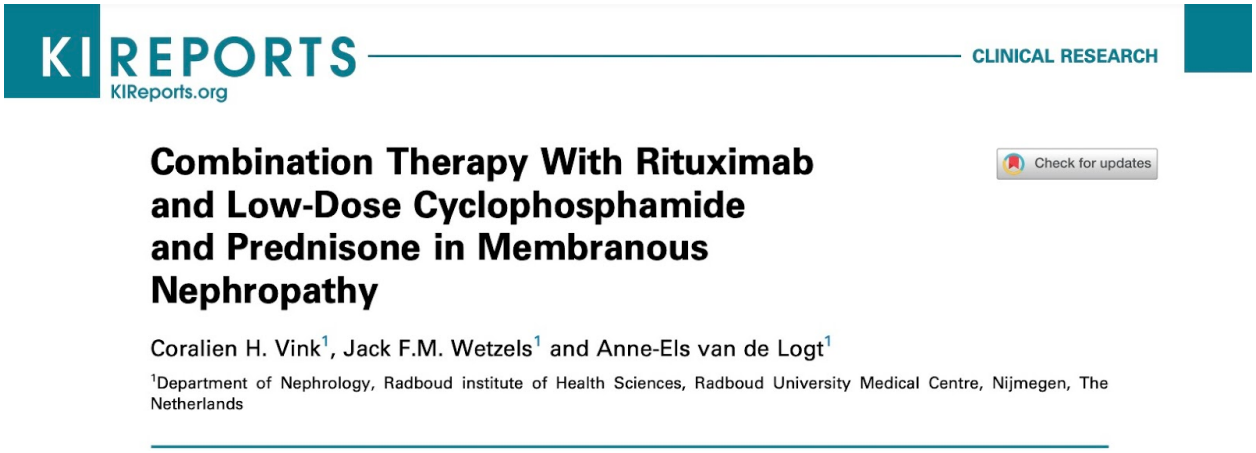
1/Hey #NephTwitter! Welcome to a 🆕 #tweetorial #xtorial brought to you by @KIReports @ISNkidneycare #XTwitter. Our author is @elbaonelida nephrologist at Hospital General de Mexico @hgm_nefrologia. Please also check out #KIReportsCommunity educational #blogposts. FOLLOW US !

2/ 🚨 Our topic🚨: Combination Therapy With Rituximab and Low Dose Cyclophosphamide and Prednisone: Rapid Immunological Remission in Membranous Nephropathy. There are no conflicts of interest. #MedTwitter #nephtwitter @ISNkidneycare

Article: https://www.kireports.org/article/S2468-0249%2824%2901921-1/fulltext



3/ 💡 Let's start with a quiz. What % of patients with Membranous Nephropathy (MN) have PLA2R abs circulating in the serum? 🧐

a) 50%
b) 60%
c) 70%
d) 90%

4/ ▶️ The correct answer is 70%.

PLA2R is present in normal podocytes and immune deposits in patients with MN. Beck Jr et al.2009 found that most patients have antibodies against a conformation-dependent epitope in PLA2R.

https://www.nejm.org/doi/pdf/10.1056/NEJMoa0810457

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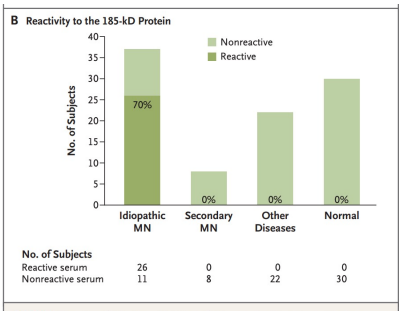


Figure 1. Results of Western Blotting of Glomerular Proteins with Serum from Patients with Idiopathic Membranous Nephropathy.

