Rituximab in Membranous Nephropathy

Philipp Gauckler 1\*, Jae Il Shin 2,3,4, Federico Alberici 5,6, Vincent Audard 7, Annette Bruchfeld 8,9, Martin Busch 10, Chee Kay Cheung 11,12, Matija Crnogorac 13, Elisa Delbarba 5, Kathrin Eller 14, Stanislas Faguer 15,16, Kresimir Galesic 13, Siân Griffin 17, Martijn W. F. van den Hoogen 18, Zdenka Hrušková 19, Anushya Jeyabalan 20, Alexandre Karras 21, Catherine King 22, Harbir Singh Kohli 23, Gert Mayer 1, Rutger Maas 24, Masahiro Muto 25, Sergey Moiseev 26, Balazs Odler 14, Ruth J. Pepper 27,Luis F. Quintana 28, Jai Radhakrishnan 20, Raja Ramachandran 23, Alan D. Salama 27, Ulf Schönermarck 29, Mårten Segelmark 30, Lee Smith 31, Vladimír Tesař 19, Jack Wetzels 24, Lisa Willcocks 32, Martin Windpessl 33,34, Ladan Zand 35, Reza Zonozi 36, Andreas Kronbichler 1\* for the *RITERM* study group

1 Department of Internal Medicine IV (Nephrology and Hypertension), Medical University Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria.

2 Department of Pediatrics, Yonsei University College of Medicine, Seoul 03722, Korea.

3 Division of Pediatric Nephrology, Severance Children's Hospital, Seoul 03722, Korea.

4 Institute of Kidney Disease Research, Yonsei University College of Medicine, Seoul 03722, Korea.

5 Nephrology Unit, ASST Spedali Civili di Brescia, Brescia, Italy.

6 Department of Medical and Surgical Specialities, Radiological Sciences and Public Health; University of Brescia, Brescia, Italy.

7 Department of Nephrology and Transplantation, Rare French Disease Centre "Idiopathic Nephrotic syndrome", Henri-Mondor/Albert-Chenevier Hospital Assistance Publique-Hôpitaux de Paris, Inserm U955, Team 21, Paris-East University, 94000 Créteil, France.

8 Department of Health, Medicine and Caring Sciences, Linköping University, Linköping, Sweden.

9 Department of Renal Medicine, CLINTEC, Karolinska Institutet at Karolinska University Hospital, Stockholm, Sweden.

10 Department of Internal Medicine III, University Hospital Jena, Friedrich-Schiller-University, Jena, Germany.

11 Department of Cardiovascular Sciences, University of Leicester, Leicester, United Kingdom.

12 John Walls Renal Unit, University Hospitals of Leicester NHS Trust, Leicester, United Kingdom.

13 Department of Nephrology and Dialysis, Dubrava University Hospital, Avenija Gojka Suska 6, 10 000, Zagreb, Croatia.

14 Clinical Division of Nephrology, Department of Internal Medicine, Medical University of Graz, Graz, Austria.

15 Département de Néphrologie et Transplantation d'Organes, Centre de Référence des Maladies Rénales Rares, Centre Hospitalier Universitaire de Toulouse, 31000 Toulouse, France.

16 Institut National de la Santé et de la Recherche Médicale, U1048 (Institut des Maladies Cardiovasculaires et Métaboliques-équipe 12), 31000 Toulouse, France.

17 Department of Nephrology and Transplantation, University Hospital of Wales, Cardiff, UK.

18 Department of Internal Medicine, Erasmus MC University Medical Centre Rotterdam, Rotterdam, Netherlands.

19 Department of Nephrology, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic.

20 Division of Nephrology, Columbia University Medical Center, New York, New York, USA.

21 Service de Néphrologie, Hôpital Européen-Georges Pompidou, Assistance Publique des Hôpitaux de Paris, 75015 Paris, France.

22 Department of Renal Medicine, Queen Elizabeth Hospital, University Hospitals Birmingham, Mindelsohn Way, Edgbaston, Birmingham, B15 2WB UK.

23 Nephrology, Post Graduate Institute of Medical Education and Research, Chandigarh, India.

24 Department of Nephrology, Radboud University Medical Center, PO Box 9101, 6500 HB, Nijmegen, Netherlands.

25 Department of Nephrology, Juntendo University Faculty of Medicine, Tokyo, Japan

26 Tareev Clinic of Internal Diseases, Sechenov First Moscow State Medical University, Moscow, Russia.

27 University College London Department of Renal Medicine, Royal Free Hospital, London, UK

28 Department of Nephrology and Renal Transplantation, Hospital Clínic, Centro de Referencia en Enfermedad Glomerular Compleja del Sistema Nacional de Salud (CSUR), Department of Medicine, University of Barcelona, IDIBAPS, Barcelona, Spain.

29 Division of Nephrology, Department of Medicine IV, University Hospital, LMU Munich, Munich, Germany.

30 Department of Clinical Sciences Lund, University, Skane University Hospital, Nephrology Lund, Lund, Sweden.

31 The Cambridge Centre for Sport and Exercise Science, Anglia Ruskin University, Cambridge CB1 1PT, UK.

32 Department of Renal Medicine, Vasculitis and Lupus Clinic, Addenbrooke's Hospital, Cambridge University Hospitals, Cambridge, UK.

33 Department of Internal Medicine IV, Section of Nephrology, Klinikum Wels-Grieskirchen, Wels, Austria.

34 Medical Faculty, Johannes Kepler University Linz, Altenberger Strasse 69, 4040 Linz

35 Division of Nephrology and Hypertension, Mayo Clinic, Rochester, Minnesota, USA.

36 Division of Nephrology, Vasculitis and Glomerulonephritis Center, Massachusetts General Hospital, 101 Merrimac Street, Boston, MA 02114, USA.

Correspondence should be addressed to:

Philipp Gauckler M.D., Department of Internal Medicine IV (Nephrology and Hypertension), Medical University Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria

*Phone*: +43 512 504 83602

*E-Mail*: philipp.gauckler@i-med.ac.at

Andreas Kronbichler M.D. Ph.D., Department of Internal Medicine IV (Nephrology and Hypertension), Medical University Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria

*Phone*: +43 512 504 81338

*E-Mail*: andreas.kronbichler@i-med.ac.at

# ABSTRACT.

Membranous nephropathy is the most common cause of primary nephrotic syndrome among adults. The identification of phospholipase A2 receptor (PLA2R) as target antigen in a majority of patients changed the management of membranous nephropathy dramatically, and provided a rationale for B cell depleting agents such as rituximab. The efficacy of rituximab in inducing remission has been investigated in several studies including three randomized controlled trials, in which complete and partial remission of proteinuria was achieved in about two thirds of treated patients. Due to its favorable safety profile, rituximab is now considered a first-line treatment option for membranous nephropathy, especially in patients at moderate and high risk of deterioration in kidney function. However, questions remain about how to best use rituximab, including the optimal dosing regimen, a potential need for maintenance therapy and assessment of long-term safety and efficacy outcomes. In this review, we provide an overview of the current literature and discuss both strengths and limitations of “the new standard”.

*Keywords*:

Membranous nephropathy, nephrotic syndrome, rituximab, B cells

# BACKGROUND

Membranous nephropathy (MN) is the most common cause of primary nephrotic syndrome (NS) among adults worldwide with a predominance in Caucasian and males. Secondary causes like underlying malignancies, infections or autoimmune disorders account for around 20 percent of all MN cases 1. The remaining 80% of cases are referred to as primary MN, which is the focus of this review. The natural course of MN is heterogeneous, implying that uniform treatment for all patients is not appropriate. Without treatment, about half of the patients attain spontaneous remission over a period of 5-10 years, while the other half sustain progressive loss of kidney function 2**.** Besides kidney outcomes, several complications, including venous thromboembolism and cardiovascular events have a significant impact on morbidity and mortality of patients with MN and therefore need to be considered in their disease management 4-6.

