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Original Article

Combination of ultra-low dose rituximab and low dose tacrolimus versus tacrolimus alone in the treatment of non-responsive idiopathic membranous nephropathy: a Chinese retrospective cohort study

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Abstract: Introduction: Some patients with idiopathic membranous nephropathy (iMN) do not respond to cyclophosphamide plus steroids treatment, and we define them as non-responsive iMN. The combined regimen of rituximab (RTX) and tacrolimus (TAC) has an excellent effect on this kind of non-responsive iMN patients; however, the optimal dose is still unclear. In this retrospective study, we comapred the efficacy and safety of ultra-low dose RTX plus low-dose TAC therapy versus standard TAC monotherapy in patients with non-responsive iMN. Materials and methods: Sixty-seven Chinese non-responsive iMN patients were included. There were 41 patients received standard tacrolimus monotherapy (TAC) and 26 patients received ultra-low dose rituximab plus low dose tacrolimus (RTX/TAC) combination therapy. All patients were observed for 12 months. Results: 18 patients (18/26, 69.2%) in the RTX/TAC group and 17 patients (17/41, 41.5%) in the TAC group achieved clinical response after 12-month follow-up (P=0.044). The median time for achieving response in the two groups was 3.0 months. As indicated by Kaplan-Meier curve, the response rate in the RTX/TAC group was higher than that in the TAC group (P=0.015). 24-hour proteinuria, serum albumin, estimated glomerular filtration rate (eGFR) and serum creatinine in the two groups were comparable at baseline; howerver, after 12-month follow up, they were significantly improved in the RTX/TAC group compared with the TAC group (P<0.05). B-cell depletion was achieved in all patients in the RTX/TAC group during the whole follow-up period. Pneumonia, urinary tract infections and glucose intolerance were the major side effects observed in this study. All adverse events were mild, and the cumulative incidence was lower in the RTX/TAC group compared with that in the TAC group (9 (34.6%) vs 27 (65.9%), P=0.023). Conclusion: The combination of ultra-low dose rituximab and low dose tacrolimus is more effective in inducing proteinuria response, improving eGFR and serum albumin in non-responsive iMN patients than standard tacrolimus monotherapy. The combined treatment also has higher safty.

Keywords: Rituximab, tacrolimus, non-responsive, idiopathic membranous nephropathy, low-dose

Introduction

Membranous nephropathy (MN) is the most common cause of the nephrotic syndrome in nondiabetic adults. Due to air pollution and increasing cases of renal biopsy, the prevalence of idiopathic membranous nephropathy (iMN) has dramatically increased in China in recent years [1]. For patients who are at high risk of progression (namely the patients who have the proteinuria above 4000 mg/d and fail to respond to a 6-month treatment with antihypertensive and antiproteinuric agents), combination therapy of a cyclophosphamide and glucocorticoids is the preferred initial therapy [2, 3]. Studies over ten years in iMN patients treated with corticosteroids combined with cyclophosphamide have reported that about 10% patients would be non-responsive to the classic regimens and were very likely to progress to ESRD [4, 5]. For these non-responsive patients, it is urgent to figure out the iMN pathogenesis and look for more effective therapies.

iMN is an antibody-mediated disease induced by deposits of immunoglobulins and complement components on the subepithelial layer of the glomerular capillary wall [6]. Antibodies

from lymphocytes (mainly B lymphocytes) in situ bind to antigen in target cells, which leads to abnormal immunoreaction, resulting in glomerular filtration barrier damage. Researchers measured specific antigens expressed in glomerular podocytes in iMN patients including the M-type phospholipase A2 receptor (PLA2R), thrombospondin type-1 domain-containing 7A (THSD7A), neural epidermal growth factor-like 1 (NELL-1), providing a better understanding of the pathogenesis of iMN and possible means of intervention.

The calcineurin inhibitor tacrolimus (TAC) reduces proliferation of intra-renal T lymphocytes and stabilizes the podocyte cytoskeleton, thus alleviates immunoreaction of iMN [7]. However, the high tendency to relapse after TAC withdrawal or tapering [8, 9] and the concern about nephrotoxicity after long-term use limited its application. As B cell plays an important role in the pathogenesis of membranous nephropathy, anti-B lymphocytes therapy using CD20 monoclonal antibody has recently been used as a more selective and promising agent for the treatment of both naïve or refractory iMN [10, 11]. However, the study from Ming-Hui Zhao's group in 2017 showed that only 41.7% of patients with non-responsive iMN acheived response in a median of 12 months of RTX treatment [8].