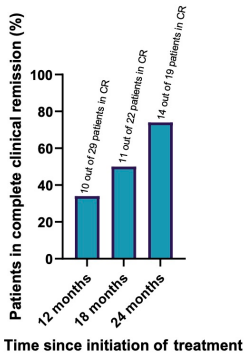
The top of Panel A shows the results of Western blotting of extract of human glomerular proteins with serum samples from each of five patients with idiopathic membranous nephropathy (MN1 through MN5) and five patients with other proteinuric conditions (two with focal and segmental glomerulosclerosis [FGS1 and FGS2] and three with diabetic nephropathy [DN1, DN2, and DN3]). Serum samples from the five patients with membranous nephropathy all recognized a band of approximately 185 kD, whereas the samples from the patients with other diseases did not. The bottom of Panel A shows the results of Western blotting, with reactive serum samples from the five patients with membranous nephropathy, of glomerular proteins that were deglycosylated with peptide *N*-glycosidase F (PNGase F+) or not deglycosylated (PNGase F–). All five samples showed the 185-kD native antigen and a deglycosylated protein of approximately 145 kD. Panel B shows the specificity of the reactivity of serum samples to the 185-kD antigen.

5/ Current tx modalities are suboptimal: standard dose cyclophosphamide (CP) & prednisone (6–12 mo) is effective, but associated with toxicity; Rituximab (RTX) 1 to 4 g i.v. is safe, with a ⬆️ primary failure rate, and tx with calcineurin inhibitors (12 mo) with ⬆️ relapse rate

6/ Why would we consider rituximab + CP ? A case series used combination therapy with RTX [cumulative dose of 8g over a period of 2 ys], oral CP [8 wks], and prednisone (24 wks) revealed very ⬆️ clinical remission (CR)

https://www.ajkd.org/article/S0272-6386%2821%2900690-9/fulltext

Figure S3. Complete clinical remission in baseline PLA2R seropositive patients.



The proportion of baseline PLA2R seropositive patients who achieved complete clinical remission at 12, 18, and 24 months of follow-up since initiating RCP was 34% (10 out of 29 patients), 50% (11 out of 22 patients), and 74% (14 out of 19 patients), respectively.

CR = complete remission

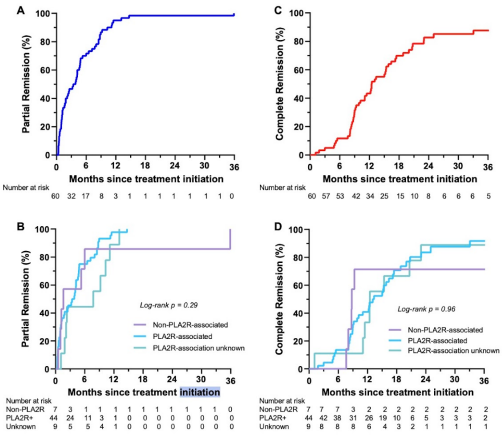


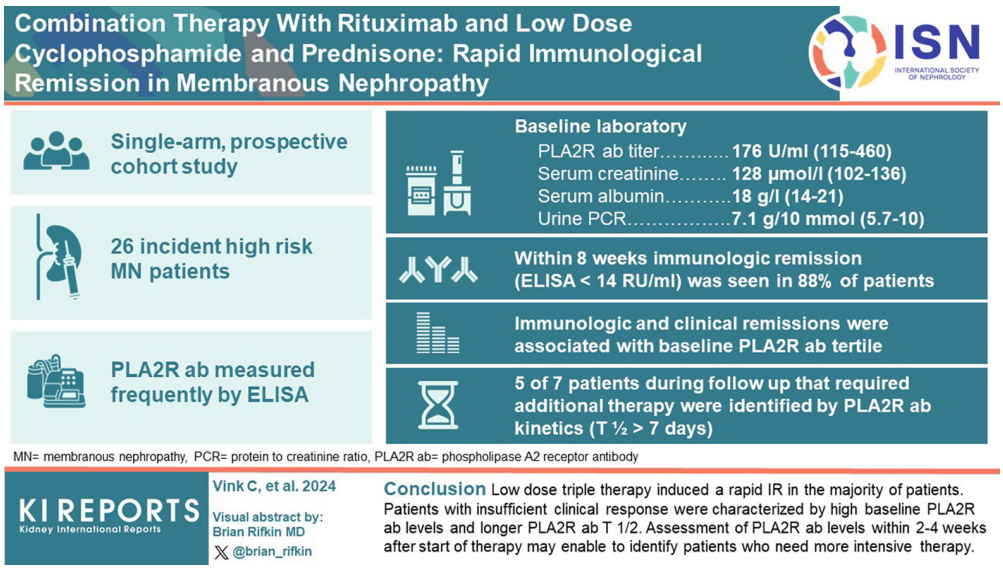
Figure 1. Kaplan-Meier curves for partial and complete remission. Kaplan-Meier curves for the overall group and when stratified by PLA_2R status are shown for partial remission (A and B) and complete remission (C and D). Abbreviation: PLA_2R, phospholipase A_2 receptor.

