Rituximab therapy in nephrotic syndrome: implications for patients' management

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Abstract | Rituximab offers an alternative to current immunosuppressive therapies for difficult-to-treat nephrotic syndrome. The best outcomes are seen in patients with steroid-dependent nephrotic syndrome who have failed to respond to multiple therapies. By contrast, the benefits of rituximab therapy are limited in patients with steroid-resistant nephrotic syndrome, particularly those with focal segmental glomerulosclerosis (FSGS). Therapy with plasma exchange and one or two doses of rituximab has shown success in patients with recurrent FSGS. Young patients and those with normal serum albumin at recurrence of nephrotic syndrome are most likely to respond to rituximab therapy. A substantial proportion of rituximab-treated patients with idiopathic membranous nephropathy show complete or partial remission of proteinuria, and reduced levels of phospholipase A_2 receptor autoantibodies, which are implicated in the pathogenesis of this disorder. Successful rituximab therapy induces prolonged remission and enables discontinuation of other medications without substantially increasing the risk of infections and other serious adverse events. However, the available evidence of efficacy of rituximab therapy is derived chiefly from small case series and requires confirmation in prospective, randomized, controlled studies that define the indications for use and predictors of response to this therapy.

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Introduction

Rituximab is a chimaeric monoclonal antibody against CD20, an antigen expressed during most stages of B-cell development. Binding of rituximab to CD20 causes rapid depletion of B-cell populations and has been used for the treatment of a number of autoimmune disorders.[1] This agent is approved for therapy in non-Hodgkin lymphoma, chronic lymphocytic leukaemia, vasculitis and rheumatoid arthritis.[2,3] In addition, rituximab has been tested in clinical trials for the treatment of systemic lupus erythematosus, and is used off-label for post-transplant lymphoproliferative disease.[2] Rituximab has also been used in the treatment of patients with nephrotic syndrome, including those with steroid-dependent nephrotic syndrome or steroid-resistant nephrotic syndrome, recurrent focal segmental glomerulosclerosis (FSGS) and membranous nephropathy (Box 1).[4–7]

This Review provides an overview of the available data on the safety and efficacy of rituximab in the treatment of paediatric and adult patients with nephrotic syndrome. Most reports are anecdotal or limited to case series, as limited data on the efficacy and safety of this treatment are available from prospective controlled trials. Owing to the retrospective nature of these reports, the indications for rituximab administration to patients are unclear and definitions of response are heterogeneous. Accordingly, we have reviewed studies on the efficacy of rituximab in patients with steroid-resistant nephrotic syndrome, recurrent FSGS or membranous nephropathy, in which achievement of complete or partial remission is reported on the basis of standard definitions, or by the authors of individual studies.[8–10] For patients with steroid-dependent nephrotic syndrome, we have reviewed studies in which the frequency of relapses, the use of corticosteroids and other immunosuppressive agents and the need for re-treatment with rituximab was reported.

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Competing interests
The authors declare no competing interests.

Mechanisms of action

CD20 is a calcium-channel protein expressed in B cells during maturation, in precursor B cells and mature B cells, but not in plasma cells.[11] Although the function of CD20 is not clear, binding of rituximab or other anti-CD20 monoclonal antibodies to this ligand *in vitro* activates apoptosis via complement-dependent cytotoxicity and antibody-dependent cytotoxicity, which leads to rapid depletion of B cells.[11,12] After binding to rituximab, CD20 is translocated into lipid rafts, where signalling through tyrosine kinases, mitogen-activated protein kinases and phospholipase Cγ mediates inhibition of B-cell growth or leads to apoptosis.[12–14]

The mechanisms by which rituximab induces remission in patients with nephrotic syndrome are unclear (Figure 1). Proteinuria in patients with minimal change disease and FSGS might be mediated by unrecognized permeability factor(s), perhaps a T-cell cytokine.[15] Since B cells activate T-helper cells through antigen presentation, depletion of B cells might alter T-cell function or

subpopulation expansion.[16] Alternatively, the beneficial effects of rituximab therapy in patients with nephrotic syndrome might be mediated through restoration of T-regulatory (T_{REG}) cell populations and/or upregulation of their functions, similar to changes seen in patients with idiopathic thrombocytopenic purpura, systemic lupus erythematosus and cryoglobulinaemic vasculitis.[17–22] Another study showed that the proportion of B-regulatory (B_{REG}) cells was increased during B-cell recovery in mice with autoimmune diabetes when they were given rituximab. Rituximab therapy may therefore induce T_{REG} and B_{REG} cell populations that restore immune tolerance.[23]

Several authors suggest that patients with nephrotic syndrome have deficient T_{REG} cell function.[24–28] In the 'two-hit' hypothesis of minimal change disease, proteinuria is initiated by podocyte expression of CD80, a T-cell co-stimulatory molecule.[29] Persistent proteinuria might result from faulty cessation of CD80 expression, owing to impaired podocyte autoregulation or impaired production of soluble CTLA4 by circulating T_{REG} cells. After rituximab treatment, patients with membranous nephropathy have transiently increased numbers of $CD3^+$ and $CD4^+$ T cells, and a persistently increased number of natural killer cells, but no change in the number of T_{REG} cells.[30] However, two reports, currently published as abstracts, indicate that therapy with rituximab produces an absolute or relative increase in the number of T_{REG} cells.[31,32]

The rapidity with which rituximab reduces proteinuria in patients with idiopathic or recurrent FSGS suggests that this treatment has direct actions on the podocyte (Figure 1). The CD20-binding region of rituximab crossreacts with an amino acid sequence within acid sphingomyelinase-like phosphodiesterase 3b (SMPDL3b).[33] CD20 engagement by rituximab upregulates sphingomyelin phosphodiesterase (also known as acid sphingomyelinase) activity in B cells.[34] The number of $SMPDL3b^+$ podocytes was lower in patients who developed recurrent FSGS after renal transplantation than in those who did not.[35] Normal human podocytes incubated with sera from patients with recurrent FSGS showed downregulation of SMPDL3b, the presence of actin stress fibres and disruption of the cytoskeleton. These findings were attenuated or reversed in the presence of rituximab.[35] Interestingly, in a podocyte model of HIV nephropathy, an altered actin cytoskeleton and diminished podocyte attachment were associated with decreased sphingomyelin phosphodiesterase activity.[36] However, the role of this enzyme in the pathogenesis of FSGS requires confirmation in further studies.

Finally, patients with recurrent FSGS have high levels of soluble urokinase plasminogen activator surface receptor (uPAR) in serum.[37] Experiments using transgenic mice and podocyte cultures show that sera from such patients activates podocyte uPAR and integrin-linked protein kinase, leading to foot process effacement, proteinuria and FSGS. Moreover, elevated serum levels of soluble uPAR are associated with a decrease in the number of T_{REG} cells.[38] The effect of rituximab on serum levels of soluble uPAR and activation of podocyte β_3 integrin is being examined in patients with FSGS.[39] These findings need confirmation in large studies and across different populations.

Key points

- Therapy with rituximab induces and maintains remission effectively in patients with difficult-to-treat, steroid-dependent nephrotic syndrome; sustained remission enables the reduction of steroid doses and withdrawal of calcineurin inhibitors
- In patients with steroid-resistant nephrotic syndrome who fail to respond to treatment with calcineurin inhibitors, the response to rituximab therapy is less efficacious
- Rituximab dose(s), the rate of B-cell recovery and clinical response are not closely correlated
- Combined therapy with rituximab and plasma exchange might be useful to prevent or treat recurrence of focal segmental glomerulosclerosis
- Therapy with rituximab should be considered in patients with idiopathic membranous nephropathy who fail to respond to treatment with cyclophosphamide or calcineurin inhibitors
- Acute infusion reactions are frequent but transient in patients who receive rituximab; serious adverse effects, including an increased risk of infections, are uncommon

Box 1 | Definitions of disease status and response to therapy

Nephrotic range proteinuria
Adults: Proteinuria >3.5 g per day.
Children: >40 mg/m^2 per hour; urinary protein:creatinine ratio >2 mg/mg or >200 mg/mmol.

Steroid-dependent nephrotic syndrome
Two consecutive relapses while receiving predniso(lo)ne on alternate days, or within 15 days of its discontinuation.

Steroid-resistant nephrotic syndrome
Lack of remission despite 4–8 weeks of therapy with daily predniso(lo)ne at a dose of 60 mg/m^2 or 2 mg/kg (maximum 60–80 mg) per day.

