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CLINICAL RESEARCH

Obinutuzumab as Initial or Second-Line Therapy in Patients With Primary Membranous Nephropathy

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Introduction: B-cell lymphocytes have been demonstrated to play a key role in the pathogenesis underlying membranous nephropathy (MN). The aim of this study was to evaluate the therapeutic efficacy and safety of Obinutuzumab, a glycoengineered type II anti-CD20 monoclonal antibody in individuals with MN.

Methods: We retrospectively analyzed data from 59 consecutive patients with primary MN who provided consent to receive Obinutuzumab and were followed for at least 6 months. The primary outcomes were complete (proteinuria $<$0.3 g/d) or partial (proteinuria $<$3.5 g/d with $\geq$ 50% reduction) remission of proteinuria.

Results: Twenty patients received Obinutuzumab as initial therapy, and 39 patients were previously treated with at least 1 immunosuppressant (second-line therapy). Fifty patients (84.7%) achieved complete remission (CR) or partial remission (PR) of proteinuria during the median follow-up of 9.4 months. The likelihood of remission was significantly higher when Obinutuzumab was used as initial therapy than as second-line therapy after adjusting for the baseline estimated glomerular filtration rate (eGFR), 24-hour urinary protein levels, and anti-phospholipase A_2 receptor (PLA_2R) status (adjusted hazard ratio [HR], 4.5; 95% confidence interval [CI]: 2.1–9.5, $P < 0.001$). Circulating $CD19^+$ B-cell count decreased to $<$5 cells/μl in all patients within 2 weeks after infusion. Serum anti-PLA_2R concentrations decreased to $<$14 relative units (RU)/ml in 43 of 48 patients with PLA_2R-related MN. After Obinutuzumab administration, a significant reduction in 24-hour urine protein and increase in serum albumin were observed. No serious adverse events were observed.

Conclusion: Obinutuzumab may represent a promising and well-tolerated therapeutic option for individuals with primary MN. The potential of Obinutuzumab was highlighted as an initial therapy for primary MN.

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Primary MN is the leading cause of nephrotic syndrome in adults without diabetes globally, constituting 20% to 37% in most kidney biopsy cohorts and rising to as high as 58% among individuals aged >65 years.[1-3] Untreated MN has a reported spontaneous CR rate ranging from 20% to 30%, with renal survival rates of 60% to 80% observed over a decade.[4-6] However, in individuals afflicted with persistent nephrotic syndrome who fail to achieve remission following therapeutic interventions, the incidence of end-stage renal disease reaches 40% to 50% within a decade.[7,8]

B-cell lymphocytes have been demonstrated to play a key role in the pathogenesis underlying MN.[6] Targeting B-cells with the anti-CD20 monoclonal antibody, such as rituximab, enabled effective depletion of B-cells and thus the selective targeting of antibody production. Based on GEMRITUX and MENTOR trials,[9,10] rituximab has emerged as a first-line therapy for primary MN that are at risk for progression to kidney failure or have persistent nephrosis despite conservative therapy.[11] However, over one-third of patients may experience treatment failure with rituximab,[10,12] highlighting the need for more potent alternative therapies.

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Obinutuzumab, a glycoengineered type II anti-CD20 monoclonal antibody, was initially developed to overcome resistance to rituximab in B-cell malignancies.[13] It has showed superior efficacy to rituximab in the treatment of B-cell lymphoma.[14,15] Obinutuzumab induces prolonged B-cell depletion with a very low risk of immunization.[16,17] In 2 case series with rituximab refractory MN, Obinutuzumab has been presented to be effective.[18,19] Successful treatment cases of Obinutuzumab in patients with MN have been increasingly documented.[20-24] Recently, the efficacy of Obinutuzumab in treating active lupus nephritis has been demonstrated,[25] and ongoing randomized controlled trials are currently investigating its potential benefits in adults with MN (NCT04629248).

Based on these findings and published case series, we have used Obinutuzumab as a rescue therapy for patients with MN who fail to achieve remission after immunosuppressant interventions. Furthermore, owing to its favorable therapeutic outcomes, we have attempted to use it as an initial treatment option for MN patients. Herein, we conducted a single-center retrospective analysis of 59 consecutive patients with primary MN undergoing Obinutuzumab therapy and presented its efficacy and tolerability.

METHODS

Study Cohort and Data Collection

This retrospective single-center cohort study included all consecutive patients who received Obinutuzumab therapy at least once during hospitalization in the nephrology department of the Second Hospital of Shanxi Medical University from January 21 to October 1, 2023. Patients were followed up for at least 3 months. Patients with secondary MN or exposure to other concurrent immunosuppressants after the administration of Obinutuzumab were excluded. Participants were categorized into an initial therapy group and a second-line therapy group according to whether they had received immunosuppressant medication before Obinutuzumab infusion. Additionally, participants were divided into rituximab-resistant and rituximab-naive groups according to their previous administration of rituximab. Resistance to rituximab was defined as the failure to achieve CR or PR of proteinuria at least 6 months after the last rituximab administration. Rituximab-naive refers to individuals who have never been exposed to rituximab.

The administration of Obinutuzumab necessitates hospitalization for all patients. Patients received Obinutuzumab infusions at a dosage of 1000 mg per administration. The decision regarding the number of Obinutuzumab infusions was made collaboratively between the clinician and the patients, based on professional consultation. The time interval between the first and second infusions typically spanned 2 to 3 weeks, whereas the duration between the second and third infusions exceeded 6 months based on B-cell levels. Obinutuzumab (Gazyvaro) was diluted in 500 ml normal saline. Patients were administered oral ibuprofen 300 mg, intravenous dexamethasone injection of 20 mg, and intramuscular diphenhydramine injection of 20 mg as systematic premedication. The infusion was administered at a rate of 10 ml/h for the first 30 minutes. The rate was then progressively increased, every 30 minutes, to a maximum of 200 ml/h.

Clinical and laboratory parameters were evaluated at baseline (within 3 days before the initial Obinutuzumab infusion) and within 1 month ($CD19^+$ B-cell counts at 2 weeks), with bimonthly monitoring of complete blood count, proteinuria, and kidney function, as well as quarterly evaluation of PLA_2R antibody levels and $CD19^+$ B-cell counts. Glomerular filtration rate was estimated according to the chronic kidney disease-epidemiology collaboration creatinine equation.[26] Serum IgG levels were not evaluated during follow-up period because of the restrictions on outpatient costs and medical insurance policies.

Prior immunosuppressive therapy was defined as any administration of immunosuppressants before the initiation of Obinutuzumab infusion. Concomitant immunosuppressive therapy referred to the combined use of immunosuppressive therapy after the initial infusion of Obinutuzumab. All immunosuppressive therapy included 6 drugs, namely glucocorticoid, calcineurin inhibitors, rituximab, cyclophosphamide, triptolide, and mycophenolate mofetil. Continuous use of immunosuppressive agents for >3 days and administration of RTX for >1 dose after the initial infusion of Obinutuzumab were excluded in our analysis.