7/ Why would we consider low-dose rituximab + CP?

💥Because many patients develop remission with less intensive therapy, a more tailor-made approach is necessary. A regimen of low-dose RTX, CP, & steroids has been successfully used in ANCA-associated vasculitis.

8/ ✨🎯This study studied the effectiveness and tolerability of a low-dose triple therapy regimen in patients with high-risk (KDIGO 2012) PLA2R ab-MN and the kinetics of the PLA2R ab response. Let's take a look at VA by @Brian_Rifkin🤩

🔗https://www.kireports.org/article/S2468-0249%2824%2901921-1/fulltext



9/ Per KDIGO 2012 High risk for progression in NM was considered with persistent proteinuria >8 g/d, independent of the degree of kidney dysfunction

https://kdigo.org/wp-content/uploads/2017/02/KDIGO-2012-GN-Guideline-English.pdf

10/ In recently guidelines KDIGO 2021 definition of High risk was improved, one of which was the addition PLA2R ab levels

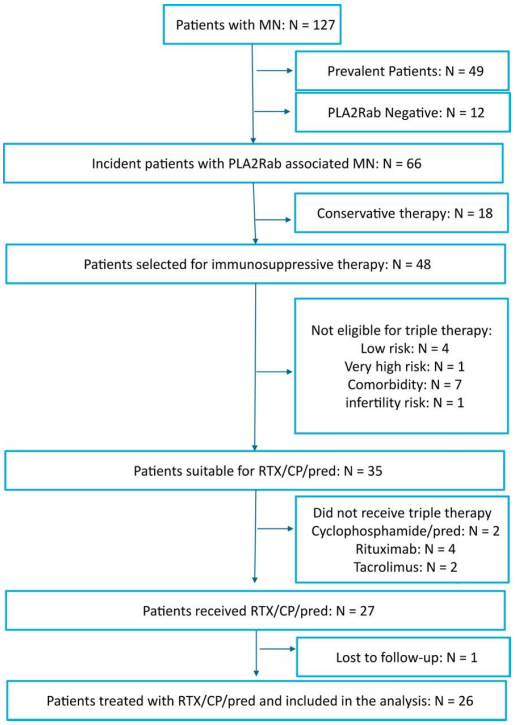
https://www.kidney-international.org/article/S0085-2538%2821%2900562-7/fulltext

Practice Point 3.2.1: In patients with MN, use clinical and laboratory criteria to assess the risk of progressive loss of kidney function (Figure 30).

Low risk	Moderate risk	High risk	Very high risk
• Normal eGFR, proteinuria <3.5 g/d and serum albumin >30 g/l OR • Normal eGFR, proteinuria <3.5 g/d or a decrease >50% after 6 months of conservative therapy with ACEi/ARB	• Normal eGFR, proteinuria >3.5 g/d and no decrease >50% after 6 months of conservative therapy with ACEi/ARB AND • Not fulfilling high-risk criteria	• eGFR <60 ml/min/1.73 m²* and/or proteinuria >8 g/d for >6 months OR • Normal eGFR, proteinuria >3.5 g/d and no decrease >50% after 6 months of conservative therapy with ACEi/ARB AND at least one of the following: • Serum albumin <25 g/l† • PLA2Rab >50 RU/ml‡ • Urinary α_1-microglobulin >40 µg/min • Urinary IgG >1 µg/min • Urinary β_2-microglobulin >250 mg/d • Selectivity index >0.20§	• Life-threatening nephrotic syndrome OR • Rapid deterioration of kidney function not otherwise explained

