

# Rituximab in Idiopathic Membranous Nephropathy

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## ABSTRACT

Selective depletion of B cells with the mAb rituximab may benefit the autoimmune glomerular disease idiopathic membranous nephropathy (IMN). Here, we describe our experience treating 100 consecutive IMN patients with persistent nephrotic syndrome with rituximab. We defined complete remission as persistent proteinuria <0.3 g/24 h and partial remission as persistent proteinuria <3 g/24 h, each also having >50% reduction in proteinuria from baseline. During a median follow-up of 29 months after rituximab administration, 65 patients achieved complete or partial remission. The median time to remission was 7.1 months. All 24 patients who had at least 4 years of follow-up achieved complete or partial remission. Rates of remission were similar between patients with or without previous immunosuppressive treatment. Four patients died and four progressed to ESRD. Measured GFR increased by a mean 13.2 (SD 19.6) ml/min per 1.73 m<sup>2</sup> among those who achieved complete remission. Serum albumin significantly increased and albumin fractional clearance decreased among those achieving complete or partial remission. Proteinuria at baseline and the follow-up duration each independently predicted the decline of proteinuria. Furthermore, the magnitude of proteinuria reduction significantly correlated with slower GFR decline ( $P=0.0001$ ). No treatment-related serious adverse events occurred. In summary, rituximab achieved disease remission and stabilized or improved renal function in a large cohort of high-risk patients with IMN.

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Idiopathic membranous nephropathy (IMN) is an antibody-mediated autoimmune glomerular disease that is found in the majority of adult patients with the nephrotic syndrome.<sup>1</sup> In an 18-year experience with 100 consecutive patients who had received no specific treatment, Schieppati *et al.*<sup>2</sup> found that at 5 years, 20% had achieved complete remission and 40% had some degree of proteinuria with stable or slowly declining renal function. The remainder had persistent nephrotic syndrome with progression to ESRD in most cases. More recently, Polanco *et al.* also found that, despite treatment with angiotensin converting enzyme (ACE) inhibitors, approximately 10% of those with persistent nephrotic syndrome die prematurely of cardiovascular events before progressing to ESRD.<sup>3</sup>

Thus far, therapeutic approaches to IMN mostly rely on nonspecific immunosuppression with steroids and alkylating agents with or without calcineurin

inhibitors.<sup>4</sup> In particular, steroids in combination with alkylating agents have been reported to reduce the rate of progression to ESRD more effectively than steroids alone in long-term randomized clinical trials.<sup>5,6</sup> Observational studies also showed that, irrespective of substantial changes in patient characteristics and concurrent advances in conservative therapies, the implementation of the above immunosuppressive regimens

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in IMN patients at high risk of progression has led to a substantial reduction in ESRD incidence in the everyday setting.<sup>7,8</sup> However, enthusiasm about more favorable outcomes achieved by steroids and alkylating agents over the last 3 decades is tempered by the side effects of these medications, which have been associated with an increased risk of lymphoproliferative disorders, cancer, infections, myelotoxicity, iatrogenic diabetes, and other serious adverse events.<sup>9</sup> The oncogenic potential of alkylating agents, although at higher doses than those currently recommended for patients with IMN, is well documented and is a major concern.<sup>10–12</sup> The risk of cancer and other serious complications is further increased when patients progressing to ESRD receive a kidney transplant and are exposed to further immunosuppressive treatment to prevent allograft rejection.<sup>13</sup>

The possibility of a specific and hopefully safer treatment for patients with IMN emerged in 1997 when rituximab, a mAb against the CD20 antigen present on B lymphocytes, was approved by the US Food and Drug Administration for the treatment of non-Hodgkin's lymphoma.<sup>14</sup> Because the CD20 antigen is not expressed on hematopoietic stem cells, normal plasma cells, or other normal tissues, selective B lymphocyte depletion by rituximab therapy was expected to inhibit the production of autoantibodies involved in the pathogenesis of the disease without the toxicity of nonspecific immunosuppression.<sup>15</sup> Thus, we initially tested the safety of rituximab in eight IMN patients with severe nephrotic syndrome unresponsive to prolonged ACE inhibitor therapy.<sup>15,16</sup> Treatment was well tolerated and led to a significant reduction in proteinuria. Other small, short-term studies confirmed these preliminary encouraging findings.<sup>17,18</sup> Thus, we decided to offer this treatment option to patients referred to our nephrology unit who were expected to progress to ESRD or to die prematurely of cardiovascular events because of persistent nephrotic syndrome.<sup>2,3</sup> Here we describe the outcome of the first 100 consecutive patients prospectively monitored for up to 10 years after rituximab administration.

## RESULTS

By September 2011, we had followed-up 100 consecutive IMN patients for at least 6 months after rituximab administration (Figure 1 and Table 1). Before referral to our unit, 32 patients had already been treated at other institutions with steroids alone or in combination with alkylating agents, calcineurin inhibitors, or other immunosuppressants. Overall, these 32 patients had been exposed to 55 courses of different immunosuppressive regimens. Nine, eight, and two patients received two, three, or four courses of treatment, respectively. Overall, 21 patients received one course and three additional patients received two courses of steroids plus alkylating agents. In 17 of them, steroids plus alkylating agents were preceded by at least one course of corticosteroid alone, cyclosporine alone, or both agents in combination. Fourteen patients received cyclosporine (combined with steroids in 7 cases), 15 received steroids alone, and 2 received adrenocorticotrophic hormone