CNI-dependent nephrotic syndrome
Remission of steroid-dependent nephrotic syndrome is achieved during therapy with CNIs (tacrolimus or ciclosporin).

CNI-resistant and steroid-resistant nephrotic syndrome
No response to therapy with predniso(lo)ne as defined above, or to CNI therapy for 3–6 months.

Complete remission
Adults: Proteinuria <0.3–1.0 g per day, normal serum albumin (>30 g/l), and stable renal function.
Children: Urinary protein:creatinine ratio <0.2–0.3 mg/mg or <30 mg/mmol and normal serum albumin (>30 g/l).

Partial remission
Adults: Proteinuria 0.3–3.5 g per day and/or ≥50% decrease in proteinuria from baseline, and stable renal function.
Children: Urinary protein:creatinine ratio 0.2–2.0 mg/mg or 30–350 mg/mmol; and serum albumin >30 g/l.

Abbreviation: CNI, calcineurin inhibitor.

Determinants of treatment efficacy

CD20 is internalized after binding to rituximab. Consequently, CD19 (another B-cell surface antigen) is used as a marker for reliable monitoring of B-cell numbers in rituximab-treated patients.[40] Pharmacokinetic studies in patients with rheumatoid arthritis show that rituximab follows a two-compartment model with a terminal half-life of 19–22 days.[41,42] The terminal

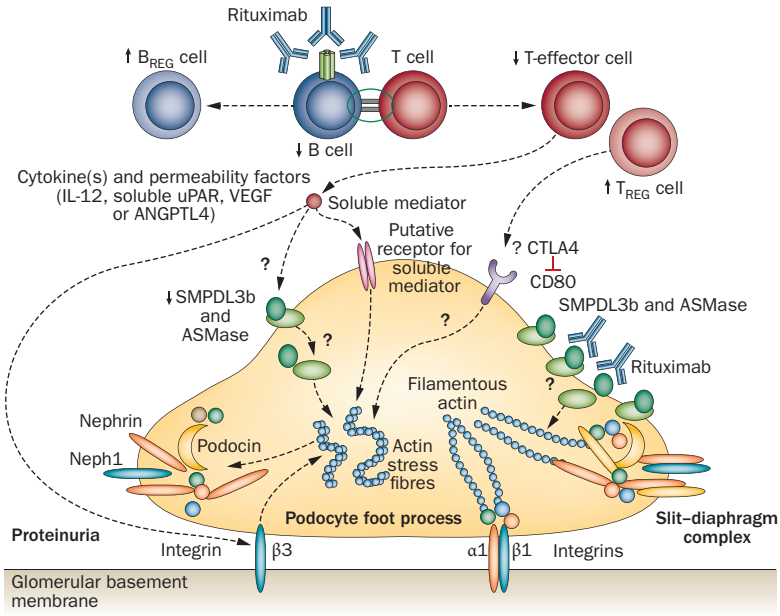

Figure 1 | Proposed mechanisms of action of rituximab in patients with nephrotic syndrome. B-cell depletion affects antigen presentation and activation of T cells through interaction with the T-cell receptor (circled in green), as well as inhibiting the production of cytokine(s) that might increase glomerular permeability. The role of rituximab in suppressing other permeability factors (for example, soluble uPAR, VEGF and ANGPTL4) is unclear. In an alternative model of minimal change disease, proteinuria is initiated by an increase in the expression of CD80 in podocytes. Production of CTLA4 by T_{REG} cells, potentially enhanced by rituximab, might inhibit CD80 activation and reduce proteinuria. The role of B_{REG} cells in mediating these effects is speculative. Finally, downregulation of SMPDL3b expression and loss of ASMase activity might lead to the formation of actin stress fibres, cytoskeletal disorganization and proteinuria, as shown in models of focal segmental glomerulosclerosis. Therapy with rituximab might reverse these changes. Abbreviations: ANGPTL4, angiopoietin-related protein 4; ASMase, acid sphingomyelinase; CTLA4, cytotoxic T-lymphocyte protein 4; REG, regulatory; SMPDL3b, sphingomyelin phosphodiesterase acid-like 3b; uPAR, urokinase plasminogen activator receptor; VEGF, vascular endothelial growth factor.

half-life of rituximab is 2.7-fold longer after the fourth dose than after the first dose.[43] Patients given four doses of rituximab also have a twofold higher serum level and prolonged serum half-life of the medication compared with patients who received a single dose.[44–46]

Prospective studies in patients with nephrotic syndrome show that one dose of rituximab results in profound B-cell depletion (<1% of leukocytes, or 0–5 cells/mm^3) in 83.3–90.0% of patients, and that B-cell depletion lasts for 4–6 months after a single dose of rituximab.[44,47] However, the relationships between the number of doses of rituximab and time to B-cell recovery, and between B-cell recovery and the occurrence of relapse of nephrotic syndrome have not been established.[48,49] After maximal B-cell depletion, which occurs by 2 weeks after therapy with two doses of rituximab, B cells start to reappear from week 16 onwards.[42] In one study, all 19 patients who responded to rituximab therapy were in remission during B-cell depletion, and the patients who relapsed showed recovery of B cells to 3–7% of leukocytes.[48] Although the above finding has been confirmed in other studies,[44,47] an equally important observation is that some patients have sustained remission despite B-cell recovery.[44] In patients with rheumatoid arthritis, the timing of B-cell recovery bears no relation to the recurrence of symptoms.[42] Similarly, in a nationwide survey of rituximab treatment for childhood-onset, difficult-to-treat nephrotic syndrome conducted in Japan, the time to B-cell recovery was similar in patients who relapsed compared with those in remission.[50]

The timing of re-treatment with rituximab is based on either the extent of B-cell recovery or the occurrence of one or more relapses. In one study, doses of rituximab were repeated in 12 of 19 patients who showed recovery of B cells after the initial clinical response.[48] Others have used multiple courses of rituximab to achieve sustained B-cell depletion for 15–18 months in patients with steroid-dependent nephrotic syndrome.[49,51] Prolonged B-cell depletion was associated with sustained remission for 2 years in two-thirds of patients, despite B-cell recovery, and without the use of additional immunosuppressive agents.[51] Other research groups have successfully used re-treatment with rituximab to treat relapses.[44,52,53]

It is speculated that proteinuria in the nephrotic range might attenuate the biological effects of rituximab by causing increased urinary loss of the drug.[48] Serum levels of rituximab were lower in patients with membranous nephropathy with substantial proteinuria than in those given rituximab during remission, or in patients with rheumatoid arthritis.[30,45] Decreased efficacy of rituximab in patients with proteinuria in the nephrotic range has been proposed in other studies,[48,53] which suggests that rituximab should be administered during remission. Although reduction of proteinuria by maximal inhibition of the renin–angiotensin–aldosterone axis might potentially reduce urinary losses of rituximab and increase its efficacy, this strategy requires prospective confirmation.

Most studies of patients with nephrotic syndrome have used a rituximab dose of 375 mg/m^2 once a week for 1–4 weeks, which is adapted from the standard regimen for lymphoma. Serum levels of rituximab are similar irrespective of whether it is administered to children or adults, and whether doses of 375 mg/m^2 or 1 g/m^2 are used.[45] Evidence from case series suggests that remission is prolonged after therapy with 2–4 doses of rituximab;[52,54,55] therefore, we propose that patients with nephrotic syndrome should receive two or more doses of this agent. Prospective studies are, however, required to examine the relationship between the number of rituximab doses and the chronology of B-cell recovery, and how this relationship affects the clinical response.