The follow-up period for all patients was defined as commencing from the initiation of Obinutuzumab therapy (date of the first dose) and continuing until the date of their final visit during the study period. Relevant patient-level data were retrospectively collected from the hospital information system and the patient's supplementary records. The final date for data collection was from April 1 to 5, 2024, during which all enrolled patients were subjected to follow-up and outcome confirmation either on-site or through telephone.

Off-label Obinutuzumab treatment and the protocol of retrospective outcome analyses were approved by the ethics committee of our institution. All participants

provided written informed consent for Obinutuzumab infusion and retrospective analyses, in accordance with the declaration of Helsinki.

Outcomes and Definitions

The clinical outcome was the incidence of remission of proteinuria, including CR or PR. CR was defined as a reduction in the 24-hour urine protein excretion to ≤0.3 g/d along with normal serum albumin concentration and stable kidney function. PR was defined as a reduction in the 24-hour urine protein ratio exceeding 50% from baseline and a final 24-hour urine protein ≤3.5 g/d, accompanied by improvement or normalization of serum albumin and stable kidney function. The others, except for CR and PR, were considered nonresponse.

Immunological outcomes were assessed only in patients with PLA_2R-related disease. Immunological remission refers to reduction of serum anti-PLA_2R levels to < 14 RU/ml via enzyme-linked immunosorbent assay (euroimmun). Enzyme-linked immunosorbent assay findings were interpreted as follows: <14 RU/ml: negative; 14–20 RU/ml: borderline; and >20 RU/ml: positive.[27] B-cell depletion (absolute CD19-positive count < 5 cells/μl) and reconstitution (absolute CD19-positive count > 5 cells/μl) was monitored by sending peripheral blood for flow cytometry based on the frequency and counts of peripheral CD19-positive B-cells at planned follow-up visits.

Safety outcomes included both serious and nonserious adverse events observed during Obinutuzumab administration as well as throughout the follow-up period. Serious adverse events included additional hospitalizations and life- or organ-threatening events.

Statistical Analysis

All patients with at least 6 months of follow-up were considered for analyses. Categorical data were presented as number (percentage). Continuous data were reported as mean ± standard deviation, or median (interquartile range [IQR]) as appropriate. The comparisons within and between groups over time were analyzed using mixed-effect models in Prism software, which accounted for repeated-measures experimental design with missing values. The Geisser-Greenhouse correction and Dunnett multiple-comparisons tests were used. Kaplan-Meier curve was used to plot the probability of achieving remission of proteinuria. Cox regression analysis was conducted to evaluate the association between patient groups, number of infusions, and anti-PLA_2R antibody status with outcomes. Multivariable-adjusted Cox regression models were used to account for clinically relevant variables, such as baseline eGFR, anti-PLA_2R antibody, and 24-hour urinary protein levels. Patients not achieving the event of interest were considered as censored on April 1, 2024. A 2-sided $P < 0.05$ was considered statistically significant. Data analyses were performed using Prism (version 9.4.1; GraphPad Software), SPSS Statistics (version 27.0, IBM), and R (version 4.1.0; R Foundation for Statistical Computing).

RESULTS

Baseline Characteristics

Between January 21 and October 1, 2023, a total of 73 patients received obinutuzumab treatment in the nephrology department at the Second Hospital of Shanxi Medical University. A comprehensive analysis was conducted on a cohort of 59 patients who were followed up for at least 6 months. Fourteen patients were excluded because of diagnosis of other glomerulonephritis, concurrent use of other immunosuppressant agents, or inadequate follow-up (Figure 1).

Baseline characteristics are summarized in Table 1. Forty-eight patients (81.4%) were men, with an average age of 50.7 years. The mean levels of 24-hour urine protein was 4.8 g per 24 hour, and the mean serum creatinine and eGFR values were 87.8 μmol/l and 92.2 ml/min per 1.73 m^2, respectively. Twenty patients received Obinutuzumab as initial therapy, and 39 patients were previously treated with at least 1 immunosuppressant, including calcineurin inhibitors (n = 28), rituximab (n = 14), cyclophosphamide (n = 11), mycophenolate mofetil (n = 1) and triptolide (n = 3). Thirty-three of 39 patients were previously administered glucocorticoid in combination. Eleven patients (28.2%) of these 39 had received at least 2 different immunosuppressive treatment regimens before receiving the Obinutuzumab therapy. The median immunosuppression-free interval was 10.2 (IQR 0–62.6)

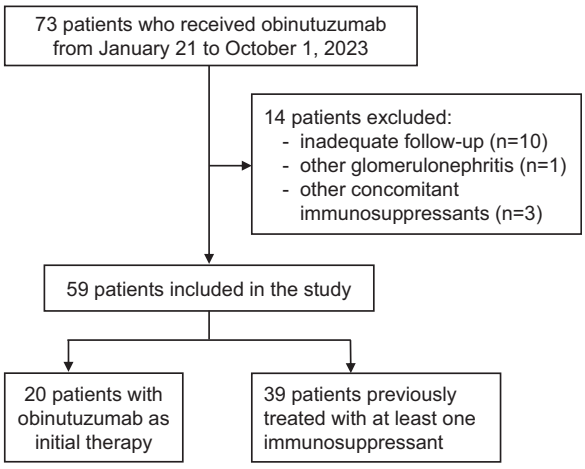

Figure 1. Study flow diagram.

Table 1. Baseline patient characteristics grouped by Obinutuzumab assecond linesecond line second line therapy