Figure 30| Clinical criteria for assessing risk of progressive loss of kidney function. eGFR and PCR are used in routine clinical care. Other biomarkers may not be available in all centers; this table provides an overview of useful biomarkers. *Most studies have used serum creatinine (SCr) values to guide management, and SCr values >1.5 mg/dl (133 µmol/l) are often used to define kidney insufficiency. An eGFR value of 60 ml/min per 1.73 m^2 defines kidney insufficiency in a young adult. It is important to realize that eGFR decreases with age, and an SCr value of 1.5 mg/dl (133 µmol/l) reflects an eGFR of 50 ml/min per 1.73 m^2 in a 60-year-old male patient and 37 ml/min per 1.73 m^2 in a 60-year-old female patient. Thus, when using eGFR in risk estimation, age should be taken into account. †Serum albumin should be measured by BCP or immunometric assay. ‡Cutoff values are not validated. Anti-PLA2R antibodies should be measured at 3-to-6-month intervals, the shorter interval being performed in patients with high anti-PLA2R antibodies levels at baseline. Changes in anti-PLA2R antibodies levels during follow-up likely add to risk estimation. Disappearance of anti-PLA2R antibodies precedes clinical remission and should lead to refraining from additional therapy. Detailed data are lacking. §Selectivity index is calculated as clearance of IgG/clearance of albumin. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BCP, bromocresol purple; eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; PLA2Rab, antibodies against the M-type phospholipase A2 receptor.

11/ 🔶This study was a single-arm prospective, cohort from October 2020 to October 2022🗒️, patients were adults with MN, PLA2R ab, and high risk for disease progression, 127 patients were evaluated for immunosuppressive therapy, 37 were eligible for combined treatment.



12/🌟Analyzed 26 incident patients who were followed up for a median of 26 months, most were men, serum creatinine was 128 umol/l (1.44 mg/dL) , and uPCR 7.1 g/10 mmol. 📉The development of IR (Immunological Remission) after 8 wks, and clinical response were associated with baseline PLA2R ab levels and kinetics.

Table 1. Clinical characteristics and outcome of treated patients, according tertiles of PLA2Rab levels

PLA2Rab tertile	All ($N = 26$)	Low ($n = 8$)	Middle ($n = 9$)	High ($n = 9$)	*P*-value
PLA2Rab (RU/ml)	176 (115–460)	16–124	131–268	273–1600	
Gender (M/F)	15/11	3/5	7/2	5/4	0.24
Age (± yr)	57 ± 14	52 ± 13	62 ± 9	58 ± 18	0.32
sCreatinine (µmol/l)	128 (102–136)	132 (84–177)	128 (115–134)	110 (97–141)	0.48
sAlbumin (g/l)	18 (14–21)	20 (15–25)	17 (14–19)	18 (14–22)	0.48
uPCR (g/10 mmol)	7.1 (5.7–10)	5.8 (4.2–10.0)	7.0 (6.6–8.0)	9.4 (6.1–10.2)	0.90
IR (<14RU/ml) after 8 wk	23	8	9	6	0.01
IR (<2RU/ml) after 8 wk	19	7	9	3	<0.01
PLA2Rab after 12 wks (RU/ml)	1.0 (range 1–65)	1.0 (IQR 1.0–1.0, range 1.0–4.0)	1.0 (IQR 1.0–1.0, range 1.0–1.0)	1.0 (IQR 1.0–10, range 1.0–65)	0.05
Albumin after 12 wk (g/l)	30 (23–32)[a]	31 (23–32)[a]	30 (26–34)	25 (22–30)[a]	0.44
uPCR after 12 wk (g/10 mmol)	4.4 (2.0–6.4)[a]	3.6 (1.5–4.0)[a]	4.2 (1.0–5.9)	6.0 (4.6–8.3)[a]	0.02
PLA2Rab $T_{1/2} > 7$ d	5	0	0	5	<0.01
FU duration (mo from start immunosuppression)	26 (21–30)	25 (24–31)	28 (20–32)	26 (18–30)	0.70
Partial remission (before additional therapy)	21	8	9	4	
Complete remission (before additional therapy)	10	4	5	1	
Partial remission EFU	25	8	8	9	
Complete remission EFU	9	4	3	2	
Additional therapy[b]	7	0	0	7	<0.01