Steroid-dependent nephrotic syndrome

Approximately 40–60% of children with idiopathic nephrotic syndrome have frequent relapses or are dependent on steroid treatment.[56] Although medications such as cyclophosphamide, levamisole and mycophenolate mofetil reduce the frequency of relapses in most patients,[57,58] the clinical management of some patients is difficult. Treatment with calcineurin inhibitors is effective, but is associated with considerable toxic effects

Table 1 | The efficacy of rituximab in steroid-dependent nephrotic syndrome

Study	Patients (*n*)	Age*; duration of disease* (years)	Rituximab therapy (375 mg/m^2 once weekly)	Follow-up* (months)	Results	
					Outcomes (number of patients)	Immunosuppressive therapy
Guigonis *et al.* (2008)[48]	22‡	14.3 (6.3–22.1); 11 (3.6–16.5)	3–4 doses in 19 patients, 2 doses in 3, 12 received >2 courses	10 (6–39)	Sustained remission 16 Relapse 3 Time to relapse 12 (7–17)* months	Mean decline by 70% of doses of all agents; ≥1 agent discontinued in 19 patients
Kamei *et al.* (2009)[44]	12	12.7 (5–19); 7. 2 (1.5–10.6)	1 dose, 7 patients received >2 courses	12	Sustained remission 3 Relapse 9 Time to relapse 4 (0.3–12)* months	Steroids stopped in all patients; other agents stopped in 8 patients
Fujinaga *et al.* (2010)[47]	10	11.6 (3.9–18.8); 4.6 (2.8–10.8)	1 dose, 1 patient received >2 courses	17 (13–21)	Sustained remission 4 Relapse 6 patients Time to relapse 9 (0–17)* months	Mean dose of steroids reduced by 63%; ciclosporin discontinued in 5 patients; restarted in 2 patients
Gulati *et al.* (2010)[52]	24	11.7 (5–17); 8.9 (2.4–14)	2 doses, 1 patient received >2 courses	17 (12–38)	Sustained remission 17 Relapse 7 Time to relapse 11.2 (8–14)*	Steroid dose reduced in all patients; all agents discontinued in 12 patients
Prytula *et al.* (2010)[54]	28	NA; NA	3–4 doses in 21 patients, 1–2 doses in 7, 5 received >2 courses	5 (1–10)	Sustained remission 10 Relapse 13 Time to relapse 6 (1–16)* months	19 of 19 patients received steroids; 16 of 19 patients received other agents
Sellier-Leclerc *et al.* (2010)[49]	22§	13.5 (6.9–19.7); 10.6 (2.6–17.5)	3–4 doses in 17 patients, 1–2 doses in 5, 19 received >2 courses	11–29	Sustained remission 13 Relapse 9 Time to relapse 3–12 months	Steroids discontinued in 19 patients; all agents discontinued in 17 patients
Hoxha *et al.* (2010)[53]	6	22.5 (19–33); 2.4–16.1	1 dose, 5 patients received >2 courses	18 (12–23)	Sustained remission 3 (partial in 1) Relapse 3 Time to relapse 4–12 months	Steroids discontinued in all patients; other agents tapered
Kemper *et al.* (2010)[55]	37	13.4 (6.4–18.2); 2.0–14.8	1–2 doses in 27 patients, 3–4 doses in 10, 19 received >2 courses	29 (9–93)	Sustained remission 12 of 29 Relapse 25 Time to relapse 10 (5–64) months	Steroids discontinued in 35 patients; all agents discontinued in 22 patients
Sugiura *et al.* (2011)[61]	9	27 (18–60); 10 (0.5–18)	1 dose, none received >2 courses	≥6	Sustained remission 9 (partial in 1) Relapse NA	Mean dose of steroids decreased by 55%
Kisner *et al.* (2012)[62]	2	32, 50; 8, 2	2 doses (1 g each), 1 patient received >2 courses	25, 22	Sustained remission 2 (partial in 1) Both relapsed, at 23 months and 9 months	≥2 agents continued
Ochi *et al.* (2012)[63]	2	27, 26; 18, 16	1 dose, both patients received >2 courses	25, 35	Sustained remission none Both relapsed, at 10 months and 35 months	≥2 agents continued
Sellier-Leclerc *et al.* (2012)[51]	30‖	12.9 (3.7–19.7); 9.5 (0.3–17.5)	1–2 doses in 12 patients; 3–4 doses in 18, 30 received >2 courses	38 (26–52)	Sustained remission 18 Relapse 12	All agents discontinued in all patients; restarted in 2 patients
Ito *et al.* (2012)[50]	55¶	4.5 (0.9–16.3); 4.8 (0.2–14.7)¶	1.8±1.4 (range 1–7) doses	24 (8–51)¶	Sustained remission 27 Relapse 28 Time to relapse 5 (1–24)* months	Steroids discontinued in 77% and ciclosporin discontinued in 60% of patients

*Median (range). ‡Includes two patients with steroid-resistant nephrotic syndrome who responded to ciclosporin. §Includes three patients from another study.[48] ‖Includes 18 patients from another study.[49] ¶Includes ≥15 patients from other reports;[44,47,67] medians (ranges) quoted refer to values reported for the entire series of 74 patients.[50] Abbreviation: NA, not available.

such as nephrotoxicity, hyperglycaemia, headaches and dyslipidaemia.[57–59] Therapies that enable steroid sparing without substantially increased adverse effects are, therefore, needed.

Rituximab was first reported to induce remission of nephrotic syndrome in a boy aged 16 years who was treated for co-existing idiopathic thrombocytopenic purpura.[60] Since then, other studies have shown the efficacy of this agent in patients with difficult-to-treat steroid-dependent nephrotic syndrome (Table 1).[44,47–55,61–63] Patients in these studies received rituximab as 'rescue therapy', several years after the onset of disease; previous therapies included three or more immunosuppressive agents in 30–100% of patients and calcineurin inhibitors in 42–100% of patients.

Clinical responses to therapy

Encouraging results have been reported for patients treated with rituximab for severe steroid-dependent or ciclosporin-dependent nephrotic syndrome. In a prospective, multicentre study, 19 of 22 patients treated with 2–4 doses of rituximab had a satisfactory response with sustained remission and successful withdrawal of one or more immunosuppressive agents.[48] Four patients (18.2%) relapsed at 7–17 months. A questionnaire survey conducted by the International Pediatric

Table 2 | The efficacy of rituximab compared to other strategies in difficult-to-treat nephrotic syndrome

Study	Patients (*n*)	Study design and duration of follow-up	Study groups	Age (years)	Outcome
Steroid-dependent nephrotic syndrome					
Ravani *et al.* (2011)[65]	54	Open-label randomized controlled trial; stratified for prednisolone or CNI toxicity; ≥3 months	Rituximab 1–2 doses plus CNI and tapered prednisolone (*n*=27) vs CNI and prednisolone; tapered if patient in remission (*n*=27)	10.2±4.0 vs 11.3±4.3	Proteinuria 69.8% lower in rituximab group (*P*=0.003) Patients with relapses: 18.5% with rituximab vs 48.1% without rituximab (*P*=0.029) Probability of successful withdrawal of prednisolone and CNI: 62.9% with rituximab vs 3.7% without rituximab (*P*<0.001) Patients who were relapse-free with rituximab: 50% at 6 months; 25% at 12 months
Ito *et al.* (2012)[67]	16	Retrospective survey, 1 year	Rituximab 1 dose plus 1 year of mycophenolate mofetil (*n*=9) vs rituximab 1 dose (*n*=7) Prednisolone tapered by 2–3 months in both groups	6.8–18.1 vs 5.0–19.9	Relapses: 3 of 9 combination-treated patients vs 6 of 7 patients (*P*<0.05) Relapse rate: 0.4 vs 2.3 per year (*P*<0.005) Prednisolone dose: 0.11 vs 0.29 mg/kg daily (*P*<0.05) Adverse effects and CD19 depletion in both groups
Sinha *et al.* (2012)[68]	23	Retrospective, 1 year	Rituximab 2–3 doses (*n*=10) vs tacrolimus for 12 months (*n*=13) Prednisolone tapered in both groups	12.2±2.3 vs 12.3±3.0	Similar decline in relapses at 6 months and 12 months Similar relapse-free survival at 6, 12 and 18 months Reduction in cumulative prednisolone dosage: 67% vs 44%
Resistance to steroids and calcineurin inhibitors					
Magnasco *et al.* (2012)[66]	31	Open-label randomized controlled trial; 15 months	Rituximab 2 doses, 2 weeks apart, with prednisolone and tacrolimus or ciclosporin (*n*=16) vs prednisolone and tacrolimus or ciclosporin (*n*=15)	8.5±4.4 vs 7.3±3.7	No difference in proteinuria at 3 months (*P*=0.77) Similar proportions of patients with initial and delayed-onset steroid-resistant nephrotic syndrome responded in both groups
FSGS undergoing kidney transplantation					
Fornoni *et al.* (2011)[35]	41	Prospective; retrospective controls; ≥1 year	Rituximab 1 dose, given within 24 h of kidney transplant (*n*=27) vs no rituximab (*n*=14) Induction therapy; triple immunosuppression in all patients None received plasma exchange	15.0±5.5 vs 12.3±5.2	Patients with recurrent nephrotic syndrome: 7 vs 9 (*P*=0.023) Patients with plasma exchange: 8 vs 10 (*P*=0.019) Change in eGFR: –5.3±18.4 vs –19±19.8 ml/min/1.73 m^2 (*P*=0.008) Renal allograft survival similar in both groups at 6 months and 12 months

Abbreviations: CNI, calcineurin inhibitor; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis.