Characteristic	Total (n = 59)	Initial therapy (n = 20)	Second-line therapy (n = 39)
Age, y	50.7 ± 14.4	50.1 ± 14.8	51.9 ± 14.0
Male, n (%)	48 (81.4%)	11 (55.1%)	37 (94.9%)
Body mass index, kg/m^2	24.5 (22.3 to −27.0)	24.6 (22.3 to −27.6)	24.5 (22.1 to −26.5)
Kidney biopsy	39 (66.1%)	14 (70.0%)	25 (64.1%)
Systolic blood pressure, mm Hg	129 ± 15	127 ± 13	130 ± 15
Diastolic blood pressure, mm Hg	77 ± 8	77 ± 6	78 ± 10
Laboratory tests, serum			
Hemoglobin, g/dl	11.9 (10.7–14.9)	11.7 (10.8–15.2)	12.0 (11.1–14.9)
Urea nitrogen, mmol/l	7.4 ± 3.3	5.6 ± 2.4	8.3 ± 3.3
Creatinine, μmol/l	87.8 ± 33.5	73.7 ± 20.7	95.1 ± 36.6
eGFR[a], ml/min/1.73 m^2	92.2 ± 23.4	98.4 ± 17.3	83.1 ± 25.7
Protein, g/l	50.9 ± 8.9	47.0 ± 6.6	52.8 ± 9.4
Albumin, g/l	28.0 ± 7.2	23.8 ± 4.5	30.1 ± 7.5
Uric acid, mmol/l	420.5 ± 114.0	413.0 ± 131.8	424.3 ± 106.1
Total cholesterol, mmol/l	6.2 ± 2.2	6.4 ± 1.6	6.2 ± 2.4
Triglycerides, mmol/l	3.2 ± 2.0	2.4 ± 1.0	3.6 ± 2.3
Low density lipoprotein, mmol/l	3.4 ± 2.2	3.2 ± 1.1	3.5 ± 2.7
High density lipoprotein, mmol/l	1.4 ± 0.6	1.7 ± 0.8	1.1 ± 0.2
Laboratory tests, urine			
ACR, mg/g	5910 (753 to −8572)	7648 (2293 to −9263)	4653 (1237 to −6740)
24-h urine protein, g/24-h	4.8 ± 2.2	5.2 ± 1.9	4.6 ± 2.6
B-cell phenotype			
CD19+ count, cells/μl	203.7 (96.2 to −314.2)	273.8 (201.1 to −402.8)	176.1 (83.1 to −261.7)
CD19+ frequency, % of lymph	9.8 (5.5 to −16.8)	16.6 (11.2 to −20.4)	7.6 (5.3 to −12.8)
Anti-PLA$_2$R antibody			
Positive history[b]	50 (84.7%)	17 (85.0%)	33 (84.6%)
Serum levels, RU/ml	85.3 (16.7 to −127.0)	81.0 (17.6 to −157.2)	89.7 (16.7 to −125.0)
Obinutuzumab, number of doses			
One	1 (1.7%)	1 (5.0%)	0 (0%)
Two	41 (69.5%)	17 (85.0%)	24 (61.5%)
Three	15 (25.4%)	2 (10%)	13 (33.3%)
Four	2 (3.4%)	0 (0%)	2 (5.1%)
Previous immunosuppressive drugs			
Glucocorticoid	33 (55.9%)	0 (0%)	33 (84.6%)
Calcineurin inhibitors	28 (47.5%)	0 (0%)	28 (71.8%)
Rituximab	14 (23.7%)	0 (0%)	14 (35.9%)
Cyclophosphamide	11 (18.6%)	0 (0%)	11 (28.2%)
Triptolide	3 (5.1%)	0 (0%)	3 (7.7%)
Mycophenolate mofetil	1 (1.7%)	0 (0%)	1 (2.6%)
Concomitant diseases			
Arterial hypertension	38 (64.4%)	13 (65.0%)	25 (64.1%)
Diabetes mellitus	7 (11.9%)	3 (15.0%)	4 (10.3%)

Data are presented as mean ± standard deviation, median (interquartile range) or number (%) where applicable.
ACR, albumin-creatinine ratio; eGFR, estimated glomerular filtration rate; PLA$_2$R, phospholipase A$_2$ receptor; RU, relative units.
[a]eGFR was estimated using the 2009 Chronic Kidney Diseases Epidemiology Collaboration (CKD-EPI) creatinine equation.
[b]Cutoff for anti-PLA$_2$R positivity: 14 RU/ml.

months (see Supplementary Table S1 for detail). Overall, patients receiving Obinutuzumab as initial therapy exhibited superior kidney function, lower levels of albumin, and elevated serum anti-PLA$_2$R antibody levels than those who underwent second-line therapy. Thirty-nine patients (66.1%) underwent kidney biopsy. In the remaining 20 patients who did not undergo kidney biopsy, all tested positive for anti-PLA$_2$R antibodies. Forty-one patients (69.5%) underwent 2 infusions, each with a dosage of 1 g, administered at a median interval of 20 (IQR 13–26) days. Three infusions were administered to 15 patients (25.4%), whereas 4 infusions were administered to 2 patients. The median interval between the second and third administrations was 7.8 (IQR 5.7–10.1) months.

Clinical Outcomes

Median follow-up time was 9.4 months (IQR 7.8–11.3 months; range 6.0–13.2 months). Fifty patients (84.7%) achieved CR or PR during the follow-up period, including 18 of 20 patients who received Obinutuzumab therapy as their initial treatment and

32 of 39 patients who received it as a second-line therapy (Table 2). The median time to remission was 4.9 (IQR 3.9–6.9) months (Figure 2a). The likelihood of remission was significantly higher when Obinutuzumab was used as initial therapy than as second line therapy (unadjusted HR, 2.5; 95% CI: 1.4–4.5; $P = 0.003$; Figure 2b).

Fourteen patients were classified as rituximab-resistant, having previously received a minimum dose of 1 g of rituximab treatment with all rituximab-free intervals exceeding 6 months. The baseline characteristics stratified by rituximab-resistant are shown in Supplementary Tables S2 and S3. Remission of proteinuria was achieved in 9 of 14 patients with resistance to rituximab, whereas the remaining 5 patients showed persistent nonresponse until the last follow-up (Table 2). Patients resistant to rituximab exhibited a lower likelihood of achieving proteinuria remission compared with those with rituximab-naive (unadjusted HR, 0.4; 95% CI: 0.2–0.9; $P = 0.02$, Figure 2c).

After adjusting for the baseline eGFR, 24-hour urinary protein levels, and anti-PLA_2R status, the significances both remains unchanged (initial therapy vs. second-line therapy: adjusted HR, 4.5; 95% CI: 2.1–9.5, $P < 0.001$; rituximab-resistant vs. rituximab-naive: adjusted HR, 0.4; 95% CI: 0.2–0.8; $P = 0.01$). Conversely, the anti-PLA_2R status ($P = 0.2$, Supplementary Figure S1) did not appear to significantly affect the remission of proteinuria.

The characteristics of 9 patients with no response are summarized in Table 3. Among them, 7 patients had previously undergone at least 1 immunosuppressant treatment, of which 5 patients had received rituximab therapy. One patient has negative anti-PLA_2R antibody. Among the 8 patients with positive anti- PLA_2R antibody, an immunological response was observed in 4 cases, whereas clinical remission had not been achieved until the last follow-up.

In whole study cohort, a significant reduction in 24-hour urine protein ($P < 0.001$ for all comparisons) and increase in serum albumin ($P < 0.001$ for all comparisons) were observed after Obinutuzumab administration compared with baseline at all time points from month 2 to the last follow-up (Figure 3a and Supplementary Figure S2a). The results of 39 patients with complete data at 4 time-points (0, 2, 4, and 6 months) were presented in Figure 3b for comparing values within the same population over time. Serum creatinine ($P = 0.6$, 0.5, 0.5, 0.4, 0.6, and 0.8 for 2, 4, 6, 8, 10, and 12 months, respectively) and eGFR ($P = 0.8$, 0.9, 0.9, 0.6, 0.8, and 0.9 for 2, 4, 6, 8, 10, and 12 months, respectively) remained stable compared with baseline throughout the follow-up period (Figure 3c and Supplementary Figure S2b).

