Rituximab for childhood-onset, complicated, frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome: a multicentre, double-blind, randomised, placebo-controlled trial

Kazumoto Iijima, Mayumi Sako, Kandai Nozu, Rintaro Mori, Nao Tuchida, Koichi Kamei, Kenichiro Miura, Kunihiko Aya, Koichi Nakanishi, Yoshiyuki Ohtomo, Shori Takahashi, Ryojiro Tanaka, Hiroshi Kaito, Hidefumi Nakamura, Kenji Ishikura, Shuichi Ito, Yasuo Ohashi, on behalf of the Rituximab for Childhood-onset Refractory Nephrotic Syndrome (RCRNS) Study Group

Summary

Background **Rituximab could be an effective treatment for childhood-onset, complicated, frequently relapsing nephrotic syndrome (FRNS) and steroid-dependent nephrotic syndrome (SDNS). We investigated the efficacy and safety of rituximab in patients with high disease activity.**

Methods **We did a multicentre, double-blind, randomised, placebo-controlled trial at nine centres in Japan. We screened patients aged 2 years or older experiencing a relapse of FRNS or SDNS, which had originally been diagnosed as nephrotic syndrome when aged 1–18 years. Patients with complicated FRNS or SDNS who met all other criteria were eligible for inclusion after remission of the relapse at screening. We used a computer-generated sequence to randomly assign patients (1:1) to receive rituximab (375 mg/m²) or placebo once weekly for 4 weeks, with age, institution, treatment history, and the intervals between the previous three relapses as adjustment factors. Patients, guardians, caregivers, physicians, and individuals assessing outcomes were masked to assignments. All patients received standard steroid treatment for the relapse at screening and stopped taking immunosuppressive agents by 169 days after randomisation. Patients were followed up for 1 year. The primary endpoint was the relapse-free period. Safety endpoints were frequency and severity of adverse events. Patients who received their assigned intervention were included in analyses. This trial is registered with the University Hospital Medical Information Network clinical trials registry, number UMIN000001405.**

Findings **Patients were centrally registered between Nov 13, 2008, and May 19, 2010. Of 52 patients who underwent randomisation, 48 received the assigned intervention (24 were given rituximab and 24 placebo). The median relapse-free period was significantly longer in the rituximab group (267 days, 95% CI 223–374) than in the placebo group (101 days, 70–155; hazard ratio: 0·27, 0·14–0·53; p<0·0001). Ten patients (42%) in the rituximab group and six (25%) in the placebo group had at least one serious adverse event (p=0·36).**

Interpretation **Rituximab is an effective and safe treatment for childhood-onset, complicated FRNS and SDNS.**

Funding **Japanese Ministry of Health, Labour and Welfare.**

Lancet 2014; 384: 1273–81

Published Online
June 23, 2014
http://dx.doi.org/10.1016/S0140-6736(14)60541-9

See Comment page 1242

Department of Pediatrics, Kobe University Graduate School of Medicine, Chuo-ku, Kobe, Japan (Prof K Iijima MD, K Nozu MD, H Kaito MD); **Division for Clinical Trials, Department of Development Strategy** (M Sako MD, H Nakamura MD), **and Department of Health Policy** (R Mori MD), **Center for Social and Clinical Research, National Research Institute for Child Health and Development, National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan; Department of General Pediatrics and Interdisciplinary Medicine** (N Tuchida MD) **and Department of Nephrology and Rheumatology** (K Kamei MD, S Ito MD), **National Center for Child Health and Development, Setagaya-ku, Tokyo, Japan; Department of Pediatrics, Graduate School of Medicine, University of Tokyo, Bunkyo-ku, Tokyo, Japan** (K Miura MD); **Chuo University, Bunkyo-ku, Tokyo, Japan** (Prof Y Ohashi PhD); **Department of Pediatrics, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Shikata-cho, Okayama, Japan** (K Aya MD); **Department of Pediatrics, Wakayama Medical University, Wakayama, Japan** (K Nakanishi MD); **Department of Pediatrics, Juntendo University Nerima Hospital, Nerima-ku, Tokyo, Japan** (Y Ohtomo MD);

Introduction

Childhood nephrotic syndrome is a disorder affecting the kidneys in which a large amount of protein passes through the glomerular filter, resulting in hypoproteinaemia and generalised oedema. Idiopathic nephrotic syndrome occurs in two or more of every 100 000 children[1] and is the most common chronic glomerular disease in paediatric nephrology practice. Minimal change nephrotic syndrome is the most common form of the disorder, for which steroid therapy is effective for most patients.[2] Those who respond well rarely progress to chronic renal failure, but up to half develop frequently relapsing nephrotic syndrome (FRNS) or steroid-dependent nephrotic syndrome (SDNS; table 1).[2] Moreover, 10–20% of patients with idiopathic nephrotic syndrome have steroid-resistant nephrotic syndrome (table 1).[2]

Standard treatments for FRNS, SDNS, and steroid-resistant nephrotic syndrome are immunosuppressive agents: cyclophosphamide, chlorambucil, ciclosporin, tacrolimus, and levamisole are used for paediatric FRNS or SDNS, and ciclosporin for paediatric steroid-resistant nephrotic syndrome.[3–5] Most children are effectively treated with these drugs; however, some have frequent relapses. In two studies,[6,7] 10–20% of children taking ciclosporin had frequent relapses, and in another study,[8] about 30% of the patients with steroid-resistant nephrotic syndrome after ciclosporin had steroid-sensitive, frequent relapses after complete remission. In addition to being ineffective in some patients, ciclosporin can cause side-effects—the most common of which is chronic nephrotoxicity[9,10]—suggesting that it should be discontinued within 24 months. However, discontinuation of ciclosporin almost always results in frequent relapses requiring long-term steroid treatment,[11]

Department of Pediatrics, Surugadai Nihon University Hospital, Chiyoda-ku, Tokyo, Japan (Prof S Takahashi MD); **Department of Nephrology, Hyogo Prefectural Kobe Children's Hospital, Suma-Ku, Kobe, Japan** (R Tanaka MD); **and Department of Nephrology, Tokyo Metropolitan Children's Medical Center, Fuchu, Japan** (K Ishikura MD)

Correspondence to: Prof Kazumoto Iijima, Department of Pediatrics, Kobe University Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan **iijima@med.kobe-u.ac.jp**

See Online for appendix

which also poses a long-term risk to children. Therefore, a new treatment that does not involve steroids or immunosuppressive agents is urgently needed.

In the past 10 years, rituximab has had some success in complicated FRNS and SDNS,[12,13] and several research groups have done single-arm or short-term studies of this drug.[14–16] The 2012 Kidney Disease: Improving Global Outcomes clinical practice guidelines[17] introduced rituximab as a treatment option for childhood-onset, complicated FRNS and SDNS. However, the efficacy and safety of rituximab for complicated FRNS and SDNS are yet to be established.[17] We aimed to assess the efficacy and safety of rituximab in patients with high disease activity.