Nephrology Association (IPNA) provided data on 28 patients given 1–4 doses of rituximab.[54] 23 patients (82.1%) had a favourable response, which was sustained in 10 patients for a median of 4.5 months. In a study of 24 patients given two doses of rituximab (375 mg/m^2 once a week) at our centre, 20 patients (83.3%) were in remission at 1 year and 17 (71%) patients were in remission at 17 months.[52] These findings were confirmed in a large cohort that included 40 patients, of whom more than two-thirds were in sustained remission at 12 months.[64] A nationwide survey conducted in Japan showed the efficacy of rituximab in 55 patients with frequently relapsing or steroid-dependent nephrotic syndrome.[50] After a mean of 1.8 ± 1.4 doses of this agent, 49.1% of patients were in remission at 7–31 months, and immunosuppressive therapy was discontinued in the majority of patients.[50]

Data on relapse rates are limited. In three studies including a total of 46 children, therapy with rituximab reduced the frequency of relapses by 62–95%.[44,47,52] The proportion of patients who achieved sustained remission depended on the dosing strategy and duration of follow-up (Table 1). In patients who received one dose of rituximab (375 mg/m^2), 25–40% were in sustained remission at 12–17 months.[44,47] Among patients who received 2–4 doses of rituximab, >70% were in sustained remission at 6–38 months.[49,52,55,62] Another study reported a significantly longer time to relapse in 11 patients given four doses of rituximab than in 16 patients who received one or two doses (23.3 ± 18.7 months versus 10.3 ± 3.5 months, respectively).[55] However, a correlation between the response to treatment and the number of doses was not seen in the IPNA questionnaire study.[54]

Treatment with rituximab enables considerable steroid sparing, with a substantial reduction in the steroid dose and discontinuation of other immunosuppressive therapies (see Table 1). Since most patients who receive rituximab have long-standing steroid-dependent disease with adverse effects related to therapy, the ability to discontinue steroid therapy is important.

Comparative studies

Few studies have compared the efficacy and safety of rituximab with that of other immunosuppressive agents (Table 2).[65,66] The results of a randomized controlled trial in 54 patients with difficult-to-treat, steroid-dependent nephrotic syndrome showed that the combination of rituximab, calcineurin inhibitors and prednisone was not inferior to standard therapy with calcineurin inhibitors and prednisone in maintaining short-term remission.[65]

Compared with standard therapy, the additional use of rituximab produced a significant decline in proteinuria, relapse rates and steroid doses. However, the duration of follow-up was short and the implications of these findings for long-term management are unclear.

Another research group compared therapeutic outcomes at 1 year in nine patients who received combination therapy consisting of a single dose of rituximab and oral mycophenolate mofetil, and seven patients given rituximab alone.[67] The combined therapy resulted in a higher proportion of patients who achieved sustained remission and a longer time to first relapse than did single-dose rituximab. This finding was confirmed in a survey of patients in Japan, which showed relapses in 15 of 40 (37.5%) rituximab-treated patients who continued to receive ciclosporin, mycophenolate mofetil or mizoribine, compared with 13 of 15 (86.7%) patients treated with rituximab alone, usually in a single dose.[50] However, another study did not show similar benefit in patients who continued to receive maintenance immunosuppression with ciclosporin after one or more doses of rituximab.[55] Further studies are necessary to examine whether the number of rituximab doses might determine the need for subsequent therapy with immunosuppressive agents. Our research group has retrospectively compared the outcomes of 23 patients with difficult-to-treat, steroid-dependent nephrotic syndrome who received either rituximab (two or three doses of 375 mg/m^2 once a week) or oral tacrolimus (0.1–0.2 mg/kg per day for 12 months) in combination with prednisolone.[68] At 12 months, the two treatments had similar efficacy: the reductions in the frequency of relapses, the proportion of patients in sustained remission and decline in the steroid dose received were comparable.[68] The results from a prospective, multicentre, open-label trial (as yet published only as an abstract), show that therapy with one or two doses of rituximab leads to a significant decline in the relapse rate; 14 of 24 (58.3%) patients with steroid-dependent or frequently relapsing nephrotic syndrome achieved remission despite withdrawal of all immunosuppressive medications.[69]

Treatment recommendations

Treatment with rituximab seems to be a promising approach for patients with difficult-to-treat, steroid-dependent nephrotic syndrome, but these positive results require confirmation in prospective studies. A double-blind placebo-controlled trial to evaluate the efficacy and safety of four doses of rituximab in patients with childhood-onset, frequently relapsing, steroid-dependent nephrotic syndrome is currently underway.[4,70] Another trial is underway to examine the efficacy of two doses of rituximab in maintaining remission in patients who are calcineurin-inhibitor dependent.[71]

While awaiting the results of these and future studies (Supplementary Box 1 online), we propose that rituximab should be used as rescue therapy in patients who have failed to respond to multiple immunosuppressive agents or in patients with calcineurin inhibitor toxicity. Treatment with rituximab might also be considered as an alternative to calcineurin inhibitors in patients with severe steroid-dependent nephrotic syndrome. Rituximab should be given during remission, at a dose of 375 mg/m^2 once a week for two or more doses, to achieve CD19 levels below 1% of leukocytes. Although additional therapy with mycophenolate mofetil for up to 12 months might enable patients to achieve sustained remission, the indications for its use need to be defined. Re-treatment with rituximab with or without mycophenolate mofetil is an option in patients who show recurrence of frequent relapses.

Steroid-resistant nephrotic syndrome

The efficacy of rituximab therapy in patients with difficult-to-treat, steroid-resistant nephrotic syndrome was first investigated in five patients (three of whom had FSGS) who received four doses of rituximab, administered once weekly.[72] Four patients achieved complete remission and one had partial remission. Subsequently, other studies have shown success with rituximab treatment in a proportion of patients with minimal change disease or FSGS refractory to treatment with steroids and calcineurin inhibitors.[73–75] Table 3 summarizes the current experience with rituximab in patients with steroid-resistant nephrotic syndrome.[50,52,54,61–63,76–79] All patients in these studies had nephrotic syndrome of long-standing duration and had received prior therapy with two or more agents, including corticosteroids, cyclophosphamide or calcineurin inhibitors.

Response to therapy

The four largest case series that evaluated the efficacy of rituximab included a total of 87 patients (11 of whom were adults, Table 3).[50,52,54,77] The participants comprised almost equal proportions of patients with minimal change disease and FSGS.[50,52,54,77] The indication for treatment was immediate or delayed unresponsiveness to calcineurin inhibitors, with or without overt toxic effects. Complete remission was seen in 0–27.3% of patients and partial remission in 21.2–37.5% of patients, for an overall remission rate of 45.6% patients.[50,52,54,77] The timing of remission was variable, usually occurring within 4–6 weeks from completion of therapy, and was sustained for 6–24 months. In the patients who achieved remission, treatment with immunosuppressive agents could be tapered or discontinued.[50,52,54,77]

One study reported complete or partial remission of proteinuria in 10 of 11 adults treated with rituximab; however, information on the efficacy of therapy was not provided separately for patients with steroid-dependent nephrotic syndrome or steroid-resistant nephrotic syndrome.[79] Other reports indicate satisfactory remission rates with rituximab therapy in patients with steroid-resistant nephrotic syndrome, although their small sample sizes, limited follow-up periods and the confounding effects of concomitant therapies make it difficult to define the efficacy of this intervention. A randomized controlled trial to evaluate the efficacy and safety of rituximab has been carried out in 31 children (aged 2–16 years) with nephrotic syndrome who had

Table 3 | Studies on the efficacy of rituximab in steroid-resistant nephrotic syndrome

