

Clinical Observation of Obinutuzumab Combined with Abemoschus manihot Tablets in the Treatment of Idiopathic Refractory Membranous Nephropathy

Xiangling Zhao

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Xuejie Chang

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Yuejuan Wang

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Duqun Chen

National Clinical Research Center for Kidney Diseases

Manshu Yu

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Jing Zhao

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Hong Zhu

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Yan Mao

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Chenxia Juan

juancx@njucm.edu.cn

Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Traditional Chinese Medicine

Keywords: Idiopathic refractory membranous nephropathy, Obinutuzumab, Abelmoschus manihot Tablets

Posted Date: September 23rd, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7499872/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Context:

Idiopathic refractory membranous nephropathy (IRMN) often progresses to ESRD with limited therapeutic options. Consequently, the development of novel and effective therapies for IRMN represents an urgent unmet need in the field of nephrology.

Objective

This study evaluated the efficacy and safety of combining Obinutuzumab and *Abelmoschus manihot* tablets for IRMN, hypothesizing synergistic actions targeting CD20 + B cells apoptosis and inflammation.

Methods

Thirty-one IRMN patients received combined therapy and were followed for 12 months. Primary endpoint was remission rate (complete/partial). Secondary outcomes included biochemical and immunological parameters and adverse events.

Results

At 12 months, the overall remission rate was 80% (complete: 16%; partial: 64%). Median time to remission was 4.5 months (IQR 2.0–7.0). Immunological remission rates were 70% (3m), 83% (6m), 90% (9m), and 93% (12m). Anti-PLA2R antibodies declined rapidly within 3 months in 80% of patients ($p < 0.001$); 64% achieved $\geq 50\%$ reduction by month 3. Antibodies became undetectable in all patients by final follow-up. Adverse events were infusion-related reactions (fever, hypotension, tachycardia, flushing). No deaths occurred.

Conclusion

The Obinutuzumab and *Abelmoschus manihot* tablets combination induced high remission rates in IRMN, with rapid immunological response and a tolerable safety profile, representing a novel therapeutic approach.

Introduction

Membranous nephropathy (MN) is a common glomerular disease, with a reported global annual incidence of 1.2 per 100,000 population[1]. It exhibits a male predominance. Approximately 60% of patients diagnosed with MN present clinically with proteinuria, hypoalbuminemia, hyperlipidemia, and

edema, often accompanied by hypertension and hematuria[2]. The onset predominantly occurs in middle-aged and elderly individuals. Another subset of patients manifests solely as asymptomatic proteinuria[2]. Idiopathic membranous nephropathy (IMN) constitutes the majority of adult MN cases[3]. Recent evidence increasingly supports a critical role for autoimmunity in IMN pathogenesis[4]. Specific biomarkers, namely anti-phospholipase A2 receptor (anti-PLA2R) antibodies, are detectable via renal biopsy in 70–80% of MN patients, and increased glomerular PLA2R staining on biopsy correlates directly with the presence of circulating PLA2R autoantibodies[5]. These anti-PLA2R antibodies are podocyte-targeting autoantibodies produced by circulating B cells. Research has demonstrated that anti-PLA2R antibodies exhibit high sensitivity for the differential diagnosis of IMN[6, 7]. Moreover, anti-PLA2R antibody titers correlate with disease activity, severity, and prognosis[8, 9]. Notably, approximately one-third of patients with anti-PLA2R antibody-associated IMN ultimately progress to chronic kidney disease (CKD)[8, 10].

Currently, the 2021 Kidney Disease: Improving Global Outcomes (KDIGO) Clinical Practice Guideline recommends rituximab (an anti-CD20 monoclonal antibody) as first-line therapy for idiopathic membranous nephropathy (IMN)[11]. This recommendation is supported by preclinical studies indicating that autoimmune injury mediated by CD20 + B cells constitutes a core pathological mechanism in IMN[12]. Anti-CD20 therapy targets and depletes precursor B cells responsible for generating anti-PLA2R antibodies, thereby preventing further autoantibody production. This approach is particularly beneficial for patients with high anti-PLA2R antibody titers[13]. Clinical studies report remission rates of approximately 65% with rituximab treatment in IMN patients[14]. However, approximately 30% of patients fail to achieve significant remission after six months of therapy. These patients are classified as having idiopathic refractory membranous nephropathy (IRMN). The clinical utility of rituximab in IRMN is limited by several factors, including delayed response, development of resistance, premature B-cells reconstitution, and renal excretion, thereby substantially reducing its therapeutic efficacy[15, 16]. Patients with IRMN[17] exhibit a particularly poor prognosis and represent a subset at high risk of progression to end-stage renal disease (ESRD). Consequently, the development of novel and effective therapies for IRMN represents an urgent unmet need in the field of nephrology.

Obinutuzumab is a next-generation anti-CD20 monoclonal antibody developed following rituximab. Compared to rituximab, which also targets CD20, Obinutuzumab features a fully humanized structure[18]. This structural difference confers enhanced B-cells depletion efficacy. Pharmacokinetic studies demonstrate that Obinutuzumab achieves significantly higher half-maximal occupancy of CD20 compared to rituximab, indicating superior binding characteristics[19]. Consequently, Obinutuzumab holds significant clinical importance as a salvage therapy for patients with membranous nephropathy (MN) who exhibit inadequate responses to prior rituximab or other conventional immunosuppressive treatments. Furthermore, *Abelmoschus manihot* tablets, a traditional Chinese patent medicine, demonstrate considerable efficacy in treating MN. The primary active component is derived from the flowers of *Abelmoschus manihot*. Research indicates that *Abelmoschus manihot* mitigates proteinuria and delays renal fibrosis through multi-target mechanisms, including anti-inflammatory effects, immunomodulation, and podocyte protection[20, 21]. Currently, evidence regarding Obinutuzumab

monotherapy for idiopathic refractory membranous nephropathy (IRMN) remains limited and inconsistent, failing to provide a definitive assessment of its true efficacy in this population. Moreover, no clinical studies have been reported on the integrated use of Obinutuzumab with traditional Chinese medicine, specifically *Abelmoschus manihot* tablets, for IRMN. Therefore, we conducted this clinical study to evaluate the efficacy of Obinutuzumab combined with *Abelmoschus manihot* tablets in the treatment of IRMN and to assess the safety profile of Obinutuzumab in this clinical setting.

Materials and Methods

1. Case collection

This study enrolled 31 patients with idiopathic refractory membranous nephropathy (IRMN) who were hospitalized in the Department of Nephrology at Jiangsu Province Hospital of Traditional Chinese Medicine between April 2023 and March 2025 and received treatment with Obinutuzumab combined with *Abelmoschus manihot* tablets. Relevant patient data were collected, and medical records were systematically archived. The inclusion criteria were: (I) age ≥ 18 years; (II) diagnosis of idiopathic refractory membranous nephropathy (IRMN) according to KDIGO guidelines; (III) failure to achieve adequate response after ≥ 6 months of prior therapy including glucocorticoids, calcineurin inhibitors (CNIs), cyclosporine A (CsA), cyclophosphamide (CTX), or rituximab (RTX), with persistent 24-hour urinary protein excretion ≥ 3.5 g/24h or $\geq 50\%$ of baseline levels; (IV) undergoing initial Obinutuzumab treatment; and (V) availability of complete clinical data with a follow-up duration ≥ 12 months. Exclusion criteria comprised: (I) secondary membranous nephropathy; (II) non-adherence to the study protocol; and (III) irregular follow-up or loss to follow-up. The study protocol received approval from the institutional ethics review board (Ethics Approval No. : (2025NL-092-02)), and written informed consent was obtained from all participants after detailed explanation of the study procedures.

