participants without epitope spreading are in remission at last follow-up whatever the protocol received and participants with epitope spreading benefit from the NICE protocol (remission rate 79% versus 55%).

Table 3. Prognosis factors of proteinuria remission at 6 months in the pooled NICE and GEMRITUX participants

Participant Characteristics	No Remission at Month 6, *n*=29	Remission at Month 6, *n*=26
Baseline characteristics		
Age	50 [38; 65]	61 [51; 70]
Sex		
Men	21 (75.0)	19 (86.0)
Women	8 (25.0)	3 (14.0)
Body mass index (kg/m^2)	25.2 [23.4; 28.6]	25.5 [23.5; 26.9]
Proteinuria (g/g of creatinine)	7.8 [4.5; 10.3]	6.0 [4.7; 8.0]
Serum creatinine (mg/dl)	1.1 [0.9; 1.4]	1.2 [0.9; 1.3]
Serum albumin (g/dl)	2.0 [1.5; 2.7]	2.1 [1.8; 2.5]
Anti-PLA2R1 antibody (RU/ml)	192.0 [45.9; 1227.0]	125.0 [62.0; 194.0]
Time to rituximab (mo)	8.0 [6.0; 12.0]	7.0 [6.0; 13.0]
Spreading[a]		
No	7 (35.0)	13 (65.0)
Yes	22 (63.0)	13 (37.0)
Epitope profile		
CysR	7 (35.0)	13 (65.0)
CysRC1	7 (50.0)	7 (50.0)
CysRC1C7	15 (71.0)	6 (29.0)
Rituximab dose and spreading[a]		
GEMRITUX protocol without spreading	4 (14)	3 (12)
NICE protocol without spreading	1 (3)	10 (38)
GEMRITUX protocol with spreading	15 (52)	5 (19)
NICE protocol with spreading	9 (31)	8 (31)
Characteristics at month 3		
Anti-PLA2R1 antibody (RU/ml)[a]	18.0 [0.0; 60.5]	0.0 [0.0; 13.5]
Spreading[b]		
No	10	22
Yes	16	3
Rituximab, μg/ml[b]	0.0 [0.0; 0.0]	2.2 [0.0; 10.9]
CD19[a]	12.1 [1.0; 25.0]	0.0 [0.0; 2.7]
Characteristics at month 6		
Anti-PLA2R1 antibody (RU/ml)[b]	9.0 [0.0; 75.5]	0.0 [0.0; 1.2]
CD19	47.5 [17.5; 92.0]	15.0 [2.0; 70.5]

NICE, XXX; GEMRITUX, XXX; PLA2R1, M-type phospholipase A2 receptor.
[a]$P<0.05$.
[b]$P<0.001$.

proteinuria, anti-PLA2R1 antibodies titers before rituximab, time from diagnosis to treatment, and residual serum rituximab levels at month 3. Only epitope spreading at diagnosis and an incomplete PLA2R1 antibody depletion at month 6 were significantly associated with relapses (eight out of eight (100%) versus 16 out of 33 (48%); P=0.01, and four out of eight (50%) versus five out of 33 (9%); P=0.05, respectively) (Table 5).

Discussion

The first aim of this study was to compare the efficacy of rituximab in term of clinical remission at 6 months and before treatment modification in two cohorts of PLA2R1-related membranous nephropathy treated with two different regimens of rituximab. We show that higher cumulative doses of rituximab (2 g in NICE cohort versus 1.4 g in GEMRITUX) combined with different timing of infusion (2-week intervals instead of 1-week intervals) induce earlier remission and higher rate of remission with more complete remissions at month 6. A shorter time to remission is clinically meaningful to reduce the risk of complications of nephrotic syndrome, particularly venous thromboembolic disease. These findings are associated with higher residual serum rituximab levels at month 3 and lower CD19 counts at month 3 and month 6, and with a greater decline in anti-PLA2R1 antibodies titer at month 6. The lack of significant difference in the outcome at last follow-up between the two treatment groups might be

Table 4. Adjusted analysis for remission at month-6

Prognosis factors tested	*P* Value	Odds Ratio	95% Wald Confidence Limits
Age	0.86	1.00	0.96 to 1.05
Anti-PLA2R1 titer at diagnosis	0.08	0.99	0.99 to 1.00
Spreading at diagnosis	0.04	4.34	1.07 to 17.54
Rituximab regimens (NICE versus GEMRITUX)	0.02	5.08	1.32 to 19.6

PLA2R1, M-type phospholipase A2 receptor.