Study	Patients; age* (years)	Duration of illness* (years)	Rituximab 375 mg/m² once weekly	Follow-up* (months); number of patients receiving concomitant therapy‡	Results	
					Response (time to response)	Final outcome
Peters *et al.* (2008)[76,§]	4; 20 (15–20)	8 (8–18)	2 doses (1 g) in 3 patients; 4 doses in 1	11.5 (6–16); 3	Complete response 1 Partial response 2 (2–4 weeks)	Remission in 3 patients
Fernandez-Fresnedo *et al.* (2009)[77]	8; 26 (19–55)	2.8 (2–8.9)	8 doses in 1 patient; 4 doses in 7	14.5 (12–24); 8	Partial response 3 (1–12 months)	Remission in 2 patients
Gulati *et al.* (2010)[52]	33; 12.7 (2–41)	6.4 (1–15)	4 doses in 28 patients; 1–2 doses in 5	21.5 (12–48); 6	Complete response 9 Partial response 7 (8–60 days)	Remission in 15 patients
Prytula *et al.* (2010)[54]	27; 3 (1.5–11)	NA	1–2 doses in 7 patients; 3–5 doses in 20	5 (1–16); 9	Complete response 6 Partial response 6 (NA)	Remission in 2 patients
Sugiura *et al.* (2011)[61]	5; 27 (24–47)	12 (0.1–18)	1 dose in all	≥6; 3	Complete response 2 Partial response 3 (1–6 months)	Remission in 5 patients
Kari *et al.* (2011)[78]	4; 10 (8–11)	2.3 (0.5–5)	1 dose in all	≥6; 1	Partial response 1 (1 month)	None in remission
Kisner *et al.* (2012)[62]	47 (31–51)	14 (0.5–13)	1 dose in 1 patient; 2 doses in 2	3 (3–8); 3	Complete response 1 Partial response 2 (3–6 months)	Remission in 3 patients
Kong *et al.* (2012)[79,‖]	11; 36 (18–89)	2.5 (0.2–39)	4 doses in 2 patients; 1–2 doses in 9	19 (7–51); 2	Complete response 7 Partial response 3 (0–12 months)	Remission in 10 patients
Ochi *et al.* (2012)[63]	2; 21, 25	12	1 dose in all	12; 2	No response	None in remission
Ito *et al.* (2012)[50]	19; NA	NA	Mean 2.3±1.4 doses	NA; 18	Complete response 6 Partial response 6; 6 (1–12)*	Complete response in 6 patients, partial response in 6, kidney transplant in 2 patients

*Median (range). ‡Concomitant therapy with mycophenolate mofetil and calcineurin inhibitors; all patients received an angiotensin-converting-enzyme inhibitor or angiotensin-receptor blocker and corticosteroids. §Includes one patient with recurrent focal segmental glomerulosclerosis, who responded to four doses of rituximab with partial remission at 7 months. ‖Includes 7 patients with steroid-dependent nephrotic syndrome (six with minimal change disease, one with focal segmental glomerulosclerosis); information on decline in relapse rates not reported. Abbreviation: NA, not available.

not responded to treatment with calcineurin inhibitors and prednisone.[66] All patients continued their existing medications, and one group was randomly assigned to receive additional therapy consisting of two doses of rituximab. Although depletion of B cells and adequate serum levels of rituximab were achieved, this therapy did not induce remission or reduce proteinuria in these patients (Table 2).

Factors affecting response to therapy

Steroid-resistant nephrotic syndrome is a heterogeneous disease and the response of patients to immunosuppressive agents is influenced by multiple factors, including the presence of minimal change disease or FSGS, duration of illness and previous therapies. Approximately 20–35% of patients respond to treatment with alkylating agents, and 60–70% respond to treatment with calcineurin inhibitors. Rituximab therapy has been used chiefly in patients who do not respond to the above agents. However, the published studies have not identified any clinical or biochemical predictors of response to rituximab, or any relationship between the number of doses and the response to this treatment.[54,77] Furthermore, many patients in these studies continued to receive one or more immunosuppressive agents, including calcineurin inhibitors, which might confound the effect of therapy with rituximab.[61,63,77]

Our experience suggests that findings on renal histology can help to predict the response of patients to treatment with rituximab: remission occurred in 64.7% of patients with minimal change disease, compared to 31.3% of patients with FSGS.[52] Collation of data from other case series suggests that patients with minimal change disease are significantly more likely to respond to treatment than those with FSGS (Figure 2).[50,54,61–63,76–79]

Treatment recommendations

Almost 60–70% of patients with steroid-resistant nephrotic syndrome treated with calcineurin inhibitors achieve complete or partial remission,[9] and at present no alternative agent shows superior efficacy. However, therapy with rituximab might be useful in two instances. First, in patients who respond to calcineurin inhibitors, treatment with 2–4 doses of rituximab might be used to maintain remission and enable withdrawal of calcineurin inhibitors, especially in the presence of drug-related toxic effects. Second, the results of retrospective case series suggest that treatment with rituximab might be effective

in some patients with nephrotic syndrome resistant to corticosteroid and calcineurin inhibitor therapy. However, the results of a randomized controlled trial that examined the efficacy of rituximab in such patients were not encouraging.[66] Adequately powered studies with long-term follow-up of groups of patients stratified for renal histology are required to clarify the benefits of these treatment strategies.

Recurrent FSGS

Patients with idiopathic FSGS and persistent proteinuria in the nephrotic range are at risk of developing kidney failure that requires renal transplantation. Approximately 30% of such patients develop recurrence of FSGS after the first allograft.[80,81] Features associated with FSGS recurrence include onset of nephrotic syndrome below the age of 15 years, rapid progression to end-stage renal disease (within 3 years from onset), mesangial proliferation on renal histopathology, white ethnicity and non-genetic (immune) forms of FSGS.[82,83] The increased risk of FSGS recurrence in children who receive renal grafts from living donors[84] is balanced by the reduced risk of rejection and decreased need for immunosuppression.[85] Recurrence of FSGS usually occurs within hours to days after the transplant procedure, and is characterized by proteinuria in the nephrotic range and progressive hypoalbuminaemia.[6] These patients are at increased risk of allograft failure (5-year kidney graft survival is 57% in patients with recurrent FSGS versus 82% in patients without recurrence).[86,87] After the loss of the first allograft, the risk of recurrence of FSGS in subsequent kidney transplants is 80–100%.[88]

Strategies for the management of patients with recurrent FSGS include the use of plasma exchange or immunoadsorption in combination with high-dose ciclosporin and cyclophosphamide. By using these strategies, 70% of children and 63% of adults with recurrent FSGS achieve complete or partial remission.[89] In one case report, a decline in proteinuria was observed after rituximab therapy in a boy aged 12 years with recurrent FSGS treated for post-transplant lymphoproliferative disorder.[90] Subsequently, some researchers have reported remission of proteinuria with the use of rituximab (2–6 doses of 375 mg/m^2, administered once every 1–2 weeks) in conjunction with plasma exchange and post-transplantation immunosuppression.

Experience with rituximab therapy in patients with recurrent FSGS has been summarized in a systematic review[6] and a multicentre report (Table 4).[54] The review included data on 39 patients with recurrent FSGS. Proteinuria in the nephrotic range and hypoalbuminaemia were present in 74.3% of patients within 1 month of kidney transplantation.[6] Combined therapy with plasmapheresis and rituximab resulted in complete or partial remission of proteinuria in 64.1% of patients. The median time to best clinical response was 2 months (range 0.6–12.0 months). On univariate analysis, a young age at transplantation, normal serum albumin level at recurrence of FSGS and the need for fewer rituximab infusions was associated with an improved response

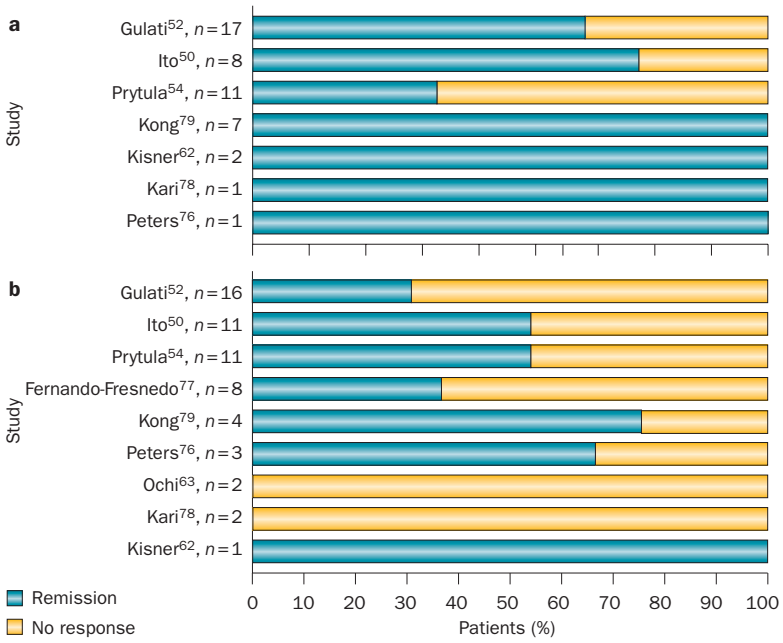

Figure 2 | Rates of remission after rituximab therapy. Data obtained from studies in patients with **a** | minimal change disease and **b** | focal segmental glomerulosclerosis. Remission was observed in 32 of 47 patients (68.1%) with minimal change disease, and 26 of 58 patients (44.8%) with focal segmental glomerulosclerosis (P=0.017).