Methods

Study design and participants

In a multicentre, double-blind, randomised, placebo-controlled trial, we enrolled patients at nine centres in Japan. Full eligibility criteria are listed in the appendix. Briefly, we screened patients aged 2 years or older experiencing a relapse of FRNS or SDNS, which had originally been diagnosed as nephrotic syndrome when aged 1–18 years (appendix). Patients with complicated FRNS or SDNS (table 1) who met all other criteria were eligible for inclusion after remission of the relapse they were experiencing at screening.

This study was approved by the institutional review boards at each centre and complied with the Declaration of Helsinki. Participants aged 20 years or older or parents of younger patients provided written informed consent.

Randomisation and masking

Once full eligibility was confirmed, patients were randomly assigned (1:1) to rituximab or placebo. We applied the minimisation method using a computer-generated sequence (SAS PROC PLAN), with age, institution, treatment history (whether a steroid or an immunosuppressive drug, or both, was given during the relapse immediately before randomisation), and the intervals between the previous three relapses as adjustment factors. Patients, patients' guardians, caregivers, treating physicians, and individuals assessing outcomes were masked to assignments. Investigators and patients (or their legal representatives) were masked to peripheral blood B-cell counts, which were centrally monitored. To maintain blinding, allocation codes were disclosed only after the entire clinical trial was completed and all data were locked. However, investigators could request the disclosure of a patient's allocation code urgently in the case of a serious adverse event that could lead to death or was life-threatening, a serious adverse event for which the information was essential to establish what treatment was necessary, or treatment failure.

Procedures

Patients received the first dose of their assigned drug within 2 weeks after randomisation. Patients assigned to rituximab received an intravenous dose of 375 mg/m² (maximum 500 mg) once weekly for 4 weeks. Because the optimum dose for paediatric FRNS and SDNS has not been established, we selected this dosing schedule on the basis of previous reports of rituximab's ability to prevent relapses in patients with immunosuppressant-resistant SDNS[12,13,18] and on the recommended dose for treating B-cell lymphoma, which has a known safety profile. Patients assigned to placebo received intravenous injections of a matched placebo at the same frequency. We used pretreatments to prevent infusion reaction (appendix). Patients could cease assigned treatment if they met discontinuation criteria (appendix).

Participants receiving prednisolone for the relapse at screening continued receiving the drug, taking 60 mg/m² orally three times a day (maximum of 80 mg per day) for 4 weeks. Participants not receiving prednisolone at screening received the same dose until 3 days after complete remission was achieved. After 4 weeks (in patients who received prednisolone at screening) or from 3 days after complete remission (in patients who did not receive prednisolone at screening), patients took 60 mg/m² prednisolone in the morning on alternate days (maximum

	Definition
FRNS	≥2 relapses of nephrotic syndrome within 6 months after initial remission, or ≥4 relapses within any 12-month period
SDNS	2 relapses of nephrotic syndrome during the reduction of steroid treatment or within 2 weeks of discontinuation of steroid treatment
SRNS	Persistent proteinuria after 60 mg/m² oral prednisolone per day for 4 weeks
Complicated FRNS or SDNS	Patients diagnosed with FRNS or SDNS when aged 2 years or older, who had ≥4 relapses in a 12-month period or steroid dependence at any point in the 2 years before relapse at screening, after completion of immunosuppressive drug treatment (eg, ciclosporin, cyclophosphamide, mizoribine, or mycophenolate mofetil); or patients diagnosed with FRNS or SDNS when aged 2 years or older, who had ≥4 relapses in a 12-month period or steroid dependence diagnosed at any point in the 2 years before relapse at screening, during immunosuppressive drug treatment (eg, ciclosporin, cyclophosphamide, mizoribine, or mycophenolate mofetil); or patients with a history of SRNS and diagnosed with FRNS or SDNS when aged 2 years or older, who had ≥4 relapses in a 12-month period or steroid dependence at any point in the 2 years before relapse at screening, during or after the completion of immunosuppressive drug treatment (eg, ciclosporin or a combination of ciclosporin and methylprednisolone)

FRNS=frequently relapsing nephrotic syndrome. SDNS=steroid-dependent nephrotic syndrome. SRNS=steroid-resistant nephrotic syndrome.

Table 1: **Definitions**

80 mg per day) for 2 weeks, then 30 mg/m² on alternate days (maximum 40 mg per day) for 2 weeks, and then 15 mg/m² on alternate days (maximum 20 mg per day) for 2 weeks. When patients had relapses during the study period (1 year of follow-up), they received 60 mg/m² oral prednisolone three times a day (maximum 80 mg per day) until 3 days after complete remission was obtained, when tapering began. If patients were receiving ciclosporin at screening, tapering of this drug began at day 85 (patients received their first dose of rituximab or placebo on day 1), with discontinuation by day 169 (figure 1). If patients were taking any other immunosuppressive drugs, these drugs were discontinued by day 85 (figure 1).

Patients were followed up for 1 year (figure 1). Study visits occurred at baseline; at weeks 1, 2, 3, and 4; and every 4 weeks from week 5. Patients were deemed to have treatment failure if a relapse had occurred by day 85, FRNS or SDNS was diagnosed between days 86 and 365, or steroid resistance was noted (figure 1, appendix). We designed the study protocol with consideration for the placebo group as much as possible. When patients had treatment failure, their allocation code was urgently disclosed. If a patient with treatment failure was in the placebo group, he or she could then choose to begin the treatment deemed the best by investigators—eg, new immunosuppressive drugs—and continue in our study, or to enter a separate rituximab pharmacokinetic study after discontinuation or completion of our trial.

Outcomes

The primary endpoint was the relapse-free period (time of randomisation to the time of first relapse after starting the study treatment). The prespecified secondary endpoints were time to treatment failure, relapse rate (number of relapses per person-year), time to four relapses of nephrotic syndrome in the study period, time to two relapses during reduction of steroid treatment or within 2 weeks of discontinuation of steroid treatment, time to transition to steroid resistance, steroid dose after randomisation, changes in steroid dose before and after randomisation, peripheral blood B-cell count, peripheral blood B-cell depletion period, human antichimeric antibody production rate, and rituximab blood concentration. Safety endpoints were frequency and severity of adverse events, and abnormal values in biochemical tests and haematology assessments. We did post-hoc analyses of the effects of age at time of treatment and age at disease onset on median relapse-free period, the effect of concomitant angiotensin-converting-enzyme inhibitors and angiotensin-receptor blockers on median relapse-free period, time to FRNS or SDNS, the proportion of patients who could discontinue steroid treatment after study drug infusion, the time between cessation of steroid treatment and first relapse, the frequency of infections that required treatment, the effect of B-cell depletion on infections that required treatment and relapses, and changes in characteristics between baseline and 1 year.

Statistical analysis

On the basis of previous reports,[12,13,18] we assumed that 40% of the patients in the rituximab group and 10% of the patients in the placebo group would maintain remission 6 months after registration. 30 patients in each group would be needed to establish the superiority of the test treatment for the primary endpoint with 90% power at a 2·5% one-sided significance level under the assumption of exponential distribution of relapse-free survival time and proportionality of hazards.

We used the log-rank test to analyse the primary endpoint and other time-to-event endpoints. We did an interim analysis (appendix) after 30 patients had relapsed, with a significance level set at 0·25% (one-sided). We summarised time-to-event data with the Kaplan-Meier method and estimated therapeutic effect hazard ratios (HRs) and their 95% CIs with Cox regression.