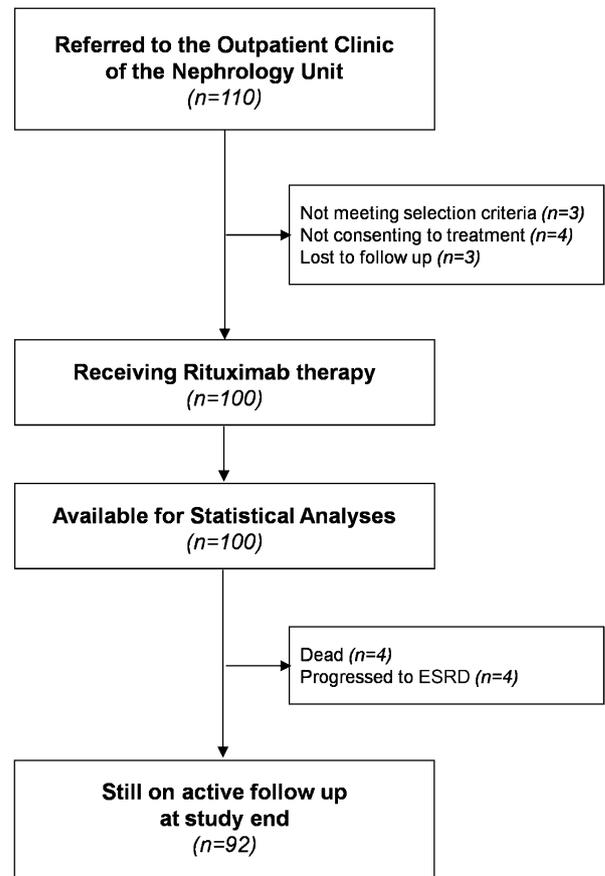


Figure 1. Patient flow chart.

(ACTH). Both patients given ACTH had been previously treated with steroids and alkylating agents. Twelve patients never achieved complete or partial remission, whereas 20 had transient partial remissions. However, no patient was on complete or partial remission after completion of the last course of immunosuppression and no one had any complete or partial remission on subsequent follow-up. Thus, the above 32 patients were persistently nephrotic since a median of 65.4 months (interquartile range [IQR], 35–80.5) before rituximab therapy (Table 1). Although previous serious adverse events had not been systematically recorded, at least 11 events had been traced in 9 patients that required definitive interruption of treatment in 4 cases. Events included four cases of severe myelotoxicity, one of amenorrhea, one of systemic infection and one of toxic hepatitis that had been associated with alkylating agents, one case of steroid-induced diabetes, one of glottis edema after ACTH administration, and two cases of severe hypertension associated with cyclosporine therapy.

At baseline evaluation, serum creatinine exceeded the normal range in 44 patients. BP was well controlled in all patients, and all were on ACE inhibitor therapy (Table 1). Median duration of persistent proteinuria before rituximab administration exceeded 2 years, and approximated 6 years among patients receiving rituximab after treatment with other immunosuppressive drugs

**Table 1.** Baseline characteristics of patients in the study group as a whole (overall) and in patients with complete, partial, or no remission or who received rituximab as first- or second-line therapy, considered separately

Characteristic	Overall (n=100)	Complete Remission (n=27)	Partial Remission (n=38)	No Remission (n=35)	First-Line Therapy (n=68)	Second-Line Therapy (n=32)
Age (yr)	51.5±5.9	48.9±16.5	51.4±15.6	53.7±15.9	55.0±16.4	44.1±12.0 <sup>a</sup>
Male sex, n (%)	72 (72)	13 (48.1) <sup>b</sup>	28 (73.7)	31 (87.5)	46 (67.6)	26 (81.3)
Clinical parameters						
body weight (kg)	76.2±13.6	70.5±15.6	78.2±12.2	78.3±12.7	76.1±12.1	76.5±16.5
systolic BP (mmHg)	130 (122–144)	129 (119.5–139)	132.5 (123–150)	135.5 (127–145)	130 (123–146)	130 (121–140)
diastolic BP (mmHg)	82 (74–90)	80 (73.5–86.5)	84 (76–90)	83 (75–90)	84 (72.5–90)	81 (76–86)
Laboratory parameters						
serum creatinine (mg/dl)	1.2 (0.97–1.7)	1.01 (0.84–1.13) <sup>b,c</sup>	1.2 (1–1.6)	1.6 (1.1–2.4)	1.2 (0.95–1.8)	1.1 (0.97–1.6)
serum albumin (g/dl)	2.2±0.6	2.5±0.6 <sup>b</sup>	2.2±0.7	2.0±0.5	2.2±0.6	2.2±0.6
total cholesterol (mg/dl)	272 (214–318)	232.5 (209–295)	277 (206–318)	288 (225–351)	257 (214–320)	288 (221–314.5)
HDL cholesterol (mg/dl)	52 (42–65)	63 (51–73) <sup>b,c</sup>	49 (42–61)	48 (39–58)	54 (42–66)	47.5 (40–60.5)
triglycerides (mg/dl)	158 (110–228)	110 (80–147) <sup>b,c</sup>	146 (130–205) <sup>b</sup>	227 (142–300)	142 (100–223)	191 (134.5–256.5)
proteinuria (g/24 h)	9.1 (5.8–12.8)	5.8 (4.3–9.6) <sup>b</sup>	8.2 (5.8–11.2) <sup>b</sup>	12.9 (9.4–18.5)	9.3 (5.8–12.7)	9 (6.4–13.3)
duration of persistent proteinuria (mo)	25.5 (11.7–67.7)	23.4 (12.3–63.6)	20.6 (11.9–66.3)	32.5 (11–76.4)	16.7 (9.2–31.4)	65.4 (35–80.5) <sup>a</sup>

Variables expressed as mean ± SD are compared using one-way ANOVA. Variables expressed as median (IQR) are compared using the Kruskal–Wallis test.

Categorical variables are expressed in percentages and compared using the chi-squared test.

<sup>a</sup>P<0.05 versus first-line therapy.

<sup>b</sup>P<0.05 versus no remission.

<sup>c</sup>P<0.05 versus partial remission.

had failed (Table 1). Concomitant treatments were similarly distributed across different considered patient groups (Supplemental Table A). During the study period, no patient received steroids or immunosuppressive drugs other than rituximab.

