



see commentary on page 811
OPEN

The STARMEN trial indicates that alternating treatment with corticosteroids and cyclophosphamide is superior to sequential treatment with tacrolimus and rituximab in primary membranous nephropathy

Gema Fernández-Juárez¹, Jorge Rojas-Rivera², Anne-Els van de Logt³, Joana Justino⁴, Angel Sevillano⁵, Fernando Caravaca-Fontán⁵, Ana Ávila⁶, Cristina Rabasco⁷, Virginia Cabello⁸, Alfonso Varela⁹, Montserrat Díez¹⁰, Guillermo Martín-Reyes¹¹, Marian Goicoechea Diezhandino¹², Luis F. Quintana¹³, Irene Agraz¹⁴, Juan Ramón Gómez-Martino¹⁵, Mercedes Cao¹⁶, Antolina Rodríguez-Moreno¹⁷, Begoña Rivas¹⁸, Cristina Galeano¹⁹, Jose Bonet²⁰, Ana Romera²¹, Amir Shabaka¹, Emmanuelle Plaisier^{22,23}, Mario Espinosa⁷, Jesus Egido², Alfonso Segarra¹⁴, Gérard Lambeau⁴, Pierre Ronco^{22,23}, Jack Wetzels³ and Manuel Praga^{5,24}; for the STARMEN Investigators²⁵

¹Nephrology Division, Hospital Universitario Fundación Alcorcón, Madrid, Spain; ²Nephrology Division, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain; ³Nephrology Division, Radboud University Medical Center, Nijmegen, The Netherlands; ⁴Institut de Pharmacologie Moléculaire et Cellulaire (IPMC), Université Côte d'Azur, Centre National de la Recherche Scientifique (CNRS), Valbonne Sophia Antipolis, France; ⁵Nephrology Division, Instituto de Investigación Hospital Universitario 12 Octubre, Madrid, Spain; ⁶Nephrology Division, Hospital Dr Peset, Valencia, Spain; ⁷Nephrology Division, Hospital Reina Sofía, Córdoba, Spain; ⁸Nephrology Division, Hospital Virgen del Rocío, Sevilla, Spain; ⁹Nephrology Division, Hospital Virgen de la Victoria de Málaga, Málaga, Spain; ¹⁰Fundació Puigvert, Nephrology Division, Institut Investigaci Biosanitaria Sant Pau, Autonomous University of Barcelona (UAB), Barcelona, Spain; ¹¹Nephrology Division, Hospital Regional Universitario de Málaga, Málaga, Spain; ¹²Nephrology Division, Hospital General Universitario Gregorio Marañón, Madrid, Spain; ¹³Centro de Referencia en Enfermedad Glomerular Compleja del Sistema Nacional de Salud (CSUR), Hospital Clínic de Barcelona, Universitat de Barcelona, Barcelona, Spain; ¹⁴Nephrology Division, Hospital Universitari Vall d'Hebron, Barcelona, Spain; ¹⁵Nephrology Division, Hospital San Pedro de Alcántara, Cáceres, Spain; ¹⁶Nephrology Division, Hospital Universitario de A Coruña, A Coruña, Spain; ¹⁷Nephrology Division, Hospital Clínico San Carlos, Madrid, Spain; ¹⁸Nephrology Division, Hospital Universitario La Paz, Madrid, Spain; ¹⁹Nephrology Division, Hospital Universitario Ramón y Cajal, Madrid, Spain; ²⁰Nephrology Division, Hospital Germans Trias i Pujol, Barcelona, Spain; ²¹Nephrology Division, Hospital de Ciudad Real, Ciudad Real, Spain; ²²Sorbonne Université, Université Pierre et Marie Curie Paris 06, and Institut National de la Santé et de la Recherche Médicale, Unité Mixte de Recherche S1155, Paris, France; ²³Centre de Référence Maladies Rares Syndrome Néphrotique Idiopathique - Hôpital de Jour Néphrologie – Hôpital TENON-Assistance Publique-Hôpitaux de Paris (APHP)- Sorbonne Université, Paris, France; and ²⁴Department of Medicine, Complutense University, Madrid, Spain

A cyclical corticosteroid-cyclophosphamide regimen is recommended for patients with primary membranous nephropathy at high risk of progression. We hypothesized that sequential therapy with tacrolimus and rituximab is superior to cyclical alternating treatment with corticosteroids and cyclophosphamide in inducing persistent remission in these patients. This was tested in a randomized, open-label controlled trial of 86 patients with primary membranous nephropathy and persistent nephrotic syndrome after six-months observation and assigned 43 each to receive six-month cyclical treatment with corticosteroid and cyclophosphamide or sequential treatment with tacrolimus (full-dose for six months and

tapering for another three months) and rituximab (one gram at month six). The primary outcome was complete or partial remission of nephrotic syndrome at 24 months. This composite outcome occurred in 36 patients (83.7%) in the corticosteroid-cyclophosphamide group and in 25 patients (58.1%) in the tacrolimus-rituximab group (relative risk 1.44; 95% confidence interval 1.08 to 1.92). Complete remission at 24 months occurred in 26 patients (60%) in the corticosteroid-cyclophosphamide group and in 11 patients (26%) in the tacrolimus-rituximab group (2.36; 1.34 to 4.16). Anti-PLA2R titers showed a significant decrease in both groups but the proportion of anti-PLA2R-positive patients who achieved immunological response (depletion of anti-PLA2R antibodies) was significantly higher at three and six months in the corticosteroid-cyclophosphamide group (77% and 92%, respectively), as compared to the tacrolimus-rituximab group (45% and 70%, respectively). Relapses occurred in one patient in the corticosteroid-cyclophosphamide group, and three patients in the tacrolimus-rituximab group. Serious adverse events were

Correspondence: Manuel Praga, Instituto de Investigación Hospital Universitario 12 Octubre, Avda. Córdoba s/n, 28041 Madrid, Spain. E-mail: mpragat@senefro.org

²⁵Members of the STARMEN Investigators are listed in the [Appendix](#).

Received 13 August 2020; revised 24 September 2020; accepted 11 October 2020; published online 7 November 2020

similar in both groups. Thus, treatment with corticosteroid-cyclophosphamide induced remission in a significantly greater number of patients with primary membranous nephropathy than tacrolimus-rituximab.

Kidney International (2021) **99**, 986–998; <https://doi.org/10.1016/j.kint.2020.10.014>

KEYWORDS: corticosteroids; cyclophosphamide; primary membranous nephropathy; rituximab; tacrolimus

Copyright © 2020, International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Primary membranous nephropathy (PMN) is one of the most common causes of nephrotic syndrome in adults.¹ In 70%–80% of cases, the disease is mediated by autoantibodies targeting the phospholipase A2 receptor (PLA2R) expressed in podocytes and in 3%–5% by autoantibodies to thrombospondin type 1 domain-containing 7A (THSD7A).^{2–4} Spontaneous remission occurs in one-third of patients,⁵ and therefore an observational period of at least 6 months is recommended.^{6–8} Conversely, about 50% of cases with persistent nephrotic syndrome eventually progress to end-stage kidney disease, and immunosuppressive therapy is recommended for these patients.

Controversy persists about the most effective type of immunosuppressive regimen. The 2012 Kidney Disease: Improving Global Outcomes (KDIGO) guidelines for glomerulonephritis recommended a 6-month cyclic regimen of alternating alkylating agents (usually cyclophosphamide) plus corticosteroids for patients at high risk of progression, since it was the only regimen that was shown to be effective in preventing end-stage kidney disease.^{8–12} However, given the important number of serious adverse events associated with cumulative doses of alkylating agents, treatment alternatives were introduced. Calcineurin inhibitors (both cyclosporine and tacrolimus) have shown efficacy in inducing remission of nephrotic syndrome in about 70% of patients.^{13,14} However, the main limitation of these drugs is the high rate of relapse after discontinuation. An observational study found a reduction in relapse rates when rituximab was administered at the time of tapering off cyclosporine or tacrolimus,¹⁵ and a pilot study reported encouraging results of a combined therapy with cyclosporine plus rituximab in high-risk PMN patients.¹⁶

More recently, the efficacy of rituximab monotherapy has received great attention.¹⁷ A single dose of rituximab was suggested to be effective for induction of remission in an observational cohort,¹⁸ although in recent clinical trials, higher doses were needed for optimal efficacy.^{19–21} Indeed, the superior efficacy of rituximab versus cyclosporine in the recent MENTOR (Membranous Nephropathy Trial of Rituximab) study was achieved using a total dose of 4 g rituximab.²⁰

The need for head-to-head trials comparing the 6-month cyclic alternating treatment with corticosteroids and

cyclophosphamide with the newer therapeutic alternatives (calcineurin inhibitors, rituximab) was stressed in a recent KDIGO conference.²² We designed the STARMEN (Sequential Treatment with Tacrolimus and Rituximab Versus Alternating Corticosteroids and Cyclophosphamide in PMN) study to compare cyclic alternating treatment of corticosteroids and cyclophosphamide with a sequential treatment of tacrolimus and rituximab in the induction and maintenance of nephrotic syndrome remission for up to 24 months. In addition, we studied the occurrence of relapses after remission and the role of anti-PLA2R autoantibodies over the course of the treatment.