We made no multiplicity adjustment in the analysis of secondary endpoints. We set the significance level at 5% (two-sided) and report two-sided p values. We calculated the relapse rate and the frequency of infection with the number of events per person-years. We compared groups with the computer-based permutation test, and calculated

For the **trial protocol** see http://www.med.kobe-u.ac.jp/pediat/pdf/rcrn01.pdf

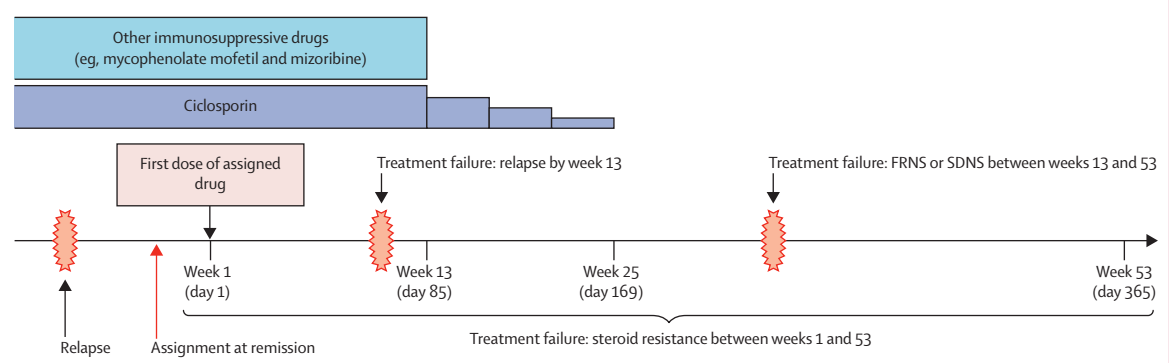

Figure 1: **Study design**
FRNS=frequently relapsing nephrotic syndrome. SDNS=steroid-dependent nephrotic syndrome.

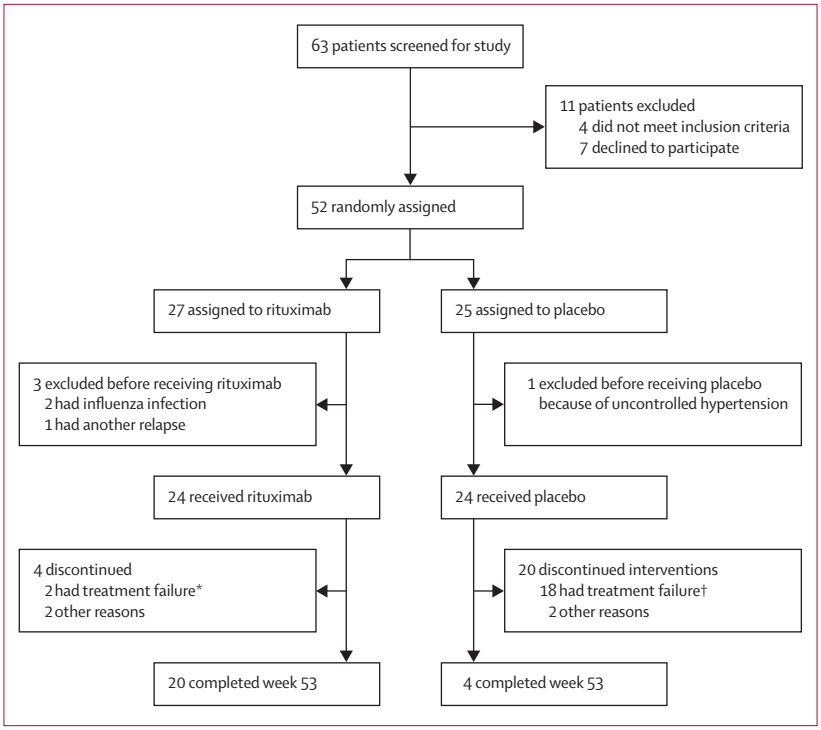

Figure 2: **Trial profile**
*One patient relapsed by week 13, and one was diagnosed with steroid resistance. †Ten relapsed by week 13, and eight were diagnosed with frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome after week 13.

the 95% CI of rate ratios fitting the negative binomial distribution and taking account of overdispersion. With the Wilcoxon rank-sum test, we compared daily steroid doses after randomisation and steroid doses before and after randomisation in both groups. We used the Kaplan-Meier method to assess the proportion of patients with human antichimeric antibody. Analyses were by modified intention to treat, including patients who received their assigned intervention. All analyses were done in SAS (version 9.1).

This trial is registered with the University Hospital Medical Information Network clinical trial registry, number UMIN000001405.

Role of the funding source

The funder of the study had no role in study design, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Nov 13, 2008, and May 19, 2010, 52 patients were randomly assigned to rituximab or placebo. Follow-up ended on Nov 10, 2011. The preplanned interim analysis showed that rituximab was superior to placebo, after which the independent data and safety monitoring committee advised us to discontinue randomisation as specified in the protocol. Therefore, randomisation ended earlier than planned, on May 21, 2010.

52 patients underwent randomisation (figure 2). 48 patients received the assigned intervention (figure 2) and were included in analyses. 20 patients given rituximab and 23 given placebo received all four doses. No patient dropped out before the first relapse. All 20 patients with treatment failure in the placebo group were enrolled into a separate rituximab pharmacokinetic study after discontinuation (n=18) or completion (n=2) of this trial.

Baseline characteristics in the two groups were similar (table 2). The predominant histological type in both groups was minimal change nephrotic syndrome (table 2). All patients were given steroids or immunosuppressants, or both, at relapse immediately before assignment (table 2). More than 70% of patients in both groups reported side-effects of steroid treatment (table 2).

By the end of 1 year of follow-up, 17 patients in the rituximab group and 23 in the placebo group had relapsed. The median relapse-free period was significantly longer in the rituximab group (267 days, 95% CI 223–374) than in the placebo group (101 days, 70–155; HR 0·27, 95% CI 0·14–0·53; p<0·0001; figure 3A). Post-hoc analyses showed that age at disease onset and age at time of treatment did not affect the median relapse-free period in the rituximab group (appendix). Concomitant angiotensin-converting-enzyme inhibitors or angiotensin-receptor blockers, or both, decreased the median relapse-free period in the rituximab group, although the difference was marginally significant (appendix).

Treatment failure was reported in ten patients in the rituximab group and 20 in the placebo group. The time to treatment failure was significantly longer in the rituximab group than in the placebo group (HR 0·27, 95% CI 0·12–0·59; p=0·0005; figure 3B). The relapse rate was significantly lower in the rituximab group (1·54 relapses per person-year [29 relapses in 18·81 person-years]) than in the placebo group (4·17 relapses per person-year [46 relapses in 11·03 person-years]; HR 0·37, 95% CI 0·23–0·59; p<0·0001). Only two patients in each group had frequent relapses in the study period. Time to two relapses during reduction of steroid treatment or within 2 weeks of discontinuation of steroid treatment was significantly longer in the rituximab group than in the placebo group (HR 0·19, 95% CI 0·07–0·54; p=0·0005). A post-hoc analysis showed that significantly more patients in the rituximab group did not experience frequent relapses or steroid dependence than in the placebo group (0·17, 0·06–0·46; p=0·0001; figure 3C). Two patients in the rituximab group had steroid-resistant relapses, compared with no patients in the placebo group.