### Primary Outcomes

Over a median follow-up of 29 months (range, 6–121) after rituximab administration, 65 patients achieved complete or partial remission (Figure 2, upper panel). Remission was achieved over a median of 7.1 months (IQR, 3.2–12.0). Twenty-seven of those achieving the combined endpoint eventually progressed to complete remission. In 20 of the 35 patients who did not achieve the combined endpoint, urinary protein excretion decreased by at least 50% versus baseline. All of the 24 patients who were alive and free of dialysis after at least 4 years of follow-up achieved complete or partial remission.

A similar proportion of patients achieved complete or partial remission among those given rituximab as first-line (47 of 68) or second-line (18 of 32) therapy (Figure 2, middle and lowest panel). Eighteen of the 65 patients achieving complete or partial remission had a relapse of proteinuria from 7 through 116 months (median 42 months) after rituximab administration. All of them received a second course of rituximab that again achieved complete or partial remission in four and seven patients, respectively (Figure 3). No other patient received additional doses of rituximab throughout the study period.

### Secondary Outcomes

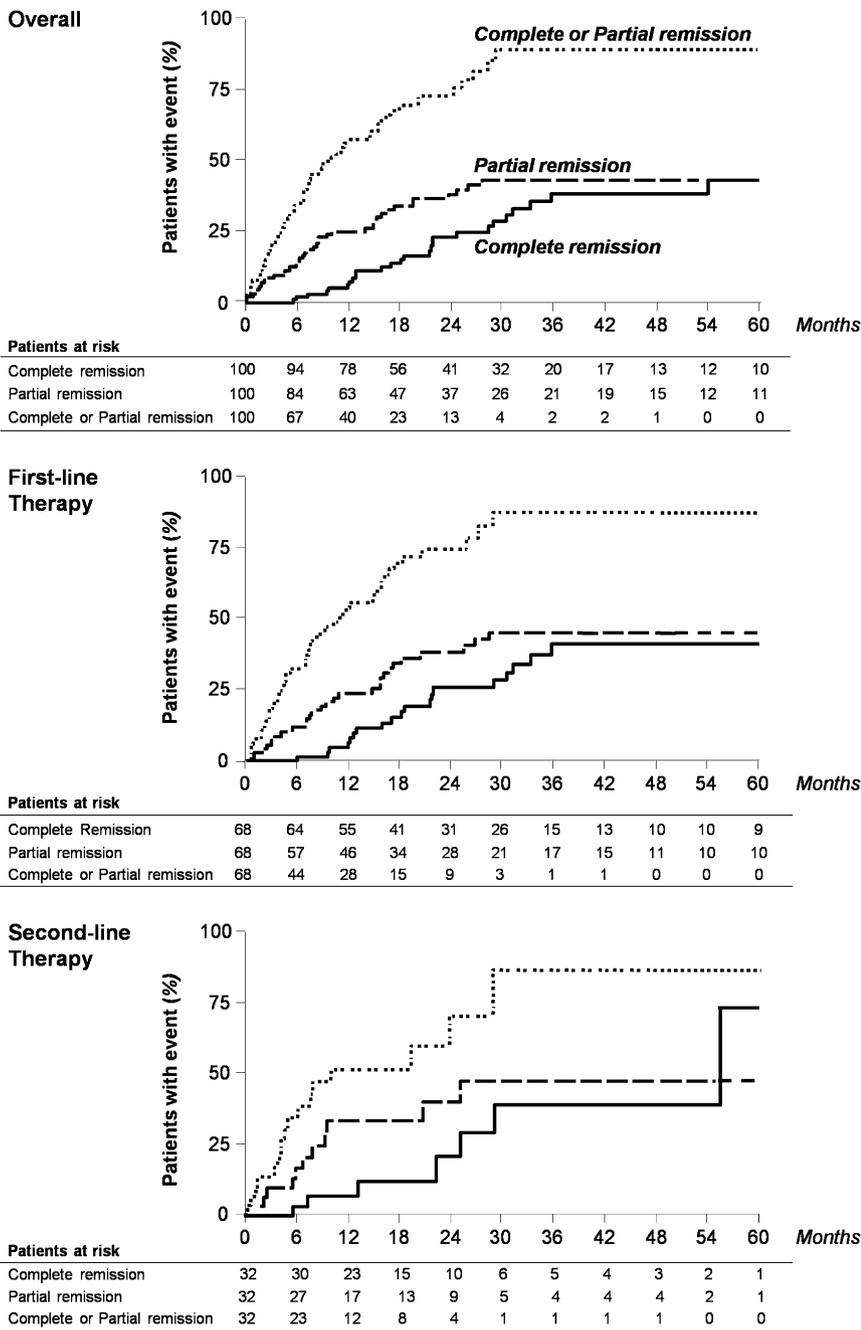
Proteinuria significantly ( $P<0.0001$ ) and progressively decreased by month 1 after rituximab infusion up to the last

available follow-up in the study group as a whole, as well as in different cohorts with homogeneous follow-up duration (Figure 4). Changes over time were paralleled by a progressive and significant increase in serum albumin concentration ( $P<0.0001$ ) and by a progressive decrease in serum cholesterol ( $P<0.0001$ ) levels that approximated normal ranges starting from month 6 after rituximab administration (Supplemental Figure A). Serum creatinine increased by >50% in 13 patients and it doubled versus baseline in 5 of them. Four patients progressed to ESRD. None of them had achieved remission during follow-up. ESRD was preceded by doubling of serum creatinine in two patients, and serum creatinine already exceeded 4 mg/dl at inclusion in one patient. The fourth patient had massive edema associated with a 24-hour proteinuria exceeding 20 g and required renal replacement therapy over 7 months.