EFU, end of follow-up; F, female; FU, follow-up; IQR, Interquartile range; IR, immunological remission; M, male; PLA2Rab, anti-PLA2R antibody level; sAlbumin, serum albumin; sCreatinine, serum creatinine; $T_{1/2}$, half-life; uPCR, urinary protein-to-creatinine ratio.
[a]Albumin and protein-to-creatinine ratio after 12 weeks are missing in 1 patient in the low tertile and 1 patient in the highest tertile, respectively.
[b]Additional therapy means repeated administration of rituximab ($n = 5$), tacrolimus ($n = 1$), rituximab/cyclophosphamide + prednisolone ($n = 1$) because of persistent nephrotic syndrome ($n = 5$), or early relapse ($n = 2$).

13/A rapid⬇️of PLA2R ab levels from 176 to 1 (Delta change 98%), IR:88%, strict IR:73% after 8 weeks. Also, there are differences in PLA2Rab kinetics, T ½ of <7 days in all patients with PLA2Rab levels in the 1st and 2nd tertiles &>7 days in 5 of 9 patients in the highest tertiles.

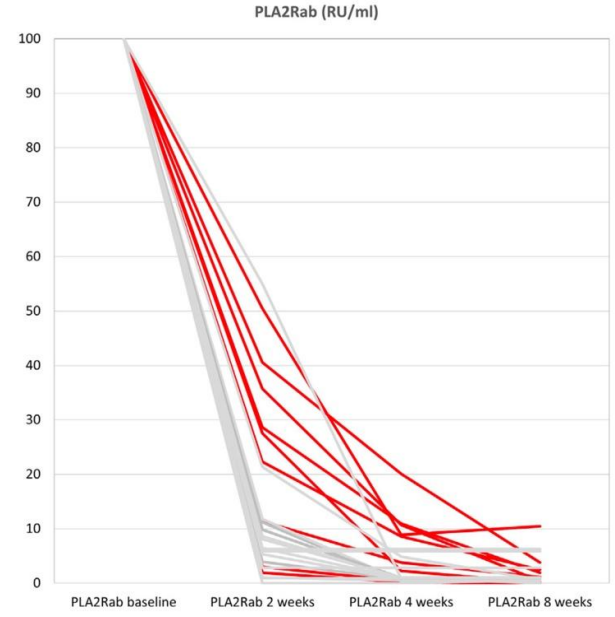


Figure 2. Percentage decrease of PLA2Rab during treatment (baseline titer is plotted as 100%). Patients in the highest tertile of PLA2Rab are depicted in red. PLA2Rab half-life is more than 7 days in 5 of 9 patients in the highest tertile (identifiable by PLA2Rab levels