Mean daily steroid dose after randomisation was significantly lower in the rituximab group than in the placebo group (9·12 mg/m² per day [SD 5·88] *vs* 20·85 mg/m² per day [9·28]; p<0·0001). Mean daily steroid (prednisolone) dose in the rituximab group

decreased significantly after randomisation, but did not change significantly in the placebo group (table 3). Exploratory analyses showed that the proportion of patients who could discontinue steroid treatment after the study drug infusion was similar in the rituximab group (21 of 24, 88%) and the placebo group (19 of 24, 79%; p=0·70). However, median time between cessation of steroid treatment and first relapse was significantly longer in the rituximab group (211 days, 95% CI 166–317) than in the placebo group (42 days, 14–98; HR 0·27, 95% CI 0·14–0·54; p<0·0001). The height-for-age *Z* score improved slightly 1 year after rituximab treatment compared with baseline, although the difference was not significant (appendix). Height *Z* score also seemed to improve in children with residual growth potential in the rituximab group, but again the difference was not significant (appendix).

Most adverse events were mild, and no patients died during the trial. Although more patients had serious adverse events in the rituximab group than in the placebo group (table 4), the difference was not significant (p=0·36). The most common grade 3–4 adverse events in the rituximab group were hypoproteinemia, lymphocytopenia, and neutropenia (table 5). Post-hoc analyses of adverse events showed that the incidence of infections that required treatment were similar in both groups (4·55 infections per person-year [105 infections in 23·08 person-years] *vs* 3·45 infections per person-year [42 infections in 12·18 person-years]; HR 1·27, 95% CI 0·77–2·07, p=0·21). More patients had mild infusion reactions in the rituximab group than in the placebo group (table 4), but the difference was not significant (p=0·12). No grade 3 or 4 infusion reactions were reported in either group (table 4).

The peripheral blood B-cell count decreased substantially immediately after the first dose of rituximab (figure 4), with a median period of B-cell depletion (<5 cells per μL) of 148 days (95% CI 131–170). B-cell counts returned to within the normal range in all patients given rituximab by day 253 (median 118 cells per μL, 95% CI 113–250). By contrast, peripheral blood B-cell count did not change in the placebo group (data not shown).

We did a post-hoc analysis of the effects of B-cell depeletion on relapses and infections. No relapses were reported in the rituximab group during the period of B-cell depletion. However, the rate of infections requiring treatment was higher during the B-cell depletion period (8·43 infections per person-year [49 infections in 5·81 person-years]) than outside of this period (3·24 infections per person-year [56 infections in 17·27 person-years]; HR 0·39, 95% CI 0·27–0·58; p<0·0001); although most were grade 1 respiratory-tract infections. The cumulative proportion of patients with human antichimeric antibody at day 365 was 14% (95% CI 5–38). Blood concentrations of rituximab are shown in table 6.

Discussion

We have shown that the relapse-free period increases with rituximab in patients with childhood-onset, complicated FRNS and SDNS. Adverse events were generally mild and the frequency of serious adverse

	Rituximab (n=24)	Placebo (n=24)
Age (years)	11·5 (5·0)	13·6 (6·9)
Duration of disease (years)	7·9 (4·7)	8·0 (5·4)
Sex		
Male	18 (75%)	16 (67%)
Female	6 (25%)	8 (33%)
Height (cm)	137·7 (21·4)	143·4 (20·4)
Height-for-age *Z* score	−0·96 (1·37)	−0·88 (1·26)
Weight (kg)	44·0 (18·6)	47·5 (15·6)
Body-mass index	22·3 (4·9)	22·6 (4·3)
Systolic blood pressure (mm Hg)	112·3 (11·0)	111·0 (9·6)
Diastolic blood pressure (mm Hg)	65·6 (9·9)	66·8 (8·2)
Serum creatinine (μmol/L)	39·78 (13·26)	44·20 (15·91)
Estimated glomerular filtration rate (mL/m per 1·73 m²)	128·9 (20·6)	126·4 (26·0)
Serum total protein (g/L)	58 (6)	59 (6)
Serum albumin (g/L)	34 (6)	34 (5)
Urinary protein to creatinine ratio (mg/mg)	0·13 (0·11)	0·11 (0·10)
Steroid and immunosuppressant use at relapse immediately before assignment		
Ciclosporin, mycophenolate mofetil, and daily steroids	1 (4%)	0
Ciclosporin, mizoribine, and daily steroids	3 (13%)	3 (13%)
Ciclosporin and daily steroids	0	1 (4%)
Mycophenolate mofetil and daily steroids	0	1 (4%)
Mizoribine and daily steroids	1 (4%)	1 (4%)
Daily steroids with no immunosuppressant	1 (4%)	0
Ciclosporin, mycophenolate mofetil, and steroids on alternate days	2 (8%)	0
Ciclosporin, mizoribine, and steroids on alternate days	6 (25%)	4 (17%)
Ciclosporin and steroids on alternate days	2 (8%)	5 (21%)
Mycophenolate mofetil and steroids on alternate days	0	0
Mizoribine and steroids on alternate days	3 (13%)	3 (13%)
Steroids on alternate days with no immunosuppressant	1 (4%)	2 (8%)
Ciclosporin and mycophenolate mofetil, with no steroids	0	0
Ciclosporin and mizoribine, with no steroids	1 (4%)	1 (4%)
Ciclosporin, with no steroids	1 (4%)	2 (8%)
Mycophenolate mofetil, with no steroids	0	0
Mizoribine, with no steroids	2 (8%)	1 (4%)
No steroids or immunosuppressant	0	0
Renal histology		
Minimal change	21 (88%)	23 (96%)
Focal segmental glomerulosclerosis	2 (8%)	1 (4%)
Unknown	1 (4%)	0
Steroid toxicity*	17 (71%)	19 (79%)
Time between relapse immediately before screening and previous relapse		
<180 days	15 (63%)	18 (75%)
≥180 days	9 (38%)	6 (25%)
Time from assignment to start of assigned intervention (days)	6·3 (2·7)	6·3 (3·4)

Data are mean (SD) or n (%). *Complications induced by steroid treatments, such as hypertension, short stature, diabetes, glaucoma, cataract, central obesity, and osteoporosis.