Median GFR slope calculated on the basis of GFR values estimated by the Modified Diet in Renal Disease (MDRD) formula was 0.008 (IQR, –0.524 to 0.272) ml/min per 1.73 m<sup>2</sup> per month in the study group as a whole. GFR was slowly increasing in patients with complete (0.076 [0.161–0.256] ml/min per 1.73 m<sup>2</sup> per month) or partial (0.065 [–0.035 to 0.395] ml/min per 1.73 m<sup>2</sup> per month) remission, whereas it progressively declined (–0.611 [–1.036 to –0.042] ml/min per 1.73 m<sup>2</sup> per month) in those without remission ( $P=0.005$  and  $P=0.001$  versus complete and partial remission, respectively).

### Clearance Parameters

Overall, the GFR increased by 6.4±20.7 ml/min per 1.73 m<sup>2</sup> compared with baseline in parallel with a significant increase



**Figure 2.** Kaplan–Meier curves for the percentages of participants with IMN achieving complete remission, partial remission, or both. Data are in the study group as a whole (overall, upper panel) and among participants with or without treatment with other immunosuppressants before rituximab administration (first-line therapy, middle panel, and second-line therapy, lower panel, respectively). Twenty-seven of the patients with partial remission eventually achieved complete remission. Thus, in the group achieving the combined end point of partial or complete remission, these patients achieved both events. In this case, time to the first event (partial remission) was considered for survival analysis.

in serum albumin levels and decrease in albumin fractional clearance (Figure 5). The GFR significantly increased by  $13.2 \pm 19.6$  ml/min per  $1.73 \text{ m}^2$  in those achieving complete remission ( $P=0.021$ ), slightly improved in those with partial

remission, and decreased by  $3.8 \pm 11.1$  ml/min per  $1.73 \text{ m}^2$  in those without remission. Serum albumin significantly increased and albumin fractional clearance decreased in both groups with complete or partial remission, but did not change significantly in those without remission (Figure 5).

### Lymphocyte Subpopulations

Circulating B lymphocytes decreased to undetectable numbers by the day after the first rituximab infusion, with the exception of one patient who achieved complete B cell depletion after the second rituximab administration, and also when a second course of rituximab was needed in patients with disease relapse.

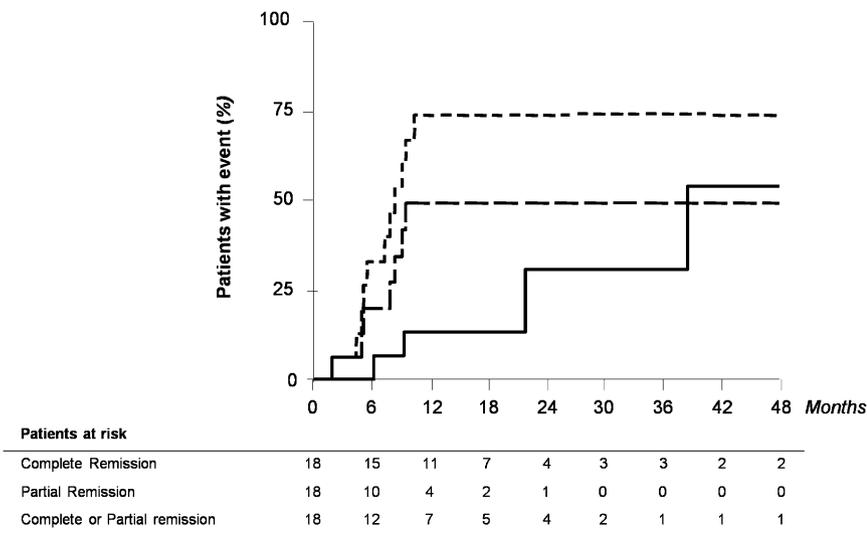
### Outcome Predictors

Multivariable Cox analyses included baseline variables that at univariable analyses were significantly ( $P<0.01$ ) associated with considered outcomes. Serum albumin and triglycerides levels were not considered because they were strongly correlated (Pearson correlation coefficient  $r \geq 0.6$ ) with proteinuria. Lower 24-hour urinary protein excretion ( $P<0.0001$ ) and serum creatinine levels ( $P=0.025$ ) significantly predicted higher probability of complete/partial remissions composite outcome (Supplemental Table B). Female sex ( $P=0.03$ ) and lower 24-hour urinary protein excretion ( $P=0.03$ ) predicted higher incidence of complete remission considered as a single outcome. Consistently, in a linear mixed-effect model including sex, creatinine, and proteinuria at baseline, proteinuria at baseline ( $P<0.0001$ ) and the length of the follow-up period ( $P<0.0001$ ) were significantly associated with decline of proteinuria over the time, whereas sex ( $P=0.063$ ) and serum creatinine ( $P=0.066$ ) were only borderline statistically significant. Larger proteinuria reduction on follow-up significantly predicted (Spearman's  $\rho=0.3772$ ;  $P=0.0001$ ) slower estimated GFR decline after rituximab administration.

### Adverse Events

#### Treatment-Related Events

Rituximab was well tolerated. Transient, nonserious adverse events were observed during rituximab administration in 28



**Figure 3.** Kaplan–Meier curves for the percentages of participants with IMN achieving complete remission, partial remission, or both within the subgroup of 18 patients who received a second course of rituximab because of disease relapse after initial complete or partial remission.

patients. Eight of them had a known history of allergy. In 17 cases, the events recovered just with temporary interruption of the infusion. In 10 patients with bronchial wheezing, but not dyspnea, and 1 patient with cutaneous rash, symptoms fully relieved after the injection of 125 or 250 mg of hydrocortisone. One patient had an episode of hypotension that recovered with treatment interruption, plasma expanders, and 500 mg of intravenous hydrocortisone.