## Pathophysiology

Major progress in the pathogenetic understanding of MN was achieved during the last decade when the M-type phospholipase A2 receptor (PLA2R) autoantibody was identified in 70-80% of patients 7. In another 3-5% of patients, autoantibodies directed against thrombospondin type-1 domain-containing 7A (THSD7A) can be identified 8. Recently, neural epidermal growth factor-like 1 (NELL-1) protein and semaphorin 3b were also identified as potential autoantigens associated with MN 9,10. While biopsy samples in primary MN usually show a predominance of IgG4 antibody deposition, IgG1 is the major IgG subtype in both NELL-1- and semaphorin 3b-associated MN, possibly indicating an underlying secondary disease cause in these cases 11. In fact, detection of NELL-1 was recently associated with concurrent malignancy 12. The remaining cases may either be caused by autoantibodies against yet unidentified antigens or reflect misclassified secondary MN. A distinct group of patients that shows an association with other autoimmune diseases may be related to accumulation of exostosin in the glomerular basement membrane and should rather be classified as secondary MN 13.

Emerging evidence shows that PLA2R antibody (Ab) titer correlates with disease activity 14. As a result, novel serology-based algorithms have been proposed to facilitate diagnosis and monitor treatment 14. Under certain conditions, diagnosis may even be established without histologic verification 15. In PLA2R-positive patients, low Ab levels predict a higher likelihood of spontaneous remission and changes of Ab titers precede changes in proteinuria by several months 15-17**.** This latency between immediate treatment-induced immunologic remission and delayed clinical remission is explained by a gradual resolution of subepithelial immune deposits observed by repeated biopsy after treatment 18.Additionally, recurrence of Ab titers after therapeutic response predicts clinical relapse. Hence, Ab titer response to immunosuppressive therapy may guide adaption of the therapeutic regimen to an individual patient 14,17. Of note, several patients with PLA2R-associated MN diagnosed on kidney biopsy do (initially) not show positive PLA2R-Ab on serologic testing. However, after saturation of tissue PLA2R with auto-Abs, seroconversion may be detected by serial serological testing on follow-up. This may explain why seroconversion can be missed in relapsing patients when they first present with a rise in proteinuria. While an enhanced glomerular staining for the PLA2R on kidney biopsy is strongly associated with primary MN (and usually goes along with positive serological testing), further diagnostic evaluation to exclude secondary disease causes should be performed in those patients with negative serological testing for PLA2R-Ab and only faintly positive histologic staining for PLA2R and/or non-IgG4 Ab deposits 19.

There is also debate whether the ability of Abs to target multiple epitopes of the PLA2R (epitope spreading) could be a poor prognostic marker 20, but most recent data calls this into question. In a prospective cohort of 150 patients, detection of epitope spreading was highly dependent on total PLA2R Ab levels and while total PLA2R Ab levels clearly predicted treatment response and outcomes, epitope-recognition patterns showed no prognostic impact by itself 21.

The recent major advances in understanding of MN have clearly established that it is an autoantibody driven disease. Given the pivotal role of B cells in producing pathogenic autoantibodies, there is a clear rationale for B cell depleting treatment modalities.

# IMMUNOSUPPRESSION IN MEMBRANOUS NEPHROPATHY

## General measures and risk stratification

Supportive treatment, such as antihypertensive, antiproteinuric and dietary measures are pivotal for all patients with proteinuric glomerular diseases 22. Additional anticoagulant measures are recommended for most patients with severe hypoalbuminemia during NS after individual risk assessment. As the disease course of MN is highly variable and a significant proportion of patients receiving supportive measures only will have a favorable outcome, benefits and harms of immunosuppressants must be carefully weighed 2. Thus, initial risk-stratification is crucial to assess the individual risk of progressive loss of kidney function. Such assessment can be performed using clinical criteria as presented in Table 1. In low-risk patients presenting without clinical signs of NS and with preserved kidney function, a “watch and wait” strategy is appropriate for up to 6 months under maximal antiproteinuric treatment. In contrast, immunomodulating treatment may be initiated immediately in patients with severe unresponsive NS or deteriorating kidney function 23.

## Immunosuppression

Given the detrimental effects of a prolonged treatment with glucocorticoids, steroid-sparing immunosuppressive agents have been used in the treatment of MN since the 1970s with some success 24. To date, the alkylating agents cyclophosphamide and chlorambucil are the only drugs with proven efficacy to prevent ESKD and death 15. Therefore a cyclical therapy of corticosteroids and alkylating agents (from here abbreviated to ‘a cyclical therapy’) was recognized as the treatment of choice for decades (“Ponticelli regimen”), consisting of a daily intravenous application of 1 g methylprednisolone for three days, followed by a daily oral dose of methylprednisolone (0.5 mg/kg/d) for 27 days in the first month and oral chlorambucil (0.15-0.2 mg/kg/d) or oral cyclophosphamide (2.0 mg/kg/d) for 30 days in the second month continued in alternating cycles over a total of 6 months 25.

Other immunosuppressive agents have only been tested in trials that used proteinuria reduction as a surrogate endpoint. Calcineurin inhibitors (CNI) have similar efficacy for remission induction as a cyclical therapy with better short-term efficacy and safety, but relapse rates after discontinuation are high (40-50%) for both, cyclosporin A and tacrolimus 26-29. High relapse rates for cyclosporine A were recently confirmed in the MENTOR trial, a randomized-controlled trial (RCT) comparing rituximab to cyclosporine A in MN 30. In the recently published STARMEN trial, a single-dose of 1g rituximab after a 6 months course of tacrolimus reduced the rate of relapses to 12%, but overall efficacy of the combined tacrolimus-rituximab regimen was lower as compared with a cyclical therapy of methylprednisolone and cyclophosphamide 31. These high relapse rates may be explained by the direct action of CNI on the actin cytoskeleton of podocytes, although they also have an immunosuppressive effect to reduce PLA2R Ab levels 32 33. CNI may be particularly useful either as supportive treatment option in low-risk patients or in addition to the standard immunosuppressive treatment for patients at very high risk in order to achieve early remission.

Data supporting the use of mycophenolate mofetil in MN are conflicting. While mycophenolate mofetil monotherapy appears to be ineffective 34, low-quality evidence tested in two small cohorts of Asian and Indian patients supports a combination with steroids as an alternative to a cyclical therapy 35,36. Adrenocorticotrophic hormone (ACTH) monotherapy appeared to be a therapeutic option after promising results in one small RCT published in 2006 37. Following that, another prospective open label cohort study could not prove any benefit of ACTH over a cyclical therapy 38. To date, the lack of evidence, associated adverse events (e.g. hyperglycemia, edema and mood disorders) and high therapeutic costs preclude widespread use 39,40.

## Side effects

Although effective immunosuppressive treatment options for MN have been established, the therapeutic options above have major disadvantages, limiting their usefulness especially for patients at moderate risk for progressive loss of kidney function.

In addition to an elevated infection risk, alkylating agents are associated with potentially fatal toxic side effects including oncogenicity, urotoxicity, myelotoxicity and infertility 41,42. While mycophenolate mofetil is mainly associated with gastrointestinal side effects and myelotoxicity, CNI exhibit a broad spectrum of side-effects (e.g. arterial hypertension, dyslipidemia, glucose intolerance and hirsutism) with CNI-related nephrotoxicity as the most relevant treatment-limiting factor 43. The concomitant use with glucocorticoids is originally designated in all mentioned immunosuppressive regimens, leading to a myriad of side effects including hyperglycemia, loss of bone density and an additional risk of infections.

# RITUXIMAB

Rituximab is a chimeric, monoclonal IgG1 antibody that exerts its B cell depleting effects via binding to CD20. Rutiximab is approved by the U.S. Food and Drug Administration and the European Medicines Agency for the treatment of non-Hodgkin’s lymphoma, rheumatoid arthritis and ANCA-associated vasculitis, and used off-label in an increasing spectrum of autoimmune diseases 44. Since the first experience of 8 MN-patients treated with rituximab was reported in 2002 by Remuzzi et al 45, two RCTs and several prospective and retrospective studies have been published.30,46. Strengths and limitations of rituximab in MN are summarized in Table 2.

## Efficacy

An overview of currently available prospective studies is given in Table 3. Several methodological differences of these studies limit direct comparability, including eligibility criteria (e.g. heterogeneous risk groups and treatment of initial disease manifestation versus patients on relapse with prior treatment courses), different treatment-protocols and inconsistent criteria to define remission. Despite these marked differences, overall remission rates (complete and partial) of rituximab at 12 months are consistently around 60-70%, ranging from 44% 47 to 85% 48. Of note, a relevant portion of patients who respond to rituximab remain proteinuric but achieve partial remission (PR). Complete remission (CR) rates vary between different studies and tend to increase with longer follow-up periods. Consequently, 30-40% of all treated patients do not respond to initial treatment with rituximab and might need additional/other treatments.