2. Definitions

Clinical remission, encompassing complete remission (CR) and partial remission (PR), was defined according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [11]. CR was defined as 24-hour urinary protein excretion < 0.3 g/24h, concomitant with a serum albumin concentration ≥ 35 g/L and stable renal function. PR was defined as 24-hour urinary protein excretion < 3.5 g/24h coupled with a $> 50\%$ reduction in proteinuria from baseline, a serum albumin concentration ≥ 30 g/L, and stable renal function. Relapse was defined as a recurrence of 24-hour urinary protein excretion > 3.5 g/24h after achieving remission. B-cells depletion was defined as a CD20 + B cell counts < 5 cells/ μ L, complete B-cells depletion as < 1 cell/ μ L, and B-cells reconstitution as a CD20 + B cell counts ≥ 5 cells/ μ L[22]. Immunological remission was defined as an anti-PLA2R antibody (aPLA2Rab) level < 14 RU/mL, with immunological complete remission similarly defined as aPLA2Rab < 14 RU/mL. Risk stratification was based on KDIGO guidelines and expert consensus; high-risk status was defined as: urinary protein excretion > 8 g/24h persisting for over 6 months; or urinary protein excretion > 5 g/24h persisting after $>$

3 months of supportive care; or urinary protein excretion > 3.5 g/24h persisting after > 6 months of supportive care with a $< 50\%$ reduction from baseline, plus at least one of the following: (1) serum albumin < 25 g/L or (2) aPLA2Rab ≥ 150 RU/mL[23]. Refractory membranous nephropathy was defined as persistent proteinuria ≥ 3.5 g/24h with or without progressive renal function decline during treatment, despite receiving at least 6 months of immunosuppressive therapy or combination therapy [17].

The primary endpoint was the overall remission rate, comprising both partial remission (PR) and complete remission (CR). Secondary endpoints included the following efficacy parameters: urinary total protein (UTP), serum albumin (ALB), serum creatinine (Scr), anti-PLA2R antibody levels, and CD20 + B cell counts; safety was assessed by monitoring the occurrence of adverse events.

3. Statistical analyses

Statistical analyses were performed using SPSS version 26.0 and GraphPad Prism version 10.0. Descriptive statistics were used for qualitative variables (e.g., demographic characteristics, presence/absence of hypertension, presence/absence of hyperlipidemia), presented as frequencies, percentages, or proportions. Quantitative variables conforming to a normal distribution were expressed as mean $\pm$ standard deviation (SD), with comparisons between groups analyzed using the independent samples t-test. Quantitative variables not conforming to a normal distribution were expressed as median (25th percentile, 75th percentile). For comparisons between two groups: if data were normally distributed and variances were homogeneous (assessed using Levene's test), the independent samples t-test was employed; if data were non-normally distributed or variances were unequal, the non-parametric Mann-Whitney U test was used. A two-sided p-value < 0.05 was considered statistically significant for all tests. Time-to-event outcomes (remission, complete remission) were analyzed using the Kaplan-Meier method, and differences between groups were compared using the log-rank test.

4. Materials and Reagents

Obinutuzumab (Gazyva®) was provided by Roche Diagnostics GmbH under clinical trial supply agreement. Obinutuzumab was diluted in 1000 mL normal saline. *Abelmoschus Manihot* tablets, a hospital-prepared traditional Chinese medicine formulation developed by Jiangsu Province Hospital of Traditional Chinese Medicine, is composed of $\geq 90\%$ *Abelmoschus manihot* flower-derived active compounds. This standardized preparation (Specification: 0.3g/tablet; Approval No.: Z04000511) is clinically indicated for the management of proteinuria associated with chronic kidney disease. *Abelmoschus manihot* tablets were administered at 1.8g three times daily.

The study was approved by the Institutional Ethics Committee at the study site and was conducted in accordance with applicable regulatory requirements (Ethics Approval No. : (2025NL-092-02)), the principles of the Declaration of Helsinki, and the International Council for Harmonisation Good Clinical Practice guidelines. Written informed consent was obtained from all participating patients. The

supplementary materials include the ethics committee approval document, study protocol for patient enrollment, and informed consent forms.

Results

1. Baseline

This study ultimately enrolled 31 patients with idiopathic refractory membranous nephropathy (IRMN) for clinical efficacy observation. The cohort comprised 25 males (80%) and 6 females (20%), with a mean age of 48.8 ± 12.5 years. All patients received the combined therapy of Obinutuzumab and *Abelmoschus manihot* tablets according to the specified treatment protocol: Obinutuzumab was administered at a dose of 1.0 g per infusion, with a total cumulative dose of 3.0 g per treatment cycle (subsequent doses administered at 2-week and 24-week intervals); *Abelmoschus manihot* tablets were administered at 1.8g three times daily. The primary baseline clinical characteristics and prior immunosuppressive regimens are summarized in Table 1.

Table 1
Baseline Characteristics of the Study Cohort

Characteristic	Total (n = 31)
Sex, Male/Female, n (%)	25(80)/6(20)
Age, years	48.8 ± 12.5
Age by Tertile, n (%)(<40/40–50/>50 years)	9/5/17
Disease Duration, months	33 ± 30
24-h Urine Protein, g/24h	7.1 ± 3.5
Serum Albumin, g/L	27.5 ± 4.7
Serum Creatinine, μmol/L	100.2 ± 58.7
anti-PLA2R Antibody Titer, RU/mL	141.5(77.2,261.2)
Comorbidities Hypertension, n(%)	20(62)
Comorbidities Hyperlipidemia, n(%)	13 (40)
Comorbidities Hyperuricemia, n(%)	8 (25)
Comorbidities Diabetes, n(%)	9 (28)
Previous Treatments before Obinutuzumab, n	
ACEI/ARB	14
Cyclophosphamide	8
Glucocorticoids	19
Calcineurin Inhibitors	16
Rituximab	10

All 31 patients were evaluated from baseline through 24 weeks of Obinutuzumab combined with *Abelmoschus manihot* tablets therapy. Prior to combination therapy, no patients met remission criteria (defined as urinary protein excretion < 0.3 g/24h). Only 3 patients (10%) exhibited urinary protein excretion levels between 0.15–3.5 g/24h, while the remaining 28 patients (90%) had nephrotic-range proteinuria, with a mean baseline value of 7.1 ± 3.5 g/24h. At baseline, all patients tested positive for anti-PLA2R antibodies. Renal biopsy had been performed in 23 patients (74%), with positive glomerular PLA2R staining confirmed in all biopsied cases. The median anti-PLA2R antibody titer was 141.5 RU/mL. Mean serum creatinine was 100.2 ± 58.7 μmol/L and mean serum albumin was 27.5 ± 4.7 g/L. Before initiating combination therapy, patients had received the following prior immunosuppressive treatments: glucocorticoids (n = 19, 61%), calcineurin inhibitors (n = 16, 52%), rituximab (n = 10, 32%), and cyclophosphamide (n = 8, 26%). Sixteen patients (52%) had received ≥ 2 combined immunosuppressive

regimens, all without achieving adequate proteinuria control. Most patients presented with comorbidities including hypertension (n = 23, 74%), dyslipidemia (n = 21, 68%), and diabetes mellitus (n = 9, 29%).

The majority of patients (30/31, 97%) received the standard obinutuzumab regimen of three 1,000 mg doses (cumulative dose 3,000 mg). One patient (3%) required an additional 1,000 mg dose beyond this regimen. Throughout obinutuzumab administration, all patients received concomitant therapy with *Abelmoschus manihot* tablets as part of the renal function stabilization protocol.

2. Clinical Efficacy and Treatment Responses

The clinical response rate for all patients is shown in Fig. 1 (A-C). At the 12-month follow-up after treatment with obinutuzumab combined with *Abelmoschus manihot* tablets, 25 patients achieved a complete response (CR) or partial response (PR), resulting in an overall response rate (ORR) of 80%. Among responders, 5 patients (16%) attained CR and 20 patients (64%) achieved PR. The median time to response for the entire cohort was 4.5 months (IQR 2.0–7.0 months). Patients were stratified by risk into intermediate-low risk (n = 13, 42%) and high-risk (n = 18, 58%) groups. A comparison of CR/PR rates between these groups is presented in Fig. 1 (D). By month 9, the intermediate-low risk group approached a 99% response rate, achieving a 100% ORR (13/13 patients) by month 12, including 4 CRs. In contrast, the high-risk group achieved an ORR of 72% (13/18 patients) at 12 months, consisting of 12 PRs and 1 CR; one relapse occurred. The response rate was significantly higher in the intermediate-low risk group compared to the high-risk group ($p = 0.03$), with a hazard ratio (HR) of 0.73 (95% CI 0.50–1.07).