Table 2: **Baseline characteristics**

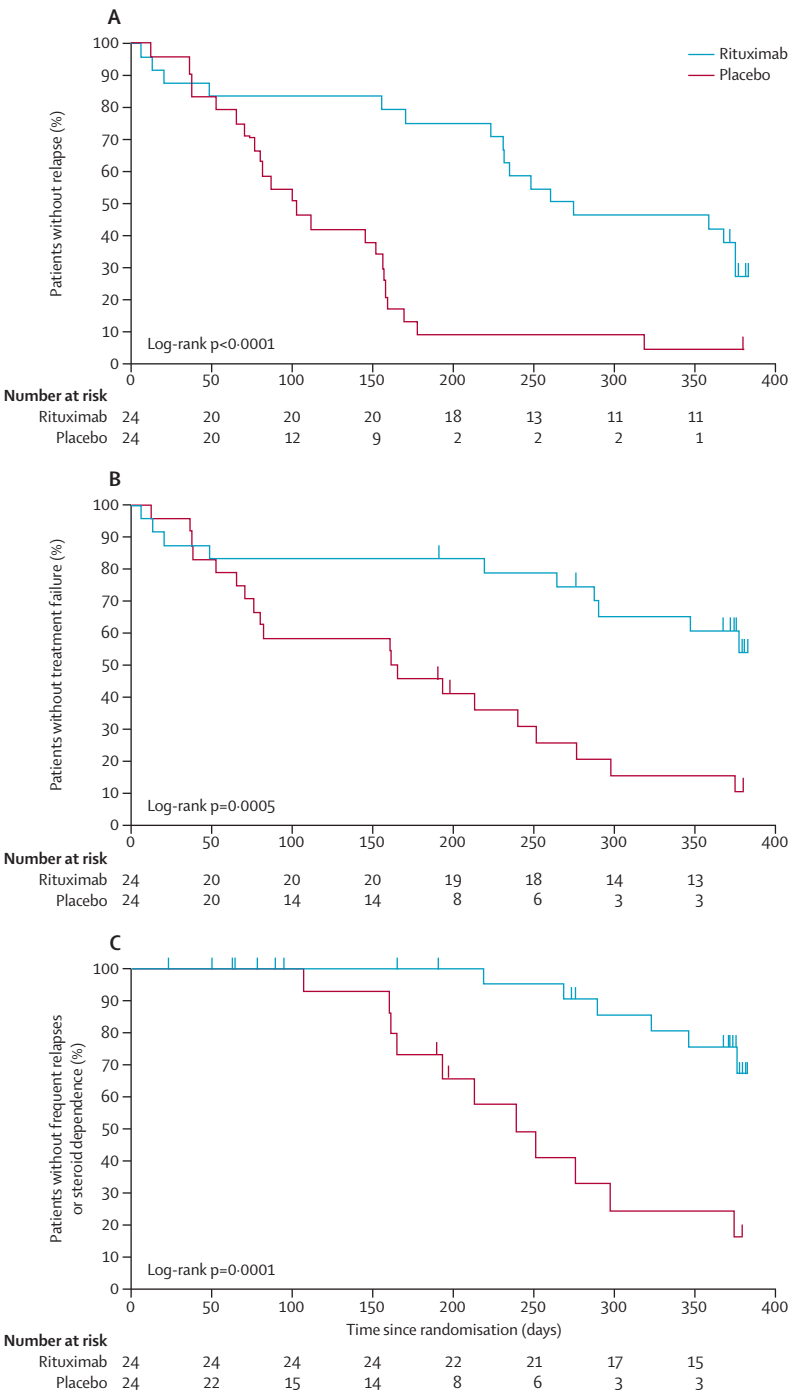

Figure 3: **Kaplan-Meier curves for primary and secondary outcomes**
(A) Patients without relapse. (B) Patients without treatment failure. (C) Patients without frequent relapses or steroid dependence. Vertical lines indicate censoring.

events did not differ significantly between groups. As far as we are aware, we are the first to show that rituximab is safe and effective for at least 1 year of treatment in a multicentre, double-blind, randomised, placebo-controlled trial (panel).

	Number of patients*	Daily prednisolone dose in the 365 days before randomisation (mg/m² per day)	Daily prednisolone dose after randomisation (mg/m² per day)	p value
Rituximab	19	19·13 (9·94)	8·37 (5·62)	<0·0001
Placebo	21	18·02 (10·15)	21·02 (9·81)	0·21

Data are mean (SD), unless otherwise stated. *Number of patients in each group for whom prednisolone doses were available for 365 days before randomisation.

Table 3: **Change in daily prednisolone dose before and after randomisation, by group**

	Rituximab (n=24)	Placebo (n=24)
Number of adverse events	357	251
Patients with ≥1 adverse event	24 (100%)	23 (96%)
Number of serious adverse events	16	7
Patients with ≥1 serious adverse event	10 (42%)	6 (25%)
Deaths	0	0
Number of grade 3 adverse events	24	15
Patients with ≥1 grade 3 adverse event	8 (33%)	3 (13%)
Number of grade 4 adverse events	3	0
Patients with ≥1 grade 4 adverse event	1 (4%)	0
Cases of infections that required treatment	105	42
Grade 1	1	0
Grade 2	101	42
Grade 3	3	0
Grade 4	0	0
Patients with ≥1 infection	23 (96%)	18 (75%)
Total number of infusion reactions	41	26
Grade 1	36	25
Grade 2	5	1
Grade 3	0	0
Grade 4	0	0
Patients with ≥1 infusion reaction	19 (79%)	13 (54%)

Data are n or n (%). Adverse events were categorised according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (version 3.0).

Table 4: **Adverse events**

Patients with complicated FRNS or SDNS usually have a long history of the disease, and many of those included in our trial were receiving fairly high daily doses of steroids with or without immunosuppressive agents to prevent frequent relapses. Therefore, some patients did not meet the usual criteria for frequent relapses or steroid dependence before randomisation. However, more than 80% of patients in our study were treated with prednisolone at the relapse immediately before randomisation, and the mean daily prednisolone dose for 1 year before randomisation was about 20 mg/m². These facts indicate that overall disease activity was high. To allow enrolment of these patients into our trial, we modified the definitions of frequent relapses and steroid dependence immediately before the trial.

A fairly large study[16] of rituximab treatment for patients with steroid-dependent and calcineurin inhibitor-dependent idiopathic nephrotic syndrome, similar to those enrolled in our trial showed that the 6-month probability of remission after the first infusion was 48%. The relapse-free period was similar to that in our study, further emphasising the efficacy of the drug. Our finding that the age at disease onset and age at time of treatment did not greatly affect the outcome is fairly consistent with data from an uncontrolled study that also included adult patients.[19] The fact that patients in the rituximab group who were concomitantly treated with angiotensin-converting-enzyme inhibitors or angiotensin-receptor blockers, or both, had earlier relapses suggests that these drugs did not prevent relapses and patients treated with those drugs had more active disease.

More than half the patients in the rituximab group could discontinue steroids for more than 200 days without relapses after receiving rituximab. A long steroid-free period would allow patients to recover from side-effects, such as impaired growth. Indeed, the height *Z* score seemed to improve 1 year after treatment in the rituximab group, although the difference was not significant. Long-term follow-up studies are needed to clarify the effects of rituximab treatment for recovery from impaired growth.

Rituximab does not increase the frequency of infection when used to treat rheumatoid arthritis.[20,21] However, the rate of infections requiring treatment was higher during the period of B-cell depletion in the rituximab group in our study than when B cells were not depleted. Therefore, attention should be paid to infections during this phase, although most infections in our study were mild and treatable. In studies of patients with complicated nephrotic syndrome who had been taking rituximab, one child died because of pulmonary fibrosis[22] and another patient with fulminant myocarditis due to enterovirus underwent heart transplantation.[23] However, we recorded no deaths or cases of pulmonary fibrosis or myocarditis.