In the GEMRITUX trial, rituximab combined with a non-immunosuppressive antiproteinuric treatment (NIAT) was compared to NIAT alone. Rituximab-treated patients received two infusions of 375 mg/m² per week. The primary outcome was reported at 6 months, , with no significant difference in the combined end point of CR or PR of proteinuria. However, in the extended follow up (median follow-up was 17 months), a significant difference was reported with remission occurring in 64.9% in the NIAT-rituximab-group but in only 34.2% in the NIAT-alone-group, respectively (p<0.01). Additionally, rates of PLA2R Ab depletion in the NIAT-rituximab and NIAT-groups were 56% and 4.3% at month 3 (p<0.001), respectively, and multivariate analysis showed that a PLA2R Ab titer <275 RU/mL at baseline was the only factor associated with remission occurring at month 6 49. This concurs with retrospective data from Ruggenenti et al., where immunologic response with PLA2R Ab titer reduction preceded proteinuria reduction by approximately 10 months 17. The recently published MENTOR trial compared rituximab to cyclosporine A in the treatment of MN. The primary composite outcome of CR or PR at 24 months was reached by 60% in the rituximab group and only 20% in the cyclosporine A group. 35% of the rituximab-treated patients and none of patients treated with cyclosporine A had CR at 24 months 30. Notably, remission rates at 12 months were not significantly different (60% versus 52% in the rituximab and the cyclosporine A group, respectively). Discontinuation of cyclosporine A after 12 months led to an increased relapse rate which explains the difference seen at 24 months 50. Rituximab therapy has both better adherence and, by inducing longer lasting remission, is overall more cost effective 30.

Non-immunosuppressive effects of CNI give a rationale for combination with rituximab. Waldman et al. tested a combination of CNI with rituximab which might accelerate time to remission and improve overall remission rates 48. Recently the authors showed superior CR rates at 24 months in a small cohort of 21 patients treated with a combination of rituximab plus cyclosporine A (57% CR) compared with those of the MENTOR trial (35% in the rituximab group, 14% in the cyclosporine A group) 51. The recently published STARMEN RCT compared a 6 months induction course with tacrolimus (followed by tapering over another 3 months) in combination with a 1 g single-dose of rituximab at month 6 with a cyclical therapy of methylprednisolone and cyclophosphamide over 6 months. At 24 months, the cyclical therapy proved to be superior (CR+PR 84%, CR 60%) to the sequential treatment with tacrolimus and rituximab (CR+PR 58%, CR 26%). Although a sequential application of 1 g rituximab may lower the relapse rate after cessation of CNI, no significant impact on the remission rate was observed 31. Conversely, addition of tacrolimus does not increase efficacy compared with recent trials of rituximab only but might reduce the cumulative rituximab dose.

Although reported efficacy of rituximab appears similar to the classical cyclical therapy, STARMEN is the only trial published to date to compare a rituximab based regimen with the conventional cyclical regimen. Comparing major RCTs from the past, RTX outcomes may be favorable 52. However, such comparison with trials conducted in a different decade is biased and bears major limitations. For instance, standards of good clinical practice and optimal supportive treatment measures expected for the control arm of such trials were implemented after the publication of historical RCTs of cyclical therapies. One large retrospective observational cohort study analyzed outcomes of 100 RTX-treated patients compared with 103 patients who received steroids plus CYC 42. Over a median follow-up of 40 months, cumulative incidence of PR was lower in the RTX group, while rates for CR and a composite end-point of doubling of serum creatinine, ESKD or death did not differ significantly. Rates of both serious and non-serious adverse events were significantly lower among RTX-treated patients. Importantly, CYC and steroids were given continuously for 6 to 12 months and not in a cyclical manner as used in the Ponticelli regimen with a cumulative period of 3 months each. A recent meta-analysis of 8 trials involving 542 patients even showed positive effects of RTX on CR rates compared with the heterogenous control groups (including supportive treatment, CSA and cyclical treatment) 53. Van den Logt et al. compared the chance to achieve immunological remission (disappearance of PLA2R Ab) 6 months after treatment with either CYC (1.5 mg/kg/d for 8-24 weeks) or RTX (cumulative dose 1.5-2.0 g). RTX, in comparison to CYC, was less effective in patients with high baseline Ab levels > 152 RU/mL 54. Nonetheless, evidence from the RI-CYCLO trial (NCT03018535) will be available soon. This RCT compares two doses of RTX (1 g each) to a cyclical therapy and preliminary, unpublished results indicate comparable remission rates at 24 months.

Taken together, solid evidence is available supporting the use of rituximab as induction treatment, achieving remission in approximately two-thirds of all patients without the need of concomitant corticosteroid therapy. A preceded course of CNI does not improve efficacy of rituximab in inducing remission while direct comparison between rituximab only with a classical cyclical therapy is still missing.

## Dosing

Initial treatment:

Different application regimens, ranging from one single-dose of 375 mg/m² to four weekly doses of 375 mg/m² repeated after 6 months, were used across various studies as illustrated in Figure 1. The recently updated KDIGO guideline (public review draft) on glomerular diseases offers a wide scope of options and recommends either two applications of 1 g fixed-dose within 2 weeks, as used for rheumatoid arthritis, or 375 mg/m² given 1 to 4 times at weekly intervals as another first-line option for the initial treatment of patients at moderate or high risk for disease progression, while a cyclical therapy is still the treatment of choice for patients at very high risk 23. Clinical criteria for risk-stratification are presented in Table 1.

There is ongoing debate whether lower doses of rituximab are safer and more cost effective with equivalent efficay. A low-dose, B cell driven protocol using only a single-dose of 375 mg/m² with re-application in case of insufficient B cell depletion was tested in a prospective, matched cohort study and compared with a historical cohort treated with the standard protocol of four weekly doses of 375 mg/m². Of 12 patients treated with the low-dose protocol, only one needed a second dose to achieve complete B cell depletion and remission rates were identical in both groups after 12 months. While the safety profile was beneficial in both groups, costs (both for rituximab and hospitalizations) could be reduced dramatically 55. In contrast, a recent retrospective analysis of patients with MN compared a higher-dose protocol of two infusions of 1 g rituximab two weeks apart (the Nice protocol) with patients receiving two times 375 mg/m² one week apart in the GEMRITUX trial. The Nice protocol was shown to be more effective, achieving higher remission rates at six months (64% versus 30%, p=0.01), a shorter median time to remission (3 months versus 9 months, p=0.01), a higher circulating level of rituximab (3.3 µg/L versus 0.0 µg/L, p<0.001) and lower CD19 counts (0.0 versus 16.5, p<0.001) at month three as well as lower levels of PLA2R Ab at month six (0.0 versus 8.3, p=0.03), respectively 20. Similarly, Moroni et al. showed in a multicentric prospective cohort of 34 consecutive patients that a low-dose protocol of 375 mg/m² rituximab administered once (18 patients) or twice (16 patients) only achieved poor remission rates in < 50% of patients at 12 and 24 months 47. Full B cell depletion was observed in all patients within 2 weeks after first rituximab infusion but assessment of both, B cell levels and PLA2R Ab titers is missing during follow-up which hinders direct comparison between the two regimens 56. Additionally, patients with high PLA2R Ab titers at baseline had a lower response rate and thus might have benefitted from a higher-dose of rituximab 47. Recently, a retrospective case-control study compared 42 patients assigned either to a low-dose rituximab protocol (375 mg/m² single-dose, n=14), a standard rituximab protocol (4x 375 mg/m² weekly, n=14) or a control group treated with a cyclical therapy (Ponticelli regimen, n=14). At 24 months, no significant differences in clinical response criteria were found (p=0.53). All patients treated with rituximab showed complete B cell depletion at month 1 but B cell recovery occurred earlier in the low-dose group (between month 3 and 6) compared with the standard group (between month 9 and 12). No relapses occurred within 24 months of follow-up 57. Importantly, inter-group comparison between rituximab and the Ponticelli regimen is limited as the latter group is a historic cohort and while all rituximab-treated patients were PLA2R-positive, and respective testing was not available for the control group. Also, baseline PLA2R Ab titers in the two rituximab-groups are not provided. Thus, the excellent response of the low-dose group may have been due to a lower immunologic activity at baseline.