Table 5. Baseline characteristics and outcome data in relapsing and nonrelapsing participants from NICE and GEMRITUX cohorts treated with rituximab

Participants Characteristics	Relapse (*n*=8)	No Relapse (*n*=33)
Baseline characteristics		
Age	60 [51; 70]	57 [46; 68]
Sex ratio (F/M)	2/6	9/24
Proteinuria (g/g of creatinine)	6.3 [5.2; 12.7]	7.0 [4.7; 9.3]
Serum albumin (g/dl)	2.0 [1.1; 2.2]	2.3 [1.8; 2.7]
Serum creatinine (mg/dl)	1.1 [0.8; 1.4]	1.1 [0.9; 1.4]
BP (mm Hg)		
Systolic	120 [108; 127]	120 [114; 140]
Diastolic	63 [60; 80]	80 [68; 81]
Anti-PLA2R1 titer (RU/ml) at first infusion	113.8 [35.3; 249.4]	100.5 [38.3; 213.7]
Spreading[a]		
No	0 (0)	17 (52)
Yes	8 (100)	16 (48)
Characteristics at month 3		
Serum rituximab (μg/ml)	3.1 [0.0; 6.6]	0.0 [0.0; 7.5]
Anti-PLA2R1 titer (RU/ml)	7.5 [0.0; 49.1]	0.0 [0.0; 11.0]
CD19 count	0.0 [0.0; 13.7]	1.0 [0.0; 17.25][b]
Characteristics at month 6		
Anti-PLA2R1 titer (RU/ml)	9.5 [0.0; 37.0]	0 [0.0; 3.0]
CD19 count	19.0 [11.7; 64.2]	34.0 [5.9; 83.5][c]
Rate of PLA2R1 antibody depletion[a]	4/8 (50)	5/33 (9)

Quantitative values are medians [interquartile ranges]; qualitative values are numbers. F/M, female/male; PLA2R1, M-type phospholipase A2 receptor.
[a]$P<0.05$.
[b]Two points are missing.
[c]Three points are missing.

explained by the small cohort size, because there was a trend toward a higher remission rate in the NICE cohort (86% versus 67%; P=0.12).

Little attention has been paid to rituximab pharmacokinetics in nephrotic patients except in one study by Fervenza *et al.*, who showed a shorter rituximab $t_{1/2}$ (11.5 days versus 18.0 days in patients with rheumatoid arthritis) with four doses of 375 mg/m^2 (day 1, day 8, day 15, and day 22). Of note, the median rituximab serum level was four-fold higher with the NICE protocol despite a substantial dispersion of individual serum rituximab concentrations, and only one of the 27 GEMRITUX participants had detectable rituximab serum levels at month 3. Residual rituximabemia, residual serum level of rituximab, levels are dependent on proteinuria loss, rituximab dosing, and genetic factors. Although rituximabemia was not correlated with baseline proteinuria in our study, there was a trend to lower levels of proteinuria in the NICE cohort, which might account for a greater loss of rituximab antibody in the urine in the GEMRITUX cohort. It has recently been suggested that such loss in urine should modify the formulation or modality of rituximab delivery to ensure efficacy of the therapy (34). Cravedi *et al.* (25) have shown that low-dose rituximab can reduce cost, whereas other groups (24,27) have found that low-dose regimens are poorly effective. Our results suggest that low doses may delay remission even if B cell depletion is achieved. Absence of remission at last follow-up was associated with lower residual serum rituximab levels, higher CD19 count, and higher anti-PLA2R1 antibody levels at month 3. These findings make a case for the use of the NICE regimen, which was also used in the Multicenter Randomized Controlled Trial of Rituximab versus Cyclosporine in the Treatment of Idiopathic Membranous Nephropathy (MENTOR), instead of the GEMRITUX regimen. They also suggest that the dose of rituximab might be insufficient in patients with heavy proteinuria in whom we have recently shown that reinfusion of rituximab could induce remission in patients that were considered "refractory" to rituximab (35).