Although we recorded no relapses during B-cell depletion, a low B-cell count could offer clues about whether relapse is likely. Because our protocol did not specify that peripheral B-cell count should be established at time of relapse, a clear correlation between B-cell count and relapse could not be identified. We believe that continued monitoring of the B-cell count throughout the study period, especially at the time of relapse, will be necessary in future investigations. Another limitation of our study was the fairly short observation period. Therefore, the long-term prognosis of patients given rituximab is unclear. Specifically, we are aware of the possibility that not all rare and serious adverse effects were detected in our study—eg, progressive multifocal leukoencephalopathy is known to be a serious side-effect of rituximab.

All patients in our trial had relapsed by 19 months after randomisation. To extend the relapse-free period, further modification of the rituximab treatment and possibly adjunct immunosuppressive therapies might be necessary.

	Rituximab (n=24)		Placebo (n=24)	
	Grade 3	Grade 4	Grade 3	Grade 4
Gastritis	1 (4%)	0	0	0
Gastroenteritis	1 (4%)	0	0	0
Gum infection	1 (4%)	0	0	0
Cellulitis	1 (4%)	0	0	0
Hypertension	1 (4%)	0	0	0
Respiratory disturbance	1 (4%)	0	0	0
Acute kidney failure	1 (4%)	0	0	0
Haemorrhagic cystitis	1 (4%)	0	0	0
Hyperuricaemia	0	1 (4%)	0	0
Hypoproteinaemia*	6 (25%)	0	6 (25%)	0
Adrenal insufficiency	1 (4%)	0	0	0
Nettle rash	1 (4%)	0	0	0
Lymphocytopenia	4 (17%)	0	4	0
Neutropenia	2 (8%)	2 (8%)	0	0
Increased aspartate aminotransferase	0	0	1 (4%)	0
Increased alanine aminotransferase	1 (4%)	0	2 (8%)	0
Increased γ-glutamyl transpeptidase	0	0	1 (4%)	0
Increased creatine phosphokinase	1 (4%)	0	0	0
Hypophosphataemia	0	0	1 (4%)	0

Data are n (%). *Not known to be a side-effect of rituximab and was probably caused by the original disease rather than by rituximab or placebo, because occurred at time of relapse in both groups; other adverse events were known to be caused by the study drug.

Table 5: **Grade 3–4 adverse events**

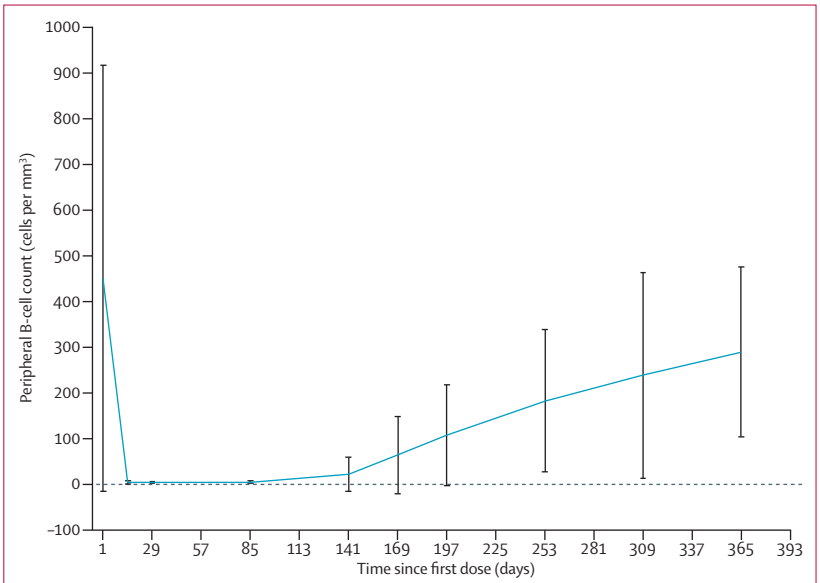

Figure 4: **Mean peripheral B-cell counts in the rituximab group**
Error bars show SD.

Additionally, a comparison of the efficacy, safety, and cost-effectiveness of various rituximab dosing regimens and B-cell-driven regimens still needs to be done.[24] An uncontrolled study[19] showed the importance of long-term follow-up after a core trial assessing the risk and benefit of rituximab treatment. We are preparing a retrospective

	Number of patients for whom data available	Mean rituximab blood concentration (ng/mL)
Day 1 (before the first infusion of rituximab)	24	0
Day 22 (before the fourth infusion of rituximab)	23	156 000 (53 700)
Day 85	24	28 800 (17 500)
Day 169	24	2320 (2680)
Day 365*	23	0

Data in parentheses are SD. *Three samples included here were not assessed on day 365; assessments occurred on days 189, 268, and 271, because these patients discontinued assigned treatment because of treatment failure. However, the values were less than the detectable range and so were included as data for day 365.

Table 6: **Blood concentrations of rituximab**

Panel: Research in context

Systematic review

On completion of our trial, we did a systematic review to identify any randomised controlled trial in which the effectiveness or safety of rituximab, or both, was assessed in children with complicated frequently relapsing nephrotic syndrome (FRNS) or steroid-dependent nephrotic syndrome (SDNS). We searched Medline, Embase, and the Cochrane Library for reports published in any language before Oct 5, 2013, with terms such as "nephrotic syndrome", "rituximab", and "child" (appendix). We identified two open-label, randomised controlled trial (appendix). Meta-analyses of remission frequency at 3 and 6 months confirmed the effectiveness of rituximab in these children, and showed an increase in the remission rate of about 50% at 3 months and of more than 300% at 6 months (appendix).

Interpretation

As far as we are aware, ours is the first randomised, placebo-controlled clinical trial in which the efficacy and safety of rituximab for childhood-onset, complicated FRNS and SDNS have been assessed. Rituximab should be considered as an effective treatment for children with these disorders.

long-term follow-up study of patients enrolled in our trial, with a focus on clinical courses, treatments after the clinical trial, growth, and late adverse effects.

The exact pathogenesis of nephrotic syndrome is unclear, but T-cell-mediated immunological abnormalities are thought to have a role.[25] Several studies[26–29] have shown that B cells can promote T-cell activation, mediate antibody-independent autoimmune damage, and provide costimulatory molecules and cytokines, which sustain T-cell activation in autoimmune diseases. Rituximab inhibits B-cell proliferation and induces B-cell apoptosis.[30] This action leads to B-cell depletion and hence suppression of interactions between B cells and T cells, which could prevent recurrences of nephrotic syndrome. Impaired function of regulatory T cells in patients with minimal change nephrotic syndrome and induction of remission in nephrotic syndrome by regulatory T cells have been reported previously.[31–33] Rituximab could induce an increase in the number and function of regulatory T cells.[34] Rituximab-maintained remission in nephrotic syndrome could be due to the restoration of function of regulatory T cells. Fornoni and colleagues reported[35] that rituximab binds directly to an acid sphingomyelinase-like phosphodiesterase 3b on the cell surface of podocytes, stabilising podocyte structure and function, which could lead to the prevention of recurrent focal segmental glomerulosclerosis. Whether a similar mechanism works in complicated FRNS and SDNS remains to be established.