Serum Anti-PLA_2R Titers

The median titer at baseline and after 1, 4, 7, 10, and 12 months of treatment was as follows: 89.8 (IQR, 24.3–137.0), 29.9 (4.5–78.3), 2.6 (1.2–7.1), 2.4 (0.5–8.3), and 2.2 (0.8–4.4), and 2.0 (0.7–3.2) RU/ml, respectively (Figure 4a and Supplementary Figure S3a); all values significantly differed from the baseline level ($P < 0.001$). The results of 36 patients with complete data at 3 time-points (0, 1, and 4 months) were presented in Figure 4b for comparing values within the same population over time. Among the 50 patients with PLA_2R-related disease, anti-PLA_2R antibody titers were not measured in 2 patients during follow-up. Immunological remission was achieved in 43 of 48 patients, including 16 of 17 patients who received Obinutuzumab as their initial treatment and 27 of 31 patients who received it as a second-line therapy (Table 2). In 43 patients with immunological remission, 14 patients exhibited undetectable anti-

Table 2. Patient clinical and immunologic outcomes grouped by Obinutuzumab as initial or second-line therapy and by rituximab exposure status

Outcomes	Overall	Grouped 1		Grouped 2	
		Initial therapy	Second-line therapy	RTX-resistant	RTX-naive
Clinical outcomes					
Number of patients	59	20	39	14	45
CR + PR	50 (84.7%)	18 (90.0%)	32 (82.1%)	9 (64.3%)	41 (91.1%)
CR	20 (33.9%)	7 (35.0%)	13 (33.3%)	3 (21.4%)	17 (37.8%)
PR	30 (50.8%)	11 (55.0%)	19 (48.7%)	6 (42.9%)	24 (53.3%)
Nonresponse	9 (15.3%)	2 (10.0%)	7 (17.9%)	5 (35.7%)	4 (8.9%)
Immunologic outcomes[a]					
Number of patients with detectable anti-PLA_2R	48[b]	17	31[b]	10	38[b]
Immunologic remission	43 (89.6%)	16 (94.1%)	27 (87.1%)	7 (70.0%)	36 (94.7%)
Immunologic nonresponse	5 (10.4%)	1 (5.9%)	4 (12.9%)	3 (30.0%)	2 (5.3%)

Data are presented as median (interquartile range) or number (%).
CR, complete remission; PLA_2R, phospholipase A_2 receptor; PR, partial remission; RTX, rituximab.
[a]only in patients with detectable anti-PLA_2R antibody.
[b]anti-PLA_2R antibody titers were not measured in 2 patients during follow-up.

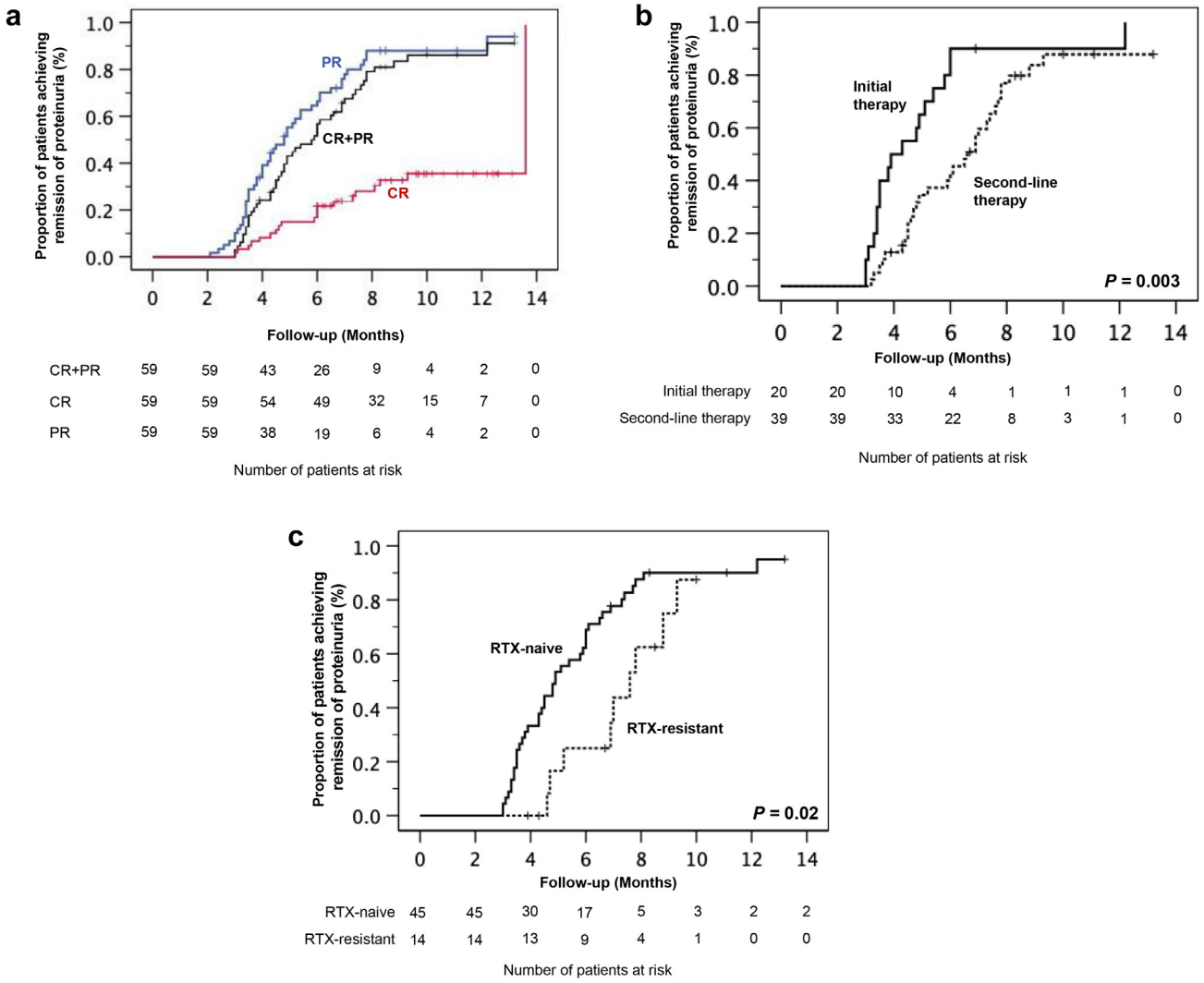

Figure 2. Remission of proteinuria after Obinutuzumab infusion. Kaplan-Meier curves for the probability of reaching the combined end point (CR + PR, blank) of CR (red) or PR (blue) of proteinuria after Obinutuzumab administration in the study group considered as a whole (a), in patients who received Obinutuzumab as initial therapy (solid line) and patients who received it as a second-line therapy (dashed line) considered separately (b) and in RTX-naive (solid line) and RTX-resistant (dashed line) patients considered separately (c). CR, complete remission; PR, partial remission; RTX, rituximab.

PLA_2R antibody (titer $<$ 0.01 RU/ml), whereas the remaining 29 patients displayed a negative range spanning from 0.8 to 12.2 (median 2.3) RU/ml.