3. Changes in Clinical Parameters

We evaluated the effects of obinutuzumab combined with *Abelmoschus manihot* tablets on urinary total protein (UTP), serum albumin (ALB), and serum creatinine (Scr) levels before and after treatment (Fig. 2). UTP demonstrated a significant downward trend, decreasing steadily after the first obinutuzumab administration from baseline levels [7.1 ± 3.5 g/24h] to [2.3 ± 2.2 g/24h] at the final follow-up ($p < 0.0001$). The mean percentage reduction in UTP reached 42.3% at month 3. By month 12, half of the patients had achieved a reduction exceeding 50%, with responders exhibiting a mean reduction of 74.8% at the last follow-up. Following risk stratification (intermediate-low risk vs. high-risk groups), both groups showed similar overall trajectories in 24-hour UTP reduction. However, the intermediate-low risk group exhibited a significantly faster decline rate during the first 3 months and maintained lower UTP levels throughout the treatment cycle compared to the high-risk group ($p < 0.05$), demonstrating the higher treatment responsiveness in this subgroup. Serum albumin levels significantly increased from baseline [27.5 ± 4.7 g/L] to [34.1 ± 5.8 g/L] at study completion ($p < 0.05$). Trend analysis revealed that while the high-risk group displayed a more gradual albumin increase, the intermediate-low risk group showed a significantly steeper rise within the initial 3 months ($p < 0.05$). Beyond month 3, greater fluctuation was observed in the intermediate-low risk group, resulting in no significant inter-group difference in albumin levels by month 12. Scr levels decreased from baseline [100.2 ± 58.7 μ mol/L] to [76.9 ± 28.2 μ mol/L]

post-final treatment. At the 12-month assessment, no significant difference in Scr was detected compared to baseline ($p = 0.438$); notably, patients with elevated baseline Scr consistently exhibited a declining trend by the end of follow-up.

4. Anti-PLA2R Antibody Dynamics and Immunological Remission

At baseline, all patients were seropositive for anti-PLA2R antibodies. By month 3 of follow-up, 9 patients had achieved anti-PLA2R antibody seronegativity. At the end of follow-up, all patients achieved seronegativity, signifying immunological remission. Compared to baseline, serum anti-PLA2R antibody concentrations were significantly reduced at months 3, 6, and 12 (all $p < 0.001$; Fig. 3A), with decreases of 133.8 RU/mL, 173.0 RU/mL, and 179.6 RU/mL, respectively; the reduction was most pronounced at month 12 (mean reduction: 179.6 RU/mL, 95% CI: 96.82 to 262.3; $P < 0.0001$). Comparing anti-PLA2R antibody levels between the high-risk and intermediate-low risk groups, the high-risk group exhibited a slightly faster rate of decline before month 3. However, by the end of follow-up, antibody levels were similar between groups, with both achieving seronegativity ($p = 0.6$, non-significant). The relative percentage decrease in anti-PLA2R antibody levels during treatment is shown in Fig. 3B. Using baseline titer as 100%, patients in the highest tertile (red) demonstrated that although 5 patients in this high-titer subgroup had anti-PLA2R levels $> 25\%$ of baseline (suggesting a half-life > 7 days) at week 4, only one patient remained seropositive by week 8, indicating obinutuzumab efficacy in high-titer patients. The overall immunological remission rate for all patients is presented in Fig. 3C: 70%, 83%, 90%, and 93% at months 3, 6, 9, and 12, respectively; the corresponding immunological complete response rates were 35%, 74%, 80%, and 80%. When stratified by baseline PLA2R antibody titer, 16 patients had levels > 100 RU/mL. Their immunological complete response rates at months 3, 6, 9, and 12 were 25%, 56%, 68%, and 68%, respectively (Figs. 3D, E). These results demonstrate that patients with lower baseline titers achieved higher response rates more readily and suggest an earlier response rate to obinutuzumab treatment.

5. B-cells Depletion Kinetics

Treatment with obinutuzumab combined with *Abelmoschus manihot* tablets induced rapid B-cells depletion, achieving complete B-cells depletion within the first 3 months. B-cells reconstitution commenced at month 6, reaching higher levels by month 12. Mean CD20 + B cell counts at months 0, 3, 6, and 12 were 201 cells/ μ L, 0.2 cells/ μ L, 6 cells/ μ L, and 30 cells/ μ L, respectively. Comparative analysis of B-cells kinetics and urinary total protein (UTP) trends (Fig. 4A) revealed that profound B-cells depletion coincided with a substantial reduction in UTP levels (compared to post-month 3 values), with B-cells depletion onset preceding UTP decline. Following B-cells reconstitution, the rate of UTP reduction decelerated, particularly between months 9–12, yet UTP levels continued a stable downward trajectory with a temporal lag. By month 12, a 65% relative reduction in UTP was achieved, indicating a favorable prognostic indicator. B-cells kinetics demonstrated synchrony with immunological remission (Fig. 4B): 80% of patients achieved immunological remission by month 3, coinciding with complete B-cells

depletion. During B-cells reconstitution, the rate of immunological remission gradually decreased but maintained a slow upward trend.

6. Network Pharmacology Analysis Reveals the Molecular Mechanism Underlying the Synergistic Effects of Obinutuzumab and *Abelmoschus manihot* Tablets in Idiopathic Refractory Membranous Nephropathy

During obinutuzumab treatment, patients received concurrent *Abelmoschus manihot* tablets as synergistic therapy. The primary active component of *Abelmoschus manihot* tablets is *Abelmoschus manihot* flower (AMF), which is widely utilized in treating kidney diseases. Clinical studies have confirmed the significant efficacy of AMF in reducing proteinuria and retarding renal fibrosis progression. Its principal bioactive constituents are flavonoids, such as quercetin and hyperin [24], known for their positive roles in anti-inflammation and promoting CD20+ B cells apoptosis. To validate the associated pharmacological mechanisms, we employed network pharmacology and molecular docking approaches. Active constituents of AMF were screened from the HERB database (<http://herb.ac.cn/>). Quercetin and hyperin were identified as the primary effective components; their SMILES structures were obtained and uploaded to SwissTargetPrediction (<http://www.swisstargetprediction.ch/>) for target prediction. MN-related and CD20-associated target genes were concurrently retrieved from the GeneCards database (<http://www.genecards.org/>). The intersection of AMF-predicted targets with MN/CD20-related targets yielded 29 overlapping genes between AMF and MN, and 14 common genes among all three categories (Figure 5A). These overlapping genes were imported into STRING (<https://string-db.org/>) for core target screening. A protein-protein interaction (PPI) network was constructed and visualized using Cytoscape 3.10.2 (Figure 5B), identifying the top five core targets: IL6, TNF, IL1B, CASP9, and JUN. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) enrichment analyses were subsequently performed using DAVID (<https://david.ncifcrf.gov/>) and a bioinformatics platform (Figure 5C, D). Integrated results indicated that AMF exerts its therapeutic effects on MN primarily by modulating cellular apoptosis (CD20+ B cells) and inflammatory responses at the functional level. At the pathway level, AMF coregulates proteinuria and mitigates renal fibrosis principally through the AGE-RAGE signaling pathway.

Furthermore, molecular docking was performed to validate the predicted interactions. Based on prior analyses, the 3D structures of quercetin and hyperin were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) and converted to PDB format using Open Babel 2.4.0. The complexes IL6-hyperin, IL6-quercetin, TNF-hyperin, and CASP9-hyperin were subjected to docking simulations in AutoDock Vina (Figures 6A-D). Results demonstrated that the binding free energies between the bioactive AMF constituents (quercetin/hyperin) and the inflammatory/CD20+ B cells apoptosis targets (IL6, TNF, CASP9) were all below $-6 \text{ kcal}\cdot\text{mol}^{-1}$, indicating strong binding affinity. These interactions were primarily mediated by hydrogen bonding and hydrophobic forces. Collectively, the findings elucidate that AMF exerts its therapeutic effects in MN through dual mechanisms: (1) promoting CD20+ B cells apoptosis to suppress inflammation, and (2) inhibiting renal fibrosis and

protecting renal function by modulating the AGE-RAGE signaling pathway, thereby reducing proteinuria. These complementary mechanisms synergize with obinutuzumab, enhancing its clinical efficacy.