Contributors

KIi and MS were responsible for the study concept. KIi, MS, and NT designed and managed the study. KNo, KK, KM, KA, KNa, YOht, ST, RT, HK, KIs, and SI collected and interpreted data. YOha did statistical analysis. RM did the systematic review. All authors were members of the writing group and agreed on the content of the report, reviewed drafts, and approved the final version.

Rituximab for Childhood-onset Refractory Nephrotic Syndrome Study Group investigators

Kandai Nozu, Hiroshi Kaito, Yuya Hashimura, Takeshi Ninchoji (Kobe University Graduate School of Medicine, Japan); Shori Takahashi, Hiroshi Saito (Surugadai Nihon University Hospital, Japan); Koichi Nakanishi, Yuko Shima (Wakayama Medical University, Japan); Ryojiro Tanaka, Kyoko Morinaga (Hyogo Prefectural Kobe Children's Hospital, Japan); Kenichiro Miura, Takashi Sekine (Graduate School of Medicine, University of Tokyo); Yoshiyuki Ohtomo, Daisuke Umino (Juntendo University Nerima Hospital, Japan); Kunihiko Aya, Takayuki Miyai (Okayama University Hospital, Japan); Kenji Ishikura, Hiroshi Hataya, Yuko Hamasaki (Tokyo Metropolitan Children's Medical Center, Japan); Shuichi Ito, Koichi Kamei, Masao Ogura, Tomohiro Udagawa, Akiko Tsutsumi (National Center for Child Health and Development, Japan).

Clinical trial steering committee

Kazumoto Iijima (Kobe University Graduate School of Medicine, Japan), Mayumi Sako (National Center for Child Health and Development, Japan), Nao Tsuchida (National Center for Child Health and Development, Japan).

Independent data and safety monitoring committee

Takashi Igarashi (Graduate School of Medicine, University of Tokyo, Japan), Masataka Honda (Tokyo Metropolitan Children's Medical Center, Japan), Satoshi Morita (Graduate School of Medicine, Yokohama City University, Japan).

Declaration of interests

KIi has received grants from the Japanese Ministry of Health, Labour and Welfare for the Large Scale Clinical Trial Network Project (Japan Medical Association Center for Clinical Trials: CCT-B-2001), research on rare and intractable diseases (H24-nanchitou (nan)-ippan-041), and clinical research (H25-iryogijutu-ippan-008); has received research grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Grant-in-Aid for Scientific Research 23591192); has received research grants from Pfizer Japan, Kyowa Hakko Kirion, Abbot Japan, Takeda Pharmaceutical, Asahi Kasei Pharma, Astellas Pharma, Terumo, Chugai Pharmaceutical, Benesis, Dainippon Sumitomo Pharma, Genzyme Japan, Novartis Pharmaceuticals, Mizutori Clinic, AbbVie, and Janssen Pharmaceutical; has received lecture fees from Novartis Pharmaceuticals, Asahi Kasei Pharma, Baxter, Sanofi, Pfizer Japan, Meiji Seika Pharma, Taisho Toyama Pharmaceutical, Kyorin Pharmaceutical, Kyowa Hakko Kirion, Dainippon Sumitomo Pharma, Astellas Pharma, and Chugai Pharmaceutical; and is a paid adviser for Zenyaku Kogyo. MS has received grants from the Japanese Ministry of Health, Labour and Welfare, and is a paid adviser for Zenyaku Kogyo. RM has received research grants from WHO; the Japanese Ministry of Health, Labour and Welfare; the Japanese Ministry of Education, Culture, Sports, Science and Technology; the Gates Foundation; the Japanese Ministry of Foreign Affairs; and Save the Children. NT has received grants from Kaketsuken, GlaxoSmithKline, and Daiichi Sankyo. KA has received grants from JCR Pharmaceuticals and Teijin Pharma, and lecture fees from Boehringer Ingelheim Japan, JMS, Asahi Kasei Pharma, Ono Pharmaceutical, and Kyorin Pharmaceutical. KNa has received lecture fees from Asahi Kasei Pharma, Novartis Pharmaceuticals, Astellas Pharma, and Takeda Pharmaceutical. YOht has received grants from Asahi Kasei Pharma, Taiho Pharmaceutical, and Pfizer Japan; and lecture fees from Kyowa Hakko Kirin, Ferring Pharmaceuticals, Asahi Kasei Pharma, and Daiichi Sankyo. ST has received grants from Sanofi and Novartis Pharmaceuticals. RT has received lecture fees from Pfizer Japan and Novartis Pharmaceuticals. HK has received grants from the Danone

Institute of Japan Foundation, the Hyogo Prefecture Health Promotion Association, and Baxter; and lecture fees from Novartis Pharmaceuticals and Daiichi Sankyo. KIs has received lecture fees and travel expenses from Novartis Pharmaceuticals and Asahi Kasei Pharma. SI has received lecture fees from Asahi Kasei Pharma, Novartis Pharmaceuticals, and Chugai Pharmaceutical. YOha has received grants from the Japanese Ministry of Health, Labour and Welfare; received unlimited educational grants from Kowa Pharmaceutical, Astellas Pharma, Kyowa Hakko Kirin, and Takeda Pharmaceutical during the study period; received lecture fees and honorarium of more than US$5000 for consultations with Chugai Pharmaceutical, Shionogi, Sanofi, and DNP Media Create in the fiscal year of 2012; and has served as the chairman of the board of directors for Statcom, owning stock. The other authors declare no competing interests.

Acknowledgments

This study was funded by the Health and Labour Sciences Research Grants for the Large Scale Clinical Trial Network Project (Japan Medical Association Center for Clinical Trials: CCT-B-2001) from the Japanese Ministry of Health, Labour and Welfare. Zenyaku Kogyo provided rituximab and placebo (which they received from Genentech) free of charge. Zenyaku Kogy was responsible for measurement of human antichimeric antibodies and rituximab blood concentrations, which they delegated to Convence. The costs for these measurements was covered by a fund from the Japanese Ministry of Health, Labour and Welfare. This study was presented at Kidney Week 2012 (San Diego, CA, USA) on Nov 3, 2012, and was reported in abstract form. We thank all our patients and their families, the physicians who participated in this study, and Emma Barber for editing assistancee.

References

1 Eddy AA, Symons JM. Nephrotic syndrome in childhood. *Lancet* 2003; **362:** 629–39.

2 Schulman SL, Kaiser BA, Polinsky MS, Srinivasan R, Baluarte HJ. Predicting the response to cytotoxic therapy for childhood nephrotic syndrome: superiority of response to corticosteroid therapy over histopathologic patterns. *J Pediatr* 1988; **113:** 996–1001.

3 The Scientific Committee of the Japanese Society for Pediatric Nephrology. The 2005 Japanese Society for Pediatric Nephrology treatment guideline for idiopathic nephrotic syndrome in children. http://www.jspn.jp/pdf/0505guideline.pdf (accessed Nov 20, 2013; in Japanese).