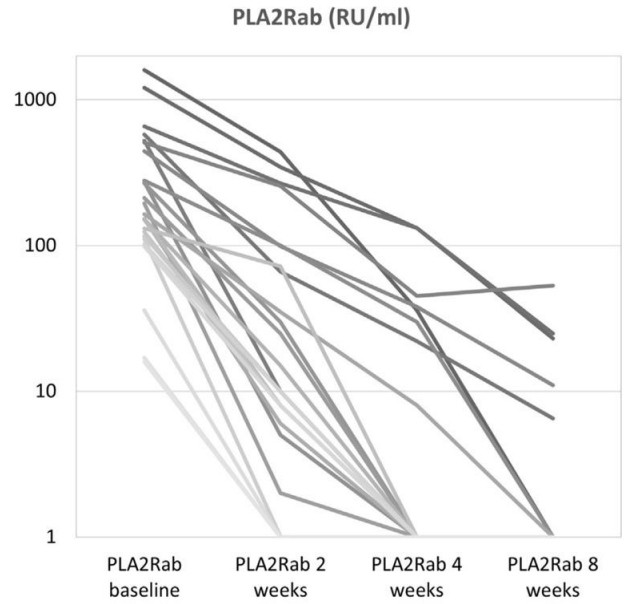


Figure 3. Course of absolute PLA2Rab levels during treatment. PLA2Rab half-life is more than 7 days in 5 of 9 patients with a baseline titer in the highest tertile.

14/💥Very rapid decrease in PLA2R ab levels; a 📉of >75% was observed in 22 of 26 patients after 2 weeks, and a reduction of >93.5% in 22 of 26 patients after 4 weeks. Extrapolating from these figures, PLA2Rab levels decreased by >50% after 1 week in 21 out of 26 patients (81 %).

15/🌟During follow-up and before the start of second-line therapy, proteinuria remission developed in 81% which persisted until the end of follow-up in all but 1 patient. A triple therapy regimen is effective in MN, though a quarter of patients require additional therapy💊 n=7

Tabel 2. Overview of 7 patients that received additional therapy in the highest tertile

	Baseline			12 wks			2nd line therapy			
Patient	PLA2Rab	sAlb	uPCR	PLA2Rab	sAlb	uPCR	Interval (mo)	Reason	Type	Rem (mo)
1	1600	17	10.1	1	21	4.5	11	Relapse	RTX 2 × 1000 mg i.v.	4
2	1206	27	4.5	12	23	13.3	9	Persisting NS	RTX 2 × 1000 mg i.v.	8
3	658	18	9.7	8	31	8.6	3	Persisting NS	Tacrolimus	3
4	525	11	6.1	1	21	4.1	6	Persisting NS	MPS 3 × 1000 mg iv + RTX 2 × 1000 mg i.v.	4
5	507	19	6.7	65	23	7.6	5	Persisting NS; renal function decline after RTX	RTX 2*1000 mg i.v.; followed by CP + Pred 2 mo	8
6	444	13	6.1	2	28	4.7	4	Persisting NS	RTX 2 × 1000 mg i.v.	6
7	273	15	11.7	1	27	7.0	14	Relapse	RTX 2 × 1000 mg i.v.	10

CP, cyclophosphamide; MPS, methylprednisolone pulses; NS, nephrotic syndrome; PLA2R, anti-PLA2R titer in RU/ml; Pred, prednisolone; Rem, Remission, interval 2nd line therapy and partial remission; RTX, rituximab; sAlb, albumin in g/l; uPCR, urine protein-to-creatinine ratio in grams/10 mmol.

16/💥 PLAR2 ab had decreased by more than 99% in most patients by week 12, and response preceded the changes in serum albumin and uPCR. 📉Changes in albumin were variable and less pronounced at this time point.

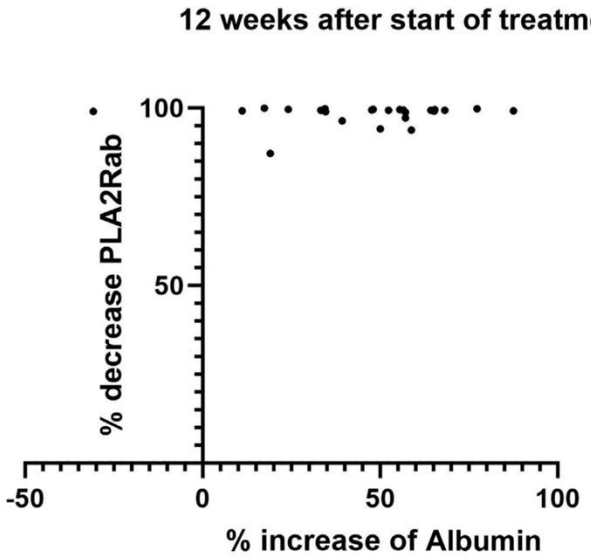


Figure 4. Percentage decrease of PLA2Rab versus percentage increase of serum albumin 12 weeks after start of treatment. Calculation: percentage change = (C12 − C0)/ (Cn − C0), where C12 = concentration at week 12, C0 is concentration at week 0, and Cn = normal concentration (for PLA2Rab = 0 RU/ml; for Salb = 40 g/l).