The second aim was to identify factors associated with remission at month 6 and last follow-up in the two combined cohorts. We confirmed that the absence of spreading at baseline was associated with higher rate of clinical remission (23). We found that the percentage of epitope spreaders tended to be lower at month 6 with the NICE protocol, which suggests that high-dose rituximab may reverse spreading. Further studies are needed to determine if this improves outcome as compared with patients that fail to experience spreading reversal. A combined analysis of rituximab protocol and spreading suggested that the NICE protocol blunted the effects of spreading on clinical remission at last follow-up. As long as epitope spreading is not routinely available, we propose that patients with anti-PLA2R1 titer >321 RU/ml (using Euroimmun ELISA) should receive high-dose rituximab. We showed that in patients with anti-PLA2R1 titer >321 RU/ml, 95% are spreaders. We previously demonstrated in the GEMRITUX cohort that 100% of patients with a titer >369.5 RU/ml are spreaders (23). Another point to notice concerns the possibility that the cut-off of anti-PLA2R1 antibodies at a low level according to the Euroimmun ELISA may need to be redefined with more sensitive assays. In fact, an indirect immunofluorescence test is still necessary for low titers (3–14 RU/ml) to confirm anti-PLA2R1 antibody positivity (36). It should be

useful to add a construct CysR-deleted domain to diagnose spreader and nonspreader in this kit. Overall, these results confirm that analysis of epitope spreading may provide an added value to quantitative serology.

This study has several limitations. First, it is a retrospective study but uses systematically collected prospective data and samples. Second, the number of participants is relatively small. Third, there is a trend for higher proteinuria in the GEMRITUX cohort (P=0.13), which could contribute to the better outcome in the NICE cohort. We cannot formally exclude a higher rate of early spontaneous remissions in the NICE cohort compared with GEMRITUX, although this is unlikely because we previously showed that spontaneous remission occurred mainly in nonspreader patients (45% versus 0.05% in spreaders) (13), and we had the same number of nonspreader patients in the two cohorts.

In conclusion, we have made several clinically relevant observations. First, rituximab dose seems to affect the early remission rate at month 6, particularly in patients with PLA2R1 epitope spreading. Second, higher residual serum rituximab levels at month 3 are associated with a higher rate of clinical remission. Third, epitope spreading is confirmed as a factor negatively associated with clinical remission, and is now potentially associated positively with relapse.

Acknowledgments

Rituximab was given by Hoffmann–La Roche to Dr. Ronco for the GEMRITUX trial.

Disclosures

Dr. Dahan reports personal fees from Amicus Therapeutics. Dr. Lambeau has a patent for Diagnosis for Membranous Nephropathy licensed with and with royalties paid to Euroimmun and a patent for Methods and Kits for Monitoring Membranous Nephropathy licensed to Euroimmun issued. Dr. Seitz-Polski and Dr. Lambeau have a patent for Prognosis and Monitoring of Membranous Nephropathy Based on the Analysis of PLA2R1 Epitope Profile and Spreading pending. Dr. Andreani, Dr. Benzaken, Dr. Bernard, Dr. Debiec, Dr. Esnault, Dr. Ronco, Dr. Rosenthal, Dr. Rousseau, Dr. Ticchioni, and Dr. Zaghrini have nothing to disclose.