#### Other Events

Eleven patients, seven in the group without remission and three and one in the groups with partial and complete remission, respectively ( $P=0.037$  for trend), had serious adverse events. Eight events were from cardiovascular causes (Table 2). One patient died from stroke at 83 years of age and two died from acute myocardial infarction when both were aged 79 years. One additional patient, who had been treated with steroids and cyclophosphamide 61 months before rituximab administration, died of lung cancer at 69 years of age (Table 2).

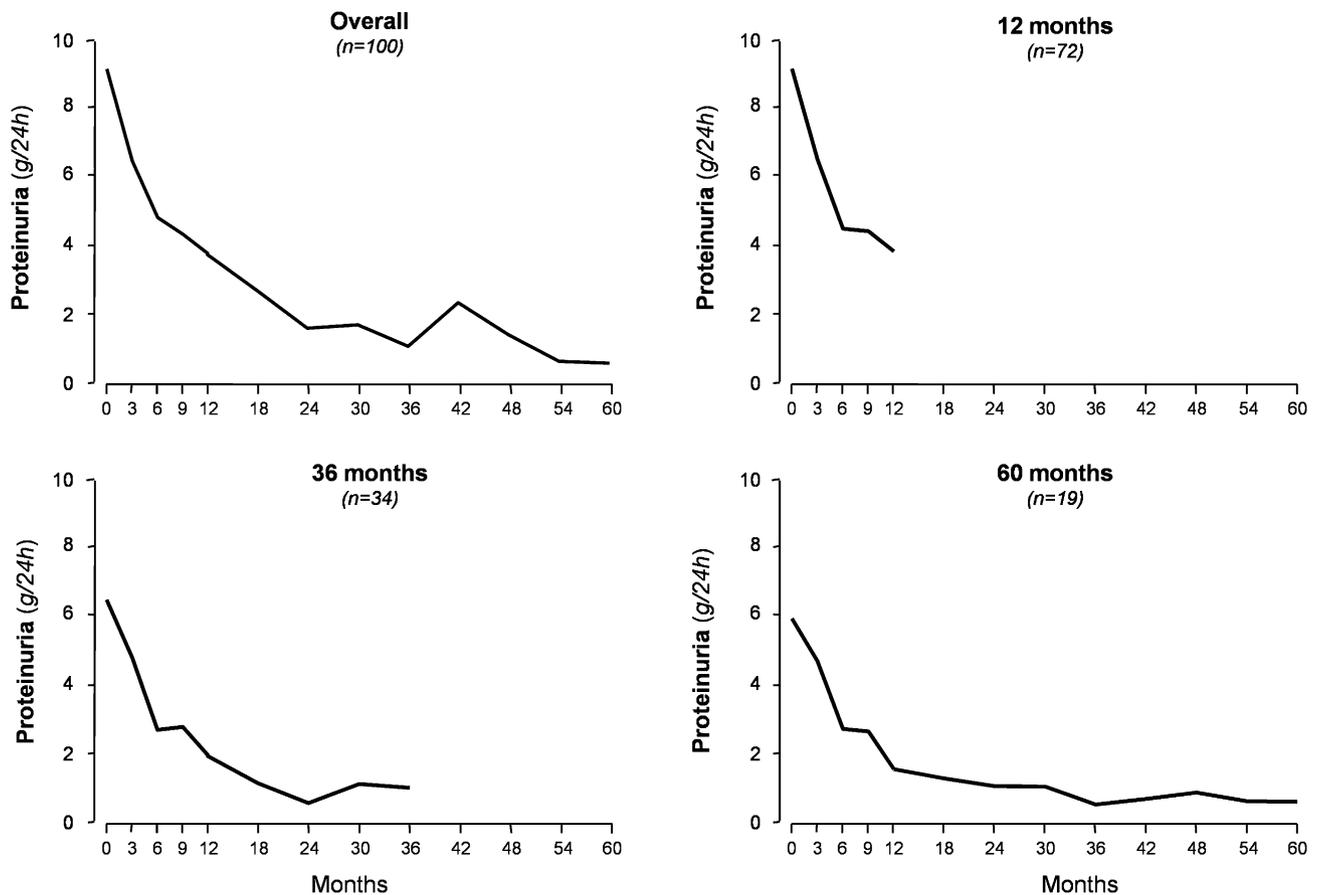
## DISCUSSION

Depletion of circulating B lymphocytes by rituximab treatment was associated with remission of the nephrotic syndrome in 65 of 100 consecutive patients with IMN who were predicted to progress to ESRD or die prematurely of disease-related complications, if untreated, due to long-lasting (from 11 to >80 months) proteinuria refractory to ACE inhibition therapy. Twenty-seven of these patients achieved complete remission with reduction of urinary protein excretion to normal range, and 20 of the 35 who failed to achieve remission had their proteinuria reduced to <50% of baseline values. Treatment

effect was time dependent and all patients with at least 4 years of follow-up achieved complete or partial remission. Rituximab was also effective when previous treatments with steroids and other immunosuppressive drugs, including cyclophosphamide and chlorambucil, had failed or a second course of rituximab was needed to treat disease recurrence after initial remission. Consistently with other studies, female sex, lower serum creatinine, and proteinuria levels at inclusion independently predicted a higher rate of disease remissions and more proteinuria reduction on follow-up. The reduction of proteinuria was progressive over time and was associated with an increase of serum albumin level to normal range and amelioration of dyslipidemia. To note, larger proteinuria reduction after rituximab administration predicted slower reduction, or even faster increase, in estimated GFR throughout the whole observation period.

Only four patients progressed to ESRD. They already had renal insufficiency at inclusion and their proteinuria did not appreciably decrease after rituximab treatment. Eight patients had major cardiovascular events. Five of them had failed to achieve remission after rituximab administration. Rituximab was well tolerated and no treatment-related serious events were observed throughout the whole study period. Nonserious events were transient and fully recovered without sequelae with interruption of rituximab infusion or, in a minority of cases, with intravenous steroids.