Subsequent dosing:

While B cell depletion is almost always achieved immediately after the first rituximab dose, immunological and especially clinical response occur mostly several months later and may persist even with fully recovered B cell counts. Rituximab serum levels on follow-up are lower in MN patients as compared with patient populations without kidney diseases, which might be related to urinary loss of rituximab due to NS 58. This appears to have clinical impact, as undetectable drug levels at month 3 were associated with active disease, early B cell recovery and ‘resistance’ to rituximab 59. For patients with PLA2R-associated MN, immunological monitoring appears to be a reasonable approach to guide rituximab therapy 17. KDIGO 2020 guidelines (public review draft) recommend PLA2R Ab monitoring at months 3 and 6 and re-dosing of patients with persisting or rising titers 23. For PLA2R-negative patients, no such guidance is possible and re-dosing must be managed by clinical response. Many patients relapse at certain time points following the last rituximab dose, accompanied by recovery of B cells as the drug effect wanes. Since these relapses are frequently seen in patients with only low PLA2R Ab levels, the question arises whether immunosuppressive maintenance strategies could be useful. Re-application at fixed-intervals comparable to the maintenance therapy in ANCA-associated vasculitis is one possible approach which should be addressed by future studies and compared to the current treatment strategies 60.

## Safety

Infusion-related reactions (IRR) are frequently observed but are mostly mild in nature and manageable if infusion speed is adjusted 61,62. Hepatitis B screening is advised since virus reactivation may occur, both in HBsAg-positive as well as in HBsAg-negative and anti-HBc–positive patients 63. Progressive multifocal leukencephalopathy due to reactivation of JC virus is a rare but fatal complication associated with rituximab treatment - mainly reported in oncologic indications and seldomly described in autoimmune diseases 64. Late-onset neutropenia is another feature reported in MN following rituximab which might be underestimated. A recently published single-center retrospective cohort study of 738 patients with autoimmune diseases treated with rituximab reported a cumulative incidence of late-onset neutropenia of 6.6% at one year. Total rates were higher in patients with lupus nephritis (25%) compared to patients with MN (8.2%) or other diseases (7.6%) 65. Hypogammaglobulinemia can either be disease-related or a consequence of rituximab but further discussions on that point are beyond the scope of this review. The risk of infectious complications depends on the indication for rituximab treatment. Comparatively high rates of up to 26 serious infections per 100 patient-years are reported in ANCA-associated vasculitis 66, while lower rates of 4.3 and 5.3 serious infections per 100 patient-years were reported in large cohorts for rheumatoid arthritis and mixed autoimmune disorders, respectively 61,67. Currently available evidence concerning safety of rituximab in MN is limited and the quality of evidence derived from the two available RCTs is considered low by the recently published KDIGO guidelines. No studies are available comparing the infection risk of rituximab with that of supportive treatment 23. In the recently published MENTOR trial, the overall number of severe infectious events per 100 patients was 7.7 for rituximab and 12.3 for cyclosporine A (p=0.23) 30. In this trial cyclosporine A was not combined with steroids unlike previous studies using cyclosporine A in MN 29. In the STARMEN trial, severe adverse events were not significantly higher in patients treated with a cyclical therapy compared with tacrolimus-rituximab (17 versus 12 events per 100 patient-years) but 4 of 5 severe infections occurred in the methylprednisolone-cyclophosphamide group 31. Van den Brand et al. compared adverse events as the primary outcome among 100 rituximab-treated and 103 patients treated with a cyclical therapy. Adverse events were less frequent in the rituximab group than in the cyclical therapy group (63 versus 173; p<0.001). No infections attributed to the treatment were observed in the rituximab group, whereas 11 serious infections occurred in the cyclical therapy group including three fatal cases of sepsis. Besides infectious complications, three blood malignancies and five solid cancers (two of them fatal) were observed and possibly related to the combined therapy of an alkylating agent (cyclophosphamide or chlorambucil) with corticosteroids during a period of 40 months follow-up of patients with MN. In comparison, 2 solid cancers were observed in the rituximab group and assessed as unrelated to treatment by physicians directly overseeing the care of these patients 42. Experience of rituximab for ANCA-associated vasculitis showed a comparable malignancy risk with the general population 68.

## Treatment options for patients with reduced kidney function

Progressive loss of kidney function with an eGFR < 30 ml/min/1.73m² is associated with scarring of the kidney and a diminished response to immunosuppressive treatment. Consequently, these patients are rarely included in clinical trials and risk-benefit assessment usually results in withholding immunosuppressants. Considering the lack of data, it remains unknown which patients with reduced kidney function may benefit from immunosuppression. In fact, certain findings on kidney biopsy, such as tubular atrophy and interstitial fibrosis, were associated with poor kidney response in a small cohort of 14 MN patients treated with rituximab and a tubulointerstitial score was proposed to discriminate patients that might benefit from initiation of rituximab 69.

One RCT compared a cyclical treatment of steroids and chlorambucil with cyclosporine A and supportive therapy alone in 108 patients with deteriorating kidney function and mean creatinine clearance at baseline of 50 mL/min. While a cyclical therapy could significantly reduce the risk of further 20% decline in kidney function, this therapy was associated with a high rate of serious adverse events, compared to patients that received cyclosporine A or supportive therapy alone (61%, 49% and 42% patients with at least one severe adverse event, respectively) 70.

In a small retrospective cohort of 28 rituximab-treated patients, univariate analysis showed reduced eGFR < 45 ml/min/1.73m² predicting lack of response to rituximab as an independent factor 71. In contrast, Hanset et al. recently reported outcomes of 13 rituximab-treated patients with PLA2R-associated MN and advanced chronic kidney disease (CKD stage 4-5). Patients received either two weekly infusions of 375 mg/m² or two doses of 1 g two weeks apart. Outcomes were quite variable, with 9 patients achieving response, while 4 patients progressed to ESKD within one year. Overall mean eGFR rose from 18 +/- 7 to 23 +/- 13 mL/min/1.73 m² and proteinuria decreased from 13 +/- 7 to 0.8 +/- 8 g/day. Four severe adverse events (3 infections, 1 IRR) were reported in three patients. In these patients, a high urine albumin/protein ratio and low urine IgG levels at baseline were predictive factors for kidney response 72.

## Beyond rituximab – options for refractory patients

Although rituximab appears to be an attractive first-line treatment option for patients with MN due to its favorable efficacy and safety profile, a non-response rate of approximately 30-40 % means there is a need for other therapies. Diagnosis of refractory disease can be made if NS persists for at least 6 months after antibody disappearance or if proteinuria persists or increases in the presence of detectable antibody levels 23. If PLA2R Ab titers remain high after a first course of rituximab, re-treatment with rituximab may be effective - as observed in a small cohort of 10 patients with elevated PLA2R Ab titers > 152 RU/mL at 6 months following the first rituximab-course 73. If true rituximab resistance is present, current KDIGO guidelines (public review draft) recommend addition of CNI if eGFR remains stable or switch to cyclophosphamide if eGFR is decreasing 23. After a first course of rituximab, neutralizing anti-rituximab Ab may be for the cause of refractory or relapsing disease. However, a second course of rituximab may achieve remission even in the setting of resistant disease and presence of anti-rituximab Ab after a first course 74. In a study of 42 patients treated with two doses of 1 g two weeks apart, anti-rituximab Ab were detectable in 10 patients. Anti-rituximab Ab neutralized rituximab in the serum in 8 of 10 patients and were associated with a higher rate of relapses (p < 0.001). 3 resistant patients were treated ofatumumab, a fully humanized anti-CD20 antibody, and all achieved remission. Alternative B cell depleting agents such as ofatumumab or type II anti-CD20 Ab obinutuzumab may prove to be a safe and effective rescue therapy for patients either refractory or sensitized against rituximab, but available evidence is limited to single case reports/series 75,76. A recently published prospective, open-label trial investigated belimumab, a monoclonal Ab inhibiting B cell production/stimulation, in a cohort of 14 patients 77. Remission was achieved in 1 (CR) and 8 (PR) patients out of 11 patients who completed a rather short follow-up period of 28 weeks. Synergistic effects of a sequential combination of rituximab with belimumab were first described in patients with systemic lupus erythematosus and will be subject of another trial (currently recruiting) in patients with MN (NCT03949855) 78. Targeting plasma cells is another approach that is currently tested in an ongoing RCT with MOR202, a human anti-CD38 antibody (NCT04145440). Promising remission rates were reported for a combination of rituximab with lower doses of cyclophosphamide and steroids, tested in a case series of 15 consecutive patients, out of which 8 had refractory or relapsing disease 79. Whether this potent but more toxic regimen is a true option for patients with refractory disease needs to be tested in larger cohorts.