There was no significant difference observed in anti-PLA_2R antibody depletion between patients receiving initial treatment and those undergoing second-line therapy (unadjusted HR, 1.7; 95% CI: 0.9–3.2; P = 0.1, Supplementary Figure S4a), as well as between patients who were rituximab-resistant and individuals who were rituximab-naive (unadjusted HR, 0.6; 95% CI: 0.3–1.4; P = 0.2, Supplementary Figure S4b). After adjusting for baseline anti-PLA_2R antibody titers, eGFR and 24-hour urinary protein levels, the significance of both comparisons remains unchanged (initial therapy vs. second-line therapy: adjusted HR, 1.9; 95% CI: 0.9–3.8, P = 0.07; rituximab-resistant vs. rituximab-naive: adjusted HR, 0.6; 95% CI: 0.2–1.3; P = 0.2).

B-Cells

Circulating $CD19^+$ B-cell count decreased to $<$ 5 cells/μl in all patients within 2 weeks after Obinutuzumab infusion, regardless of clinical outcome. The median $CD19^+$ B-cell count at baseline and after 0.5, 4, 7, 10, and 12 months of treatment was as follows: 203.7 (IQR, 96.2–341.2), 0 (0–0), 0.5 (0–1.2), 3.4 (0.6–6.4), 25.4 (1.8–35.2), and 75.2 (1.3–123.2) RU/ml, respectively (Figure 4c and Supplementary Figure S3b). The results of 36 patients with complete data at 3 time-points (0, 0.5, and 7 months) were presented in Figure 4d. Circulating CD19 B-cell reconstitution (i.e., cell counts increased to $>$ 5 cells/μl) was observed in only 1 of 18 (5.6%) available measurements within 4 months, in 10 of 37 available measurements within 7 months (27.0%), in 10 of 14 available measurements within 10 months (71.4%). Fifteen patients received their third

Table 3. Summary of the patients who did not achieve proteinuria remission following Obinutuzumab infusions

Case	Age and gender	Kidney biopsy	Follow-up, months	24-h urine protein (g/d)		Albumin (g/l)		Anti-PLA_2R titer (RU/ml)		CD19 + B cell count (/μl)		Immunologic remission	Treatments prior to Obinutuzumab	Dose of Obinutuzumab	Adverse event
				Initial	Final	Initial	Final	Initial	Final	Initial	Final				
1	21 M	Yes	10.2	6.7	3.8	25.3	37.8	164.0	39.3	572.1	18.7	No	GC, RTX	3 g	None
2	49 M	Yes	8.9	6.4	4.2	20.3	33.2 38.9	381.3	34.6	206.0	0.8	No	GC, CNI, RTX	3 g	None
3	53 M	No	8.5	4.7	3.1	37.7	37.1	112.6	<0.01	510.0	0.0	Yes	GC, CNI, CYC, RTX	3 g	Neutropenia
4	43 M	Yes	6.7	6.9	3.4	29.3	38.0	104.0	18.9	18.0	0.9	No	GC, CNI, CYC, MMF, Triptolide, RTX	2 g	Neutropenia; Upper respiratory infections
5	69 M	No	7.4	8.5	6.7	25.6	33.2	63.1	11.2	550.0	3.2	Yes	GC, RTX	2 g	Bronchitis
6	52 M	Yes	11.1	7.6	3.7	23.7	37.3	89.7	<0.01	54.9	0.0	Yes	GC, CNI, CYC	3 g	Neutropenia
7	62 M	No	13.2	4.6	4.1	31.4	38.2	36.2	<0.01	103.8	0.0	Yes	GC, CNI, CYC	3 g	Bronchitis
8	63 F	Yes	6.9	9.1	6.5	21.0	31.5	Negative	Negative	203.7	134.2	NA	None	0.5 g[a]	Termination of infusion due to intolerable malaise
9	20 F	Yes	8.3	8.9	3.9	22.5	34.7	93.3	89	83.3	0.0	No	None	2 g	Mild rash and itching

CNI, calcineurin inhibitors; CYC, Cyclophosphamide; F, female; GC, glucocorticoid; M, male; MMF, Mycophenolate mofetil; PLA_2R, phospholipase A_2 receptor; RU, relative units; RTX, rituximab.
[a]about 50% of 1 g infusion when termination.

Obinutuzumab infusion with an average interval of 7.4 months after the second infusion.

Safety

Twenty-six adverse events were reported in 23 patients (Table 4). Infusion-related reactions, such as headache, eyelid swelling, rash and itching, oropharyngeal discomfort, nausea, and vomiting, occurred in 9 patients (15.2%) with 10 events. All 9 patients, with the exception of 1 who experienced intolerable malaise and discontinued the infusion, exhibited mild reactions that fully resolved without any subsequent complications after a temporary interruption of the infusion. Neutropenia was observed in 6 patients (10.2%), with a median onset time of 2.6 months after infusion. Recovery from neutropenia was spontaneous (3 patients) or occurred after stopping sulfamethoxazole treatment (3 patients). Five patients experienced a total of 7 events of bronchitis and urinary tract infections, all of which were completely resolved after oral antiinfective drugs outside the hospital. No patients required admission for infection. No serious adverse events were observed. One patient exhibited slightly elevated hepatic aminotransferases, which normalized within 1 month after atorvastatin treatment was stopped. No patient developed malignancy and death during the study period.

DISCUSSION

This retrospective, single-center analysis demonstrates that the infusion of Obinutuzumab can induce B-cell depletion, the clinical and immunological remission in patients with MN who received Obinutuzumab as initial or second-line therapy. During the median 9.4-month follow-up period, CR or PR of proteinuria was achieved by 84.7% of patients (50 of 59), including 90% of patients (18 of 20) with initial therapy and 82.1% of patients (32 of 39) with second-line therapy. Immunological remission was observed in 89.6% of patients (43 of 48) with detectable serum PLA_2R antibody, including 94.1% of patients (16 of 17) with initial therapy and 87.1% (27 of 31) with second-line therapy. Furthermore, there was a significant increase in serum albumin levels alongside the maintenance of kidney function. The safety profile of Obinutuzumab was generally acceptable. The observed infusion reactions related to the treatment were mostly mild, with complete recovery after a temporary interruption.

This study provides compelling evidence supporting the favorable efficacy of Obinutuzumab as an initial immunosuppressive agent in MN. Remissions of proteinuria were achieved in 18 of 20 patients as the initial therapy, demonstrating a superior