Recent reports revealed an increased response rate when different doses of rituximab were added to calcineurin inhibitors in refractory iMN, lupus nephritis and other autoimmune diseases. The combination therapy presented better toleration, less relapse and almost no additional side effect due to the quick calcineurin inhibitor tapering [12, 13]. Thus, we launched a research of comparison between TAC therapy and combination therapy of ultra-low dose RTX and low dose TAC to observe the response and relapse rate in patients with non-responsive iMN.

Materials and methods

Patients

For this retrospective and single-center study, we collected data of adult non-responsive iMN patients at Tongji Hospital of Tongji Medical College of HuaZhong University of Science and Technology in Wuhan from November 2015 to January 2021, and divided them into two groups: the RTX/TAC group (patients received low dose tacrolimus in combination with ultra-low dose rituximab) and the TAC group (standard tacrolimus therapy). Inclusion criteria: (i) biopsy-proved MN, (ii) proteinuria above 4000 mg/d and non-responsive to previous therapy, defined as not meeting response level after at least 6-month treatmen, and (iii) estimated glomerular filtration rate (eGFR) >30 mL/min/1.73 m^2. Exclusive criteria: (i) membranous nephropathy secondary to other causes: positivity for Hepatitis B, C or HIV; malignancy; autoimmune diseases; untreated infection, (ii) with hepatitis B or diabetes, (iii) pregnant or breast feeding females and those planning a pregnancy or using unreliable contraception, (iiii) malignancy or had a life expectancy of less than 1 year. All patients provided written informed consent. The research was approved by the Ethics Committee of Tongji Hospital of Tongji Medical Colleage of HuaZhong University of Science and Technology (No. TJ-IRB20210632). Informed consent was obtained for sampling tissue and blood.

Treatment and follow-up

Rituximab: The ultra-low dose RTX was defined as infusion dosage of 100 mg. The RTX was reconstituted in saline to a final concentration of about 0.4 mg/mL (general infusion volume was 250 ml) and infused at a controlled speed, the whole time-span for fulfilling the infusion was above 2 hours according to each patient's tolerability. The circulative B cells were monitored in the next morning after administration, once a month for the first 3 months and then once every three months. B-cell depletion was defined as <5 B cells/ul and <1% of total lymphocytes in the circulation. If circulating B cells didn't achieve the standard, addintional ultra-low dose RTX was applied in that month to maintain circulating B cell depletion until 12 months. The dosing interval was adjusted at the discretion of the attending physician.

Tacrolimus

TAC group: Tacrolimus monotherapy was given at an initial dose of 0.05 mg/kg/day p.o. divided into 2 equal doses given at 12 h intervals and adjusted to a serum concentration level at 4 to 10 ng/mL for 6 months. Then the dose was tapered gradually to 0.5 mg per day at the end of 12 months. TAC dosage was reduced by 30% when 30% increase in serum creatinine was detected as compared with the baseline

value, and TAC was withdrawn if the renal function was not improved after 2 weeks. Two patients in the TAC group once reached complete response by TAC monotherapy but relapsed within half a year.

RTX/TAC group: Low dose tacrolimus was given at 0.025 mg/kg/d initially, once the patients have achieved partial or complete response, the dose was tapered to 0.5 mg per day. When proteinuria level was maintained or the relapse occured, the tacrolimus was kept at the initial dose or even increased. Therefore, the duration of the taper varied individually.

Baseline data was established with a serial of laboratory measurerments before rituximab administration. The patients visited doctors every month for the first three months and then once every three months. The follow-up lasted for at least 12 months. Follow-up was terminated when the patients switched to other immunosuppressant or started regular renal replacement therapy. The final follow-up for this study ended in January, 2021.

Outcomes

The primary clinical outcome was complete or partial response in 12 months after initial therapy. Secondary clinical outcomes included time to treatment response up to 12 months, time for anti-PLA2R antibody to turn negative and adverse events. The data of proteinuria, creatinine clearance and serum albumin were systematically assessed. Complete response was defined as urinary protein <0.3 g/day and serum albumin >35 g/L. Partial response was defined as proteinuria <3.5 g/day and >50% reduction from baseline. The remaining ones were defined as no response. Relapse was charactered as recurrence of proteinuria >3.5 g/day after a period of partial or complete response. We consider end-stage renal disease (ESRD) as the end points.