17/ ⚠️There were 4 serious adverse events. The patient who developed pneumocystis jiroveci pneumonia was not prescribed prophylaxis. Another patient experienced a pulmonary embolism due to noncompliance with oral anticoagulation.💊

Table 3. (Serious) adverse events

Serious adverse events	$n = 1$ pneumocystis jerovici pneumonia (2 mo after start) $n = 1$ pneumosepsis (3 mo after start) $n = 1$ Influenza A (2 mo after start) $n = 1$ pulmonary embolism (1 mo after start)
Adverse events	$n = 6$ leukopenia $n = 1$ liver test abnormalities $n = 1$ urinary tract infection $n = 3$ poor sleep quality $n = 2$ loss of hair $n = 2$ nausea $n = 1$ low IgG

18/🧵Initial therapy combining low-dose RTX, CP, and prednisone resulted in a high rate of IR (88%) and CR (73%) vs initial RTX treatment (4 g i.v. in MENTOR, with IR and CR 60%) vs low-dose CP (42% CR with 9 wks of CP and steroids) 👇

🔗https://www.nejm.org/doi/10.1056/NEJMoa1814427

🔗https://www.kireports.org/article/S2468-0249%2822%2901898-8/fulltext

Study in MN	Type of study	Admission criteria	Treatment	Response
Fervenza et al 2019 (Mentor study)	Open- label, randomized, multicenter, non-inferiority trial	- Proteinuria ≥ 5 g per 24 horas - eTFG ≥ 40ml/min/1.73m2. - Had been receiving RAAS blockade (3mo)	RTX (4 g i.v.) 2 infusions, 1000 mg each, administered 14 days apart; repeated at 6 months in case of partial response vs oral cyclosporine (starting at a dose of 3.5 mg per kilogram of body weight per day for 12 months)	Immunological and clinical response (60%) Rituximab was noninferior to cyclosporine in inducing complete or partial remission of proteinuria at 12 months and was superior in maintaining proteinuria remission up to 24 months
Vink et al. 2023	Cohort	- High risk of progression - Persisting nephrotic syndrome for more than 6 months despite conservative treatment, and - ↑ urinary excretion of β-2-microglobulin (>1000 ng/min) and/or ↑ excretion of α-1 –macroglobulin (> 40ug/min) or - deteriorating kidney function, or - severe symptoms related to nephrotic syndrome.	Low dose CP 1.5 mg/kg/d and steroids in cycles of 8 wks 1000 mg for 3 days at day 1–3, in some cases also at days 61–63 and 121–123), oral prednisone (0.5 mg/kg on alternate days)	42% clinical response
Vink et al. 2024	Single-arm prospective cohort study	PLA2R ab, and high risk for disease progression	Low-dose RTX (2 x 1000mg), CP (1.5mg/kg/d x 8 weeks), and prednisone (I.V. 2 X 1g + 3 weeks oral [1 wk 0.5 mg/kg and 1 wk 0.25 mg/kg]	Immunological response (88%) Clinical remission (73%)

Infographic by Elba Medina @elbaonelida

19/ 🤔Data of PLA2R ab kinetics are limited. Here are some studies📊👇

Author	% Decrease in PLA2Rab titers around 1 week	% or number of patients with decrease	Treatment
Mahmud et al. 2019[1]	50%	Less than 50 %	Rituximab
Rosenzwajg et al.2017[2]	≥ 50%	2 out of 8 patients	Rituximab
Vink, C.H, et al .2022[3]	50%	<50% (22 of 42)	CP and steroids
Robin BH, et al. 2024 [4]	> 50% decrease	less than 50%	Feltarzamab (anti-CD38)