Altogether, the above findings converge to indicate that rituximab has a remarkably good risk-benefit profile for the treatment of IMN and that disease remission achieved by rituximab treatment, in addition to prevent terminal kidney failure, may also help reduce the excess cardiovascular risk in this population, particularly in most severe forms. The potential benefits of the immunosuppressive treatments for IMN used thus far<sup>4,19–24</sup> must be weighed against their risks. To note, the rate of treatment-related serious adverse events observed in real life in the 32 patients exposed to other immunosuppressants before rituximab administration—a rate that was surely underestimated because these events were recorded retrospectively without the systematic assessment that should characterize any controlled clinical trial—largely exceeded the figures reported in previous trials.<sup>23,24</sup> The risk of late adverse events has also been underestimated thus far because serious events such as acute myeloid leukemia, bladder and skin cancer, and other neoplasia may occur as late as 10–20 years after treatment exposure<sup>11</sup> and therefore they were not captured by the above trials.<sup>21</sup> In addition, this study was too short to capture long-term side effects of rituximab therapy. However, data in patients with autoimmune diseases or lymphoproliferative disorders exposed to extremely higher



**Figure 4.** Median 24-hour urinary protein excretion at baseline (month 0), at 3, 6, 9, and 12 months and at 6-monthly evaluations after rituximab administration in the study group as a whole (overall) and in different cohorts with homogeneous follow-up durations.

doses than in this study, or receiving long-life rituximab therapy for chronic lymphomas, consistently show that rituximab is remarkably safe, particularly compared with other immunosuppressants, over  $\geq 10$  years.<sup>25–27</sup>

An important difference between previous studies and this study is that selection of patients with long-lasting nephrotic syndrome despite ACE inhibitor therapy allowed us to reasonably exclude any appreciable confounding effect of spontaneous remissions. Moreover, none of our patients had evidence of myelotoxicity, lymphoproliferative disorders, or life-threatening opportunistic infections, and the three cases of cancer observed in 100 patients over a median follow-up of 29 months reflects the age-adjusted incidence of neoplastic disease in the general population.<sup>28</sup> To note, the patient who eventually died of lung cancer had been previously exposed to steroids and cyclophosphamide. The above data confirm and extend previous evidence of a remarkable safety profile of rituximab not only from small studies in IMN<sup>15,18,29</sup> but also from large, long-term trials in lymphoproliferative disorders,<sup>30</sup> rheumatoid arthritis,<sup>31</sup> and other immune-mediated diseases.<sup>32,33</sup> Evidence that in our patients proteinuria reduction was preceded by complete B cell depletion and disease recurrence by B lymphocyte re-emergence in the circulation,

**Table 2.** Patients with at least one serious adverse event in the study group as a whole (overall) and according to disease outcome (complete, partial, or no remission)

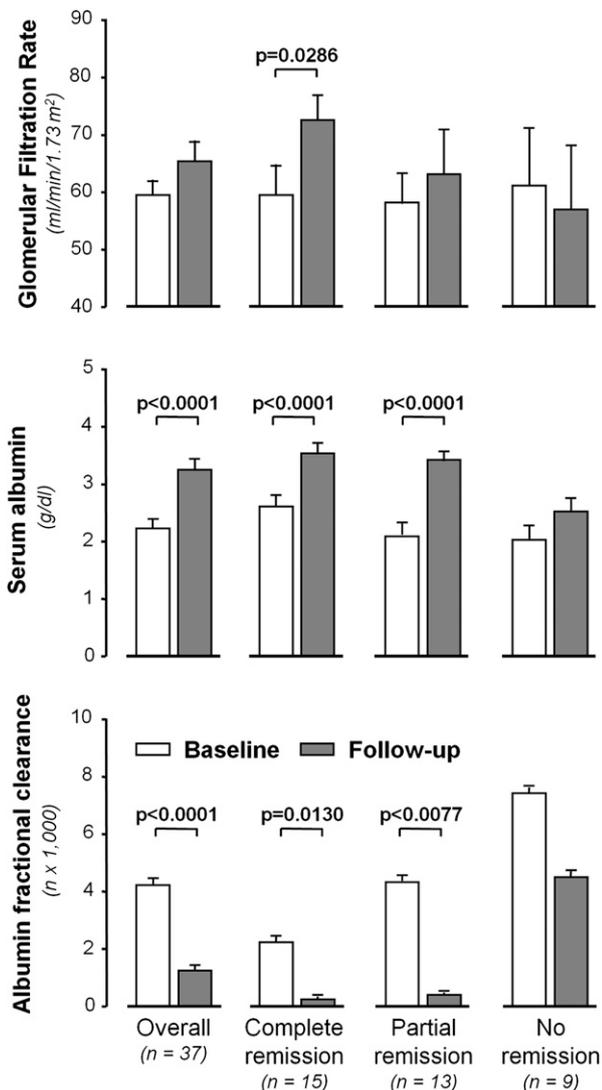
Serious Adverse Event	Overall	Complete Remission	Partial Remission	No Remission
Fatal	4	0	1	3
cardiovascular	3	0	1	2
stroke	1	0	1	0
acute MI	2	0	0	2
cancer <sup>a</sup>	1	0	0	1
Nonfatal	7	1	2	4
cardiovascular	5	1	1	3
stroke	2	0	0	2
acute MI <sup>b</sup>	1	0	1	0
TIA	2	1	0	1
cancer <sup>c</sup>	2	0	1	1
Total	11	1	3	7

The incidence of events in participants with complete remission was lower than in those with partial or no remission ( $P=0.037$ , Cochran–Armitage test for trend). MI, myocardial infarction; TIA, transitory ischemic attack.

<sup>a</sup>Lung cancer.

<sup>b</sup>Event during a relapse of nephrotic syndrome.

<sup>c</sup>Breast and prostate cancer.