# CONCLUSION

As recommended by the currently updated KDIGO guideline for glomerular diseases, rituximab is a promising new first-line treatment option for patients with primary MN. While a classical cyclical therapy consisting of alkylating agents and corticosteroids is still recommended for a certain subset of patients at very high risk for progressive kidney disease, rituximab might be the treatment of choice for a majority of patients at moderate and high risk. Nonetheless, important questions such as long-term efficacy and safety, the optimal dosing-regimen and application-timing or strategies for patients with advanced CKD or MN refractory to rituximab still remain unanswered. For now, it appears reasonable that treatment with rituximab is adapted individually to each patient’s disease course. Monitoring disease activity by serial measurement of PLA2R Ab levels may allow such tailored long-term treatment and low-dose protocols with titrated rituximab applications according to B cell counts and PLA2R Ab levels may be appropriate in selected scenarios to reduce side effects and costs. While a sequential induction strategy of tacrolimus followed by a rituximab single-dose appears inferior to a cyclical therapy of steroids and alkylating agents, direct comparison between rituximab alone and the cyclical therapy is still based on contraposition of rituximab with historical cohorts and thus afflicted by severe limitations. Results by the ongoing RI-CYCLO trial may provide answers to this critical question helping to find the optimal treatment modality for selected patients. Meanwhile RITERM, a multicenter, international retrospective study, will address several central issues in a large cohort.

## Disclosures

FA reports other from Baxter, outside the submitted work; VA reports personal fees from ADDMEDICA, outside the submitted work; AB reports personal fees from Chemocentryx, personal fees from Astra-Zeneca, personal fees from Vifor, personal fees from Bayer, outside the submitted work; MvdH. reports personal fees from Amgen, personal fees from Astellas, from Genzyme, from MSD, from Sanofi, from Vifor, outside the submitted work; GM reports personal fees from Astra Zeneca, personal fees from Böhringer Ingelheim, personal fees from Vifor, from Eli Lilly, outside the submitted work; US reports grants and non-financial support from Alexion Pharma, grants and non-financial support from Ablynx, grants and non-financial support from Chemocentryx, outside the submitted work; VT reports other from Calliditas, other from Retrophin, other from Omeros, personal fees from Boehringer Ingelheim, other from Astra-Zeneca, other from Mundipharma, outside the submitted work; JW reports participating in ERA-EDTA funded STARMEN study which evaluated rituximab therapy outside the submitted work; AK reports personal fees from Vifor Pharma, personal fees from TerumoBCT, personal fees from Novartis, outside the submitted work;.all other authors report nothing to disclose.

# Scientific References:

1. Couser WG. Primary Membranous Nephropathy. Clin J Am Soc Nephrol 2017;12(6):983-997. DOI: 10.2215/CJN.11761116.

2. van den Brand JA, van Dijk PR, Hofstra JM, Wetzels JF. Long-term outcomes in idiopathic membranous nephropathy using a restrictive treatment strategy. J Am Soc Nephrol 2014;25(1):150-8. DOI: 10.1681/ASN.2013020185.

3. van den Brand JA, Hofstra JM, Wetzels JF. Low-molecular-weight proteins as prognostic markers in idiopathic membranous nephropathy. Clin J Am Soc Nephrol 2011;6(12):2846-53. DOI: 10.2215/CJN.04020411.

4. Barbour SJ, Greenwald A, Djurdjev O, et al. Disease-specific risk of venous thromboembolic events is increased in idiopathic glomerulonephritis. Kidney Int 2012;81(2):190-5. DOI: 10.1038/ki.2011.312.

5. Lee T, Derebail VK, Kshirsagar AV, et al. Patients with primary membranous nephropathy are at high risk of cardiovascular events. Kidney Int 2016;89(5):1111-1118. DOI: 10.1016/j.kint.2015.12.041.

6. Plaisier E, Ronco P. Screening for Cancer in Patients with Glomerular Diseases. Clin J Am Soc Nephrol 2020;15(6):886-888. DOI: 10.2215/CJN.09000819.

7. Beck LH, Jr., Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med 2009;361(1):11-21. DOI: 10.1056/NEJMoa0810457.

8. Tomas NM, Beck LH, Jr., Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med 2014;371(24):2277-2287. DOI: 10.1056/NEJMoa1409354.

9. Sethi S, Debiec H, Madden B, et al. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. Kidney Int 2020;97(1):163-174. DOI: 10.1016/j.kint.2019.09.014.

10. Sethi S, Debiec H, Madden B, et al. Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. Kidney Int 2020. DOI: 10.1016/j.kint.2020.05.030.

11. Ohtani H, Wakui H, Komatsuda A, et al. Distribution of glomerular IgG subclass deposits in malignancy-associated membranous nephropathy. Nephrol Dial Transplant 2004;19(3):574-9. DOI: 10.1093/ndt/gfg616.

12. Caza T, Hassen S, Dvanajscak Z, et al. NELL1 is a target antigen in malignancy-associated membranous nephropathy. Kidney Int 2020. DOI: 10.1016/j.kint.2020.07.039.

13. Sethi S, Madden BJ, Debiec H, et al. Exostosin 1/Exostosin 2-Associated Membranous Nephropathy. J Am Soc Nephrol 2019;30(6):1123-1136. DOI: 10.1681/ASN.2018080852.

14. 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(2):421-430. DOI: 10.1681/ASN.2016070776.

15. 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(2):268-280. DOI: 10.1016/j.kint.2018.10.018.

16. Radice A, Trezzi B, Maggiore U, et al. Clinical usefulness of autoantibodies to M-type phospholipase A2 receptor (PLA2R) for monitoring disease activity in idiopathic membranous nephropathy (IMN). Autoimmun Rev 2016;15(2):146-54. DOI: 10.1016/j.autrev.2015.10.004.

17. Ruggenenti P, Debiec H, Ruggiero B, et al. Anti-Phospholipase A2 Receptor Antibody Titer Predicts Post-Rituximab Outcome of Membranous Nephropathy. Journal of the American Society of Nephrology : JASN 2015;26(10):2545-2558. DOI: 10.1681/ASN.2014070640 [doi].

18. Ruggenenti P, Cravedi P, Sghirlanzoni MC, et al. Effects of rituximab on morphofunctional abnormalities of membranous glomerulopathy. Clin J Am Soc Nephrol 2008;3(6):1652-9. DOI: 10.2215/CJN.01730408.

19. Hoxha E, Kneissler U, Stege G, et al. Enhanced expression of the M-type phospholipase A2 receptor in glomeruli correlates with serum receptor antibodies in primary membranous nephropathy. Kidney Int 2012;82(7):797-804. DOI: 10.1038/ki.2012.209.

20. 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. DOI: 10.2215/CJN.11791018.

21. Reinhard L, Zahner G, Menzel S, Koch-Nolte F, Stahl RAK, Hoxha E. Clinical Relevance of Domain-Specific Phospholipase A2 Receptor 1 Antibody Levels in Patients with Membranous Nephropathy. J Am Soc Nephrol 2020;31(1):197-207. DOI: 10.1681/ASN.2019030273.

22. Floege J, Amann K. Primary glomerulonephritides. Lancet 2016;387(10032):2036-48. DOI: 10.1016/S0140-6736(16)00272-5.

23. KDIGO. KDIGO CLINICAL PRACTICE GUIDELINE ON GLOMERULAR DISEASES. PUBLIC REVIEW DRAFT (JUNE 2020)2020.

24. Ponticelli C, Patrizia P, Del Vecchio L, Locatelli F. The evolution of the therapeutic approach to membranous nephropathy. Nephrol Dial Transplant 2020. DOI: 10.1093/ndt/gfaa014.