Statistical analysis

Statistical analysis was performed using statistical software SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Parametric data were expressed as means ± standard deviation (SD). Nonparametric data were expressed as median [interquartile range (IQR)]. Response rates were expressed as frequencies or percentages and their 95% CIs. T-test was used for analysis of normally distributed data in different groups. Kruskal-Wallis test or the Mann-Whitney U-test was used for analysis of semi-quantitative data. 'Chi-squared' test, Fisher's exact test or one-way analysis of variation (ANOVA) was used for analysis of qualitative data. All probabilities were two-tailed and the level of significance was set at 0.05.

Results

Patients

Since November 2015, in Tongji Hospital of Tongji Medical Colleage of HuaZhong University of Science and Technology, there were 621 patients with biopsy-proved idiopathic membranous nephropathy. Among them, 156 had proteinuria <4000 mg/24 h, 223 showed a complete or partial response after the initial immunosuppressive treatment, 66 lost to follow-up (paritially due to COVID-19 pandemic in 2020), and 75 did not use immunosuppressors for sufficient time. There were 101 eligible patients, 33 of them switched to other immunosuppressors such as MMF, Leflunomide, etc. The remaining 68 were assigned to the RTX/TAC combination therapy group (26 patients) and the TAC monotherapy group (41 patients). One patient in RTX/TAC group withdrew the consent before administration (shown in **Figure 1**).

Basic information

Mean age of the participants was 41.7 (RTX/TAC) vs 44.3 (TAC) years in the two groups. There were 7 and 10 females in the two groups, respectively (RTX/TAC vs TAC). They had experienced a median of 22.43 (IQR 12.4-24.9 months) vs 19.95 (IQR 12.7-21.9 months) months of iMN history since their percutaneous renal biopsy operation. The positive rates of anti-PLA2R antibody on pathological specimens were 88.9% vs 85.2% in RTX/TAC group and TAC group.

All the patients had been administered at least one course (mean 2.23 vs 1.85 courses, range 1-4) of immunosuppressant therapy, including glucocorticoids + cyclophosphamide, cyclosporine and/or TAC + small dose glucocorticoids, mycophenolate mofetil and leflunomide. Among all the participants, 14 of 26 in the RTX/TAC group and 26 of 41 patients in the TAC group achieved complete or partial response during previous therapies but relapsed within one year. Other baseline characteristics in the

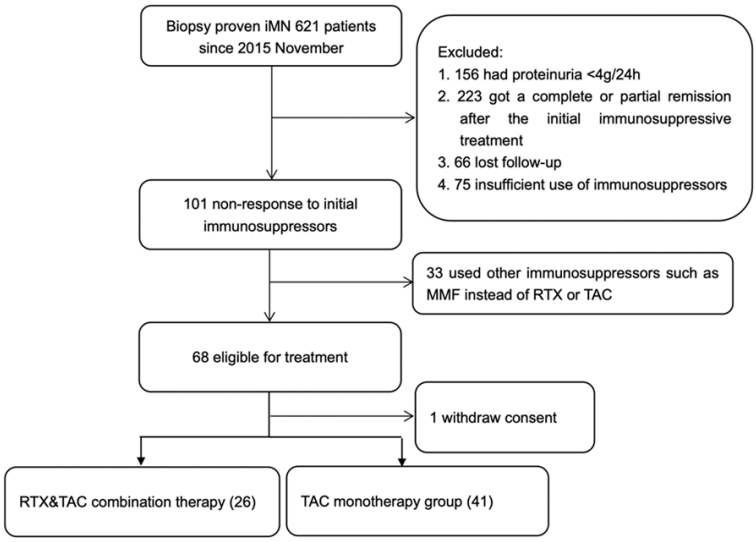

Figure 1. Flow chart of the trial. The flowchart shows the process of patient selection in this retrospective cohort study. The cohort included 621 patients who were biopsy-proved iMN in Tongji Hospital from November 2015 to March 2020. After screening under the conditions shown in this figure, 67 eligible patients (26 in the RTX&TAC group and 41 in the TAC group) were included in the final analysis.

two groups were comparable with no significant differences (**Table 1**).