[1]Mahmud, M. et al. Role of phospholipase A2 receptor 1 antibody level at diagnosis for long-term renal outcome in membranous nephropathy, *PLoS One*. 2019; 14, e0221293.
[2] Rosenzwajg, M. et al. B- and T-cell subpopulations in patients with severe idiopathic membranous nephropathy may predict an early response to rituximab *Kidney Int.* 2017; 92:227-237
[3] Vink, C.H. et al. Antibody-guided therapy in phospholipase A2 receptor-associated membranous nephropathy
Kidney Int Rep. 2022; 8:432-441
[4] Rovin, BH. et al. Phase 1b/2a study assessing the safety and efficacy of felzartamab in anti-phospholipase A2 receptor autoantibody-positive primary membranous nephropathy *Kidney Int Rep.* 2024; 9:2635-2647

20/👀In this cohort, 27% of patients showed an insufficient clinical response to the low-dose therapy. However, after renewed therapy (primarily with additional RTX pulses), the CR rate improved to 100%📉

21/ The treatment was not guided by PLA2R ab levels🧪. All patients received triple therapy, with CP withdrawn after 8 weeks. Renewed therapy was provided only to patients with insufficient clinical response or with relapse.

22/ KDIGO 2021 recommends measuring PLA2R ab at 3-to-6-month intervals, the shorter interval being performed in patients with high anti-PLA2R antibodies levels at baseline; however this study suggests measuring PLA2R ab at 1, 2, and 4 weeks after therapy initiation.

https://kdigo.org/guidelines/gd/

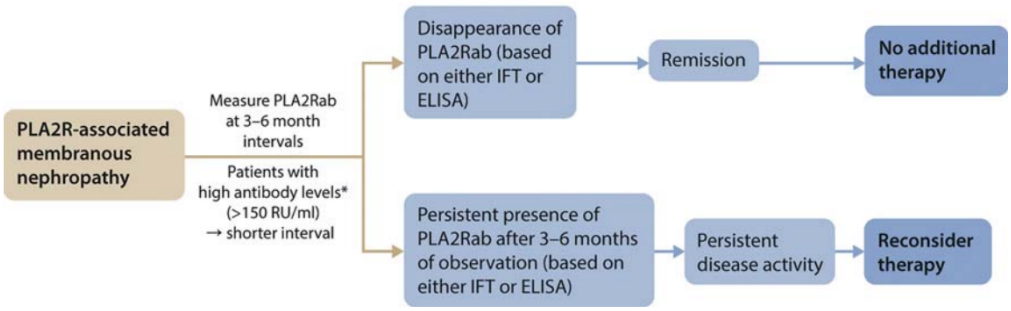


Figure 27 | Guidance for the use and interpretation of the anti-PLA2R antibody assay in patients with known anti-PLA2R-associated MN. *High titers (ELISA) are associated with lower likelihood of spontaneous remission and higher likelihood of nonresponse to low-dose rituximab. ELISA, enzyme-linked immunosorbent assay; PLA2Rab, antibodies against the M-type phospholipase A2 receptor.

23/ Why is PLA2R ab important to measure before ?

✅May help identify nonresponders:PLA2R ab T ½ >7 days identified 5 out of 7 patients who required renewed therapy

✅Early prediction of response (after 2–4 wks)

✅This facilitates further individualization of tx and timely

24/⚠️Limitation: The cohort is small and lacks a validation cohort, was calculated PLA2R ab T ½ assuming linear kinetics.

25/ 🤷‍♀️Debatable aspects: High-dose methylprednisolone pulses (1000mg) based on the original Ponticelli protocol vs lower doses and the use of CP oral vs i.v.

26/ Thank you! Please share this #tweetorial with your followers and friends! Thanks to @Sophia_kidney for authoring & @, @, @ for great feedback!

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