**Figure 5. Baseline and follow-up GFR, serum albumin, and albumin fractional clearance in the subgroup of participants with clearance evaluations.** GFR, measured by the iohexol plasma clearance technique (upper panel), serum albumin levels (middle panel), and albumin fractional clearance (lower panel) at baseline and after rituximab infusion in 37 participants with IMN. Data are in the study group as a whole (overall) or in patients with complete remission, partial remission, or no remission considered separately. Data are mean  $\pm$  SEM.

corroborated the hypothesis of a cause and effect relationship between the production of nephritogenic antibodies by autoreactive B lymphocytes and disease activity.<sup>34–38</sup> An additional unique aspect of our study, compared with previous trials in IMN, is that a subgroup of patients had their GFR measured by a gold standard technique.<sup>39</sup> This allowed discovering that complete remission was associated with a significant improvement of the GFR that approximated 13 ml/min per 1.73 m<sup>2</sup> versus baseline. The GFR marginally improved in patients achieving partial remission but, as expected, decreased

by approximately 4 ml/min per 1.73 m<sup>2</sup> in those who failed to achieve disease remission. Consistently, analyses of slopes calculated on the basis of estimated GFR values showed that in the whole study group, complete and partial remission were also associated with improving or stable kidney function, whereas persistent nephrotic syndrome was invariably associated with progressive renal function loss over time. To note, the finding that in patients achieving complete or partial remission serum albumin increase to normal range was associated with significant reduction of albumin fractional clearance, allowed us to conclude that amelioration of the signs of the nephrotic syndrome was likely explained by improved glomerular sieving function in this population. Combined to previous evidence that remission of proteinuria is also associated with regression of kidney structural and ultra-structural changes,<sup>40</sup> these findings can be taken to suggest that rituximab therapy might have protective effects against renal disease progression also in the long term.<sup>41</sup> By and large, the above findings support the notion that rituximab therapy is at least as effective and remarkably safer than immunosuppressive regimens including steroids and alkylating agents. This conclusion is consistent with evidence that in this study, despite the inclusion of patients with more severe disease, the rate of complete or partial remissions was similar to that observed in previous series of patients given methylprednisolone and chlorambucil or cyclophosphamide,<sup>5,6</sup> and renal function loss was slower<sup>42</sup> or even prevented (Figure 5). Moreover, steroids and alkylating agents were associated with serious adverse events, requiring treatment withdrawal in some cases, never observed after rituximab administration (Table 3).

A limitation to rituximab treatment may be the drug cost. Independent of treatment benefits that in any cost/effectiveness analyses are prioritized to pure treatment costs, one 375-mg/m<sup>2</sup> dose of rituximab (the current standard therapy for IMN at our center<sup>43</sup>) in an average 70-kg patient costs €3100 (\$4130). Six-month alternate treatment with intravenous plus oral steroids and oral cyclophosphamide at currently recommended doses costs approximately €450 (\$600). Six-month treatment with cyclosporin A or mycophenolate mofetil would be much more expensive. Considering that 1-day hospitalization in a nonintensive care unit costs €300–500 (\$400–666), just one admission because of a treatment-related adverse event would largely offset the costs saved with steroids plus alkylating agents versus rituximab therapy.

### Limitations and Strengths

The major limitation of this study is the lack of controls given other immunosuppressive drugs or conservative therapy alone. Per center practice, we never used cyclophosphamide or chlorambucil due to the associated risk of serious adverse events. Conceivably, patients with severe side effects upon exposure to immunosuppressive therapy in other centers were preferably referred to our unit for rituximab therapy. There were no controls on conservative therapy alone due to the concern of withholding a potentially effective treatment in

**Table 3.** Number of patients with treatment-related adverse events that were serious and/or required treatment interruption in different series of patients with IMN and the nephrotic syndrome

Adverse Event	Rituximab (n=100)	Prednisone + Chlorambucil (n=42)	Prednisone + Cyclophosphamide (n=47)
Infections	0	1 (2.4)	10 (21.3)
pneumonia	0	1 (2.4) <sup>a</sup>	3 (6.4)
disseminated tuberculosis	0	0	1 (2.1)
other	0	0	6 (12.8)
Other	0	7 (16.7)	0
myelotoxicity	0	2 (4.8)	0
gastric/liver toxicity	0	4 (9.5) <sup>a</sup>	0
diabetes	0	1 (2.4)	0
Total	0	8 (19.0)	10 (21.3)

Data are presented as n (%).

<sup>a</sup>Treatment was stopped in four patients because of pneumonia (n=1), peptic ulcers (n=2), and gastric intolerance to chlorambucil (n=1).

patients with very poor prognosis.<sup>2,3</sup> Thus, the uncontrolled design did not allow us to definitely exclude the possibility of spontaneous remissions, at least in those with less severe proteinuria at inclusion. The above limitation was addressed by testing the effect of rituximab per protocol in participants who had still >3.5 g of 24-hour proteinuria after a 6-month run-in period on ACE inhibitors. On the basis of our previous findings,<sup>44</sup> 20–25 of our 100 IMN patients were expected to progress to ESRD over the observation period, a figure that largely exceeded the number of those (n=4) who actually progressed to ESRD after rituximab administration. To note, in patients given rituximab as second-line therapy, previous duration of the nephrotic syndrome approximated 6 years. Both the extremely long duration of proteinuria, which exceeded the time window after disease onset that is normally associated with spontaneous remissions,<sup>3</sup> and its persistency despite previous treatments could be taken to rule out the possibility of remissions not related to rituximab as a confounding variable. On the other hand, similar rates of remissions in those receiving rituximab as first-line therapy can be taken to exclude any appreciable role of spontaneous remissions in both groups. Finally, evidence that the remission rate after rituximab re-treatment because of disease recurrence was similar to that observed after the first course of rituximab therapy further corroborated the hypothesis of a cause and effect relationship between treatment and observed outcomes.