7. Safety Profile

We documented all adverse events occurring in the study cohort. Based on previous reports detailing common adverse reactions associated with biological agents[25] , which include infusion-related reactions (e.g., chills, fever, rash), infections, neutropenia, and hypogammaglobulinemia, the combination therapy of obinutuzumab and *Abelmoschus manihot* tablets demonstrated a favorable safety profile in our observational cohort. Notably, no patients progressed to end-stage renal disease or died. During the follow-up period, only four patients experienced mild, transient adverse reactions, including fever, hypotension, tachycardia, and facial flushing (**Table2**); these reactions resolved spontaneously and did not recur during subsequent infusions.

Table 2 Adverse Events Observed in the Study Cohort

Adverse Event	Total (n=31) n (%)
Fever	1
Hypotension	1
Tachycardia	1
Facial Flushing	1

Discussion

This study represents the first investigation employing a combination of obinutuzumab and *Abelmoschus manihot* tablets, an integrated traditional Chinese and Western medicine approach, for the treatment of idiopathic refractory membranous nephropathy. The therapeutic mechanism was explored using network pharmacology and validated through molecular docking. Throughout the treatment period, no pulse steroid therapy or other immunosuppressants were administered; treatment strictly adhered to the obinutuzumab prescribing guidelines, ensuring a systematic and standardized regimen. Clinically, the overall response rate (ORR) was 80% and the complete response (CR) rate was 16% in our cohort, consistent with historical obinutuzumab data[22, 26], and compares favorably with the reported rituximab response rate of approximately 68% for idiopathic MN[27–29]. Furthermore, immunological responses demonstrated high reactivity, with 41% of patients achieving response within just 1 month, preceding the clinical response – a finding aligning with other studies[30]. Notably, seven out of nine patients who were previously unresponsive to rituximab achieved remission after the study treatment, representing a significant advance. Importantly, only one recurrence was observed in this study. While recurrence rates have not been statistically analyzed in prior obinutuzumab studies for MN, reported recurrence rates for rituximab regimens are approximately 27%[14, 31]. The combination therapy of obinutuzumab and *Abelmoschus manihot* tablets was associated with a markedly lower recurrence rate.

The complete response (CR) rate in our cohort was 9% at 3 months and increased to 40% by 12 months. These rates are comparable to those reported by Klomjit et al. [32] (which included 3 patients, all achieving immunological response, with 2 partial responses and a CR rate of 33%) and Sethi et al. [33] (who observed a CR rate of 40% [4/10 patients] at a median follow-up of 6 months), despite our study enrolling a substantially larger patient population. Proteinuria level significantly influences the treatment efficacy and prognosis in membranous nephropathy. Our results demonstrated significant reductions in 24-hour urinary total protein (24hUTP) excretion from baseline: 4.5 g/24h in the high-risk group and 3.79 g/24h in the intermediate/low-risk group. Given our specific focus on obinutuzumab for idiopathic refractory membranous nephropathy, enrolled patients presented with refractory, persistently high baseline UTP levels, inherently posing a greater treatment challenge. Notably, the overall UTP level had decreased to below 50% of baseline by month 3. Furthermore, serum albumin (ALB) levels significantly increased during follow-up. Although baseline serum creatinine (Scr) levels were largely within the normal range for the cohort, Scr levels were effectively controlled in patients with elevated baseline Scr, and the overall Scr level at the end of follow-up was lower than baseline. Collectively, these findings underscore the significant therapeutic potential of obinutuzumab in the management of idiopathic refractory membranous nephropathy.

Currently, according to the 2021 KDIGO Clinical Practice Guideline for Glomerular Diseases interpretation for membranous nephropathy, anti-PLA2R antibody status has been established as an indicator for diagnosis, treatment response, and prognosis[34]. In our study, anti-PLA2R antibody levels declined rapidly following the first dose of obinutuzumab in 25 patients (80%); a $\geq 50\%$ reduction was achieved in 20 patients (64%), with levels exhibiting a sustained downward trend, and 12 patients (38%) achieved seroconversion. By the end of follow-up, anti-PLA2R antibody levels had significantly decreased in both the high-risk and intermediate/low-risk groups ($p > 0.05$, difference not statistically significant). Furthermore, the complete response (CR) rate at 12 months was lower in patients with high baseline anti-PLA2R antibody titers (> 100 RU/mL) compared to those with lower titers (≤ 100 RU/mL) (68% vs. 86%, respectively), a finding consistent with the report by Roads et al.[35]. These results collectively suggest a favorable prognostic trajectory. When compared to the first-generation anti-CD20 biologic rituximab for refractory MN, a study by Lin et al.[36] similarly demonstrated a rapid decline in anti-PLA2R antibodies as early as week 6 following obinutuzumab treatment.

Regarding the safety profile of the obinutuzumab combined with *Abelmoschus manihot* tablets regimen, published clinical studies specifically investigating obinutuzumab for membranous nephropathy remain limited. Consequently, we also considered safety data from rituximab, which shares a similar mechanism of action. The most frequently documented adverse event associated with rituximab is infusion-related reactions[37]. Throughout our follow-up period, monitoring the cumulative incidence of adverse events during treatment revealed no serious adverse events. Only a subset of patients experienced transient adverse reactions at the initiation of infusion, predominantly manifested as fever, tachycardia, hypotension, and facial flushing.

Abelmoschus manihot tablets have been increasingly utilized in recent years for the clinical treatment of membranous nephropathy, demonstrating significant efficacy in reducing proteinuria[24]. The primary active constituents of *Abelmoschus manihot*, classified as flavonoids, are quercetin and hyperoside[38]. In our study, network pharmacology and molecular docking were employed to predict the mechanism of action of *Abelmoschus manihot* tablets. Key therapeutic targets identified for MN treatment include IL6, TNF, IL1B, CASP9, and JUN. KEGG and GO enrichment analyses revealed that these targets modulate pathways such as “Apoptosis - multiple species” and “response to tumor necrosis factor”, thereby promoting CD20 + B cells apoptosis and suppressing inflammatory responses. The apoptosis of CD20 + B cells impedes the autoimmune response, reducing the production of autoantibodies (e.g., anti-PLA2R antibodies). This mechanism aligns with that of obinutuzumab, suggesting that the combination therapy synergistically enhances therapeutic efficacy. Furthermore, as inflammation is a primary driver of renal fibrosis progression, results indicated that the active components of *Abelmoschus manihot* tablets exert protective effects by modulating the AGE-RAGE signaling pathway, leading to decreased levels of pro-inflammatory cytokines TNF- α and IL-6, and consequently inhibiting renal fibrosis progression. This finding is consistent with prior research on *Abelmoschus manihot* in chronic kidney disease[39], ultimately achieving the goals of ameliorating renal injury and reducing proteinuria.

Our protocol included the use of low-dose intravenous dexamethasone as adjunctive therapy. The rationale for adding this corticosteroid was specifically to suppress the immune system and mitigate potential immune activation, including immunogenic/allergic reactions, triggered by biologic agents like obinutuzumab. Whether combining obinutuzumab with higher-dose corticosteroids yields superior efficacy remains debatable; consequently, we employed only low-dose dexamethasone to inhibit such immune-mediated reactions. Furthermore, prior to initiating the obinutuzumab regimen, enrolled patients had received treatments including cyclophosphamide and calcineurin inhibitors. Upon enrollment, these prior therapies were discontinued. Remarkably, significant therapeutic effects were observed with the obinutuzumab and *Abelmoschus manihot* tablets combination alone. Nevertheless, despite achieving an overall response rate of 80% with obinutuzumab for idiopathic refractory membranous nephropathy in this study, 20% of patients exhibited either a lack of response or disease recurrence. The potential benefit of extending the obinutuzumab treatment duration or employing additional combination strategies for this subset of patients warrants further investigation.