RESULTS

Patients

From June 2014 through June 2017, 130 patients were assessed for eligibility, of whom 44 (33%) were excluded from the study. Main reasons for screening failure were not meeting the eligibility criteria (21 patients), unwillingness to participate in the study (14 patients), and diagnosis of a secondary cause of membranous nephropathy (9 patients). The remaining 86 patients who met the eligibility criteria were randomly assigned to the corticosteroid-cyclophosphamide group (43 patients) or to the tacrolimus-rituximab group (43 patients) (Figure 1). As presented in Table 1, no significant differences between groups were observed at baseline. Baseline stored serum samples were available in 69 patients, and anti-PLA2R was positive in 53 (77%). Sensitivity analyses showed no differences at baseline between anti-PLA2R-positive and anti-PLA2R-negative patients nor between patients with or without anti-PLA2R determinations at baseline (Supplementary Tables S1 and S2). One patient was anti-THSD7A positive. Kidney biopsies were performed 8 (range, 6–18) months before randomization. Seventy-three patients (85%) had de novo PMN and 13 (15%) relapsing PMN (Table 1).

All patients received at least 1 month of the assigned therapeutic intervention. Two patients in the corticosteroid-cyclophosphamide group (4.6%) and 6 (13.9%) in the tacrolimus-rituximab group discontinued the intervention (Figure 1). The remaining 41 in the corticosteroid-cyclophosphamide group and 37 in the tacrolimus-rituximab group received the complete intervention. Patients assigned to the corticosteroid-cyclophosphamide group received a median dose of oral methylprednisolone of 0.49 ± 0.05 mg/kg/day in months 1, 3, and 5, with a total cumulative dose of 3.4 ± 0.9 g. The total cumulative dose of intravenous methylprednisolone was 8.2 ± 1.4 g. The total cumulative dose of cyclophosphamide was 10 ± 3.5 g. Doses and blood levels of tacrolimus in the tacrolimus-rituximab group are presented in Supplementary Table S3. Three patients in this group received a second dose of rituximab (0.5 g in 2 patients, 1 g in 1 patient) at months 12, 12, and 18, respectively, and 2 others received additional doses of tacrolimus beyond month 9.

During the follow-up period after the end of the assigned intervention, 5 patients in each group were

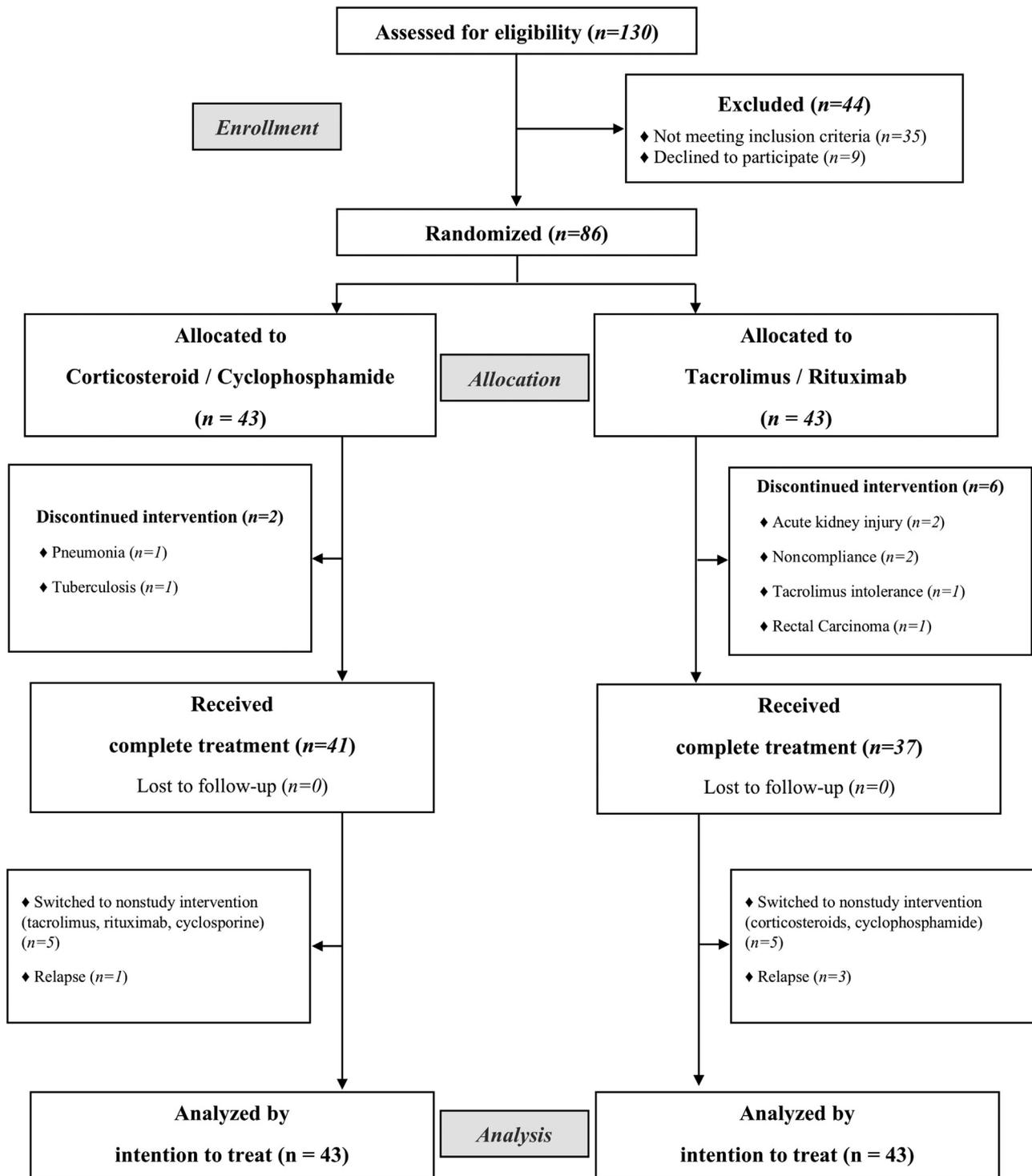


Figure 1 | Enrollment, randomization, and follow-up. Patients were randomly assigned in a 1:1 ratio to receive corticosteroid-cyclophosphamide or tacrolimus-rituximab. Premature discontinuation occurred in 2 patients in the corticosteroid-cyclophosphamide group and 6 patients in the tacrolimus-rituximab group. The remaining patients (41 in the corticosteroid-cyclophosphamide group and 37 in the tacrolimus-rituximab group) received the complete intervention. During the follow-up after the end of the assigned intervention, 5 patients in each group were switched to a nonstudy intervention owing to lack of efficacy of the assigned treatment and were considered to be nonresponders. Relapses occurred in 1 patient in the corticosteroid-cyclophosphamide group and 3 patients in the tacrolimus-rituximab group.

switched to a nonstudy intervention owing to a lack of efficacy of the assigned treatment. In the corticosteroid-cyclophosphamide group, 2 patients were treated with

rituximab at month 14, 2 patients received tacrolimus from month 18, and 1 patient cyclosporine from month 16. In the tacrolimus-rituximab group, the 5 patients received a

Table 1 | Baseline characteristics according to treatment group

| Characteristic | All patients (n = 86) | Corticosteroid-cyclophosphamide (n = 43) | Tacrolimus-rituximab (n = 43) | P value |
|---|-----------------------|--|-------------------------------|---------|
| Age, yr | 55.7 ± 11.4 | 56.2 ± 12.0 | 55.2 ± 10.8 | 1 |
| Male sex | 55 (64) | 24 (55) | 31 (72) | 0.12 |
| De novo/relapsing PMN | 73 (85) / 13 (15) | 36 (83)/7 (17) | 37 (86)/6 (14) | 0.70 |
| Weight, kg | 78.5 ± 16.0 | 76.9 ± 17.0 | 80.0 ± 15.0 | 0.37 |
| Blood pressure, mm Hg | | | | |
| Systolic | 128.3 ± 15.9 | 129.4 ± 17.7 | 127.1 ± 14.0 | 0.25 |
| Diastolic | 76.0 ± 9.8 | 75.1 ± 10.7 | 77.0 ± 8.8 | 0.37 |
| Serum creatinine, mg/dl | 1.0 ± 0.3 | 1.0 ± 0.3 | 1.0 ± 0.28 | 0.48 |
| eGFR, ml/min per 1.73 m ^{2a} | 79.8 ± 23.5 | 79.1 ± 25.5 | 80.5 ± 21.6 | 0.78 |
| Serum albumin, g/dl | 2.6 (2.2–2.9) | 2.6 (2.3–2.9) | 2.6 (2.0–2.9) | 0.47 |
| Serum cholesterol, mg/dl | 263.9 ± 64.0 | 264.1 ± 57.8 | 263.8 ± 70.2 | 0.34 |
| Anti-PLA2R-positive patients ^b | 53 of 69 (77) | 29 of 37 (78) ^c | 24 of 32 (75) ^d | 0.38 |
| Anti-PLA2R, RU/ml | 80 (44–149) | 59 (37–150) | 113 (61–151) | 0.1 |
| Urinary protein, g/24 h | 7.4 (5.2–11.5) | 7.4 (4.8–11.3) | 7.4 (6.7–11.6) | 0.22 |
| Concomitant treatment | | | | |
| ACEis and/or ARBs | 82 of 83 (99) | 39 of 43 (91) | 43 of 43 (100) | 0.48 |
| Diuretics | 70 of 83 (84) | 35 of 43 (81) | 35 of 43 (81) | 0.45 |

ACEis, angiotensin-converting enzyme inhibitors; Anti-PLA2R, anti-phospholipase A2 receptor autoantibodies; ARBs, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate; RU, relative units.

^aeGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation.

^bAnti-PLA2R positivity defined as >14 RU/ml.

^cIn 6 of 43 patients (16%) anti-PLA2R was not determined at baseline.

^dIn 11 of 43 patients (25.5%) anti-PLA2R was not determined at baseline.

Values are presented as mean ± SD, n (%), or median (interquartile range).

6-month cyclic treatment with corticosteroid and cyclophosphamide at month 12 (2 patients), month 14 (2 patients), or month 21 (1 patient). All the patients who were switched to a nonstudy intervention were considered to be nonresponders (Figure 1). Follow-up was complete in all of the 86 enrolled patients.

Primary outcome

Thirty-six patients (83.7%) in the corticosteroid-cyclophosphamide group and 25 patients (58.1%) in the tacrolimus-rituximab group had a primary outcome of complete/partial remission at 24 months (relative risk [RR] 1.44, 95% confidence interval [CI] 1.08 to 1.92) (Table 2; Figure 2a). The per-protocol analysis confirmed significant differences in the primary outcome between groups: 35 of 41 (85%) in the corticosteroid-cyclophosphamide group and 22 of 37 (59%) in the tacrolimus-rituximab group (RR 1.44, 95% CI 1.07 to 1.93) (Table 2). The difference in the number of patients with complete or partial remission in both groups was already significant at month 3 and was maintained throughout the study (Table 2; Figure 2a).

As shown in Table 3 and Figure 2b, 26 patients (60%) in the corticosteroid-cyclophosphamide group achieved complete remission at 24 months. In the tacrolimus-rituximab group, 11 patients (26%) achieved complete remission at 24 months (RR 2.36, 95% CI 1.34 to 4.16).

A tendency for a greater efficacy of the corticosteroid-cyclophosphamide treatment was found across different non-prespecified subgroups defined by baseline values of proteinuria, serum albumin, serum creatinine, anti-PLA2R levels, and age, although no differences were

found in female patients (Supplementary Figure S1). When baseline characteristics of patients were compared between those who achieved complete/partial remission and those with no response, a significantly higher proportion of men (80% vs. 57%) and a significantly higher

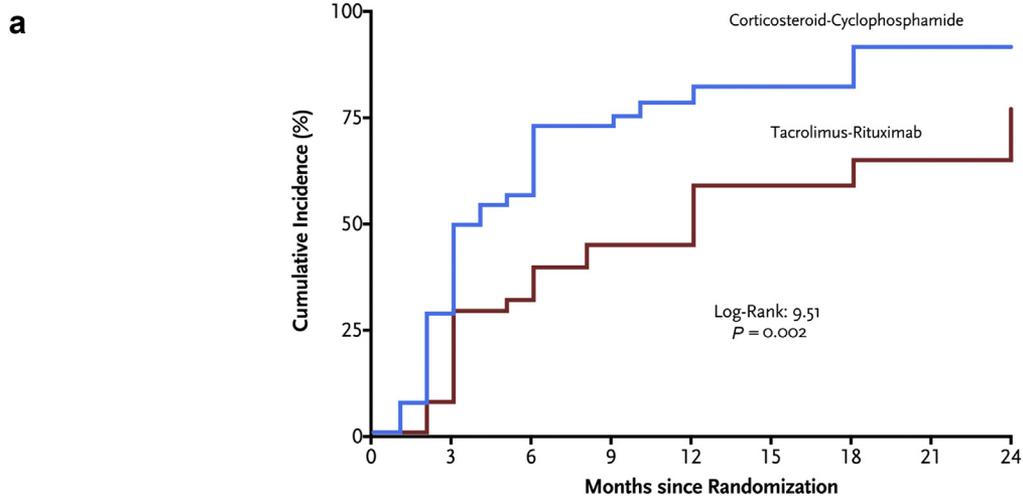
Table 2 | Composite outcome of complete or partial remission at 3 to 24 months

| Intention-to-treat analysis | | | |
|-----------------------------|--|-------------------------------|------------------------|
| Time from randomization | Corticosteroid-cyclophosphamide (n = 43) | Tacrolimus-rituximab (n = 43) | Relative risk (95% CI) |
| 3 mo | 22 of 43 (51) | 12 of 43 (28) | 1.83 (1.04–3.22) |
| 6 mo | 32 of 43 (74) | 19 of 43 (44) | 1.68 (1.15–2.46) |
| 12 mo | 34 of 43 (79) | 22 of 43 (51) | 1.55 (1.11–2.15) |
| 18 mo | 36 of 43 (84) | 23 of 43 (53) | 1.57 (1.15–2.13) |
| 24 mo | 36 of 43 (84) | 25 of 43 (58) | 1.44 (1.08–1.92) |
| Per-protocol analysis | | | |
| Time from randomization | Corticosteroid-cyclophosphamide (n = 41) | Tacrolimus-rituximab (n = 37) | Relative risk (95% CI) |
| 3 mo | 22 of 41 (51) | 12 of 37 (32) | 1.65 (0.96–2.85) |
| 6 mo | 31 of 41 (75) | 18 of 37 (48) | 1.55 (1.07–2.26) |
| 12 mo | 33 of 41 (80) | 20 of 37 (54) | 1.49 (1.07–2.08) |
| 18 mo | 35 of 41 (85) | 20 of 37 (54) | 1.58 (1.14–2.18) |
| 24 mo | 35 of 41 (85) | 22 of 37 (59) | 1.44 (1.07–1.93) |

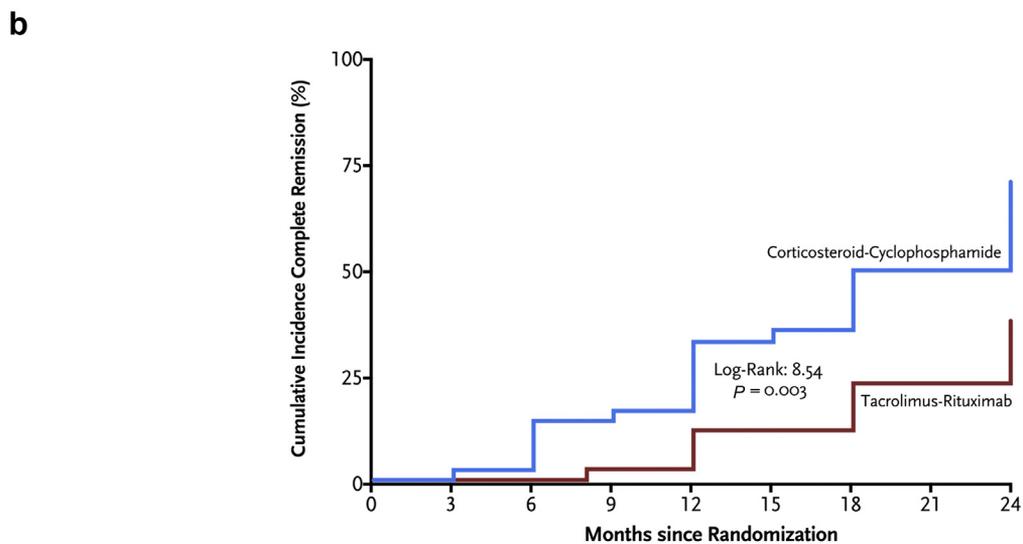
CI, confidence interval.

The intention-to-treat analyses included all the patients who underwent randomization, whereas the per-protocol analyses included all the patients who received the full course of trial medications. In both analyses, the primary outcome was the composite of complete or partial remission at 24 months.

Data are n (%) or 95% CI.



| No. at Risk | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 |
|---------------------------------|----|----|----|----|----|----|----|----|----|
| Corticosteroid-Cyclophosphamide | 43 | 31 | 19 | 12 | 6 | 4 | 4 | 1 | 1 |
| Tacrolimus-Rituximab | 43 | 39 | 27 | 20 | 16 | 7 | 7 | 6 | 6 |



| No. at Risk | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 |
|---------------------------------|----|----|----|----|----|----|----|----|----|
| Corticosteroid-Cyclophosphamide | 43 | 43 | 42 | 37 | 31 | 24 | 23 | 17 | 17 |
| Tacrolimus-Rituximab | 43 | 42 | 40 | 36 | 32 | 24 | 24 | 21 | 21 |

Figure 2 | Kaplan-Meier analysis of complete or partial remission. Kaplan-Meier estimates of (a) remission (complete or partial) and (b) complete remission in the corticosteroid-cyclophosphamide and tacrolimus-rituximab groups.

proteinuria were observed among nonresponders (Supplementary Table S4).