Rituximab regimen

All of the 26 patients in RTX/TAC group used RTX under a B-cell driven protocol. They were followed up every 1-3 month, an additional dose was decided based on the number of circulating B cells measured at each follow-up. At the 12th month of follow-up, the cumulative dose per person was 219.2±112.9 mg (**Table 2**), with an average of 3 injections per patient in 12th month. All 26 patients in the RTX/TAC group achieved B-cell depletion during follow-up (**Figure 2**).

Tacrolimus regimen

In this study, patients of the two groups were both treated with TAC, but the dose of TAC used in the RTX/TAC group was less compared with that in the TAC group. The initial dose of the two groups was 1.33±0.41 mg/d and 2.62±0.72 mg/d respectively, P<0.01 (**Table 1**). The TAC concentration of all patients in the TAC group reached the target range.

Kidney outcomes

The level of urinary protein in the RTX/TAC group decreased from 5875.31±1548.62 mg/24 h to 2158.1±2412.9 mg/24 h (*P*<0.001) and from 5580.43±1510.02 to 3732.1±3190.3 mg/24 h (*P*=0.015) in the TAC group (**Table 2**). The 24 h-proteinuria in both groups decreased significantly and the improvement of proteinuria in the RTX/TAC group was better than that in the TAC group at the end of follow-up (**Figure 3**). The serum albumin, eGFR and serum creatinine of the two groups were similar to 24 h-proteinuria, and they were improved significantly from the baseline (P<0.05), but the improvement was better in the RTX/TAC group at the last one or two follow-ups (P<0.05) (**Figure 3**).

To obtain similar observation periods in the two groups, we truncated the follow-up at 12 months. 18 of 26 (69.2%) in the RTX/TAC group vs 17 of 41 patients (41.5%) in the TAC group achieved partial or complete response at 12th month, *P*=0.044 (**Table 3**). There were significant differences in the current and cumulative response rates after 6th month of follow-up between the two groups, the data are shown in **Table 3**. We presented the cumulative response of the two groups using Kaplan-Meier curve, and the RTX/TAC group showed a better response (P=0.015) (**Figure 4**).

The average time for anti-PLA2R antibody to turn negative in the RTX/TAC group was 3.13 (1.97-7.25) months, which was shorter than 4.89 (2.65-7.89) months in the TAC group (*P*=0.021). 2 patients maintained positive of anti-PLA2R antibody in the RTX/TAC group while there were 7 patients in the TAC group (*P*=0.465) at the end of follow-up.

Relapse

In this study, 1 patient in the RTXTAC group and 3 patients in the TAC group relapsed after partial response. No relapse happened after complete response in both groups.

Adverse effects

Rituximab was well tolerated in most patients. No severe adverse events were observed during infusions in all the patients. Only 2 patients presented chills and rash during the infusion

Table 1. Baseline characteristics of patients with non-responsive iMN in RTX/TAC and TAC groups

Patients	RTX/TAC	TAC	*P* value
Female/Total (n)	7/26	10/41	0.744
Age (years) (mean ± SD)	41.73±14.89	44.34±10.57	0.404
History (months) [median (IQR)]	22.43 (12.4-24.9)	19.95 (12.7-21.9)	0.128
Diabetes mellitus/Total (n)	3/26	6/41	0.999
Prior immunosuppressive therapy (n) (%)			
1	7 (26.9%)	20 (48.8%)	0.770
2	9 (34.6%)	12 (29.3%)	0.788
≥3	10 (38.5%)	9 (22.0%)	0.172
Previous therapies			
Prednisone + cyclophosphamide, n	26	41	
Prednisone + cyclosporine, n	2	3	
Prednisone + tacrolimus, n	6	2	
Mycophenolate mofetil, n	7	13	
Prednisone + leflunomide, n	8	6	
Immunoglobulin G infusion, n	9	11	
Systolic blood pressure (mean ± SD)	132.94±14.23	128.70±14.41	0.349
Diastolic blood pressure (mean ± SD)	86.77±9.35	82.98±9.83	0.122
Proteinuria (g/24 h) (mean ± SD)	5875.31±1548.62	5580.43±1510.02	0.441
Circulation B cells (mean ± SD)	219.2±112.91	208.7±49.39	0.607
Albumin (g/L) (mean ± SD)	25.07±5.56	25.85±5.92	0.592
eGFR (mL/min/1.73 m^2) (mean ± SD)	94.23±24.67	88.48±19.75	0.324
Creatinine (umol/L) (mean ± SD)	84.50±21.62	84.39±22.74	0.984
Cholesterol (mmol/l) (mean ± SD)	6.13±1.67	7.05±2.06	0.424
PLA2R positive/Total (pathology) (%)	24/26 (92.3)	36/41 (87.8)	0.697
PLA2R positive/Total (plasma) (%)	18/26 (69.2)	31/41 (75.6)	0.584
Tacrolimus initial dose (mg)	1.41±0.36	2.48±0.47	0.01
Diuretics, n (%)	7/26 (26.9)	12/41 (29.3)	0.997
Previous remission, n (%)	14/26 (53.8)	26/41 (63.4)	0.456