Conclusion

The Obinutuzumab and *Abelmoschus manihot* tablets combination induced high remission rates in IRMN, with rapid immunological response and a tolerable safety profile, representing a novel therapeutic approach.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Jiangsu Provincial Hospital of Traditional Chinese Medicine (Ethical Approval No. 2025NL-092-02), and written informed consent was obtained from all participants after thorough explanation of the study procedures.

Consent for publication

No applicable.

Competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Fundings

This work was supported by to Y. M. and C. J. This study was supported by grants from the National Natural Science Foundation of China (grant 82400796 to Y. M.), the National Natural Science Foundation of China (grant 82100753 to C. J.), and the Excellent Young Doctoral Training Program of Jiangsu Province Hospital of Chinese Medicine (grant 2024QB023 to Y. M.).

Author Contribution

Author contributionsC. J. and Y. M. performed study concept, design, project administration, writing - review & editing, and funding acquisition.X. Z. and X. C. conceived the study design, performed all statistical analyses, conducted patient recruitment and follow-up, curated the clinical database, wrote the original draft, and visualized the results.Y. W. , D. C. , M. Y. , H. Z. and J. Z. participated in clinical investigations, secured essential resources, validated research data, and contributed to manuscript revision.

Acknowledgements

We are grateful for all the listed authors who participated in this project.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

References

1. McGrogan A, Franssen CF, de Vries CS. The incidence of primary glomerulonephritis worldwide: a systematic review of the literature. *Nephrol Dial Transpl.* 2011;26(2):414–30.
2. Fervenza FC, Sethi S, Specks U. Idiopathic membranous nephropathy: diagnosis and treatment. *Clin J Am Soc Nephrol.* 2008;3(3):905–19.
3. Couser WG. Primary Membranous Nephropathy. *Clin J Am Soc Nephrol.* 2017;12(6):983–97.
4. Yu Y, Liu G, Zhang R, Chen B, Luan Z. Association between immune cell subtypes and membranous nephropathy: A bidirectional Mendelian randomization study. *Med (Baltim).* 2025;104(23):e42774.
5. McDonnell T, Wu HHL, Sinha S, Chinnadurai R. The Role of PLA2R in Primary Membranous Nephropathy: Do We Still Need a Kidney Biopsy? *Genes (Basel)* 2023; 14(7).
6. 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.
7. Du Y, Li J, He F, et al. The diagnosis accuracy of PLA2R-AB in the diagnosis of idiopathic membranous nephropathy: a meta-analysis. *PLoS ONE.* 2014;9(8):e104936.
8. Hofstra JM, Beck LH Jr., Beck DM, Wetzels JF, Salant DJ. Anti-phospholipase A₂ receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol.* 2011;6(6):1286–91.
9. Provatopoulou S, Kalavrizioti D, Stangou M, et al. Circulating anti-phospholipase A2 receptor antibodies as a diagnostic and prognostic marker in Greek patients with idiopathic membranous nephropathy - a retrospective cohort study. *Rom J Intern Med.* 2019;57(2):141–50.
10. Cattran D. Management of membranous nephropathy: when and what for treatment. *J Am Soc Nephrol.* 2005;16(5):1188–94.
11. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. *Kidney Int.* 2021;100(4s):S1–276.
12. Zhang Z, Shi Y, Yang K, Crew R, Wang H, Jiang Y. Higher frequencies of circulating ICOS(+), IL-21(+) T follicular helper cells and plasma cells in patients with new-onset membranous nephropathy. *Autoimmunity.* 2017;50(8):458–67.
13. Ruggenti P, Reinhard L, Ruggiero B, et al. Anti-Phospholipase A2 Receptor 1 and Anti-Cysteine Rich Antibodies, Domain Recognition and Rituximab Efficacy in Membranous Nephropathy: A Prospective Cohort Study. *Am J Kidney Dis.* 2024;83(5):588–e6001.
14. Ruggenti P, Cravedi P, Chianca A, et al. Rituximab in idiopathic membranous nephropathy. *J Am Soc Nephrol.* 2012;23(8):1416–25.
15. Beers SA, French RR, Chan HT, et al. Antigenic modulation limits the efficacy of anti-CD20 antibodies: implications for antibody selection. *Blood.* 2010;115(25):5191–201.
16. Golay J, Semenzato G, Rambaldi A, et al. Lessons for the clinic from rituximab pharmacokinetics and pharmacodynamics. *MAbs.* 2013;5(6):826–37.
17. Rojas-Rivera JE, Ortiz A, Fervenza FC. Novel Treatments Paradigms: Membranous Nephropathy. *Kidney Int Rep.* 2023;8(3):419–31.

18. Meena P, Ramachandran R. Obinutuzumab as a Promising Treatment for Membranous Nephropathy. *Indian J Nephrol.* 2025;35(3):322–3.
19. Grimm HP, Schick E, Hainzl D, et al. PKPD Assessment of the Anti-CD20 Antibody Obinutuzumab in Cynomolgus Monkey is Feasible Despite Marked Anti-Drug Antibody Response in This Species. *J Pharm Sci.* 2019;108(11):3729–36.
20. Li N, Tang H, Wu L, et al. Chemical constituents, clinical efficacy and molecular mechanisms of the ethanol extract of *Abelmoschus manihot* flowers in treatment of kidney diseases. *Phytother Res.* 2021;35(1):198–206.
21. Wei C, Wang C, Li R, et al. The pharmacological mechanism of *Abelmoschus manihot* in the treatment of chronic kidney disease. *Heliyon.* 2023;9(11):e22017.
22. Su X, Wu B, Tie X, et al. Obinutuzumab as Initial or Second-Line Therapy in Patients With Primary Membranous Nephropathy. *Kidney Int Rep.* 2024;9(8):2386–98.
23. Radhakrishnan Y, Zand L, Sethi S, Fervenza FC. Membranous nephropathy treatment standard. *Nephrol Dialysis Transplantation.* 2024;39(3):403–13.
24. Zhang L, Li P, Xing CY, et al. Efficacy and safety of *Abelmoschus manihot* for primary glomerular disease: a prospective, multicenter randomized controlled clinical trial. *Am J Kidney Dis.* 2014;64(1):57–65.
25. Scolari F, Delbarba E, Santoro D, et al. Rituximab or Cyclophosphamide in the Treatment of Membranous Nephropathy: The RI-CYCLO Randomized Trial. *J Am Soc Nephrol.* 2021;32(4):972–82.
26. Su W, Li J, Men J, et al. Obinutuzumab is effective for the treatment of rituximab-refractory PLA2R-associated membranous nephropathy. *Clin Kidney J.* 2025;18(5):sfaf026.
27. Chen P, Mao M, Wang C, et al. Preliminary study on the efficacy of rituximab in the treatment of idiopathic membranous nephropathy: A single-centre experience. *Front Endocrinol (Lausanne).* 2023;14:1044782.
28. Huang L, Dong QR, Zhao YJ, Hu GC. Rituximab for the management of idiopathic membranous nephropathy: a meta-analysis. *Int Urol Nephrol.* 2021;53(1):111–9.
29. Zhang S, Huang J, Dong J, et al. Efficacy and safety of rituximab for primary membranous nephropathy with different clinical presentations: a retrospective study. *Front Immunol.* 2023;14:1156470.
30. Ruggenenti P, Debiec H, Ruggiero B, et al. Anti-Phospholipase A2 Receptor Antibody Titer Predicts Post-Rituximab Outcome of Membranous Nephropathy. *J Am Soc Nephrol.* 2015;26(10):2545–58.
31. 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):348–58.
32. Klomjit N, Fervenza FC, Zand L. Successful Treatment of Patients With Refractory PLA(2)R-Associated Membranous Nephropathy With Obinutuzumab: A Report of 3 Cases. *Am J Kidney Dis.* 2020;76(6):883–8.