Secondary outcomes

Proteinuria decreased from a median 7.4 g/24 h (interquartile range 4.8–11.3) at baseline to 0.35 g/24 h (0.2–9) at 24 months in the corticosteroid-cyclophosphamide group and from 7.4 g/24 h (6.7–11.6) at baseline to 1 g/24 h (0.3–3.3) at 24 months in the tacrolimus-rituximab group (between-

group difference $P = 0.005$) (Figure 3a; Supplementary Table S5). Serum albumin increased from a mean 2.6 ± 0.1 g/dl at baseline to 4 ± 0.1 g/dl at 24 months in the corticosteroid-cyclophosphamide group and from 2.6 ± 0.1 g/dl at baseline to 3.9 ± 0.1 g/dl at 24 months in the tacrolimus-rituximab group (between-group difference $P = 0.2$) (Figure 3b; Supplementary Table S5). There was a nonsignificant trend for higher values of estimated glomerular filtration rate (eGFR) in the corticosteroid-

Table 3 | Complete remission at 3 to 24 months

| Intention-to-treat analysis | | | |
|-----------------------------|--|-------------------------------|------------------------|
| Time from randomization | Corticosteroid-cyclophosphamide (n = 43) | Tacrolimus-rituximab (n = 43) | Relative risk (95% CI) |
| 3 mo | 1 of 43 (2) | 0 of 43 (0) | |
| 6 mo | 6 of 43 (14) | 0 of 43 (0) | |
| 12 mo | 14 of 43 (33) | 4 of 43 (9) | 3.50 (1.25–9.78) |
| 18 mo | 19 of 43 (44) | 7 of 43 (16) | 2.71 (1.27–5.78) |
| 24 mo | 26 of 43 (60) | 11 of 43 (26) | 2.36 (1.34–4.16) |
| Per-protocol analysis | | | |
| Time from randomization | Corticosteroid-cyclophosphamide (n = 41) | Tacrolimus-rituximab (n = 37) | Relative risk (95% CI) |
| 3 mo | 1 of 41 (2) | 0 of 37 (0) | |
| 6 mo | 5 of 41 (13) | 0 of 37 (0) | |
| 12 mo | 13 of 41 (32) | 4 of 37 (11) | 2.93 (1.05–8.21) |
| 18 mo | 18 of 41 (44) | 7 of 37 (19) | 2.31 (1.09–4.92) |
| 24 mo | 25 of 41 (61) | 10 of 37 (27) | 2.26 (1.26–4.04) |

CI, confidence interval.

The intention-to-treat analyses included all the patients who underwent randomization, whereas the per-protocol analyses included all the patients who received the full course of trial medications. In both analyses, the primary outcome was the composite of complete or partial remission at 24 months.

Data are n (%) or 95% CI.

cyclophosphamide group than in the tacrolimus-rituximab group throughout the follow-up (Figure 3c; Supplementary Table S6). At 24 months, the numbers of patients with a $\geq 50\%$ increases of baseline serum creatinine were 1 (2%) in the corticosteroid-cyclophosphamide group and 5 (12%) in the tacrolimus-rituximab group ($P = 0.2$). The numbers of patients with preserved renal function ($eGFR \geq 45$ ml/min per 1.73 m²) at 24 months were 40 (93%) in the corticosteroid-cyclophosphamide group and 37 (86%) in the tacrolimus-rituximab group ($P = 0.48$). The only patient who developed end-stage kidney disease was a 73-year-old man who had been assigned to the corticosteroid-cyclophosphamide group. Three months after treatment completion he was excluded from the study because of persistent massive proteinuria and declining renal function and switched to rituximab (2 doses of 1 g, 15 days apart). No response was observed, and chronic dialysis was initiated 16 months after randomization.

Anti-PLA2R levels showed a significant decrease in both groups (Table 4; Figure 3a). The proportions of anti-PLA2R-positive patients who achieved immunologic response at 3 and 6 months were significantly higher in the corticosteroid-cyclophosphamide group (77% and 92%, respectively) than in the tacrolimus-rituximab group (45% and 70%, respectively) (Table 4). Most patients (80%) who achieved immunologic response during the study presented remission of nephrotic syndrome at 24 months. Immunologic response at 3 months ($P = 0.036$) and 6 months ($P = 0.005$) was associated with remission at 24 months. Nonresponding patients showed a slower decline in anti-PLA2R levels and a significantly lower proportion of immunologic responses,

compared with patients who achieved a complete or partial remission of nephrotic syndrome (Supplementary Table S7).

One out of the 36 patients (2.7%) in the corticosteroid-cyclophosphamide group, and 3 out of the 25 patients (12%) in the tacrolimus-rituximab group who had achieved partial remission presented a relapse (Supplementary Table S8). The 3 relapses in the tacrolimus-rituximab group occurred at month 12, 3 months after tacrolimus discontinuation. Two of them occurred in anti-PLA2R-positive patients who had reached an immunologic response at month 6 and were not accompanied by a reappearance of anti-PLA2R antibodies. The remaining relapse occurred in a patient in whom anti-PLA2R antibodies had not been measured at baseline. Tacrolimus was restarted in 2 patients and the other one was treated with rituximab. The relapse in the corticosteroid-cyclophosphamide group occurred at month 9 in an anti-PLA2R-positive patient who had reached immunologic response at month 3. In this case, the relapse was accompanied by a reappearance of anti-PLA2R antibodies and the patient was treated with tacrolimus.

Adverse events

All patients except 6 (1 from the corticosteroid-cyclophosphamide group and 5 from the tacrolimus-rituximab group) had at least 1 adverse event (Table 5). Most adverse events were of low (345, 84%) or medium (56, 13%) severity, but 17 (4%) were serious. There were more adverse events and more adverse events per patient in the corticosteroid-cyclophosphamide group than in the tacrolimus-rituximab group ($P = 0.04$). More patients in the corticosteroid-cyclophosphamide group had leukopenia and Cushing syndrome, while acute kidney injury, hyperkalemia, diarrhea, and distal tremor were more common in the tacrolimus-rituximab group. There was a statistically significant association between the presence of leukopenia and the development of infections, both in the total trial cohort and in each treatment group ($P < 0.0001$ for the total group and for the cyclophosphamide group, $P = 0.041$ for the tacrolimus/rituximab group). Most adverse events occurred within the first 9 months of the trial (304 of 409, 74%), but only 5 of the 17 serious adverse events occurred within this period.

There were no statistically significant differences in the frequency of serious adverse events between groups. The only case of serious acute kidney injury occurred in the tacrolimus-rituximab group, while 4 of the 5 serious infections occurred in the corticosteroid-cyclophosphamide group. Three cases of cancer were reported, although none of them was deemed to be related to the treatment received. Two cancers occurred in the corticosteroid-cyclophosphamide group (gastric adenocarcinoma and breast carcinoma, detected at 12 and 11 months, respectively) and 1 in the tacrolimus-rituximab group (rectal carcinoma, detected at 1 month). Anti-PLA2R was positive at baseline in the 3 patients with cancer. At the time of tumor detection, the 2 patients in the corticosteroid-cyclophosphamide group were in clinical remission.

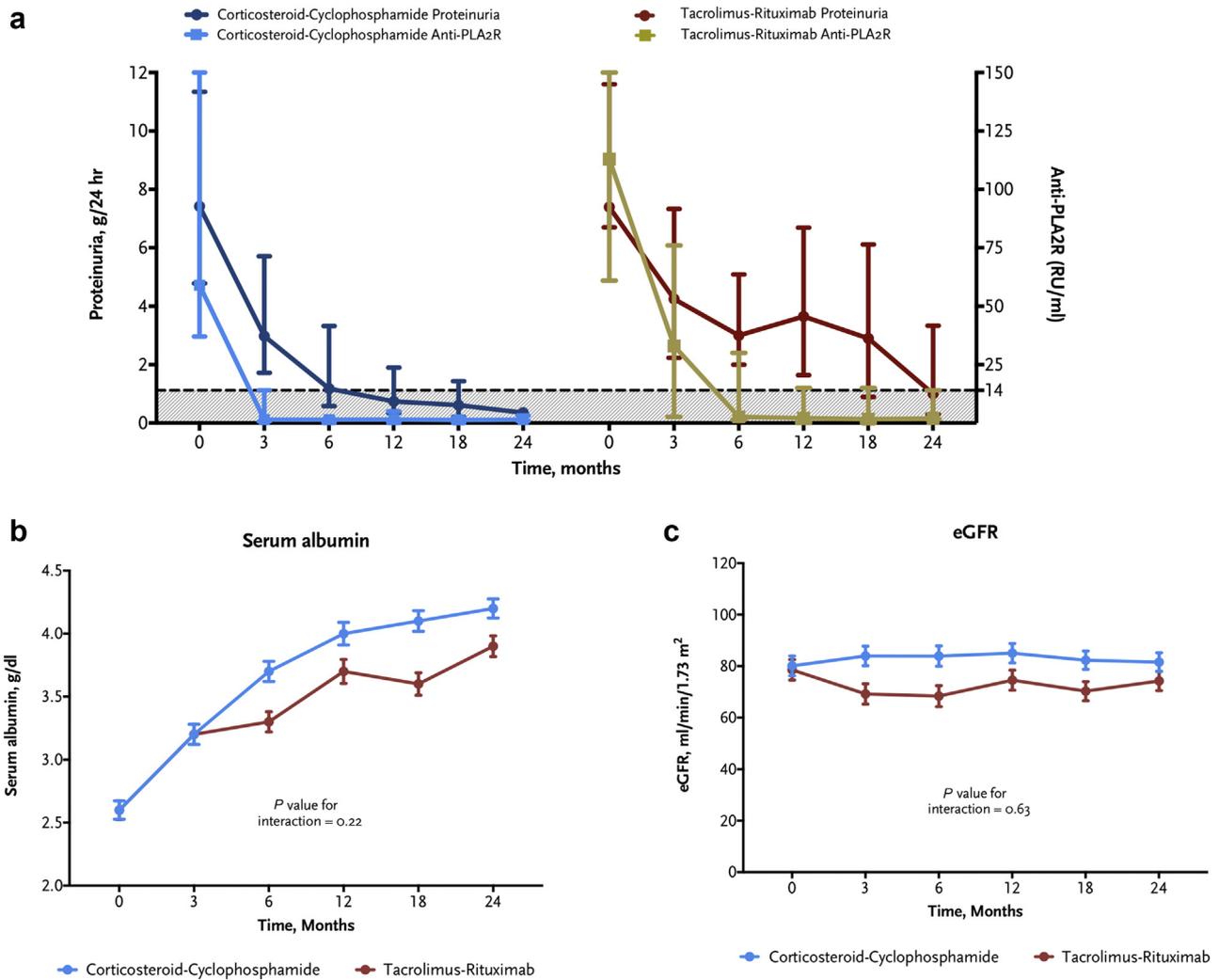


Figure 3 | Evolution of proteinuria and anti-phospholipase A2 receptor (PLA2R), serum albumin, and estimated glomerular filtration rate (eGFR). Data are presented as median (interquartile range) over time (albumin, proteinuria, anti-PLA2R) or mean ± SD by assigned treatment.