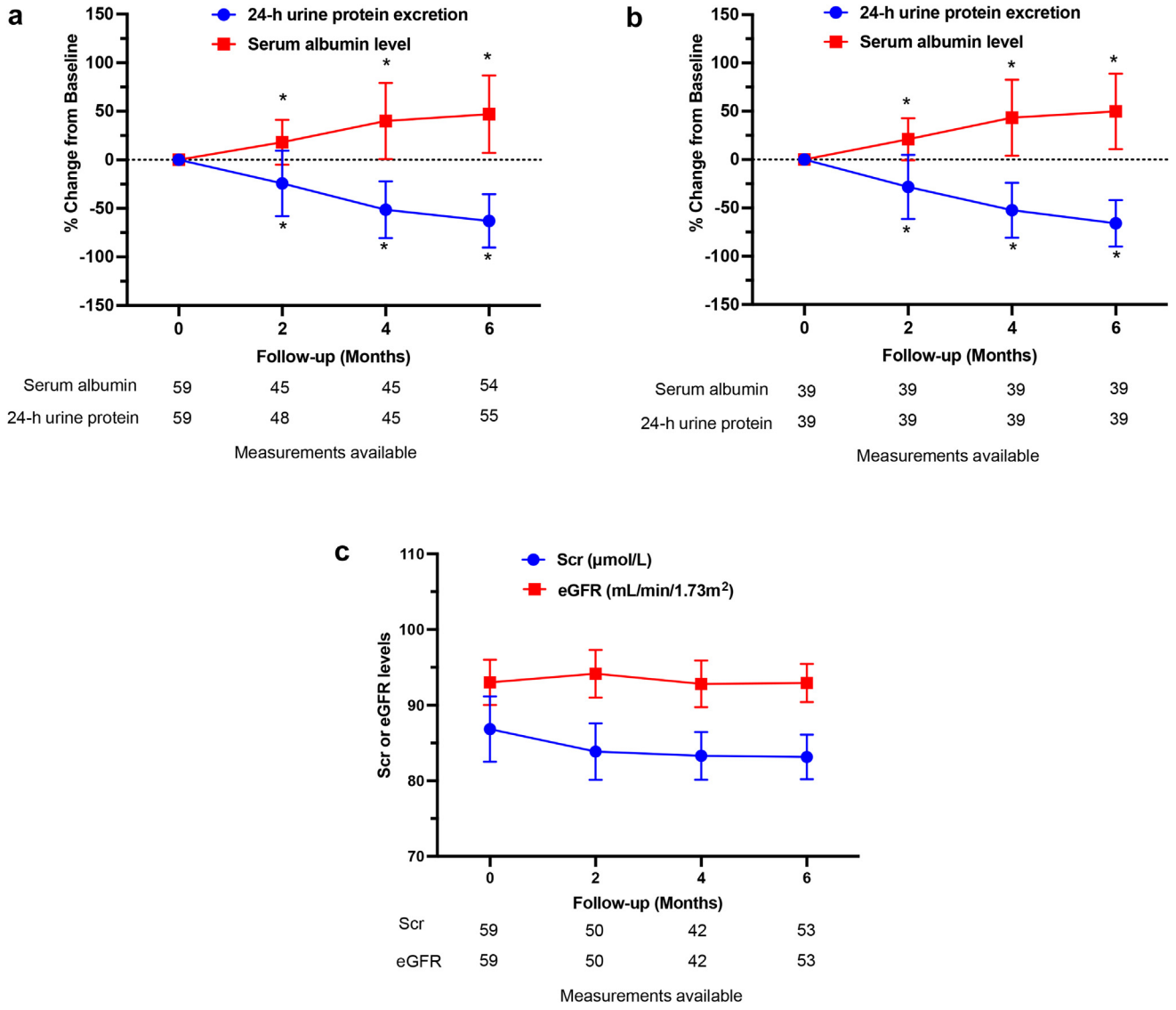

Figure 3. Trends in proteinuria and kidney function within 6 months after Obinutuzumab administration. (a and b) Twenty-four-hour urinary protein excretion significantly decreased (blue) and serum albumin levels significantly increased (red) after Obinutuzumab administration. Data represents mean ± SD. (a) provided measurements available from the whole cohort, whereas (b) presented the results from a subset of thirty-nine patients with complete data at four time-points (0, 2, 4, and 6 months). (c) Serum creatinine and eGFR remained stable compared to baseline after Obinutuzumab administration. Data represent means ± SEM. eGFR, estimated glomerular filtration rate; Scr, serum creatinine. *$P < 0.05$ versus baseline.

response to second-line treatment. One patient with no remission discontinued the infusion of Obinutuzumab owing to intolerable malaise, terminating ~50% of the 1 g infusion. The cost-effectiveness of Obinutuzumab compared with rituximab and the avoidance of long-term oral use of glucocorticoids and other immunosuppressive drugs are both the important reasons of selecting Obinutuzumab for patients undergoing initial anti-CD20 treatment or experiencing relapse. At present, medical insurance services do not provide coverage for any anti-CD20 treatments for MN in our region. Obinutuzumab was administered at a cost of CNY 9300 per 1 g dose, where rituximab required 2 doses (CYN 12,600) at a cost of CNY 6300 per dose. In the RI-CYCLO study, patients who had previously received immunosuppressive therapy were excluded, and CR or PR was achieved in 23 of 37 participants in RTX group.[28] Other studies[29-32] have reported remission rates ranging from 32% to 89% when RTX is administered as an initial therapy for primary MN (Table 5). Obinutuzumab is a highly potent type II humanized monoclonal antibody against CD20 with increased direct and antibody-dependent cell-mediated cytotoxicity.[33] Its glycosylated Fc portion provides specific in vitro activities. Obinutuzumab exhibits epitope recognition distinct from that of rituximab, specifically designed to overcome several postulated