eGFR: Estimated glomerular filtration rate; *P* value <0.05 statistically significant; RTX: Rituximab; TAC: Tacrolimus.

Table 2. Comparison of laboratory indices in the two groups after 12 months follow-up

Patients	RTX + TAC	TAC	*P* value
Rituximab dose (mg) (mean ± SD)	219.2±112.9	0.00	0.00
Proteinuria (g/24 h) (mean ± SD)	2158.1±2412.9	3732.1±3190.3	0.037
Albumin (g/L) (mean ± SD)	36.45±8.04	32.08±8.65	0.046
Creatinine (umol/L) (mean ± SD)	77.81±19.01	92.49±23.01	0.011
eGFR (mL/min/1.73 m^2) (mean ± SD)	98.25±25.06	81.74±21.50	0.006
PLA2R positive rates (positive/total) (%)	2/26 (7.69)	7/41 (17.1)	0.465
Median time for achieving partial response (month) (IQR)	3 (1.0-6.0)	3 (1.5-8.25)	0.272
Time for antibody to turn negative (month) (IQR)	3.13 (1.97-7.25)	4.89 (2.65-7.89)	0.021
24 h-proteinuria reduction of non-responsive iMN	-1585±2842	588.7±2316.3	0.033

eGFR: Estimated glomerular filtration rate; *P* value <0.05 indicates statistically significant difference; RTX: Rituximab; TAC: Tacrolimus.

which diminished quickly after administration of anti-allergic drugs, and the discomforts did not reappear in the same patients in the next injection (**Table 4**). Long-term use of TAC may

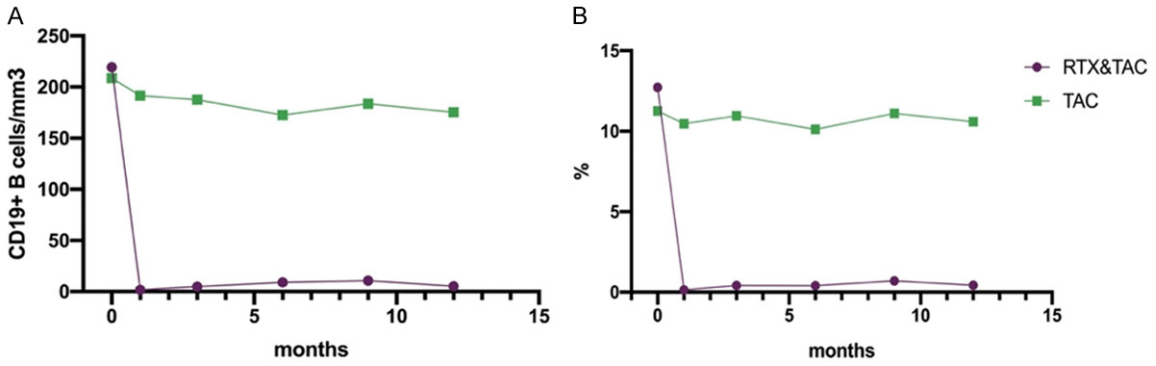

Figure 2. Changes in peripheral B lymphocytes (A) and proportion of peripheral B lymphocytes (B) after administration of RTX/TAC or TAC in the two groups.