33. Sethi S, Kumar S, Lim K, Jordan SC. Obinutuzumab is Effective for the Treatment of Refractory Membranous Nephropathy. *Kidney Int Rep.* 2020;5(9):1515–8.
34. Timmermans SA, Abdul Hamid MA, Cohen Tervaert JW, Damoiseaux JG, van Paassen P. Anti-PLA2R Antibodies as a Prognostic Factor in PLA2R-Related Membranous Nephropathy. *Am J Nephrol.* 2015;42(1):70–7.
35. Rodas LM, Matas-García A, Barros X, et al. Antiphospholipase 2 receptor antibody levels to predict complete spontaneous remission in primary membranous nephropathy. *Clin Kidney J.* 2019;12(1):36–41.
36. Lin Y, Han Q, Chen L, et al. Obinutuzumab in Refractory Membranous Nephropathy: A Case Series. *Kidney Med.* 2024;6(8):100853.
37. Ou J-Y, Chen Y-W, Li T-L, et al. Evaluation of efficacy of rituximab for membranous nephropathy: A systematic review and meta-analysis of 11 studies. *Néphrologie Thérapeutique.* 2022;18(2):104–12.
38. Guo JM, Lu YW, Shang EX, et al. Metabolite identification strategy of non-targeted metabolomics and its application for the identification of components in Chinese multicomponent medicine *Abelmoschus manihot* L. *Phytomedicine.* 2015;22(5):579–87.
39. Ge J, Miao J-J, Sun X-Y, Yu J-Y. Huangkui capsule, an extract from *Abelmoschus manihot* (L.) medic, improves diabetic nephropathy via activating peroxisome proliferator–activated receptor (PPAR)- α/γ and attenuating endoplasmic reticulum stress in rats. *J Ethnopharmacol.* 2016;189:238–49.

Figures

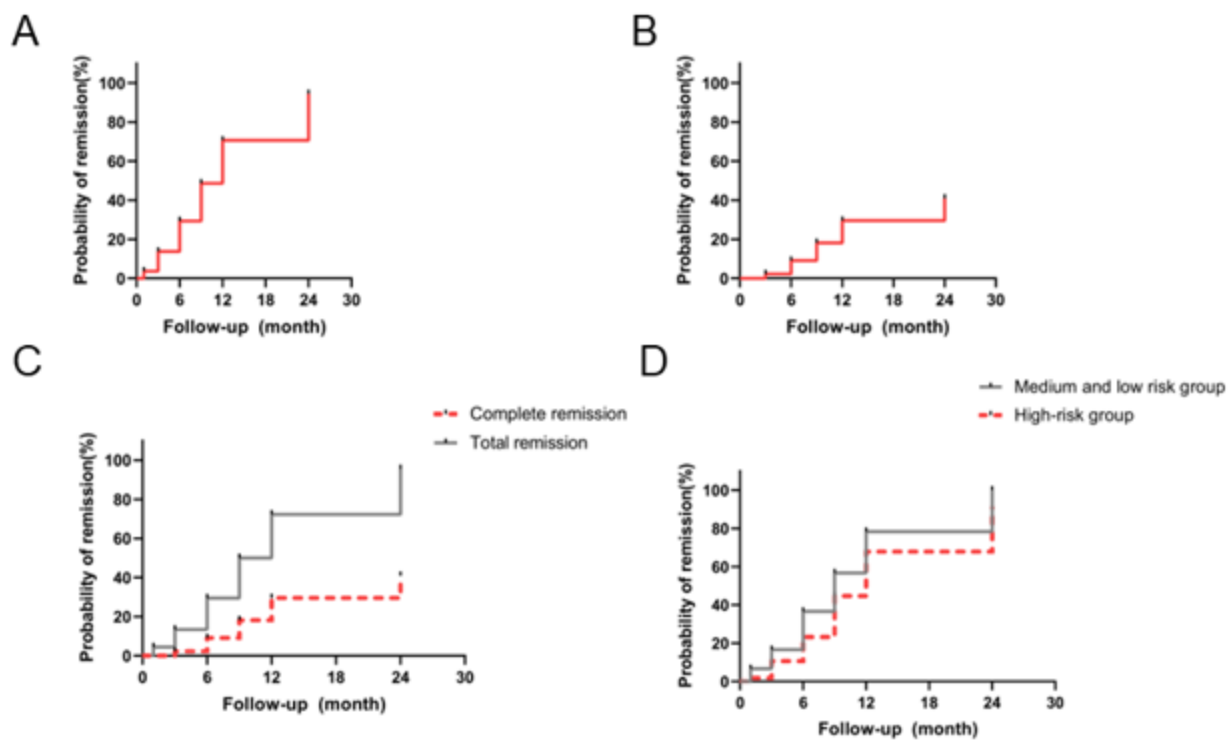


Figure 1

Clinical Efficacy. A. Overall response rate (ORR) in all patients; B. Complete response rate (CR) in all patients; C. ORR versus CR in the study cohort; D. Comparison of ORR and CR between high-risk and intermediate/low-risk groups.

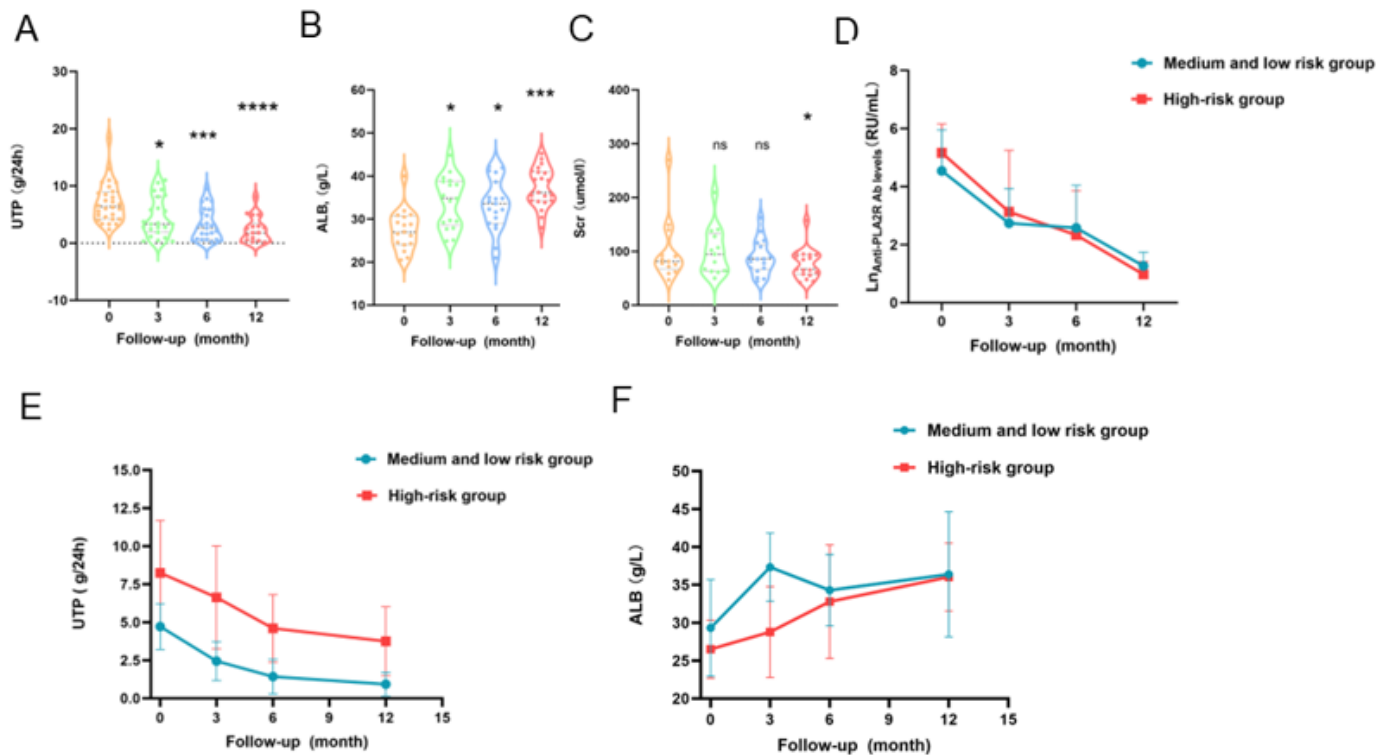


Figure 2

Change Trend Graph of Efficacy Indicators. A. 24-hour urinary protein (UTP) changes over time (0, 3, 6, 12 months) in all patients; B. Serum albumin (ALB) recovery over time in all patients; C. Serum creatinine (Scr) evolution over time. Subgroup analysis: Trend comparison of anti-PLA2R antibody titers (D), UTP (E), and ALB (F) between high-risk and low-risk groups over time. Abbreviations: UTP, 24-hour urinary protein excretion; ALB, serum albumin; Scr, serum creatinine.