DISCUSSION

The study failed to support the hypothesis that sequential therapy with tacrolimus and rituximab was superior to cyclic alternating treatment with corticosteroids and cyclophosphamide in inducing persistent remission in patients with PMN. In fact, we found that treatment with corticosteroid and cyclophosphamide was more effective than sequential

treatment with tacrolimus and rituximab in inducing remission. The occurrence of remissions was also faster in the corticosteroid-cyclophosphamide group, with a significant difference in the number of remissions already at 3 months. Moreover, most remissions were complete in the corticosteroid-cyclophosphamide group, whereas most remissions were partial in the tacrolimus-rituximab group.

Table 4 | Evolution of anti-PLA2R antibodies and percentage of immunologic response according to treatment group

| Time from randomization | Anti-PLA2R, RU/ml, median (IQR) | | | Immunologic response, % | | | |
|-------------------------|---------------------------------|---------------------------------|----------------------|-------------------------|---------------------------------|----------------------|---------|
| | All patients | Corticosteroid-cyclophosphamide | Tacrolimus-rituximab | P value | Corticosteroid-cyclophosphamide | Tacrolimus-rituximab | P value |
| Baseline | 80 (44–149) | 59 (37–150) | 113 (61–151) | 0.1 | | | |
| 3 mo | 2.3 (1.3–65) | 1.4 (0.7–14) | 33 (2.6–76) | 0.003 | 77 | 45 | 0.03 |
| 6 mo | 1.4 (0.7–7.4) | 1.4 (0–1.7) | 2.6 (1.4–30) | 0.02 | 92 | 70 | 0.04 |
| 12 mo | 1.6 (0–8.1) | 1.5 (0–5) | 2.1 (0–15) | 0.5 | 88 | 79 | 0.31 |
| 18 mo | 1.5 (0–2) | 1.3 (0–2.3) | 1.6 (0–15) | 0.3 | 88 | 83 | 0.88 |
| 24 mo | 1.6 (1.3–5.1) | 1.4 (0.9–3.2) | 1.9 (1.7–14) | 0.06 | 88 | 83 | 0.91 |

IQR, interquartile range; PLA2R, phospholipase A2 receptor; RU, relative units. Differences of median were compared with the Mann-Whitney test.

Table 5 | Adverse events according to treatment group

| Event | Corticosteroid-cyclophosphamide (n = 43) | | Tacrolimus-rituximab (n = 43) | | P value ^a |
|--|--|-----------|-------------------------------|-----------|----------------------|
| | Patients | Events | Patients | Events | |
| Any adverse event | 42 (98) | 239 (411) | 39 (91) | 170 (280) | 0.04 |
| Serious adverse events | 8 (19) | 10 (17) | 6 (14) | 7 (12) | 0.93 |
| Fatal | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1.00 |
| Nonfatal | 8 (19) | 10 (17) | 6 (14) | 7 (12) | 0.56 |
| Nonserious adverse events | 34 (79) | 229 (394) | 33 (77) | 163 (268) | 0.04 |
| Adverse events in at least 5% of patients or any serious adverse event | | | | | |
| Systemic/general | | | | | |
| Headache | 3 (7) | 5 (9) | 5 (12) | 7 (12) | 0.48 |
| Asthenia | 7 (16) | 9 (16) | 4 (10) | 4 (7) | 0.52 |
| Depression | 3 (7) | 3 (5) | 1 (2) | 2 (3) | 0.62 |
| Anxiety | 8 (19) | 14 (24) | 4 (10) | 4 (7) | 0.35 |
| Metabolic | | | | | |
| Cushing syndrome | 7 (16) | 8 (14) | 0 (0) | 0 (0) | 0.01 |
| Hyperglycemia | 4 (9) | 4 (7) | 2 (5) | 2 (3) | 0.68 |
| Hyperlipidemia | 5 (12) | 7 (12) | 2 (5) | 2 (3) | 0.43 |
| Gastrointestinal | | | | | |
| Diarrhea | 4 (9) | 4 (7) | 13 (31) | 13 (21) | 0.02 |
| Abdominal complaint | 7 (16) | 7 (12) | 3 (7) | 4 (7) | 0.31 |
| Infections | | | | | |
| Upper respiratory infection | 10 (23) | 14 (24) | 10 (24) | 14 (23) | 1.00 |
| Pneumonia | 4 (9) | 4 (7) | 2 (5) | 2 (3) | 0.68 |
| Urinary tract infection | 4 (9) | 4 (7) | 1 (2) | 1 (2) | 0.36 |
| Tuberculosis | 1 (2) | 1 (2) | 0 (0) | 0 (0) | 1.00 |
| Musculoskeletal | | | | | |
| Bone pain | 10 (23) | 12 (21) | 9 (21) | 12 (20) | 1.00 |
| Myalgia | 7 (16) | 9 (16) | 2 (5) | 2 (3) | 0.16 |
| Cramps | 5 (12) | 6 (10) | 5 (12) | 5 (8) | 1.00 |
| Bone fracture | 1 (2) | 1 (1) | 2 (5) | 2 (3) | 0.62 |
| Neurologic | | | | | |
| Tremor | 2 (5) | 2 (3) | 7 (17) | 7 (12) | 0.09 |
| Paresthesia | 2 (5) | 2 (3) | 2 (5) | 2 (3) | 1.00 |
| Hematologic | | | | | |
| Anemia | 13 (30) | 17 (29) | 9 (21) | 10 (17) | 0.46 |
| Leukopenia | 13 (30) | 22 (38) | 2 (5) | 2 (3) | 0.003 |
| Cardiovascular and blood pressure | | | | | |
| Acute coronary syndrome | 1 (2) | 1 (2) | 1 (2) | 1 (2) | 1.00 |
| Venous thrombosis | 5 (12) | 5 (9) | 2 (5) | 2 (3) | 0.43 |
| Hypertension | 5 (12) | 7 (12) | 6 (14) | 6 (10) | 0.76 |
| Hypotension | 4 (9) | 4 (7) | 0 (0) | 0 (0) | 0.12 |
| Renal | | | | | |
| Acute kidney injury | 8 (19) | 8 (14) | 14 (33) | 16 (26) | 0.14 |
| Hyperkalemia | 1 (2) | 1 (2) | 6 (14) | 6 (10) | 0.06 |
| Edema | 6 (14) | 6 (10) | 4 (10) | 5 (8) | 0.74 |
| Dermatologic | | | | | |
| Skin eruption | 4 (9) | 4 (7) | 0 (0) | 0 (0) | 0.12 |
| Miscellaneous | | | | | |
| Drug infusion reaction | 1 (2) | 1 (2) | 4 (9) | 4 (7) | 0.36 |
| Cancer | 2 (5) | 2 (3) | 1 (2) | 1 (2) | 0.62 |
| Gastric adenocarcinoma | 1 (2) | 1 (2) | 0 (0) | 0 (0) | |
| Breast carcinoma | 1 (2) | 1 (2) | 0 (0) | 0 (0) | |
| Rectal carcinoma | 0 (0) | 0 (0) | 1 (2) | 1 (2) | |

Results for patients are presented as n (%), for events as n (rate per 100 patient-years).

^aP values are for the differences in number of events between groups.

Previous studies with calcineurin inhibitors and rituximab have demonstrated that these drugs are effective at inducing remission in PMN. It has been postulated that

the favorable effect of calcineurin inhibitors in PMN could be largely attributed to their effects on podocyte cytoskeleton, resulting in a nonspecific proteinuria

reduction.²³ However, we found that tacrolimus induced a rapid decrease in anti-PLA2R levels, in agreement with previous studies.²⁴

The main limitation of calcineurin inhibitors is the high relapse rate after discontinuation, occurring in 40%–60% of patients. In the MENTOR study,²⁰ rituximab (1 g at days 1 and 15 after randomization, and 2 other infusions at month 6 if the patient had not reached complete remission) was compared with cyclosporine given for 12 months. There were no significant differences in the number of remissions at 12 months. However, a higher proportion of patients in the cyclosporine group relapsed after treatment withdrawal, and the number of patients in remission at 24 months was significantly higher in the rituximab group (60% vs. 20%).