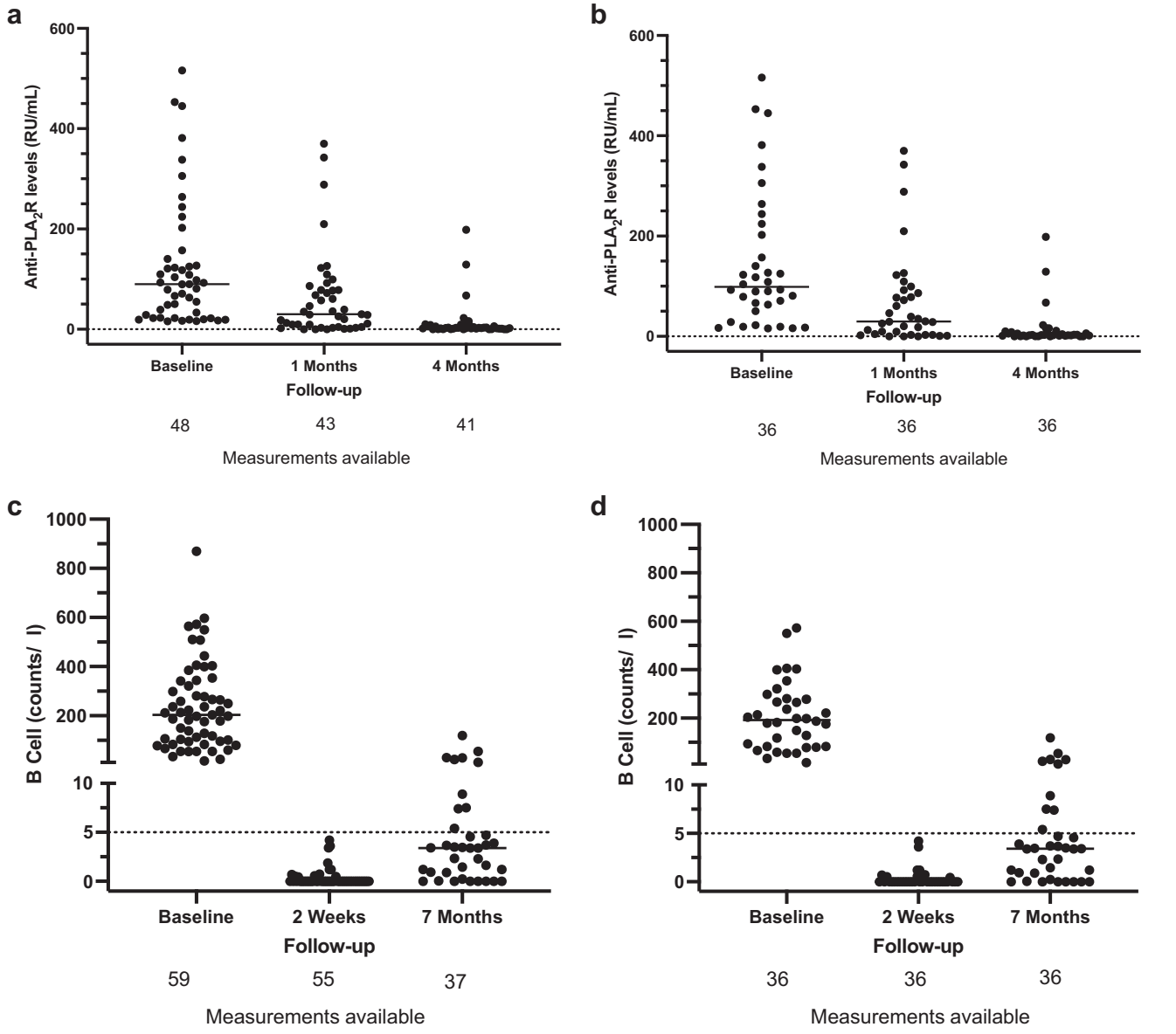

Figure 4. Changes in anti-PLA_2R antibody levels and circulating $CD19^+$ B-cells within 6 months after Obinutuzumab administration. (a and b) Serum anti-PLA_2R antibody levels at 0, 4, and 7 months after Obinutuzumab administration in the patients with PLA_2R-related membranous nephropathy. (a) provided measurements available from the whole cohort, whereas (b) presented the results from a subset of thirty-six patients with complete data at four time-points (0, 1 and 4 months). (c and d) Changes in absolute counts of total circulating $CD19^+$ B cells at 0, 0.5, and 7 months after Obinutuzumab infusion. Horizontal solid lines represent median. (c) provided measurements available from the whole cohort, whereas (b) presented the results from a subset of 36 patients with complete data at 4 time-points (0, 0.5, and 7 months). PLA_2R, phospholipase A_2 receptor.

mechanisms of rituximab resistance.[13] The results from a review of 20 cases of MN treated with Obinutuzumab in 7 published studies demonstrated that 17 of the total patients achieved CR or PR of proteinuria, providing limited evidence to support the efficacy of Obinutuzumab in treating MN (Table 6).[18-24] A case series included 10 patients with nephrotic syndrome who were unresponsive to multiple immunosuppressive agents. The findings showed that 90% patients and 85.7% of patients who were rituximab-refractory achieved either CR or PR with 2 doses of Obinutuzumab In our cohort, 64.3% (9/14) of patients with resistance to rituximab achieved remission of proteinuria, with immunological remission attained by 70% (7/10) among them (Table 2). These preliminary studies and our analysis suggested that even in patients with MN who were unresponsive to rituximab, reinitiating anti-CD20 therapy using Obinutuzumab may still have over 60% chance of achieving disease remission. In comparison to ofatumumab, another type I anti-CD20 therapy, the remission rate was 30% (3 of 10 patients with

Table 4. Adverse events grouped by Obinutuzumab as initial or second-line therapy

	Total (n = 59)		Initial therapy (n = 20)		Second-line therapy (n = 39)	
Adverse Event	Incidence	No. of events	Incidence	No. of events	Incidence	No. of events
Neutropenia	6 (10.2%)	6	1 (5.0%)	1	5 (12.8%)	5
Bronchitis	3 (5.1%)	4	1 (5.0%)	1	2 (5.1%)	3
Urinary tract infections	2 (3.4%)	3	1 (5.0%)	1	2 (5.1%)	3
Abnormal liver function	1 (1.7%)	1	0	0	1 (2.6%)	1
Acute gastroenteritis	2 (3.4%)	2	0	0	2 (5.1%)	2
Infusion-related reaction						
Headache	1 (1.7%)	1	0	0	1 (2.6%)	1
Eyelid swelling	2 (3.4%)	2	2 (10.0%)	2	0	0
Rash and itching	3 (5.1%)	4	2 (10.0%)	2	1 (2.6%)	2
Digestive symptoms	1 (1.7%)	1	1 (5.0%)	1	0	0
Malaise	2 (3.4%)	2	1 (5.0%)	1	1 (2.6%)	1

resistance to rituximab achieved remission).[34] Ongoing phase 2/3 clinical trials (ClinicalTrials.gov identifiers NCT04629248 and NCT05050214) are currently evaluating the efficacy and safety of Obinutuzumab in patients with MN who were treatment-naive and those who were rituximab-resistant, which will likely provide valuable insights and further evidence in managing MN.

Notably, among the 7 patients (1–7) with nonresponse who received Obinutuzumab as second-line therapy, varying degrees of serum albumin elevation were observed, despite no achievement of PR in proteinuria. We postulated that the relatively limited duration of follow-up might have partially contributed to these outcomes. Three of 7 patients were severely rejected for kidney biopsy despite of repeated suggestion from doctors. Other diseases could not be ruled out as the possible cause of proteinuria although anti-PLA_2R antibody was positive.

We observed that Obinutuzumab effectively induced complete depletion of B-cells in all patients. Even after 7 months, the median B-cell count remained <5 cells/μl. These findings are consistent with previous studies investigating the efficacy of Obinutuzumab for various indications, including lupus nephropathy[16,35,36] and MN.[18,19] Indeed, the glyco-engineered Fc region of Obinutuzumab was developed to enhance its binding affinity to the FcγRIII receptor on immune effector cells, thereby augmenting direct cell death and antibody-dependent cell-mediated cytotoxicity or phagocytosis (ADCC/ADCP) activities, while concurrently reducing complement-dependent cytotoxicity effects.[37]

The mechanism of action of obinutuzumab, classified as type II, sets it apart from classical type I anti-CD20 monoclonal antibodies, such as rituximab and ofatumumab.[37] Rituximab primarily functions through complement-dependent cytotoxicity by clustering CD20 within lipid rafts and ADCC/ADCP, whereas direct cell death plays a lesser role.[38] Ofatumumab also predominantly acts via complement-dependent cytotoxicity after binding both loop domains of CD20 at a distinct epitope compared to rituximab.[39] Ofatumumab may represent an effective and safe treatment for rituximab-intolerant cases of MN. In a recent case series, 7 patients who were rituximab intolerant and 10 patients who were rituximab resistant received ofatumumab and over a median follow-up of 5.0 months, all 7 patients who were rituximab intolerant and 3 of the 10 patients who were rituximab resistant achieved CR or PR of nephrotic syndrome.[34] These novel anti-CD20 monoclonal antibodies could serve as valuable alternatives for a subset of patients with MN who have exhausted other therapeutic options.