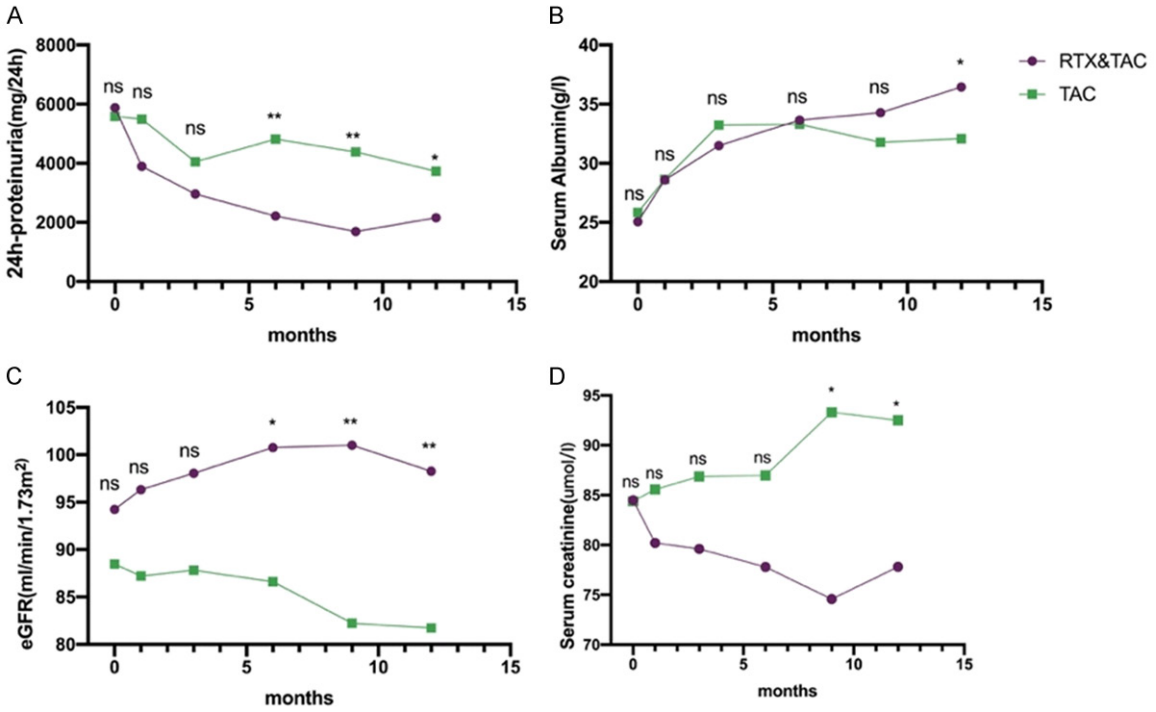

Figure 3. Changes in 24 h-proteinuria (A), serum albumin (B), eGFR (C) and serum creatinine (D) in the two groups at baseline, during the periods of therapy and follow-up. Compare the two groups at different time points (ns, no significant difference, *P<0.05, **P<0.01, P<0.05 indicates significant difference).

cause pneumonia, urinary tract infections and glucose intolerance (**Table 4**). The cumulative incidence of the adverse events were lower in the RTX/TAC group compared with that in the TAC group (9 (34.6) vs 27 (65.9), *P*=0.023), as shown in **Table 4**.

Discussion

In this retrospective study, we exhibited the therapeutic and side effects in the RTX/TAC group and the TAC group. Results showed that the combination therapy of ultra-low dose RTX and low dose TAC had better response rate than monotherapy of standard TAC. The response rate in the RTX/TAC group was consistent with the data (60-80%) reported in a previous paper adopting different doses of RTX [14]. Otherwise, the patients in the RTX/TAC group presented fewer adverse effects. Data above demonstrated that the RTX/TAC combination therapy seemed to be a better choice for non-responsive iMN patients.

A cytotoxic agent (commonly cyclophosphamide) combined with glucocorticoids is the pre-