Funding

Dr. Esnault and Dr. Seitz-Polski are recipients of grants from the Centre Hospitalier Universitaire de Nice and the Direction générale de l'Offre de soins of the French Ministry of Health (PHRC2011-A01302-39, NCT01897961). Dr. Lambeau is a recipient of grants from CNRS, the National Research Agency (ANR) through the Investments for the Future LABEX SIGNALIFE programme (MNaims reference #ANR-17-CE17-0012-01), the Fondation pour la Recherche Médicale (FRMING20140129210, SPF20150934219, FDT201805005509, and DEQ20180339193). Dr. Ronco is a recipient of grants from the European Research Council ERC-2012-ADG_20120314 (grant agreement 322947) and the Seventh Framework Programme of the European Community contract 2012-305608 (European Consortium for High-Throughput Research in Rare Kidney Diseases). Dr. Seitz-Polski is recipient of a grant of Les Amis de la faculté de médecine de Nice. Dr. Zaghrini is a recipient of grants the National Research Agency (ANR) through the Investments for the Future LABEX SIGNALIFE programme (reference #ANR-11-LABX-0028-01). The sponsor of the GEMRITUX cohort (NCT01508468) was Assistance Publique-Hôpitaux de Paris (Département de la Recherche Clinique et du Développement). The GEMRITUX study received legal, monitoring, and administrative management support from the Assistance Publique-Hôpitaux de Paris. The sponsor of the NICE cohort was Centre Hospitalier de Nice (NCT02199145). This work has been developed and supported through the FHU Oncoage, Nice.

Supplemental Material

This article contains the following supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.11791018/-/DCSupplemental.

Supplemental Table 1. Prognosis factors of proteinuria remission before treatment modification in the pooled NICE and GEMRITUX participants.

Supplemental Table 2. Individual biological data of participants with membranous nephropathy relapses.

Supplemental Figure 1. (A) Time from initiation of rituximab to remission of membranous nephropathy in participants according to epitope profile. (B) Time from initiation of rituximab to remission of membranous nephropathy in participants according to proteinuria level at baseline (T1: low tertile, T2: medium tertile, T3: high tertile). (C) Time from initiation of rituximab to remission of membranous nephropathy in participants according to PLA2R1 titer at baseline (T1: low tertile versus T2-T3: medium and high tertile).

Supplemental Figure 2. Correlation between anti-PLA2R1 titer and epitope spreading.

Supplemental Figure 3. (A) Remission at month 6 according titer of PLA2R1 (T1: low tertile versus T2-T3: medium and high tertile) at baseline and rituximab protocol received. (B) Remission before treatment modification according to titer of PLA2R1 at baseline and rituximab protocol received.

Supplemental Figure 4. (A) Remission at month 6 according titer of PLA2R1 (at baseline and rituximab protocol received. (B) Remission before treatment modification according to titer of PLA2R1 at baseline and rituximab protocol received.

References

1. Debiec H, Guigonis V, Mougenot B, Decobert F, Haymann JP, Bensman A, Deschênes G, Ronco PM: Antenatal membranous glomerulonephritis due to anti-neutral endopeptidase antibodies. *N Engl J Med* 346: 2053–2060, 2002
2. Beck LH Jr., Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ: M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med* 361: 11–21, 2009
3. Tomas NM, Beck LH Jr., Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, Dolla G, Hoxha E, Helmchen U, Dabert-Gay AS, Debayle D, Merchant M, Klein J, Salant DJ, Stahl RAK, Lambeau G: Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. *N Engl J Med* 371: 2277–2287, 2014
4. Hofstra JM, Beck LH Jr., Beck DM, Wetzels JF, Salant DJ: Anti-phospholipase A_2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 6: 1286–1291, 2011
5. Hofstra JM, Debiec H, Short CD, Pellé T, Kleta R, Mathieson PW, Ronco P, Brenchley PE, Wetzels JF: Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. *J Am Soc Nephrol* 23: 1735–1743, 2012
6. Hoxha E, Harendza S, Zahner G, Panzer U, Steinmetz O, Fechner K, Helmchen U, Stahl RA: An immunofluorescence test for phospholipase-A_2-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. *Nephrol Dial Transplant* 26: 2526–2532, 2011
7. Ruggenenti P, Debiec H, Ruggiero B, Chianca A, Pellé T, Gaspari F, Suardi F, Gagliardini E, Orisio S, Benigni A, Ronco P, Remuzzi G: Anti-phospholipase A2 receptor antibody titer predicts