to treatment. Response was not related to the kidney donor type (living or deceased), the use of pretransplant or post-transplant plasmapheresis, other immunosuppressive therapy or the post-transplant severity of proteinuria. On stepwise regression analysis, normal serum albumin level at recurrence (which implies mild FSGS) and young age predicted a favourable response to rituximab therapy.[6] The IPNA study showed that therapy with 1–4 doses of rituximab in combination with plasmapheresis resulted in complete or partial remission in nine of 15 (60%) patients (Table 4).[54] Five of the seven patients treated with B-cell depletion showed complete or partial remission; two patients (one with and one without B-cell depletion) were nonresponders.[54] In a review of data from 25 patients with recurrent FSGS treated with rituximab infusions and plasma exchange, the 12 patients who achieved remission had received rituximab infusions significantly earlier than the 13 patients who did not respond (mean 100.5 ± 95.4 days from the onset of recurrence versus 468.1 ± 379.8 days, respectively).[91]

In another report, six of eight patients with recurrent FSGS refractory to plasmapheresis achieved complete or partial remission with 1–4 doses of rituximab.[92] Furthermore, patients who had relapses of proteinuria responded to re-treatment with rituximab. Treatment with plasmapheresis and two doses of rituximab induced complete or partial remission in four adults with recurrent FSGS (Table 4).[93] Other reports indicate similar findings that rituximab is effective in sustaining remission in patients who either do not respond to or who are dependent on plasmapheresis.[94–96] Although the possibility that these patients had a delayed response

Table 4 | Efficacy of rituximab in treatment and prevention* of recurrent FSGS

Characteristics	Tsagalis *et al.* (2010)[93]	Araya *et al.* (2011)[6]	Prytula *et al.* (2009)[54]	Audard *et al.* (2012)[98]*	Kumar *et al.* (2012)[92]
Patients (*n*)	4	39	15	4	8
Age (range)	32–57 years	5–48 years; 19 children (age not specified)	All children (age not specified)	28–43 years	5–17 years
Time to recurrence (range)	2–72 months	1–3, 513 days	NA	No recurrence	Immediate in 7 patients; at 4 years in 1
Pretransplant plasma exchange (patients)	None	9	NA	NA	4
Post-transplant plasma exchange (patients); number of sessions or duration	4; 20–69 sessions	38; mean 21 sessions	10; NA	2; 6 and 15 sesssions	All; 3 days to 61 months
Rituximab regimen (375 mg/m^2 infusion, unless specified)	1 g; 2 doses every 2 weeks, repeated at 1 year	1–6 doses	1–4 doses every 1–2 weeks	1–2 doses on day 0 (*n*=4) and day 7 (*n*=2)	1–10 doses (including repeat courses)
Median interval between recurrence and rituximab therapy (range)	3 (1–12) weeks	149 (3–1,086) days	NA	No recurrence	26.5 (0.25–63) months
Patients in remission (%)	Complete 2 Partial 2	Complete 17 (43.5%) Partial 8 (20.5%) No response 14 (35.9%)	Complete 6 (40%) Partial 3 (20%) No response 6 (40%)	No recurrence	Complete 2 Partial 4 No response 2
Duration of follow-up	18–60 months	NA	5–84 months	12–54 months	1–41 months
Outcome at last follow-up	Complete remission 2 Partial remission 2	Complete remission 17 Partial remission 8	Complete or partial remission 5 Recurrence 3 NA 1	No recurrence	Complete or partial remission 5 1 death

*Prevention of recurrent FSGS in second transplant. Abbreviations: FSGS, focal segmental glomerulosclerosis; NA, not available.

to plasmapheresis cannot be excluded, the timing of response correlated with that of rituximab therapy.

Prevention of recurrent FSGS

Combined therapy with rituximab and pretransplant plasmapheresis is useful in kidney transplant recipients at high risk of recurrence of FSGS. Therapy with 1–2 doses of rituximab and multiple sessions of plasmapheresis prevented recurrent FSGS in recipients of a second kidney transplant who were followed up for 12–54 months.[97] In a report, a girl aged 7.9 years with a history of recurrent FSGS was successfully managed using four sessions of plasmapheresis and a single dose of rituximab (375 mg/m^2) 21 days before transplantation.[98] In another report, administration of rituximab within 24 h after transplantation prevented recurrence of FSGS (Table 2).[35] Recurrent FSGS was noted in 25.9% of 27 patients treated with rituximab, compared with 64.3% of 14 patients who did not receive the medication.[35] However, the overall incidence of FSGS recurrence in this study was considerably higher than that reported in the literature; the recurrence rate in patients receiving rituximab was close to that cited elsewhere for untreated patients.

Treatment recommendations

All patients with FSGS who are about to undergo renal transplantation should be advised about the possibility of recurrence of this disease. Pre-emptive plasma exchange should be planned in advance of living-donor transplantation.[99] Early and aggressive therapy with immunosuppression and plasma exchange should be initiated if proteinuria develops after transplantation.

The efficacy of rituximab in patients with recurrent FSGS is difficult to estimate owing to confounding effects from other concomitant therapies. Combination therapy with plasma exchange and rituximab has shown promise in the prevention and treatment of recurrent FSGS, but the efficacy of this approach needs to be examined in prospective controlled studies.[82] Rituximab is cleared from the body by plasma exchange;[100] we suggest, therefore, an interval of 36–48 h between rituximab infusion and plasmapheresis.

Idiopathic membranous glomerulonephritis

Membranous nephropathy is the principal cause of nephrotic syndrome in adults,[101,102] among whom idiopathic membranous nephropathy accounts for 32–80% of diagnoses. Secondary causes of membranous nephropathy include systemic lupus erythematosus, chronic infections (especially hepatitis B), medications or malignancy.[103,104] Spontaneous remission occurs in 30–40% of patients within 12–24 months.[105] Current therapies include inhibitors of the renin–angiotensin–aldosterone system, and combinations of corticosteroids with alkylating agents or calcineurin inhibitors.[106] The results of randomized trials indicate that therapy with the above-mentioned agents can induce remission in 59–90% of patients; however, relapse rates are high and patients with proteinuria in the nephrotic range can develop progressive renal failure.[107,108] The efficacy of rituximab was initially reported in eight patients with

Table 5 | Efficacy of rituximab in patients with idiopathic membranous glomerulonephritis

Study	Number of patients (number given prior therapies*)	Age (years)‡	Duration of disease‡	Follow-up (months)‡	Results	
					Outcomes	Decline in proteinuria from baseline
Bomback *et al.* (2009)[117,§]	69 (8)	NA	0.8–30 months	5–60	Complete response 23 Partial response 21 Relapse NA	48–99%
Segarra *et al.* (2009)[118,‖]	13 (13)	45 (26–71)	64 (35–96) months	35 (31–54)	Complete response 4 Partial response 9 Relapse 3	65.6% at 6 months; 71.8% at 12 months
Fervenza *et al.* (2010)[30]	20 (11)	48.6 (29–80)	29.7 (4–144) months	24	Complete response 4 Partial response 12 Relapse 1	8.5±6.6% per month for 24 months
Sprangers *et al.* (2010)[119,¶]	4 (4)	NA	3.4 (0.2–8) months	20–27	Complete response 1 Partial response 2 Relapse 0	NA
Sugiura *et al.* (2011)[61]	4 (3)	69 (54–74)	19 (7–25) years	6	Complete response 0 Partial response 2 Relapse 0	32.6%
Cravedi *et al.* (2011)[120,#]	22 (11)	50.1±12.3 vs 48.6±13.9	9 (6–17) vs 51 (26–55) months	24	Complete response 3 vs 2 Partial response 5 vs 5 Relapse 1 vs 1	69.4±40.4% vs 60.9±17.4%
Hoxha *et al.* (2011)[116]	5 (5)	55 (51–66)	28 (12–144) months	15 (9–18)	Complete response 0 Partial response 3 Relapse 1	NA
Kong *et al.* (2011)[79]	11 (6)	45 (33–79)	34 (1–108) months	34 (9–74)	Complete response 4 Partial response 3 Relapse 2	NA
Michel *et al.* (2011)[114]	28 (8)	44.4 (18.5–82.4)	14 (0.5–74) months	11.9 (6–50)	Complete response 6 Partial response 13 Relapse 3	86.8% (80.3–99.0%)
Ruggenenti *et al.* (2012)[121]	100 (32)	51.5±5.9	25.5 (11.7–67.7) months	29 (6–121)	Complete response 27 Partial response 38 Relapse 18	NA

*Alkylating agents, mycophenolate mofetil or calcineurin inhibitors; almost all patients were treated with angiotensin-converting-enzyme inhibitors and/or angiotensin-receptor blockers. ‡Median (range). §Includes two patients with disease recurrence after transplantation. ‖Includes patients with calcineurin-inhibitor-dependent nephrotic syndrome and partial remission who had not relapsed in the past 2 months. ¶Includes patients with disease recurrence after transplantation. #11 patients who received rituximab as first-line therapy were compared with 11 patients who failed to respond to prior immunosuppressive therapies. Abbreviation: NA, not available.

membranous nephropathy,[109] but this agent has since been used in the treatment of both idiopathic and secondary forms of the disease, as first-line therapy and after patients fail to respond to standard therapy.