25. KDIGO. Chapter 7: Idiopathic membranous nephropathy. Kidney Int Suppl (2011) 2012;2(2):186-197. DOI: 10.1038/kisup.2012.20.

26. Qiu TT, Zhang C, Zhao HW, Zhou JW. Calcineurin inhibitors versus cyclophosphamide for idiopathic membranous nephropathy: A systematic review and meta-analysis of 21 clinical trials. Autoimmun Rev 2017;16(2):136-145. DOI: 10.1016/j.autrev.2016.12.005.

27. Ramachandran R, Yadav AK, 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(4):610-616. DOI: 10.1016/j.ekir.2017.02.004.

28. Alfaadhel T, Cattran D. Management of Membranous Nephropathy in Western Countries. Kidney Dis (Basel) 2015;1(2):126-37. DOI: 10.1159/000437287.

29. Cattran DC, Appel GB, Hebert LA, et al. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. Kidney Int 2001;59(4):1484-90. DOI: 10.1046/j.1523-1755.2001.0590041484.x.

30. Fervenza FC, Appel GB, Barbour SJ, et al. Rituximab or Cyclosporine in the Treatment of Membranous Nephropathy. N Engl J Med 2019;381(1):36-46. DOI: 10.1056/NEJMoa1814427.

31. Fernández-Juárez G, Rojas-Rivera J, Logt A-Evd, et al. The STARMEN trial indicates that alternating treatment with corticosteroids and cyclophosphamide is superior to sequential treatment with tacrolimus and rituximab in primary membranous nephropathy. Kidney International. DOI: 10.1016/j.kint.2020.10.014.

32. 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(6):1357-66. (In eng). DOI: 10.1681/ASN.2013040430.

33. 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(9):931-8. (In eng). DOI: 10.1038/nm.1857.

34. Dussol B, Morange S, Burtey S, et al. Mycophenolate mofetil monotherapy in membranous nephropathy: a 1-year randomized controlled trial. Am J Kidney Dis 2008;52(4):699-705. DOI: 10.1053/j.ajkd.2008.04.013.

35. Chan TM, Lin AW, Tang SC, et al. Prospective controlled study on mycophenolate mofetil and prednisolone in the treatment of membranous nephropathy with nephrotic syndrome. Nephrology (Carlton) 2007;12(6):576-81. DOI: 10.1111/j.1440-1797.2007.00822.x.

36. Senthil Nayagam L, Ganguli A, Rathi M, et al. Mycophenolate mofetil or standard therapy for membranous nephropathy and focal segmental glomerulosclerosis: a pilot study. Nephrol Dial Transplant 2008;23(6):1926-30. DOI: 10.1093/ndt/gfm538.

37. Ponticelli C, Passerini P, Salvadori M, et al. A randomized pilot trial comparing methylprednisolone plus a cytotoxic agent versus synthetic adrenocorticotropic hormone in idiopathic membranous nephropathy. Am J Kidney Dis 2006;47(2):233-40. DOI: 10.1053/j.ajkd.2005.10.016.

38. van de Logt AE, Beerenhout CH, Brink HS, van de Kerkhof JJ, Wetzels JF, Hofstra JM. Synthetic ACTH in High Risk Patients with Idiopathic Membranous Nephropathy: A Prospective, Open Label Cohort Study. PLoS One 2015;10(11):e0142033. (In eng). DOI: 10.1371/journal.pone.0142033.

39. Kittanamongkolchai W, Cheungpasitporn W, Zand L. Efficacy and safety of adrenocorticotropic hormone treatment in glomerular diseases: a systematic review and meta-analysis. Clin Kidney J 2016;9(3):387-96. DOI: 10.1093/ckj/sfw045.

40. Duarte-Garcia A, Matteson EL, Shah ND. Older Drugs With Limited Trial Evidence: Are They Worth the Expense? The Case of Repository Corticotropin Marketed as H.P. Acthar Gel. Ann Intern Med 2019. DOI: 10.7326/M18-3513.

41. Ponticelli C, Glassock RJ. Treatment of membranous nephropathy in patients with renal insufficiency: what regimen to choose? J Nephrol 2013;26(3):427-9. DOI: 10.5301/jn.5000289.

42. van den Brand J, Ruggenenti P, Chianca A, et al. Safety of Rituximab Compared with Steroids and Cyclophosphamide for Idiopathic Membranous Nephropathy. J Am Soc Nephrol 2017;28(9):2729-2737. DOI: 10.1681/ASN.2016091022.

43. Jefferson JA. Complications of Immunosuppression in Glomerular Disease. Clin J Am Soc Nephrol 2018;13(8):1264-1275. DOI: 10.2215/CJN.01920218.

44. MacIsaac J, Siddiqui R, Jamula E, et al. Systematic review of rituximab for autoimmune diseases: a potential alternative to intravenous immune globulin. Transfusion 2018;58(11):2729-2735. DOI: 10.1111/trf.14841.

45. Remuzzi G, Chiurchiu C, Abbate M, Brusegan V, Bontempelli M, Ruggenenti P. Rituximab for idiopathic membranous nephropathy. Lancet 2002;360(9337):923-4. DOI: 10.1016/S0140-6736(02)11042-7.

46. Dahan K, Debiec H, Plaisier E, et al. Rituximab for Severe Membranous Nephropathy: A 6-Month Trial with Extended Follow-Up. J Am Soc Nephrol 2017;28(1):348-358. DOI: 10.1681/ASN.2016040449.

47. Moroni G, Depetri F, Del Vecchio L, et al. Low-dose rituximab is poorly effective in patients with primary membranous nephropathy. Nephrol Dial Transplant 2017;32(10):1691-1696. DOI: 10.1093/ndt/gfw251.

48. Waldman M, Beck LH, Jr., Braun M, Wilkins K, Balow JE, Austin HA, 3rd. Membranous nephropathy: Pilot study of a novel regimen combining cyclosporine and Rituximab. Kidney Int Rep 2016;1(2):73-84. DOI: 10.1016/j.ekir.2016.05.002.

49. Dahan K, Debiec H, Plaisier E, et al. Rituximab for Severe Membranous Nephropathy: A 6-Month Trial with Extended Follow-Up. Journal of the American Society of Nephrology : JASN 2017;28(1):348-358. DOI: 10.1681/ASN.2016040449 [doi].

50. Ponticelli C, Moroni G. Rituximab or Cyclosporine for Membranous Nephropathy. N Engl J Med 2019;381(17):1688-1689. DOI: 10.1056/NEJMc1910393.

51. Waldman M, Austin HA, 3rd, Balow JE. Rituximab or Cyclosporine for Membranous Nephropathy. N Engl J Med 2019;381(17):1688. DOI: 10.1056/NEJMc1910393.

52. Rojas-Rivera JE, Carriazo S, Ortiz A. Treatment of idiopathic membranous nephropathy in adults: KDIGO 2012, cyclophosphamide and cyclosporine A are out, rituximab is the new normal. Clin Kidney J 2019;12(5):629-638. DOI: 10.1093/ckj/sfz127.

53. Lu W, Gong S, Li J, Luo H, Wang Y. Efficacy and safety of rituximab in the treatment of membranous nephropathy: A systematic review and meta-analysis. Medicine (Baltimore) 2020;99(16):e19804. DOI: 10.1097/MD.0000000000019804.

54. 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(4):1016-1017. DOI: 10.1016/j.kint.2017.12.019.

55. 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(5):932-7. DOI: 10.2215/CJN.01180307.

56. Cravedi P. Rituximab in Membranous Nephropathy: Not All Studies Are Created Equal. Nephron 2017;135(1):46-50. DOI: 10.1159/000450659.

57. Fenoglio R, Baldovino S, Sciascia S, et al. Efficacy of low or standard rituximab-based protocols and comparison to Ponticelli's regimen in membranous nephropathy. J Nephrol 2020. DOI: 10.1007/s40620-020-00781-6.

58. Fogueri U, Cheungapasitporn W, Bourne D, Fervenza FC, Joy MS. Rituximab Exhibits Altered Pharmacokinetics in Patients With Membranous Nephropathy. Ann Pharmacother 2019;53(4):357-363. DOI: 10.1177/1060028018803587.