Table 3. Remission in patients of the two groups

Months	Current remission rate			Cumulative remission rate		
	RTX/TAC	TAC	*p* value	RTX/TAC	TAC	*p* value
1-month (RR/T) (%)	7/26 (26.9)	5/41 (12.2)	0.191	7/26 (26.9)	5/41 (12.2)	0.191
1-month (CR/T) (%)	0	0	1	0	0	1
3-month (RR/T) (%)	13/26 (50.0)	13/41 (31.7)	0.198	13/26 (50.0)	13/41 (31.7)	0.198
3-month (CR/T) (%)	1/26 (3.85)	0	0.388	2/26 (7.69)	0	0.147
6-month (RR/T) (%)	19/26 (73.1)	14/41 (34.2)	0.003	19/26 (73.1)	15/41 (36.6)	0.006
6-month (CR/T) (%)	6/26 (23.1)	2/41 (4.9)	0.048	6/26 (23.1)	2/41 (2.44)	0.048
9-month (RR/T) (%)	18/26 (69.2)	17/41 (41.5)	0.044	20/26 (76.9)	18/41 (43.9)	0.011
9-month (CR/T) (%)	7/26 (26.9)	4/41 (9.76)	0.092	8/26 (30.8)	4/41 (9.76)	0.048
12-month (RR/T) (%)	18/26 (69.2)	17/41 (41.5)	0.044	20/26 (76.9)	20/41 (48.8)	0.040
12-month (CR/T) (%)	9/26 (34.6)	3/41 (7.32)	0.028	9/26 (34.6)	4/41 (9.76)	0.024

CR: Complete remission; PR: Partial remission; *P* value <0.05 indicates statistically significant differnce; RR=CR + PR, T: Total number of patients in each group.

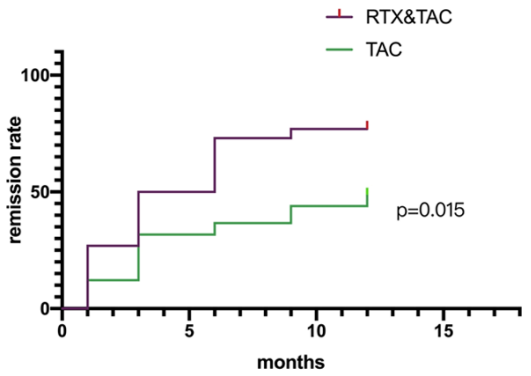

Figure 4. Cumulative response of the two groups. Shown are Kaplan-Meier estimates of the time to response of different treatment groups. Using the Log-rank (Mantel-Cox) test, there was a significant difference in response rate between the two groups (P=0.015).

ferred initial therapy for patients who are at high risk of progression. Cyclophosphamide is an alkylating agent that prevents cell mitosis by cross-linking DNA strands and decreasing DNA synthesis [15]. Nonspecific and selective inhibitor for cell cycle phase mainly plays inhibitory and subtractive effects on B lymphocytes, with many side effects, especially infection and reproductive toxicity [16, 17]. When Cyclophosphamide treatment fails to achieve response, it is generally recommended to switch to calcineurin inhibitors (TAC or cyclosporine, now we prefer TAC). TAC selectively inhibits calcineurin and impairs the transcription of interleukin-2 (IL-2) and several other cytokines in T lymphocytes, resutling in reducing T cell proliferation and improving iMN [7]. Calcineurin inhibitors can generally induce a quicker relief of clinical symptoms, especially in nephrotic syndrome patients and are widely used in clinical therapy in recent years. However, its related nephrotoxicity and relapse after tapering are important issues of concern, which have limited its clinical applications in long-term stability of the patient's remission [11].

Selective CD20 monoclonal antibody-RTX was first invented in 1997 and used for non-Hodgkin lymphoma, chronic lymphocytic leukemia, and it is now recommended for iMN as well. RTX seems to be safer and shows a better patient compliance. However, the optimal dose for rituximab is still uncertain. There are two mainstreams of RTX in the treatment of iMN, one was given in the MENTOR trial [11], namely 1 g initially and another 1 g 14 days later. The other regimen was 375 mg/m^2 every week and lasted for four weeks [18, 19]. Some studies suggested that even low-dose treatment with RTX can lead to expectant treatment response in patients with iMN [19]. In the RTX-treated autoimmune diseases, it is reported that ultra-low dose RTX maintenance could also deplete circulating B lymphocytes and exert therapeutic effects [20]. Although it is easy to achieve the depletion of circulating B lymphocytes with low-dose rituximab, the therapeutic effect was not satisfying [14]. Considering the different action mechanisms of the two drugs and the side effects brought by high-dose drugs, we tried RTX combined with TAC to treat non-responsive iMN.

Table 4. Adverse events

Adverse events, n	RTX/TAC	TAC	*P* value
Allergic reaction, n (%)	4 (15.4)	3 (7.3)	0.417
Hypertension, n (%)	0	5 (12.2)	0.148
Infection, n (%)	3 (11.5)	11 (26.8)	0.217
Urinary tract infection, n (%)	2 (7.7)	5 (12.2)	0.697
Pneumonia, n (%)	1 (3.8)	6 (14.6)	0.234
IGT/Diabetes, n (%)	2 (7.7)	8 (19.5)	0.295
Total, n (%)	9 (34.6)	27 (65.8)	0.023

IGT: IGT: Impaired glucose tolerance.