Strengths of our study are that patients were prospectively monitored with standardized evaluations, including GFR measurements by a gold standard technique, and that proteinuria was averaged from three consecutive urine sample collections.

In summary, B lymphocyte depletion by rituximab treatment achieved disease remission and stabilized or even improved renal function in a large cohort of IMN patients at high risk of poor outcomes because of persistent nephrotic syndrome, and was

remarkably safe. Our present findings in IMN provide a good landmark for further research and a future, formal comparison of therapeutic regimens in those patients with nephrotic syndrome unresponsive to conservative therapy.

## CONCISE METHODS

Since April 2001, we have elected rituximab treatment for all consecutive patients referred to our nephrology unit presenting with the following criteria: biopsy-proven membranous nephropathy, creatinine clearance >20 ml/min per 1.73m<sup>2</sup>, 24-hour proteinuria persistently exceeding 3.5 g despite at least 6-month therapy with full-dose ACE inhibitors and optimized conservative therapy, and no circulating hepatitis B surface antigens.<sup>44,45</sup> Patients with secondary forms of membranous nephropathy were not considered. The treatment protocol was approved by the Ethical Committee of the Clinical Research Center of the Mario Negri Institute and of the Azienda Ospedaliera, Ospedali Riuniti, Bergamo, Italy. Patients gave written informed consent to rituximab treatment according to the Declaration of Helsinki. Rituximab was supplied by the Pharmacy of the Azienda Ospedaliera. No pharmaceutical company was involved.

### Treatment and Monitoring

Before rituximab administration, proteinuria was measured in three consecutive 24-hour urine collections and the average value was recorded. Creatinine excretion was measured in all collections to assess their correctness and the last measurement was considered for the calculation of creatinine clearance. A blood sample was collected for hematochemistry and blood cell counts. Rituximab (375 mg/m<sup>2</sup>) was reconstituted in saline to a final concentration of 1 mg/ml and was infused at an initial rate of 40 ml/h, progressively increased to 200 ml/h according to tolerability. An intensivist was alerted in the case of adverse events during the infusion. Up to October 2005, patients received four weekly rituximab doses. Thereafter, patients received a second rituximab infusion only when >5 circulating B cells per mm<sup>3</sup> were detected the morning after completion of the first rituximab administration.<sup>43</sup> All of the clinical and laboratory evaluations at baseline were then repeated at months 1, 2, 3, 6, 9, and 12 after rituximab administration and at least every 6 months thereafter.

### Clearance Studies

Before rituximab infusion and at the final visit, all of the 37 patients who consented to clearance evaluations had their GFR measured by the iothexol plasma clearance technique.<sup>39</sup> During the clearance studies, albumin concentrations in plasma and urine samples were measured to calculate albumin fractional clearance (albumin clearance/GFR).

### Primary Outcomes

Primary outcomes were complete or partial remission defined as 24-hour urinary protein excretion <0.3 or 3.0 g (with at least 50% reduction versus baseline), respectively, in at least two consecutive visits.<sup>20</sup> Patients not fulfilling the above criteria were considered as nonresponders. A relapse was diagnosed whenever 24-hour

proteinuria increased to  $\geq 3.5$  g after a period of partial or complete remission.<sup>46</sup>

### Secondary Outcomes

Secondary outcomes included changes over time in 24-hour proteinuria, estimated GFR (by the MDRD formula), and in other components of the nephrotic syndrome serially monitored throughout the observation period as well as in GFR, serum albumin, and albumin fractional clearance, all considered as continuous variables. Safety parameters included any serious and nonserious adverse event and unexpected changes in clinical or laboratory parameters observed throughout the whole follow-up period.

### Statistical Analyses

All patients with at least 6 months of follow-up were considered for the analyses. The Kaplan–Meier method was used to plot the probability of achieving complete remission, partial remission without subsequent complete remission, or the complete and/or partial remission composite endpoint. Survival time was determined from the beginning of the first treatment until the event of interest. Patients not achieving remission were considered as censored at the time of the last visit with a nonmissing value of proteinuria. In the complete/partial remission composite endpoint the survival time for participants was time to event until partial remission. In a multivariable context, the Cox regression model was carried out to test predictors of achieving complete remission and complete/partial remission composite endpoint (Supplemental Material).

The multivariable Cox model included proteinuria at baseline and all of the baseline covariates in the univariate Cox analysis that were significantly ( $P < 0.01$ ) associated with the outcome, with the exception of variables that were strongly correlated with proteinuria. Due to their skewed distribution proteinuria, creatinine, triglycerides, and duration of persistent proteinuria were log-transformed before statistical analyses. To test the robustness of results, predictors of proteinuria considered as continuous outcome were identified using linear mixed-effect models to assess the effect of the baseline covariates on the decline of proteinuria over time. This model incorporates random effects (with an unstructured covariance matrix) to account for correlated observations on the same patient. The rate of estimated GFR decline was evaluated by a single linear model by using at least three GFR values, including baseline, estimated with the MDRD formula, and compared across groups using the Wilcoxon rank-sum test. The correlation between proteinuria reduction on follow-up and estimated GFR decline after rituximab administration was evaluated by using the Spearman's rank correlation test.

Analyses were carried out using SAS (version 9.1) and Stata (version 11) software. The data of baseline characteristics were presented as numbers and percentages, means and SDs, or medians and IQRs, as appropriate. Comparisons between groups were made using one-way ANOVA, the Kruskal–Wallis test, the chi-squared test, or the Cochran–Armitage test for trend, as appropriate. The multiple comparisons issue was addressed by means of Bonferroni adjustment. The follow-up data were expressed as medians and ranges or IQRs. Normality for continuous variables was assessed by means of the Q-Q plot. All  $P$  values were two-sided.

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### DISCLOSURES

None.

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