59. Boyer-Suavet S, Andreani M, Cremoni M, et al. Rituximab bioavailability in primary membranous nephropathy. Nephrol Dial Transplant 2019. DOI: 10.1093/ndt/gfz041.

60. Guillevin L, Pagnoux C, Karras A, et al. Rituximab versus azathioprine for maintenance in ANCA-associated vasculitis. N Engl J Med 2014;371(19):1771-80. DOI: 10.1056/NEJMoa1404231.

61. van Vollenhoven RF, Emery P, Bingham CO, 3rd, et al. Longterm safety of patients receiving rituximab in rheumatoid arthritis clinical trials. J Rheumatol 2010;37(3):558-67. DOI: 10.3899/jrheum.090856.

62. Kronbichler A, Windpessl M, Pieringer H, Jayne DRW. Rituximab for immunologic renal disease: What the nephrologist needs to know. Autoimmun Rev 2017;16(6):633-643. DOI: 10.1016/j.autrev.2017.04.007.

63. Loomba R, Liang TJ. Hepatitis B Reactivation Associated With Immune Suppressive and Biological Modifier Therapies: Current Concepts, Management Strategies, and Future Directions. Gastroenterology 2017;152(6):1297-1309. DOI: 10.1053/j.gastro.2017.02.009.

64. Focosi D, Tuccori M, Maggi F. Progressive multifocal leukoencephalopathy and anti-CD20 monoclonal antibodies: What do we know after 20 years of rituximab. Rev Med Virol 2019;29(6):e2077. DOI: 10.1002/rmv.2077.

65. Zonozi R, Wallace ZS, Laliberte K, et al. Incidence, Clinical Features, and Outcomes of Late-onset Neutropenia from Rituximab for Autoimmune Disease. Arthritis Rheumatol 2020 (In eng). DOI: 10.1002/art.41501.

66. Kronbichler A, Kerschbaum J, Gopaluni S, et al. Trimethoprim-sulfamethoxazole prophylaxis prevents severe/life-threatening infections following rituximab in antineutrophil cytoplasm antibody-associated vasculitis. Ann Rheum Dis 2018;77(10):1440-1447. DOI: 10.1136/annrheumdis-2017-212861.

67. Tony HP, Burmester G, Schulze-Koops H, et al. Safety and clinical outcomes of rituximab therapy in patients with different autoimmune diseases: experience from a national registry (GRAID). Arthritis Res Ther 2011;13(3):R75. DOI: 10.1186/ar3337.

68. van Daalen EE, Rizzo R, Kronbichler A, et al. Effect of rituximab on malignancy risk in patients with ANCA-associated vasculitis. Ann Rheum Dis 2017;76(6):1064-1069. DOI: 10.1136/annrheumdis-2016-209925.

69. Ruggenenti P, Chiurchiu C, Abbate M, et al. Rituximab for idiopathic membranous nephropathy: who can benefit? Clin J Am Soc Nephrol 2006;1(4):738-48. DOI: 10.2215/CJN.01080905.

70. Howman A, Chapman TL, Langdon MM, et al. Immunosuppression for progressive membranous nephropathy: a UK randomised controlled trial. Lancet 2013;381(9868):744-51. (In eng). DOI: 10.1016/S0140-6736(12)61566-9.

71. Michel PA, Dahan K, Ancel PY, et al. Rituximab treatment for membranous nephropathy: a French clinical and serological retrospective study of 28 patients. Nephron Extra 2011;1(1):251-61. DOI: 10.1159/000333068.

72. Hanset N, Esteve E, Plaisier E, et al. Rituximab in Patients With Phospholipase A2 Receptor-Associated Membranous Nephropathy and Severe CKD. Kidney Int Rep 2020;5(3):331-338. DOI: 10.1016/j.ekir.2019.12.006.

73. 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 2019;95(1):233-234. DOI: 10.1016/j.kint.2018.08.045.

74. Boyer-Suavet S, Andreani M, Lateb M, et al. Neutralizing Anti-Rituximab Antibodies and Relapse in Membranous Nephropathy Treated With Rituximab. Front Immunol 2019;10:3069. DOI: 10.3389/fimmu.2019.03069.

75. Klomjit N, Fervenza FC, Zand L. Successful Treatment of Patients With Refractory PLA2R-Associated Membranous Nephropathy With Obinutuzumab: A Report of 3 Cases. Am J Kidney Dis 2020. DOI: 10.1053/j.ajkd.2020.02.444.

76. Podesta MA, Ruggiero B, Remuzzi G, Ruggenenti P. Ofatumumab for multirelapsing membranous nephropathy complicated by rituximab-induced serum-sickness. BMJ Case Rep 2020;13(1). DOI: 10.1136/bcr-2019-232896.

77. Barrett C, Willcocks LC, Jones RB, et al. Effect of belimumab on proteinuria and anti-phospholipase A2 receptor autoantibody in primary membranous nephropathy. Nephrol Dial Transplant 2020;35(4):599-606. DOI: 10.1093/ndt/gfz086.

78. Dorner T, Furie R. Novel paradigms in systemic lupus erythematosus. Lancet 2019;393(10188):2344-2358. DOI: 10.1016/S0140-6736(19)30546-X.

79. Cortazar FB, Leaf DE, Owens CT, Laliberte K, Pendergraft WF, 3rd, Niles JL. Combination therapy with rituximab, low-dose cyclophosphamide, and prednisone for idiopathic membranous nephropathy: a case series. BMC Nephrol 2017;18(1):44. DOI: 10.1186/s12882-017-0459-z.

80. Tencer J, Torffvit O, Thysell H, Rippe B, Grubb A. Proteinuria selectivity index based upon alpha 2-macroglobulin or IgM is superior to the IgG based index in differentiating glomerular diseases. Technical note. Kidney Int 1998;54(6):2098-105. (In eng). DOI: 10.1046/j.1523-1755.1998.00205.x.

81. Fervenza FC, Cosio FG, Erickson SB, et al. Rituximab treatment of idiopathic membranous nephropathy. Kidney Int 2008;73(1):117-25. DOI: 10.1038/sj.ki.5002628.

82. 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(6):1083-8. DOI: 10.2215/CJN.06041108.

83. Fervenza FC, Abraham RS, Erickson SB, et al. Rituximab therapy in idiopathic membranous nephropathy: a 2-year study. Clin J Am Soc Nephrol 2010;5(12):2188-98. DOI: 10.2215/CJN.05080610.

84. Busch M, Ruster C, Schinkothe C, Gerth J, Wolf G. Rituximab for the second- and third-line therapy of idiopathic membranous nephropathy: a prospective single center study using a new treatment strategy. Clin Nephrol 2013;80(2):105-13. DOI: 10.5414/CN107912.

85. Ruggenenti P, Debiec H, Ruggiero B, et al. Anti-Phospholipase A2 Receptor Antibody Titer Predicts Post-Rituximab Outcome of Membranous Nephropathy. J Am Soc Nephrol 2015;26(10):2545-58. DOI: 10.1681/ASN.2014070640.

86. Roccatello D, Sciascia S, Di Simone D, et al. 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 2016;15(6):529-38. DOI: 10.1016/j.autrev.2016.02.014.

87. Fiorentino M, Tondolo F, Bruno F, et al. Treatment with rituximab in idiopathic membranous nephropathy. Clin Kidney J 2016;9(6):788-793. DOI: 10.1093/ckj/sfw091.

88. Cravedi P, Sghirlanzoni MC, Marasa M, Salerno A, Remuzzi G, Ruggenenti P. Efficacy and safety of rituximab second-line therapy for membranous nephropathy: a prospective, matched-cohort study. Am J Nephrol 2011;33(5):461-8. DOI: 10.1159/000327611.

89. Ruggenenti P, Cravedi P, Chianca A, et al. Rituximab in idiopathic membranous nephropathy. J Am Soc Nephrol 2012;23(8):1416-25. DOI: 10.1681/ASN.2012020181.