In our study, the number of remissions at 6 months was lower in the tacrolimus-rituximab group than in the corticosteroid-cyclophosphamide group. Rituximab infusion at month 6 did not attenuate the difference in remission rates, although the number of complete remissions increased after rituximab infusion. An important finding was the low number of relapses after tacrolimus discontinuation. This finding agrees with a previous observational study that reported a beneficial effect of rituximab, infused at the onset of calcineurin inhibitor tapering, to prevent relapses in PMN patients who had responded to cyclosporine or tacrolimus.¹⁵

The pathogenesis of relapses after tacrolimus discontinuation in PMN and the mechanisms through which rituximab can prevent this complication are unclear. Notably, rituximab is also effective in reducing the number of nephrotic syndrome relapses in other kidney diseases, such as minimal-change disease and focal and segmental glomerulosclerosis.^{25,26} In the present study, the low number of relapses compensated for the lower than expected number of remissions at 24 months. Consequently, the final response rate of remissions in the tacrolimus-rituximab group was 58%, similar to that obtained with rituximab-only in recent trials^{19,20} and better than that observed after cyclosporine or tacrolimus discontinuation without the coverage of rituximab infusion.^{13,14,24} On the other hand, it should be considered that the tacrolimus-rituximab group had 72% male subjects (vs. 55% in the corticosteroid-cyclophosphamide group) and there were trends toward higher anti-PLA2R levels, a higher interquartile range of proteinuria, and a lower interquartile range of serum albumin in this group. Although not significant, these differences at baseline may have biased the result toward an inferior outcome in the tacrolimus-rituximab group.

Since the discovery of anti-PLA2R autoantibodies as a pathogenic driver of the disease in 70%–80% of patients with PMN, a cumulative number of studies have confirmed the crucial role of serial measurement of anti-PLA2R levels to predict clinical outcomes, to evaluate the therapeutic response, and to help guiding the length of immunosuppressive therapy.^{2,27–31} We found that both corticosteroid-cyclophosphamide and tacrolimus-rituximab regimens induced a significant reduction in anti-PLA2R levels,

although immunologic response occurred faster in the corticosteroid-cyclophosphamide group. Cyclophosphamide induces generalized leukocyte and mature plasma cell ablation, resulting in a more drastic reduction in antibody production than more specifically targeted drugs such as rituximab or calcineurin inhibitors, and previous studies have demonstrated the effectiveness of alkylating agents in the most aggressive cases of PMN.^{32–34} Early immunologic response was followed by clinical remission in the vast majority of patients, which confirms the usefulness of anti-PLA2R monitoring for a personalized treatment of the disease.

The rate of adverse effects was significantly higher among patients treated with corticosteroid-cyclophosphamide, although there were no differences in the number of serious adverse events. Some studies have shown that cyclophosphamide-based therapies in PMN are accompanied by serious untoward complications.^{35,36} However, cumulative doses of cyclophosphamide were higher than those used in our study. Observational studies have suggested that noncyclic schemes of cyclophosphamide-based treatments, using lower doses of the drug, can achieve satisfactory results in PMN.³⁷ Anti-PLA2R monitoring could help to reduce treatment length in patients in which a rapid immunologic response is observed. Other retrospective studies have reported encouraging results with the use of intravenous pulses of cyclophosphamide as a substitute for oral cyclophosphamide in patients treated with the alternating cyclic regimen of corticosteroids and cyclophosphamide.^{38,39} On the other hand, it would be important to explore whether the use of lower doses of corticosteroids can reduce side-effects without decreasing the effectiveness of the treatment. These new schemes of treatment with corticosteroids and cyclophosphamide should ideally be compared with treatments based on calcineurin inhibitors and rituximab. Whether treatment with tacrolimus beyond 6 months or higher and earlier doses of rituximab could increase the remission rates in patients treated with tacrolimus-rituximab should be explored in future studies.

The present study has important limitations. There was lack of blinding regarding interventions and outcome assessment. The limited sample size prevented analysis by prespecified subgroups or detailed analysis of anti-PLA2R kinetics, and anti-PLA2R antibodies were not measured in a number of patients. Because CD19+ B cells were not measured, no information about the adequacy of rituximab dose was available. However, this study was a head-to-head prospective controlled trial that compared the classic cyclic alternating treatment with corticosteroids and cyclophosphamide versus the newer therapeutic alternatives (sequential treatment with tacrolimus and rituximab), with a sample size and follow-up period that allowed us to draw important conclusions about the treatment of PMN in clinical practice.

In conclusion, treatment with corticosteroid-cyclophosphamide induced remission (mostly complete) of

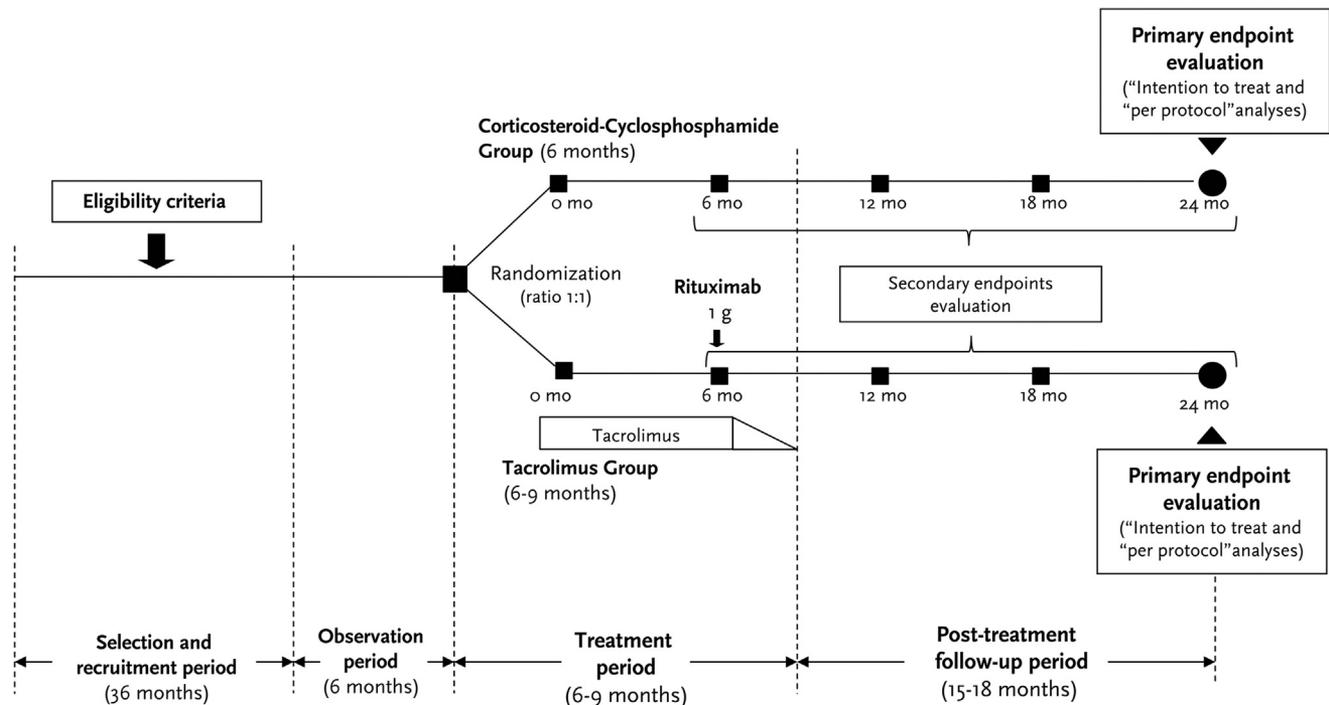


Figure 4 | Design of the study. During a 6-month observational period, treatment with angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and supportive treatment was optimized. Study visits were scheduled at screening (–1 month), at enrollment, monthly from month 1 to month 9, and at months 12, 15, 18, 21, and 24 after randomization. Clinical data and medications were recorded at every study visit. Routine laboratory tests were obtained at every study visit. Blood and urine samples were collected at baseline and at months 3, 6, 12, 18, and 24. Primary outcome evaluation was performed at 24 months per intention-to-treat and per-protocol analyses, and secondary outcomes were evaluated at 6, 12, 18, and 24 months.

nephrotic syndrome in a significantly greater number of patients than treatment with tacrolimus-rituximab, although side-effects were more frequent in the former group.

METHODS

Study design

This multicenter, prospective, open-label, randomized, controlled trial with a 2-group parallel design (Figure 4) was designed by the principal investigators and conducted at 20 sites (19 in Spain, 1 in the Netherlands). A complete list of study sites and investigators is provided in the Supplementary material. Members of the steering committee and other study committees for the trial are listed in the Supplementary material. The study protocol was previously published.⁴⁰ A data and safety monitoring board ensured the safety of the participants and the integrity of the trial. Appropriately authorized ethics committees approved the trial at all participating sites. The trial is registered at ClinicalTrials.gov: NCT01955187.