Pathogenesis of membranous glomerulonephritis

Membranous glomerulonephritis is thought to result from an immune response in which podocyte antigens are targeted by circulating IgG4 antibodies. Subepithelial deposition of these immune complexes results in podocyte injury and loss of the filtration barrier. Of particular interest is an autoantibody against the M-type isoform of the phospholipase A2 receptor (PLA2R).[110] Support for a pathogenetic role of this autoantibody comes from findings of associations between titres of this antibody and nephrotic range proteinuria or post-transplant recurrence of idiopathic membranous nephropathy.[111–113] Positive staining for PLA2R was found in immune deposits in kidney biopsy samples from patients with membranous nephropathy.[114] Patients with membranous nephropathy and autoantibodies against PLA2R often also have elevated levels of antibodies against other antigens, including neutral endopeptidase, aldose reductase, superoxide dismutase 2 and α-enolase.[115] Although rituximab therapy does not remove circulating autoantibodies, treated patients show a decline in the titre of anti-PLA2R antibodies and remission of proteinuria.[45,111,114,116] Table 5 and Supplementary Figure 1 online show the clinical response of patients with membranous nephropathy to rituximab.[30,61,114,116–121] However, heterogeneity in previous therapies, dosing schedules of rituximab and definitions of response (Box 1) make it difficult to collate data from these studies.

The efficacy of rituximab therapy has been assessed in a systematic review of data from 21 studies, including 69 patients with idiopathic membranous nephropathy.[117] The complete response rate was 15–20% and the partial response rate was 35–40%. Although these figures are inferior to those reported for treatment with alkylating agents and calcineurin inhibitors, many patients in these studies had previously failed to respond to the above therapies.[117] In a prospective cohort study of 100 consecutive patients with membranous nephropathy who were followed up for 2.5 years after treatment with 1–4 doses of rituximab, complete and partial remission occurred in 27 and 38 patients, respectively, at a median

of 7.1 months (range 3.2–12.0 months).[121] One-third of patients in this cohort had failed to respond to previous therapy with other medications. Remission was seen in 47 of 68 (69.1%) patients given rituximab as the primary immunosuppressive agent, and in 18 of 32 (56.3%) patients who received rituximab after the failure of an alternative immunosuppressive agent. Remission rates were similar in a prospective cohort of patients treated with rituximab as first-line therapy and a retrospective cohort of patients who received this agent as second-line therapy and were matched for age, sex and severity of proteinuria.[120] Three separate reports provide information on the efficacy of rituximab in the treatment of patients with post-transplantation recurrence of membranous nephropathy.[117,119,122] Therapy with 1–8 doses of rituximab stabilized renal function and induced complete or partial remission in 13 of 14 patients,[117,119,122] including those who required re-treatment.[122]

A prospective study indicates that the proportion of patients with membranous nephropathy who achieve complete and partial remission increases over time, indicating a delayed response.[121] Other studies have also reported increasing rates of remission during extended follow-up[30,114,120] and that remission is sustained after B-cell recovery. These observations might reflect gradual clearance of glomerular immune deposits, correction of pathological abnormalities and/or resolution of proteinuria. Although these patients might relapse, re-treatment with rituximab is effective in inducing further remission.[114,116,118–121] Most studies do not provide information on the effect of rituximab therapy on kidney function; however, in one study, the glomerular filtration rate (GFR) increased in patients with complete remission, but progressively declined in those who did not respond to therapy.[121] Other studies confirm that remission of membranous nephropathy improves[30,118] or stabilizes[114,119,120] renal function.

Predictors of a lack of response to rituximab therapy in patients with membranous nephropathy include a high tubulointerstitial score, tubular atrophy and interstitial fibrosis in kidney biopsy samples,[123] and impaired renal function at baseline (estimated GFR <45 ml/min/1.73 m^2).[114] By contrast, in another case series, no relationship was noted between the response to therapy and histological findings.[30] In one study, urinary excretion of α1 microglobulin, retinol-binding protein, albumin and IgG at baseline correlated well with the rate of response to rituximab at 12 months but not at 24 months.[124]

Reports demonstrate that anti-PLA2R antibody titres decline after therapy with rituximab. Antibodies to PLA2R were detected at baseline in 10 of 28 patients treated with rituximab, and were absent in all five patients tested for these antibodies after complete or partial remission.[114] In a case series, a decline in anti-PLA2R antibody titre was associated with a reduction in proteinuria in two patients, whereas the three patients in whom anti-PLA2R antibody titres increased did not enter remission.[115] These findings were confirmed in another study, which showed that patients with decreasing anti-PLA2R antibody levels after rituximab treatment had increased rates of remission at 12 and 24 months.[108]

Treatment recommendations

The 2012 Kidney Disease: Improving Global Outcomes guidelines recommend that immunosuppressive therapy should be initiated in patients with idiopathic membranous nephropathy who have nephrotic syndrome, proteinuria of ≥4 g per day and estimated GFR ≥30 ml/min/1.73 m^2 who fail to respond to 6 months of treatment with antihypertensive and antiproteinuric agents.[107] An initial immunosuppressive regimen that combines corticosteroids and an alkylating agent is recommended, if that fails calcineurin inhibitors should be used. Therapy with 2–4 doses of rituximab is likely to result in complete remission in 20–33% of patients and partial remission in 20–60% of patients who fail to respond to treatment with cyclophosphamide or calcineurin inhibitors.[7] The results of ongoing prospective studies are expected to clarify the benefits of this medication.[125,126]

Adverse effects of rituximab

Therapy with rituximab is well tolerated in most patients; however, a number of adverse affects have been documented (Table 6).[4,52,55,62,76,77,127]

Infusion reactions

Acute reactions can occur within the first 30–120 min of an infusion,[5,117] with an incidence that ranges from 9.1% to 56.3%.[30,47,48,50,51,54,67,79,114,121] Commonly reported reactions include flu-like symptoms, chills, fever, headache, myalgia, itching, erythematous rash,[30,44,47,48,54,55,66,68,117,120,121] cough, sore throat, nasal congestion, tachycardia,[44,48] and hypotension[44,48,121] or hypertension.[44,76] These symptoms can be minimized by pretreatment with antihistamines and/or corticosteroids. The intensity of symptoms declines on stopping the infusion and they rarely recur. Anaphylaxis[54,128] and/or bronchospasm[44,48,65,66,117,121] are rarely reported in association with rituximab infusions. In view of the risk of infusion reactions, cautious administration is advised, beginning with a slow infusion rate and increasing the rate at 30 min intervals. In adult oncology practice, the first dose of rituximab is infused over 6 h and subsequent doses are given over a 4 h period.[129]