# Tables and legends.

**Table 1.** Criteria for risk-assessment of progressive loss of kidney function.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Low risk** | **Moderate risk** | **High risk** | **Very high risk** |
| eGFR | normal | normal | < 60 ml/min/1.73m² | Rapid deterioration |
| Proteinuria | < 3.5 g/d and/or serum albumin > 30 g/L | > 4 g/d and no decrease > 50% after 6 months of supportive therapy | > 8 g/d for > 6 months | Life-threatening nephrotic syndrome |
| PLA2R Ab\* |  | < 50 RU/mL | > 150 RU/mL |  |
| Low molecular weight proteinuria |  | Mild | High | High (in two urine samples collected with interval of 6-12 months) |
| Urinary IgG |  | < 250 mg/d | > 250 mg/d |  |
| Selectivity index\*\* |  | < 0.15 | > 0.20 |  |

Modified according to provisional KDIGO guidelines (public review draft) 23.

*eGFR, estimated glomerular filtration rate; IgG, immunoglobulin G; PLA2R Ab, M-type phospholipase A2 receptor antibody*

\* Serial measurement every 3 to 6 months should be performed, as changes of PLA2R Ab levels precede signs. Dynamics of PLA2R Ab levels therefore may be of additional value for risk estimation.

\*\* Ratio of clearance of high molecular weight molecules (IgG, IgM, α2-macroglobulin) to that of albumin80

**Table 2. Strengths and limitations of rituximab in membranous nephropathy**

|  |  |  |
| --- | --- | --- |
|  | **Pro** | **Con** |
| **Efficacy** | Remission in two-thirds of treated patients | Possibly lower CR rates compared with a cyclical therapy |
|  | Low relapse rates |  |
| **Application** | Simple dosing | Frequent IRR |
|  | Long intervals (≥ 6 months) and in PLA2R Ab-positiv patients serologic monitoring option for maintenance treatment | Delicate scheduling due to persistent B cell depletion e.g. during COVID-19 pandemic |
| **Side effects** | Overall beneficial safety profile; Low rates of SAE | No long-term experience, late-onset neutropenia |
| **Long-term sequelae** | No increased malignancy risk, no increase in cardiovascular mortality | Treatment-associated long-lasting hypogammaglobulinemia |

*COVID-19, coronavirus disease 2019; CR, complete remission; IRR; infusion-related reactions; PLA2R Ab, M-type phospholipase A2 receptor antibody; SAE, severe adverse events;*

**Table 3.** Overview of prospective rituximab trials in membranous nephropathy

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First author, (study)** | **Year** | **Design** | **n** | **RTX / Immunosuppression** | **Mean FU [months]** | **Complete + partial remission rates\*** | **Complete remission rates** | **% SAE [% infections]** |
| Fervenza 81 | 2008 | Uncontrolled,  open label pilot trial | 15 | RTX (1g day 1 & 15; + 2nd course at 6m if proteinuric + B cell recovery) | 12 | 12m: 57% | 12m: 14% | No SAE |
| Segarra 82 | 2009 | Uncontrolled | 13 | RTX (4 weekly 375mg/m²) in CNI-dependent patients +/- prior IS | 30 | 30m: 100% (vs. 100% before RTX)\*\* | 12m: 31% | No AE |
| Fervenza 83 | 2010 | Uncontrolled | 20 | RTX (4 weekly 375mg/m² + second course at 6m regardless of response) | 24 | 12m: 50%  24m: 80% | At 12 months: 0%  24m: 22% | No SAE |
| Busch 84 | 2013 | Uncontrolled | 14 | RTX (4 monthly 375mg/m²) + prior IS | 36 (median; range 1-6y) | 12m: 71% | 12m: 21% | 7% [7%, central venous catheter infection] |
| Ruggenenti 85  \*\*\* | 2015 | Uncontrolled | 132 | RTX (4 weekly 375mg/m²) +/- prior IS +/- RTX re-application | 30.8 (median, 6-145.4) | Overall: 64% | Overall: 33% | Not reported. |
| Roccatello 86 | 2016 | Uncontrolled | 17 | RTX (4 weekly 375mg/m²) +/- RTX re-application | 36.3 (range 24-48) | 6m: 65%  12m: > 80%  Overall: 88% | 6m: 41%  12m: > 45%  Overall: 82% | Not reported. |
| Fiorentino 87 | 2016 | Uncontrolled | 38 | RTX (4 weekly (n=36) or 2 weekly (n=2) 375mg/m²) +/- prior IS | 15 (median, IQR 7.7-30.2) | Overall: 76% | Overall: 40% | No SAE |
| Waldman 48 | 2016 | Phase 2 pilot study (single arm) | 13 | RTX (1g day 1 & 15) +/- re-application + CSA (6m + 18m tapering) | 41 (range 24-56) | 12 & 24m: 85% | 12 & 24m: 54% | 30% (5 episodes of late-onset neutropenia in 3 patients) |
| Moroni 47 | 2017 | Uncontrolled, observational | 34 | Low-dose RTX (single dose (n=18) or 2x (n=16) 375mg/m²) +/- prior IS | 23.9 (+/- 18.6) | 12 & 24 m: 44%  No difference between one or two doses of RTX | 12m: 15% | No SAE |
| Dahan (GEMRITUX) 49 | 2017 | RCT | 37 (vs. 38) | NIAT +/- RTX (2 weekly 375mg/m²) | 17 (median) | 6m (primary end point): no difference  17m (post-hoc): 64.9% (RTX) vs. 34.2% (control), OR, 3.5; 95% CI, 1.7-9.2; p<0.01) | 17m (post-hoc): 19% (RTX) vs. 0% (control) | 22% (RTX) vs. 21% (control) [3% (prostatitis; RTX) vs. 0%] |
| Fervenza (MENTOR) 30 | 2019 | RCT | 65 (vs. 65) | RTX (1g day 1 & 15) +/- RTX re-application vs. CSA | 24 | RTX vs. CSA  12m: 60% vs. 52%  24m: 60% vs. 20% (risk difference, 40 PP, 95% CI, 25-55; p>0.001) | 24m: 35% vs. 0% | RTX vs. CSA  17% vs. 31% [6% vs. 12%] |
| Fernández-Juárez (STARMEN) 31 | 2020 | RCT | 43 (vs. 43) | Oral TAC for 6m (+ 3m tapering) + RTX (1g single-dose) at month 6 vs. MP (months 1,3,5) and CYC (months 2,4,6) | 24 | TAC-RTX vs. MP-CYC  24m: 58% vs. 84% | 24m: 26% vs. 60% | TAC-RTX vs. MP-CYC  14% vs. 19% |

*AE, adverse event; CI, confidence interval; CNI, calcineurin inhibitors; CSA, cyclosporine A; CV, cardiovascular; FU, follow-up; IQR, interquartile range; IRR, infusion-related reaction; IS, immunosuppression; m, months; MP, methylprednisolone; NIAT, non-immunosuppressive antiproteinuric treatment; OR, odds ratio; PP percentage points; RCT, randomized-controlled trial; RTX, rituximab; SAE, serious adverse events; TAC, tacrolimus*

*\* varying definitions for remission in the listed studies (e.g. proteinuria cut-off for PR < 3g (e.g. 81) or 3.5g (e.g. 30,46,47,83,84) per g creatinine or 24h) limit direct comparability.*

*\*\* all patients were in remission before receiving RTX; proteinuria decreased from 2,5 +/- 0,76 at baseline to 0.85 +/- 0.17 at 6 months (p=0.0003); CNI and other IS could be withdrawn in all patients; GFR increased significantly (from 95.4 +/- 11 to 110 +/- 13 at month 6; mean percent increase of 15.3%); 3/13 patients suffered relapse and received a second course of RTX (titrated to B cell-depletion); proteinuria cut-offs for remission were not defined in this study.*

*\*\*\* Prior prospective studies from the center Bergamo (Italy)45,55,88,89 likely report overlapping cases and thus are not listed separately in the table.*

**Figure 1. Overview of different dosing-regimens used in clinical trials (blue boxes) and a potential algorithm for subsequent dosing as recommended by current KDIGO guidelines (green boxes).**

*CYC, cyclophosphamide; PLA2R Ab, M-type phospholipase A2 receptor antibody; PR, partial remission*

\* In ‘high-dose regimens’ using a second course of the initial rituximab dosing after 6 months, KDIGO recommendations for subsequent dosing in the first 6 months are not applicable (grey arrows). Nonetheless, subsequent dosing may be guided similarly thereafter.