Patients

Adult patients (≥ 18 years old) with biopsy-proven PMN were eligible. All eligible patients were followed for an observational period of at least 6 months. Inclusion criteria were eGFR ≥ 45 ml/min per 1.73 m², nephrotic-range proteinuria (>4 g/24 h, without a decrease of $>50\%$ during the observational period), and hypoalbuminemia (≤ 3.5 g/dl during the observational period). All patients received the standard of care (therapy with angiotensin-converting enzyme inhibitors/angiotensin receptor blockers for at least 2 months and controlled blood pressure ($\leq 150/90$ mm Hg) for at least 3 months, with exceptions in case of intolerance,

contraindications, or low blood pressure, before the screening period. In the case of fertile women, a negative urine pregnancy test was mandatory. Exclusion criteria were secondary causes of membranous nephropathy (autoimmune or infectious diseases, neoplasms, etc.), HIV infection, liver disease, treatment with another investigational drug, suspected or known hypersensitivity or allergy reaction to drugs from study, previous treatment with corticosteroids (3 months before screening), other immunosuppressive agent (6 months before screening), rituximab, or any other biologic agent (2 years before screening), nonresponse to previous immunosuppressants, other severe condition or abnormal laboratory test with a potential risk for the patient outcome, and current drug or alcohol dependence. Full eligibility criteria as well as inclusion and exclusion criteria are provided in [Supplementary Methods](#).

Interventions and follow-up

We used a random number-producing algorithm in central computer systems for simple randomization, with an equal allocation ratio (1:1) to intervention with corticosteroid-cyclophosphamide or tacrolimus-rituximab. The subject numbers were assigned sequentially as each subject entered the study.

In the corticosteroid-cyclophosphamide group, patients received methylprednisolone at months 1, 3, and 5 (1 g intravenously at days 1, 2, and 3, then 0.5 mg/kg/day orally from day 4 to day 30). At months 2, 4, and 6, patients received oral cyclophosphamide adjusted for age and renal function (1.0–2.0 mg/kg/day for 30 days) ([Supplementary Methods](#)).

In the tacrolimus-rituximab group, patients received oral tacrolimus (0.05 mg/kg/day), to reach target blood levels of 5–7 ng/ml, for 6 months. At day 180, patients received intravenous rituximab (1 g

and tacrolimus dosage was reduced by 25% per month, with complete withdrawal at the end of month 9 (Supplementary Methods).

Tacrolimus dose was reduced in case of kidney function impairment (Supplementary Methods). To minimize infusion reactions with rituximab, patients received 100 mg methylprednisolone, 1 g acetaminophen, and 50 mg diphenhydramine. Both groups received prophylaxis with 160/800 mg oral trimethoprim/sulfamethoxazole 3 times a week during the treatment period. Patients were switched to nonstudy interventions in case of lack of response, and patients with a relapse of nephrotic syndrome were classified as “no response.”

Outcomes and definitions

The primary outcome was complete or partial remission (composite endpoint) at 24 months. Secondary end points included the rate of complete and partial remission at 3, 6, 12, 18, and 24 months; relapse of nephrotic syndrome at 6, 12, 18, and 24 months; immunologic response at 3 to 24 months; and the percentage of patients free of $\geq 50\%$ increases of serum creatinine and with preserved kidney function (eGFR ≥ 45 ml/min per 1.73 m²) at 24 months; and adverse events. Safety end points were the proportion of patients with drug-related adverse events during the study and total cumulative dose received of each study treatment.

Complete remission was defined as a reduction of proteinuria from baseline to a value ≤ 0.3 g/24 h plus stable kidney function (eGFR ≥ 45 ml/min per 1.73 m²); partial remission as a reduction of proteinuria $> 50\%$ from baseline; and a value < 3.5 g/24 h plus stable renal function (eGFR ≥ 45 ml/min per 1.73 m²). No response was defined as a proteinuria reduction of $< 50\%$ from baseline values.

Relapses were defined as a reappearance of proteinuria > 3.5 g/24 h and at least $\geq 50\%$ increase from the lowest value in 3 or more consecutive visits in patients with previous partial or complete remission. Patients were considered to be positive for anti-PLA2R when baseline serum levels were > 14 RU/ml as measured with a standardized commercial enzyme-linked immunosorbent assay⁴¹ (Euroimmune, Lubeck, Germany). Immunologic response was defined by a level of anti-PLA2R ≤ 14 RU/ml. eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation.

Statistical analysis

Based on the results of previous studies, we hypothesized a remission rate of 60% at 2 years for the corticosteroid-cyclophosphamide group and 85% for the tacrolimus-rituximab group. We planned to include 94 patients, assuming a statistical power of 80%.

Primary outcome (complete/partial remission at 24 months), was analyzed by intention-to-treat and per-protocol analyses estimating the RR with 95% CI and compared with Pearson χ^2 or Fisher exact test. Hazards for complete or partial remission at 3, 6, 12, and 18 months were also estimated for the evaluation of secondary objectives. Subgroup analyses of the primary outcome were undertaken to determine whether the difference between treatments varied according to subgroups of baseline characteristics: sex, age, albumin, proteinuria, creatinine, eGFR, and anti-PLA2R. Risk ratios and 2-side interaction *P* values were calculated with the use of multivariate modified Poisson regression models (Poisson regression with robust error variance)

For secondary outcomes, differences between the 2 groups in continuous variables were analyzed with unpaired Student *t* test or Wilcoxon rank sum test, as appropriate. Differences between

categorical variables were analyzed with likelihood χ^2 or Fisher exact test as appropriate.

Longitudinal data, such as serum albumin, serum creatinine, eGFR, and other repeated measures, from randomization until months 3, 6, 9, 12, 18, and 24 were analyzed with the use of multivariate linear mixed models. The models included time, treatment, and their interaction as fixed effect and subject as random effect, with unstructured covariance matrix. Proteinuria and anti-PLA2R levels were analyzed as median and interquartile range.

Time-to-event analyses (time to remission, time to nephrotic syndrome relapse) were performed with the use of Kaplan-Meier curves, log-rank test, and Cox proportional hazards regression models. Those patients who dropped out of the study without reaching the primary outcome were censored.

To test the proportional hazards assumption, a time-dependent covariate was defined as an interaction of the time variable and the covariate in question. The proportional hazards assumption was accepted as reasonable when the significance of the coefficient of the time dependent covariate was statistically significant. Baseline factors associated with major outcomes were determined with the use of a Cox proportional hazards regression model. The magnitude of association was reported as the hazard ratio with 95% CI.

A detailed description of the statistical methods is provided in Supplementary Methods. All statistical analyses were performed by statisticians blinded for treatment groups and using Stata 15 and SPSS 25.

APPENDIX

STARMEN Investigators

Spain. Hospital Universitario 12 Octubre, Madrid: Manuel Praga, Angel Sevillano, Fernando Caravaca-Fontan, Hernando Trujillo, Eduardo Gutiérrez; Hospital Universitario Fundación Alcorcón, Madrid: Gema Fernandez Juarez, Amir Shabaka; Hospital Universitario Fundación Jiménez Díaz, Madrid: Jorge Rojas-Rivera, Jesus Egido, Alberto Ortiz; Hospital General Universitario Gregorio Marañón, Madrid: Marian Goicoechea, Úrsula Verdalles; Hospital Universitario Vall d’Hebron, Barcelona: Alfons Segarra, Irene Agraz; Hospital Regional Universitario de Málaga: Guillermo Martín-Reyes, Lara Perea; Hospital Virgen de la Victoria de Málaga: Ildefonso Valera, Mónica Martín; Hospital Virgen del Rocío, Sevilla: Virginia Cabello, Miguel Angel Pérez Valdivia; Hospital Clinic, Barcelona: Luis F. Quintana, Miquel Blasco; Hospital Universitario de A Coruña, A Coruña: Mercedes Cao, Andrés López Muñiz; Hospital Dr. Peset, Valencia: Ana Avila, Tamara Malek; Hospital Reina Sofía, Córdoba: Mario Espinosa, Cristina Rabasco; Fundación Puigvert, Barcelona: Montserrat Diaz, Iara DaSilva; Hospital Germans Trias i Pujol, Barcelona: Jordi Bonet, Maruja Navarro; Hospital San Pedro de Alcántara, Cáceres: Juan Ramón Gómez-Martino; Hospital Universitario La Paz, Madrid: Begoña Rivas; Hospital Clínico San Carlos, Madrid: Antolina Rodríguez-Moreno; Hospital Universitario Ramón y Cajal, Madrid: Cristina Galeano; Hospital de Ciudad Real, Ciudad Real: Ana Romera; Hospital Universitario Puerta de Hierro, Madrid: Ana Huerta; Hospital Universitario de Getafe: Ezequiel Rodríguez-Paternina; Hospital Universitario Severo Ochoa, Leganes: Ana Vigil; Hospital Universitario Infanta Leonor, Madrid: Roberto Alcázar; Hospital del Henares: Vicente Paraiso; Hospital Universitario Infanta Sofía, Madrid: Vicente Barrio.

The Netherlands. Radboud University Medical Center, Nijmegen: Jack Wetzels, Anne-Els van de Logt, Julia Hofstra.

DISCLOSURE

GL has received royalty fees from Euroimmune. MP has received consultancy and speaker’s fees from Otsuka, Alexion, Fresenius, and Retrophin and research grants from Alexion. All the other authors declared no competing interests.

DATA SHARING

Data from the STARMEN trial, including patient-level data, can be made available on request from established research groups with an appropriate

data-sharing agreement. Please contact the corresponding author for data sharing.