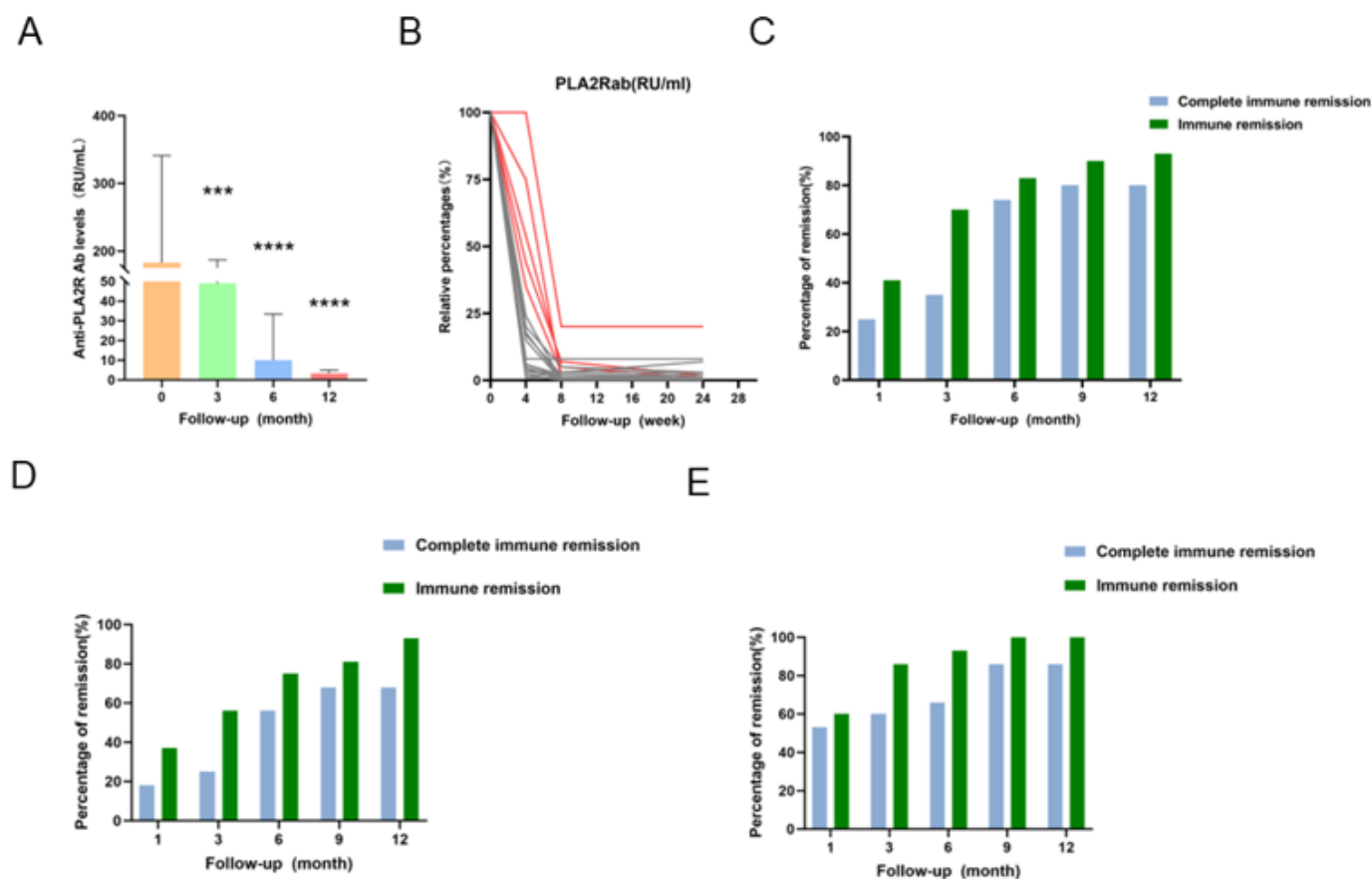


Figure 3

Anti-PLA2R Antibody Dynamics and Immunological Remission. A. Bar plot showing longitudinal changes in anti-PLA2R antibody titers for all patients. B. Line graph depicting relative percentage change in anti-PLA2R levels (baseline set as 100%). C. Immunological complete/partial remission rates in the overall cohort. D. Immunological remission rates in patients with baseline aPLA2Rab >100 RU/mL. E. Immunological remission rates in patients with baseline aPLA2Rab ≤100 RU/mL.

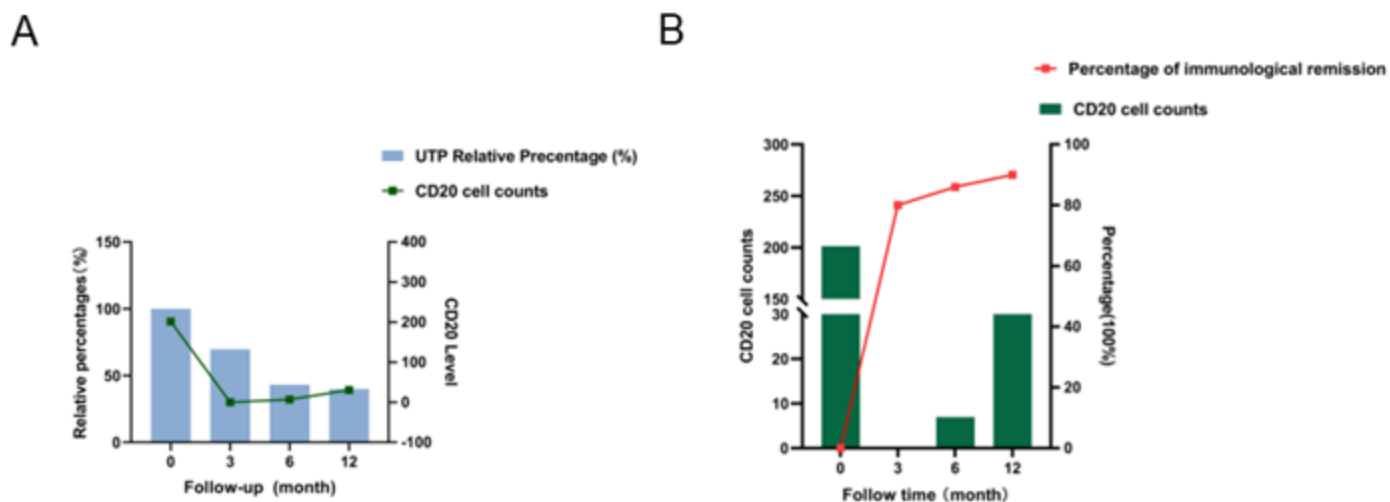


Figure 4

B-Cell Depletion Kinetics. A. Dual-axis plot of CD20⁺ cell count versus relative percentage change in 24-hour urinary protein (UTP). B. Dual-axis correlation between CD20⁺ cell count and immunological remission rate.