Risk of infections

Patients receiving rituximab are at increased risk of infections with usual or unusual organisms, including pyogenic infections, tuberculosis and cytomegalovirus.[51,117,130] The risk of infections is also increased by concomitant use of other immunosuppressive agents and the underlying disorder. Since CD20 is not present on plasma cells, immunoglobulin production is not altered and the risk of hypogammaglobulinaemia is low (Table 6).[44,47,48,54,61,118,131,132] However, low serum levels of immunoglobulins have been noted in a few patients receiving maintenance rituximab after haematopoietic stem cell transplantation,[133] malignancies[134]

or autoimmune cytopenias.[135] A meta-analysis of data from three placebo-controlled randomized trials (including 938 patients) of rituximab therapy in patients with rheumatoid arthritis showed no increase in the incidence of infections.[136] Systematic reviews of rituximab therapy in patients with lymphomas show that this treatment is associated with a twofold increase in the risk of neutropenia and infections[137–139] compared with standard therapy, perhaps as a result of the increased dosage of rituximab and the underlying disease The dose specified is higher than usually used for nephrotic syndrome. Patients with nephrotic syndrome treated with rituximab may show transient leukopenia associated with bacterial sepsis or pneumonia caused by *Pneumocystis jiroveci* (formerly known as *P. carinii*),[48,51,52,54,76,140] similar to that reported in patients with haematologic malignancies or rheumatoid arthritis.[141] Therefore, the use of cotrimoxazole prophylaxis should be considered in patients with nephrotic syndrome who are receiving rituximab therapy and concomitant immunosuppression. A report on 19 patients with difficult-to-treat systemic lupus erythematosus who were given two doses of rituximab showed exacerbation of herpes zoster infection in five patients.[142] Another study reported increased infection-related mortality in 77 kidney transplant recipients treated with rituximab.[143] By contrast, infection rates were similar in 170 highly sensitized kidney transplant recipients treated with rituximab and intravenous immunoglobulin and in 191 nonsensitized patients who did not receive this combination therapy.[144]

Patients with non-Hodgkin lymphoma and collagen vascular disorders treated with rituximab are at risk of reactivation of hepatitis B infection, resulting in fulminant liver failure.[145] The use of rituximab should, therefore, be avoided in patients who are positive for hepatitis B antigens.[146]

Progressive multifocal leukoencephalopathy

Evidence indicates a possible association between long-term rituximab therapy and progressive multifocal leukoencephalopathy (PML), an encephalitis caused by JC polyomavirus.[147] Multiple case reports describe PML in rituximab-treated patients with haematological malignancies, systemic lupus erythematosus and rheumatoid arthritis, which have prompted modification of current prescribing information (including a 'black box' warning).[148] Patients with PML show a gradual onset of cognitive impairment, motor weakness, speech problems and deterioration of vision. The diagnosis is made on the basis of these clinical features, MRI findings and demonstration of polyomavirus in brain biopsy samples or cerebrospinal fluid.

PML has not been reported in patients with nephrotic syndrome treated with rituximab. Screening of blood and urine specimens from 11 children with nephrotic syndrome treated with rituximab and eight controls showed the presence of JC virus in the urine of one patient and one control.[149] BK virus was found in the urine of seven patients and two controls, and in blood samples from four patients.[149] The above findings suggest the need for increased awareness regarding these infections in immunocompromised patients.

Table 6 | Rituximab-associated adverse effects

Adverse effect	Patients (% of total)	Reference(s)
Infusion-related reactions	140 of 624 (22.4)	30,44,47,48,50–52,54,55,64–67, 76,79,114,117,120,121,126
Acute reactions	84 of 379 (22.2)	30,47,48,50,51,54,67,79, 114,121
Anaphylaxis	4 of 77 (5.2)	54,128
Rash, flushing or itching	27 of 511 (5.3)	30,44,47,48,50,54,55,64,66,117, 120,121
Bronchospasm or dyspnoea	30 of 321 (9.4)	44,48,50,65,66,117,121
Hypotension	4 of 193 (2.1)	44,48,50,121
Hypertension	2 of 75 (2.7)	44,50,76
Abdominal pain and vomiting	7 of 97 (7.2)	48,50,66
Chills	9 of 152 (5.9)	52,64,117
Myalgia	3 of 152 (2.0)	52,64,117
Tachycardia or arrhythmia	4 of 93 (4.3)	44,48,50
Arthritis or serum sickness	3 of 112 (2.7)	65,117
Infections		
Septicaemia	3 of 214 (1.4)	50,54,117
Pneumonia	7 of 222 (3.2)	30,48,54,79,117,140
Diarrhoea	2 of 52 (3.9)	48,51
Fever of infectious origin	3 of 89 (3.4)	50,51
Human herpesvirus 6 infection	1 of 30 (3.3)	51
Catheter-related sepsis, BK-virus-associated nephropathy	1 of 2	130
Herpes zoster reactivation	1 of 85 (1.2)	117
Laboratory tests		
Neutropenia*	10 of 248 (4.0)	48,50–52,54,76,93
Thrombocytopenia	1 of 30 (3.3)	51
Elevation of transaminases	1 of 89 (1.1)	50,51
Hypogammaglobulinaemia	5 of 156 (3.2)	44,47,48,54,61,72,118
Anecdotal reports‡		
Acute lung injury or interstitial pneumonitis	4	55,92,152,153
Colitis or pancolitis	2	44,127
Cardiomyopathy	1	44
Acute kidney injury	2	44,92
CNS glioma	1	92

*Associated with infections in two patients. ‡Precise number not available. Abbreviation: CNS, central nervous system.

Rituximab-associated lung injury

Rituximab can cause delayed pulmonary toxicity, chiefly in adults treated for B-cell lymphoma[150] and other disorders.[151] Two systematic reviews on rituximab-induced

lung disease (in 121[151] and 30[152] patients, respectively) suggest a mortality rate of 14.9–29.0%. Pulmonary symptoms usually occur after a mean of four (range 1–12) doses of rituximab, 1–3 months after the last infusion. Clinical features include dyspnoea, fever, nonproductive cough and hypoxaemia, with bilateral lung infiltrates, reduced diffusion capacity and a restrictive ventilatory pattern. Rarely, patients have hypoxaemia and respiratory insufficiency after the first infusion.[153] Pathological findings include bronchiolitis obliterans organizing pneumonia, interstitial pneumonitis, acute respiratory distress syndrome and hypersensitivity pneumonitis.[154] Acute lung injury with thrombotic microangiopathy, multiorgan dysfunction and death was reported in one patient treated with rituximab for recurrent FSGS.[92]

Rituximab should be used cautiously in patients with underlying lung disease, and a diagnosis of rituximab-related lung injury should be considered in any patient who develops respiratory symptoms or new radiographic changes after therapy. Affected patients are treated with supportive care and corticosteroids, although the efficacy of steroids is not proven.[152]

Human antichimaeric antibodies

Repeated use of rituximab in patients with systemic lupus erythematosus and lymphoma is associated with the formation of human antichimaeric antibodies.[155,156] The presence of host antibodies to the murine peptide sequences in rituximab might cause an anaphylactic reaction in some patients or, by increasing rituximab clearance, reduce its efficacy.[73,157,158] The presence of human antichimaeric antibodies in six of 14 patients with idiopathic membranous nephropathy did not affect the response to rituximab.[30] Development of human antichimaeric antibodies is thought to be linked to the rituximab dosing schedule, since they are more common in patients who received one rather than four doses of this agent.[2,158,159] The development of human antichimaeric antibodies might be minimized with the use of new humanized or fully human monoclonal antibodies against CD20.[160]

Conclusions

Rituximab has mild to moderate efficacy and a favourable safety profile in patients with difficult-to-treat nephrotic syndrome. However, the current literature on its efficacy in this setting is based chiefly on case series and data from very few randomized controlled trials. For this reason, firm recommendations cannot yet be presented for its use in clinical practice. Despite the limitations of the available evidence, satisfactory results have been obtained with rituximab therapy in patients with refractory steroid-dependent nephrotic syndrome, recurrent FSGS and membranous nephropathy. By contrast, this treatment has limited benefits in patients with steroid-resistant nephrotic syndrome. Prospective, randomized controlled studies with adequate sample sizes and appropriate follow-up are necessary to define the indications for therapy with rituximab therapy, and to determine outcomes and predictors of response.

Review criteria

We searched PubMed and EMBASE for full-text articles mainly in the English language published from January 2002 to October 2012, using the following terms: "nephrotic syndrome", "rituximab", "CD20", "minimal change", "focal segmental glomerulosclerosis", "recurrent FSGS", "membranous glomerulonephritis" and "membranous nephropathy". The reference lists of selected publications were reviewed to identify additional relevant articles. To reduce publication bias, we included data from systematic reviews and studies including four or more patients, except in the case of papers relating to recurrent focal segmental glomerulosclerosis and adverse effects, for which even single case reports were considered. Studies by the same group of authors were scrutinized to avoid overlap in the reporting of findings derived from the same patients.

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Supplementary information
Supplementary information is linked to the online version of the paper at www.nature.com/nrneph.