ACKNOWLEDGMENTS

Work in this study was supported by the Instituto de Salud Carlos III/Fondo Europeo de Desarrollo Regional (ISCIII/FEDER) grants PI13/02495 and ICI14/00350, Red de Investigación Renal (RedInRen) (RD12/0021/0029), European Renal Association-European Dialysis and Transplant Association (ERA-EDTA), Fundación Renal Íñigo Álvarez de Toledo (FRIAT), and Fundación para la Investigación Biomédica Hospital 12 de Octubre (i+12). Funding agencies had no participation in any of the phases of the study. GL received grants from Centre National de la Recherche Scientifique, Fondation Maladies Rares (LAM-RD_20170304), National Research Agency (ANR, grants MNains ANR-17-CE17-0012-01), "Investments for the Future" Laboratory of Excellence SIGNALIFE, a network for innovation on signal transduction pathways in life sciences (ANR-11-LABX-0028-01) with allocated PhD fellowships for JJ, Initiative of Excellence (IDEX; UCAJedi ANR-15-IDEX-01), and Fondation pour la Recherche Médicale (FRM, ING20140129210, DEQ20180339193, and FDT201805005509 [to GL and JJ]).

We thank the trial staff at all participating sites and the patients who participated in the trial.

AUTHOR CONTRIBUTIONS

GF-J, JR-R, GL, PR, JW, and MP drafted the manuscript. GF-J, JR-R, ASevillano, FC-F, AA, CR, VC, AV, MD, GM-R, MGD, LFQ, IA, JRG-M, MC, AR-M, BR, CG, JB, AR, AShabaka, EP, ME, JE, ASegarra, PR, JW, and MP participated in data collection. FC-F performed the statistical analysis and contributed to the writing of the manuscript. All authors agreed on the content of the manuscript, reviewed drafts, and approved the final version.

SUPPLEMENTARY MATERIAL

[Supplementary File \(PDF\)](#)

Investigators and Committee Members.

Supplementary Methods.

Table S1. Sensitivity analyses according to anti-PLA2R positivity.

Table S2. Sensitivity analyses in patients with or without anti-PLA2R determination at baseline.

Table S3. Mean doses and mean blood levels of tacrolimus in the tacrolimus-rituximab group.

Table S4. Baseline characteristics of patients who achieved complete or partial remission at any time of the study and nonresponder patients.

Table S5. Proteinuria and serum albumin by group and time from randomization.

Table S6. Serum creatinine and eGFR by group and time from randomization.

Table S7. Evolution of anti-PLA2R and development of immunologic response in nonresponder patients.

Table S8. Evolution of proteinuria and anti-PLA2R in patients who presented a relapse.

Figure S1. Subgroup analyses of the primary composite outcome (complete/partial remission) at 24 months by non-prespecified characteristics of patients at baseline.

CONSORT Checklist.

REFERENCES

- Couser W. Primary membranous nephropathy. *Clin J Am Soc Nephrol.* 2017;12:983–997.
- 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;361:11–21.
- Ronco P, Debiec H. Molecular pathogenesis of membranous nephropathy. *Annu Rev Pathol.* 2020;15:287–313.
- 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;371:2277–2287.
- Polanco N, Gutiérrez E, Covarsí A, et al. Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy. *J Am Soc Nephrol.* 2010;21:697–704.
- Waldman M, Austin HA. Treatment of idiopathic membranous nephropathy. *J Am Soc Nephrol.* 2012;23:1617–1630.
- Hofstra JM, Fervenza FC, Wetzels JFM. Treatment of idiopathic membranous nephropathy. *Nat Rev Nephrol.* 2013;9:443–458.
- Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO clinical practice guidelines for glomerulonephritis. *Kidney Int Suppl.* 2012;2, 139–27.
- Ponticelli C, Zucchelli P, Passerini P, et al. A randomized trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. *N Engl J Med.* 1989;320:8–13.
- Ponticelli C, Zucchelli P, Passerini P, et al. A 10-year follow-up of a randomized study with methylprednisolone and chlorambucil in membranous nephropathy. *Kidney Int.* 1995;48:1600–1604.
- 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;9:444–450.
- Jha V, Ganguli A, Saha TK, et al. A randomized, controlled trial of steroids and cyclophosphamide in adults with nephrotic syndrome caused by idiopathic membranous nephropathy. *J Am Soc Nephrol.* 2007;18:1899–1904.
- Cattran DC, Appel GB, Hebert LA, et al. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int.* 2001;59:1484–1490.
- Praga M, Barrio V, Juárez GF, Luño J. Tacrolimus monotherapy in membranous nephropathy: a randomized controlled trial. *Kidney Int.* 2007;71:924–930.
- Segarra A, Praga M, Ramos N, et al. Successful treatment of membranous glomerulonephritis with rituximab in calcineurin inhibitor-dependent patients. *Clin J Am Soc Nephrol.* 2009;4:1083–1088.
- Waldman M, Beck LH Jr, Braun M, et al. Membranous nephropathy: pilot study of a novel regimen combining cyclosporine and Rituximab. *Kidney Int Rep.* 2016;1:73–84.
- Ruggenenti P, Cravedi P, Chianca A, et al. Rituximab in idiopathic membranous nephropathy. *J Am Soc Nephrol.* 2012;23:1416–1425.
- 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.* 2007;2:932–937.
- 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;28:348–358.
- Fervenza FC, Appel GB, Barbour SJ, et al. Rituximab or cyclosporine in the treatment of membranous nephropathy. *N Engl J Med.* 2019;381:36–46.
- Seitz-Polski B, Dahan K, Debiec H, et al. High-dose rituximab and early remission in pla2r1-related membranous nephropathy. *Clin J Am Soc Nephrol.* 2019;14:1173–1182.
- Floege J, Barbour SJ, Cattran DC, et al. Management and treatment of glomerular diseases (part 1): conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int.* 2019;95:268–280.
- Faul C, Donnelly M, Merscher-Gomez S, et al. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. *Nat Med.* 2008;14:931–938.
- Ramachandran R, Kumar A, Kumar V, et al. Two-year follow-up study of membranous nephropathy treated with tacrolimus and corticosteroids versus cyclical corticosteroids and cyclophosphamide. *Kidney Int Rep.* 2017;2:610–616.
- Iijima K, Sako M, Nozu K, et al. Rituximab for childhood-onset, complicated, frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome: a multicentre, double-blind, randomised, placebo-controlled trial. *Lancet.* 2014;384:1273–1281.
- Ruggenenti P, Ruggiero B, Cravedi P, et al. Rituximab in steroid-dependent or frequently relapsing idiopathic nephrotic syndrome. *J Am Soc Nephrol.* 2014;25:850–863.
- van de Logt AE, Fresquet M, Wetzels JF, Brenchley P. The anti-PLA2R antibody in membranous nephropathy: what we know and what remains a decade after its discovery. *Kidney Int.* 2019;96:1292–1302.
- 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.* 2017;28:421–430.
- 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*. 2014;25:1357–1366.
30. 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;26:2545–2558.
 31. Glasscock RJ. Antiphospholipase A2 receptor autoantibody guided diagnosis and treatment of membranous nephropathy: a new personalized medical approach. *Clin J Am Soc Nephrol*. 2014;9:1341–1343.
 32. van de Logt AE, Dahan K, Rousseau A, et al. Immunological remission in PLA2R-antibody-associated membranous nephropathy: cyclophosphamide versus rituximab. *Kidney Int*. 2018;93:1016–1017.
 33. Howman A1, Chapman TL, Langdon MM, et al. Immunosuppression for progressive membranous nephropathy: a UK randomised controlled trial. *Lancet*. 2013;381:744–751.
 34. Oleinika K, Mauri C, Salama AD. Effector and regulatory B cells in immune-mediated kidney disease. *Nat Rev Nephrol*. 2019;15:11–26.
 35. 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;9:1066–1073.
 36. 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;28:2729–2737.
 37. Eriguchi M, Oka H, Mizobuchi T, et al. Long-term outcomes of idiopathic membranous nephropathy in Japanese patients treated with low-dose cyclophosphamide and prednisolone. *Nephrol Dial Transplant*. 2009;24:3082–3088.
 38. Mathrani V, Alejmi A, Griffin S, Roberts G. Intravenous cyclophosphamide and oral prednisolone is a safe and effective treatment option for idiopathic membranous nephropathy. *Clin Kidney J*. 2017;10:450–454.
 39. Kanigicherla DAK, Hamilton P, Czaplak K, Brenchley PE. Intravenous pulse cyclophosphamide and steroids induce immunological and clinical remission in new-incident and relapsing primary membranous nephropathy. *Nephrology (Carlton)*. 2018;23:60–68.
 40. Rojas-Rivera J, Fernández-Juárez G, Ortiz A, et al. A European multicentre and open-label controlled randomized trial to evaluate the efficacy of sequential treatment with tacrolimus-rituximab versus steroids plus cyclophosphamide in patients with primary membranous nephropathy: the STARMEN study. *Clin Kidney J*. 2015;8:503–510.
 41. Dahnrich C, Komorowski L, Probst C, et al. Development of a standardized ELISA for the determination of autoantibodies against human M-type phospholipase A2 receptor in primary membranous nephropathy. *Clin Chim Acta*. 2013;421:213–218.