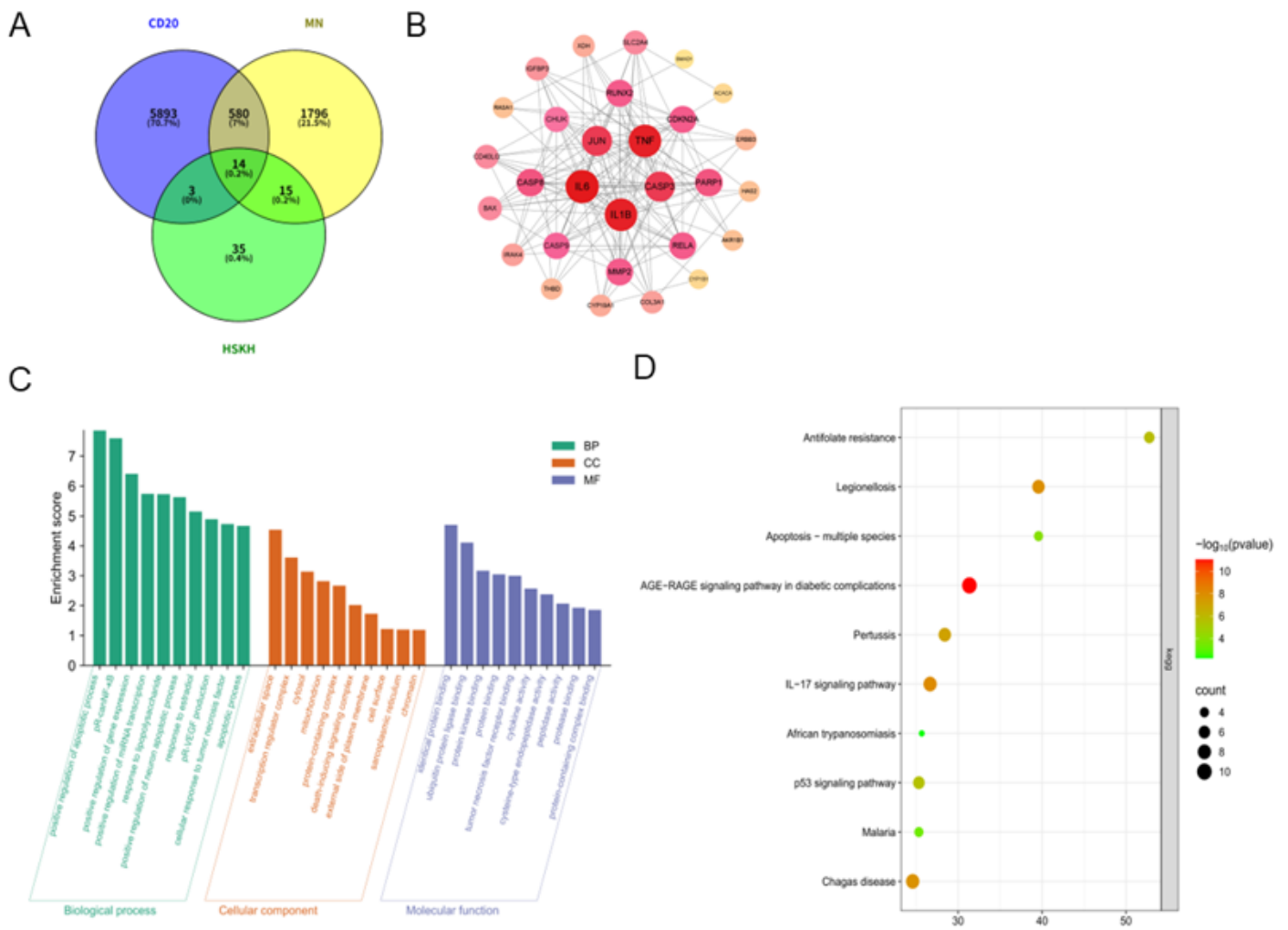


Figure 5

Network pharmacology analysis. A. Venn diagram displaying distribution of 14 overlapping targets among membranous nephropathy-associated genes, potential therapeutic targets of *Abelmoschus manihot*, and CD20⁺ cell-related genes, with 29 shared targets between the first two categories. B. Protein-protein interaction (PPI) network of targets common to membranous nephropathy and *A. manihot*. C. Cytoscape visualization with node size scaled by degree centrality, showing 5 core targets and 8 common targets clustered in two inner-layer circles. D. GO enrichment and KEGG pathway analysis of *A. manihot* targets relevant to membranous nephropathy treatment.

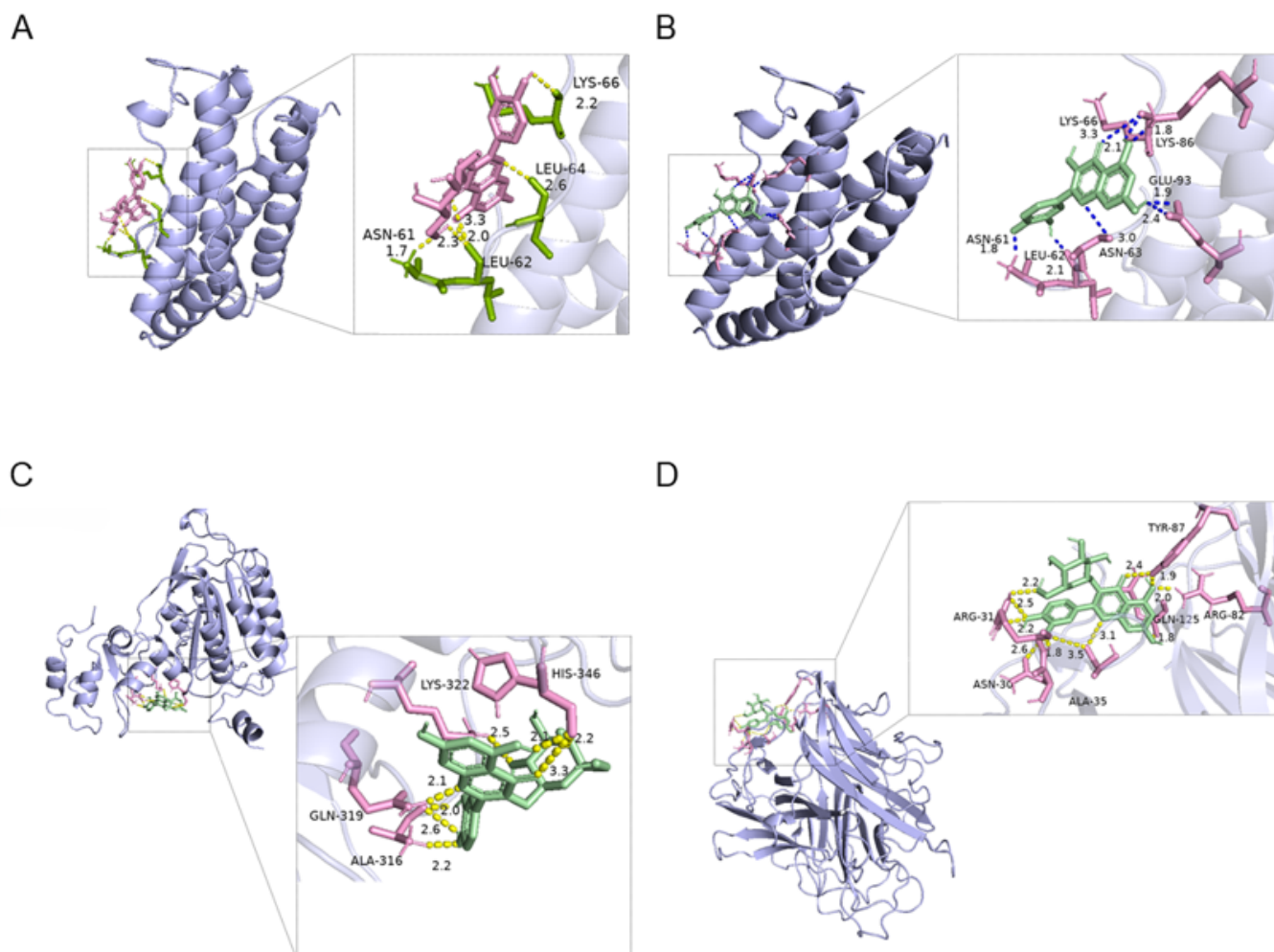


Figure 6

Molecular docking analysis. A-D. Binding poses of ligand-receptor complexes: A. IL-6 with hyperoside, B. IL-6 with quercetin, C. TNF- α with hyperoside, D. Caspase-9 with hyperoside.