4 Hodson EM, Willis NS, Craig JC. Non-corticosteroid treatment for nephrotic syndrome in children. *Cochrane Database Syst Rev* 2013; **10:** CD002290.

5 Hodson EM, Willis NS, Craig JC. Interventions for idiopathic steroid-resistant nephrotic syndrome in children. *Cochrane Database Syst Rev* 2010; **11:** CD003594.

6 Ishikura K, Ikeda M, Hattori S, et al. Effective and safe treatment with cyclosporine in nephrotic children: a prospective, randomized multicenter trial. *Kidney Int* 2008; **73:** 1167–73.

7 Ishikura K, Yoshikawa N, Hattori S, et al, and Japanese Study Group of Renal Disease in Children. Treatment with microemulsified cyclosporine in children with frequently relapsing nephrotic syndrome. *Nephrol Dial Transplant* 2010; **25:** 3956–62.

8 Hamasaki Y, Yoshikawa N, Hattori S, et al, and the Japanese Study Group of Renal Disease. Cyclosporine and steroid therapy in children with steroid-resistant nephrotic syndrome. *Pediatr Nephrol* 2009; **24:** 2177–85.

9 Inoue Y, Iijima K, Nakamura H, Yoshikawa N. Two-year cyclosporin treatment in children with steroid-dependent nephrotic syndrome. *Pediatr Nephrol* 1999; **13:** 33–38.

10 Iijima K, Hamahira K, Tanaka R, et al. Risk factors for cyclosporine-induced tubulointerstitial lesions in children with minimal change nephrotic syndrome. *Kidney Int* 2002; **61:** 1801–05.

11 Ishikura K, Yoshikawa N, Nakazato H, et al. Two-year follow-up of a prospective clinical trial of ciclosporin for frequently relapsing nephrotic syndrome in children. *Clin J Am Soc Nephrol* 2012; **10:** 1576–83.

12 Benz K, Dötsch J, Rascher W, Stachel D. Change of the course of steroid-dependent nephrotic syndrome after rituximab therapy. *Pediatr Nephrol* 2004; **19:** 794–97.

13 Gilbert RD, Hulse E, Rigden S. Rituximab therapy for steroid-dependent minimal change nephrotic syndrome. *Pediatr Nephrol* 2006; **21:** 1698–700.

14 Guigonis V, Dallocchio A, Baudouin V, et al. Rituximab treatment for severe steroid- or cyclosporine-dependent nephrotic syndrome: a multicentric series of 22 cases. *Pediatr Nephrol* 2008; **23:** 1269–79.

15 Ravani P, Magnasco A, Edefonti A, et al. Short-term effects of rituximab in children with steroid- and calcineurin-dependent nephrotic syndrome: a randomized controlled trial. *Clin J Am Soc Nephrol* 2011; **6:** 1308–15.

16 Ravani P, Ponticelli A, Siciliano C, et al. Rituximab is a safe and effective long-term treatment for children with steroid and calcineurin inhibitor-dependent idiopathic nephrotic syndrome. *Kidney Int* 2013; **84:** 1025–33.

17 Kidney Disease: Improving Global Outcomes. KDIGO clinical practice guideline for glomerulonephritis. June, 2012. http://kdigo.org/home/glomerulonephritis-gn/ (accessed Nov 20, 2013).

18 Kemper MJ, Gellermann J, Habbig S, et al. Long-term follow-up after rituximab for steroid-dependent idiopathic nephrotic syndrome. *Nephrol Dial Transplant* 2012; **27:** 1910–15.

19 Ruggenenti P, Ruggiero B, Cravedi P, et al, for the Rituximab in Nephrotic Syndrome of Steroid-Dependent or Frequently Relapsing Minimal Change Disease Or Focal Segmental Glomerulosclerosis (NEMO) Study Group. Rituximab in steroid-dependent or frequently relapsing idiopathic nephrotic syndrome. *J Am Soc Nephrol* 2014; **25:** 850–63.

20 Kelesidis T, Daikos G, Boumpas D, Tsiodras S. Does rituximab increase the incidence of infectious complications? A narrative review. *Int J Infect Dis* 2011; **15:** e2–16.

21 Buch MH, Smolen JS, Betteridge N, et al, and the Rituximab Consensus Expert Committee. Updated consensus statement on the use of rituximab in patients with rheumatoid arthritis. *Ann Rheum Dis* 2011; **70:** 909–20.

22 Chaumais MC, Garnier A, Chalard F, et al. Fatal pulmonary fibrosis after rituximab administration. *Pediatr Nephrol* 2009; **24:** 1753–55.

23 Sellier-Leclerc AL, Belli E, Guérin V, Dorfmüller P, Deschênes G. Fulminant viral myocarditis after rituximab therapy in pediatric nephrotic syndrome. *Pediatr Nephrol* 2013; **28:** 1875–79.

24 Cravedi P, Ruggenenti P, Sghirlanzoni MC, Remuzzi G. Titrating rituximab to circulating B cells to optimize lymphocytolytic therapy in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 2007; **2:** 932–37.

25 Shalhoub RJ. Pathogenesis of lipoid nephrosis: a disorder of T-cell function. *Lancet* 1974; **2:** 556–60.

26 Liu K, Mohan C. Altered B-cell signaling in lupus. *Autoimmun Rev* 2009; **8:** 214–18.

27 Chan OT, Hannum LG, Haberman AM, Madaio MP, Shlomchik MJ. A novel mouse with B cells but lacking serum antibody reveals an antibody-independent role for B cells in murine lupus. *J Exp Med* 1999; **189:** 1639–48.

28 Sfikakis PP, Boletis JN, Lionaki S, et al. Remission of proliferative lupus nephritis following B cell depletion therapy is preceded by down-regulation of the T cell costimulatory molecule CD40 ligand: an open-label trial. *Arthritis Rheum* 2005; **52:** 501–13.

29 Bugatti S, Codullo V, Caporali R, Montecucco C. B cells in rheumatoid arthritis. *Autoimmun Rev* 2007; **7:** 137–42.

30 Maloney DG. Mechanism of action of rituximab. *Anticancer Drugs* 2001; **12** (suppl 2)**:** S1–4.

31 Araya C, Diaz L, Wasserfall C, et al. T regulatory cell function in idiopathic minimal lesion nephrotic syndrome. *Pediatr Nephrol* 2009; **24:** 1691–98.

32 Hashimura Y, Nozu K, Kanegane H, et al. Minimal change nephrotic syndrome associated with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *Pediatr Nephrol* 2009; **24:** 1181–86.

33 Le Berre L, Bruneau S, Naulet J, et al. Induction of T regulatory cells attenuates idiopathic nephrotic syndrome. *J Am Soc Nephrol* 2009; **20:** 57–67.

34 Stasi R, Cooper N, Del Poeta G, et al. Analysis of regulatory T-cell changes in patients with idiopathic thrombocytopenic purpura receiving B cell-depleting therapy with rituximab. *Blood* 2008; **112:** 1147–50.

35 Fornoni A, Sageshima J, Wei C, et al. Rituximab targets podocytes in recurrent focal segmental glomerulosclerosis. *Sci Transl Med* 2011; **3:** 85ra46.