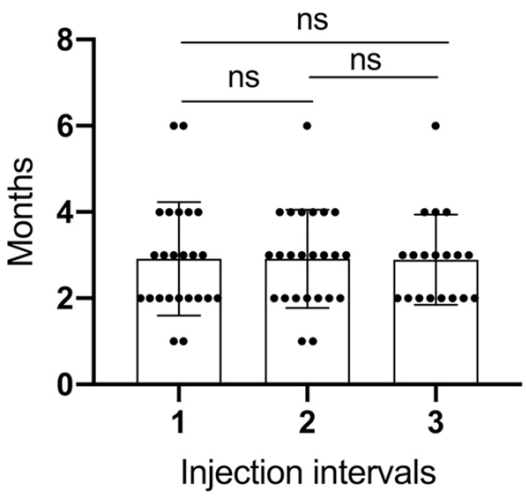

Figure 5. The first three dosing intervals for patients receiving rituximab. There is no significant statistical difference between the three dosing intervals.

This study showed that the RTX/TAC treatment was effective in 76.9% of non-responsive iMN patients during the 12-month follow-up period, which was better than the 41.7% of Chinese patients with non-responsive iMN achieving response in a median of 12 months of RTX monotherapy [10]. Compared with other regimes, the combination of ultra-low dose RTX and TAC has consistent response rate which varied from 60% to 76% in first- or second-line therapy [11, 14, 21]. This may have contributed to the dual inhibitory effects on both B and T lymphocytes with the combination therapy.

RTX/TAC therapy in this cohort maintained the number of circulative B lymphocytes below 5 cells/ul for 12 months, which is a full time low level of B lymphocytes state in peripheral circulation. As for the other method of administration, the number of B lymphocytes generally increased from about 6 months [22]. CD20 is expressed on pre-B to fully differentiated B lymphocytes, however, ultimately differentiated plasma cells do not express CD20 marker. The histologic lesion of iMN under light microscope is diffuse thickening of the glomerular basement membrane (GBM) throughout all glomeruli with a diffuse granular pattern of immunoglobulin G (IgG) and C3 deposits along the GBM. These deposits are mainly produced by plasma cells. However, after the process of affinity maturation in germinal centers, plasma cells have an indeterminate lifespan, ranging from days to months [23]. Thus, prolonging the low level of circulative B lymphocytes may bring more benefits to patients in response period. Although there were 8 patients in RTX/TAC group who did not achieve response (including those who relapsed after response), the 24 h-proteinuria of these non-responsive patients were still significantly reduced (**Table 2**). Thus, ultra-low dose rituximab plus low dose tacrolimus showed better effect on non-responsive iMN patients.

RTX eliminated B lymphocytes, which may weaken the immunity. However, as RTX was at ultra-low doses, there was no significant difference between the two groups in the concern of infection. In the first 12 months of the study, 83 patients received intravenous RTX, with an average injection interval of 3.8 months. Multiple times of RTX injection may lead to the production of circulative rituximab antibodies and affect its efficacy. In this study, we counted the intervals between the first three doses. The mean interval was 3.17, 2.92, and 3.05 months (**Figure 5**), and there was no statistical difference among each other. For at least 12 months, we did not observe decreased effect of RTX on circulating B lymphocytes due to antibody production.

In this study, relapse was observed in one patient in the RTX/TAC group while 3 in the TAC group after achieving partial response. Some studies pointed out that RTX could maintain iMN remission up to 24 months [11]. TAC usually induces more rapid remission [11], and the combination therapy may have advantages of both.

Limitations: This study also had two limitations. One is the small sample size. The other is that most of the patients used qualitative measure-

ment to monitor serum level of anti-PLA2R antibody, which did not reflect the trend of antibody titers. The patient's anti-PLA2R antibody titers will be measured in later follow up.

In conclusion, ultra-low dose rituximab plus low dose tacrolimus therapy is more effective and safer than standard tacrolimus therapy, implying that it might be a potential option for patients with non-responsive iMN.

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Disclosure of conflict of interest

None.

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