post-rituximab outcome of membranous nephropathy. *J Am Soc Nephrol* 26: 2545–2558, 2015
8. De Vriese AS, Glassock RJ, Nath KA, Sethi S, Fervenza FC: A proposal for a serology-based approach to membranous nephropathy. *J Am Soc Nephrol* 28: 421–430, 2017
9. Glassock RJ: Diagnosis and natural course of membranous nephropathy. *Semin Nephrol* 23: 324–332, 2003
10. Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, Nikam M, Poulton K, McWilliam L, Short CD, Venning M, Brenchley PE: Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. *Kidney Int* 83: 940–948, 2013
11. Hoxha E, Thiele I, Zahner G, Panzer U, Harendza S, Stahl RA: Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. *J Am Soc Nephrol* 25: 1357–1366, 2014
12. Ruggenenti P, Fervenza FC, Remuzzi G: Treatment of membranous nephropathy: Time for a paradigm shift. *Nat Rev Nephrol* 13: 563–579, 2017
13. Seitz-Polski B, Dolla G, Payré C, Girard CA, Polidori J, Zorzi K, Birgy-Barelli E, Jullien P, Courivaud C, Krummel T, Benzaken S, Bernard G, Burtey S, Mariat C, Esnault VL, Lambeau G: Epitope spreading of autoantibody response to PLA2R associates with poor prognosis in membranous nephropathy. *J Am Soc Nephrol* 27: 1517–1533, 2016
14. Semac I, Palomba C, Kulangara K, Klages N, van Echten-Deckert G, Borisch B, Hoessli DC: Anti-CD20 therapeutic antibody rituximab modifies the functional organization of rafts/microdomains of B lymphoma cells. *Cancer Res* 63: 534–540, 2003
15. Bonavida B: Rituximab-induced inhibition of antiapoptotic cell survival pathways: Implications in chemo/immunoresistance, rituximab unresponsiveness, prognostic and novel therapeutic interventions. *Oncogene* 26: 3629–3636, 2007
16. Manches O, Lui G, Chaperot L, Gressin R, Molens JP, Jacob MC, Sotto JJ, Leroux D, Bensa JC, Plumas J: In vitro mechanisms of action of rituximab on primary non-Hodgkin lymphomas. *Blood* 101: 949–954, 2003
17. Wang SY, Racila E, Taylor RP, Weiner GJ: NK-cell activation and antibody-dependent cellular cytotoxicity induced by rituximab-coated target cells is inhibited by the C3b component of complement. *Blood* 111: 1456–1463, 2008
18. Roccatello D, Sciascia S, Di Simone D, Solfietti L, Naretto C, Fenoglio R, Baldovino S, Menegatti E: New insights into immune mechanisms underlying response to Rituximab in patients with membranous nephropathy: A prospective study and a review of the literature. *Autoimmun Rev* 15: 529–538, 2016
19. Remuzzi G, Chiurchiu C, Abbate M, Brusegan V, Bontempelli M, Ruggenenti P: Rituximab for idiopathic membranous nephropathy. *Lancet* 360: 923–924, 2002
20. Fervenza FC, Cosio FG, Erickson SB, Specks U, Herzenberg AM, Dillon JJ, Leung N, Cohen IM, Wochos DN, Bergstralh E, Hladunewich M, Cattran DC: Rituximab treatment of idiopathic membranous nephropathy. *Kidney Int* 73: 117–125, 2008
21. Beck LH Jr., Fervenza FC, Beck DM, Bonegio RG, Malik FA, Erickson SB, Cosio FG, Cattran DC, Salant DJ: Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol* 22: 1543–1550, 2011
22. Dahan K, Debiec H, Plaisier E, Cachanado M, Rousseau A, Wakselman L, Michel PA, Mihout F, Dussol B, Matignon M, Mousson C, Simon T, Ronco P; GEMRITUX Study Group: Rituximab for severe membranous nephropathy: A 6-month trial with extended follow-up. *J Am Soc Nephrol* 28: 348–358, 2017