Furthermore, with regard to overall safety, we observed that the infusion-related reactions occurred in 9 patients (15.3%) with systematic premedication. Neutropenia was reported in 10.2% patients. In this phase 2 trial (NOBILITY), in which participants with lupus nephritis received 4 infusions of Obinutuzumab

Table 5. Summary of published studies on the administration of rituximab as an initial therapy for primary membranous nephropathy

References	Year	Initial therapy	Number of patients	CR + PR n (%)	CR (n)	PR (n)	PLA_2R titers (RU/ml)	Median follow-up
Cravedi et al.[29]	2011	Rituximab	11	7 (64)	2	5	NA	12 mo
Moroni et al.[30]	2017	Rituximab	19	6 (32)	4	2	NA	12 mo
RI-CYCLO[28]	2021	Rituximab	37	23 (62)	6	17	0 (0 to −54)	12 mo
Zhang et al.[31]	2023	Rituximab	36	32 (89)	12	20	2 (2 to −2)	12 mo
Chen et al.[32]	2023	Rituximab	19	16 (84)	2	14	0 (0 to −2.6)	10 mo
This cohort	2024	Obinutuzumab	20	18 (90)	7	11	1.3 (0 to −2.5)	9.4 mo

CR, complete remission; mo, months; NA, not available; PLA_2R, phospholipase A_2 receptor; PR, partial remission.

Table 6. Summary of published studies on the administration of obinutuzumab in patients with primary membranous nephropathy

				Baseline					
References	Year	Number of Patients	CR + PR (*n*)	Serum anti-PLA_2R positive (*n*)	Mean age	Mean serum albumin (g/dl)	Mean Proteinuria	Mean Serum creatinine (mg/dl)	Previous treatment
Sethi S et al.[18]	2020	10	9	5	58	2.9	UP CR 7.6 g/g	1.4	CNI, RTX, GC
Klomjit N et al.[19]	2020	3	2	3	56	2.5	24-h UP 16.4 g/d	1.8	CNI, RTX, CYC, GC
Ginthör NE et al.[20]	2021	1	1	0	55	NA	UP CR 6.4 g/g	NA	CNI, RTX, CYC, MMF, GC
Hudson R et al.[21]	2022	2	1	2	35	1.8	24-h UP 11.1 g/d	0.8	CNI, RTX, CYC, GC
Naik S et al.[22]	2023	2	2	2	50	1.8	24-h UP 10.9 g/d	3.6	CNI, RTX, CYC, GC
Conversano E et al.[23]	2024	1	1	0	2	NA	UP CR 18.6 g/g	NA	CNI, RTX, GC
Zhang Y et al.[24]	2024	1	1	1	31	1.7	24-h UP 19.8 g/d	1.1	CNI, RTX, CYC, MMF, GC
This Cohort	2024	59	48	48	51	2.8	24-h UP 4.8 g/d	1.0	See Supplementary Table S1

CNI, calcineurin inhibitors; CR, complete remission; CYC, Cyclophosphamide; GC, glucocorticoid; MMF, mycophenolate mofetil; NA, not available; PLA_2R, phospholipase A_2 receptor; PR, partial remission; RTX, rituximab; UPCR, urine protein-to-creatinine ratio; 24hUP, urinary protein excretion.

at a dosage of 1000 mg/1.73 m^2, only a small proportion (5%) of patients experienced neutropenia, which was similar to the occurrence rate in the placebo group.[25] We postulated that the higher prevalence of neutropenia in this study might be partly attributed to the sulfamethoxazole. The neutrophil counts normalized after sulfamethoxazole withdrawal in 3 patients, similar to a recent study on Obinutuzumab in children with refractory nephrotic syndrome.[40] The safety profile of Obinutuzumab was generally acceptable. This means that it may be a more tolerable option for some patients who cannot tolerate or experience adverse effects from other treatments. Although no serious adverse events were observed during the study period, it is important to acknowledge that potential treatment-related complications may arise in the future owing to the limited duration of follow-up.

Our study possesses several strengths and limitations. The primary strength lies in the inaugural cohort in which Obinutuzumab was used as an initial immunosuppressive agent for MN. Additionally, this study encompasses a relatively large sample size of 59 patients with primary MN who received Obinutuzumab, which is currently 1 of the largest samples available. The main limitations are the short duration of follow-up time, potentially leading to an underestimation of both proteinuria remission and relapse rates, as well as treatment-related adverse events. Despite this limitation, an impressive 84.7% of patients achieved remission of proteinuria within a median duration of 9.4 months, instilling anticipation for these findings. The second limitation is inherent to data collection in retrospective studies, the use of data derived from a single center, and the absence of a parallel control drug. The immunoglobulin monitoring was not conducted during the follow-up period because of its lack of coverage by health insurance. Additionally, some patients were reluctant to monitor B-cell and anti PLA_2R antibodies because of the high cost of the tests, which contribute to the incompleteness of the data. Our ongoing prospective multicenter study aims to address these gaps and provide comprehensive evidence in the future. This study has been registered with the Chinese Clinical Trial Registry (https://www.chictr.org.cn, ChiCTR2400082133). Finally, kidney biopsy was performed in only 66% of the patients. Despite positive anti-PLA_2R results in all patients who did not undergo biopsy, other potential causes for proteinuria could not be definitively excluded.

In conclusion, this retrospective cohort showed that Obinutuzumab may be an effective and safe therapeutic option for patients with primary MN. Patients who received Obinutuzumab as their initial treatment demonstrated superior efficacy to those who received it as a second-line treatment. However, even in these patients with second-line therapy, the probability of achieving remission of proteinuria may exceed 80%. Our results suggested that Obinutuzumab may serve as a valuable therapeutic option for patients with MN, not only as an initial treatment but also for nonresponsive cases to other therapies. Further prospective randomized controlled trials are needed to confirm our findings and fully understand its efficacy and safety profile in different patient populations and settings.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

XS and LW research idea and study design. XS, BW and XT data acquisition. XS, BW and XG data analysis/

interpretation. XS, WB and XG statistical analysis. XQ and LW supervision or mentorship. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated, and resolved, including with documentation in the literature if appropriate.

SUPPLEMENTAL MATERIAL

Supplementary File (PDF)

Figure S1. Remission of proteinuria after Obinutuzumab infusion.

Figure S2. Trends in proteinuria and kidney function over time after Obinutuzumab administration.

Figure S3. Changes in anti-PLA_2R antibody levels and circulating $CD19^+$ B-cells after Obinutuzumab administration.

Figure S4. Immunologic remission after Obinutuzumab infusion.

Table S1. Prior immunosuppressive therapy in patients with second-line therapy.

Table S2. Baseline patient characteristics grouped by RTX-naive or RTX-resistant.

Table S3. Clinical details of 14 patients with RTX-resistant.

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