23. Seitz-Polski B, Debiec H, Rousseau A, Dahan K, Zaghrini C, Payré C, Esnault VLM, Lambeau G, Ronco P: Phospholipase A2 Receptor 1 epitope spreading at baseline predicts reduced likelihood of remission of membranous nephropathy. *J Am Soc Nephrol* 29: 401–408, 2018
24. Cattran D, Brenchley P: Membranous nephropathy: Thinking through the therapeutic options. *Nephrol Dial Transplant* 32[Suppl 1]: i22–i29, 2017
25. Cravedi P, Ruggenenti P, Sghirlanzoni MC, Remuzzi G: Titrating rituximab to circulating B cells to optimize lymphocytolytic therapy in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 2: 932–937, 2007
26. Fervenza FC, Abraham RS, Erickson SB, Irazabal MV, Eirin A, Specks U, Nachman PH, Bergstralh EJ, Leung N, Cosio FG, Hogan MC, Dillon JJ, Hickson LJ, Li X, Cattran DC; Mayo Nephrology Collaborative Group: Rituximab therapy in idiopathic membranous nephropathy: A 2-year study. *Clin J Am Soc Nephrol* 5: 2188–2198, 2010
27. Moroni G, Depetri F, Del Vecchio L, Gallelli B, Raffiotta F, Giglio E, Brunini F, D'Amico M, Longhi S, Radice A, Messa P, Sinico RA: Low-dose rituximab is poorly effective in patients with primary membranous nephropathy. *Nephrol Dial Transplant* 32: 1691–1696, 2017
28. Piro LD, White CA, Grillo-López AJ, Janakiraman N, Saven A, Beck TM, Varns C, Shuey S, Czuczman M, Lynch JW, Kolitz JE, Jain V: Extended Rituximab (anti-CD20 monoclonal antibody) therapy for relapsed or *refractory low-grade or* follicular non-Hodgkin's lymphoma. *Ann Oncol* 10: 655–661, 1999
29. Cartron G, Blasco H, Paintaud G, Watier H, Le Guellec C: Pharmacokinetics of rituximab and its clinical use: Thought for the best use? *Crit Rev Oncol Hematol* 62: 43–52, 2007
30. Cartron G, Trappe RU, Solal-Céligny P, Hallek M: Interindividual variability of response to rituximab: From biological origins to individualized therapies. *Clin Cancer Res* 17: 19–30, 2011
31. Thurlings RM, Teng O, Vos K, Gerlag DM, Aarden L, Stapel SO, van Laar JM, Tak PP, Wolbink GJ: Clinical response, pharmacokinetics, development of human anti-chimaeric antibodies, and synovial tissue response to rituximab treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 69: 409–412, 2010
32. Boye J, Elter T, Engert A: An overview of the current clinical use of the anti-CD20 monoclonal antibody rituximab. *Ann Oncol* 14: 520–535, 2003
33. Dähnrich C, Komorowski L, Probst C, Seitz-Polski B, Esnault V, Wetzels JF, Hofstra JM, Hoxha E, Stahl RA, Lambeau G, Stöcker W, Schlumberger W: Development of a standardized ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. *Clin Chim Acta* 421: 213–218, 2013
34. Jacobs R, Langer-Jacobus T, Duong M, Stahl K, Haller H, Schmidt RE, Schiffer M: Detection and quantification of rituximab in the human urine. *J Immunol Methods* 451: 118–121, 2017
35. Dahan K, Johannet C, Esteve E, Plaisier E, Debiec H, Ronco P: Retreatment with rituximab for membranous nephropathy with persistently elevated titers of anti-phospholipase A2 receptor antibody. *Kidney Int* 95: 233–234, 2019
36. Liu Y, Li X, Ma C, Wang P, Liu J, Su H, Zhuo H, Kong X, Xu D, Xu D: Serum anti-PLA2R antibody as a diagnostic biomarker of idiopathic membranous nephropathy: The optimal cut-off value for Chinese patients. *Clin Chim Acta* 476: 9–14, 2018

Received: October 3, 2018 **Accepted:** June 17, 2019

B.S.-P., K.D., and H.D., and G.L., P.R., and V.L.M.E. are cofirst and colast authors.

Published online ahead of print. Publication date available at www.cjasn.org.