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Long-Term Efficacy and Safety of Rituximab Versus Tacrolimus in Children With Steroid Dependent Nephrotic Syndrome

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Introduction: In the Rituximab for Relapse Prevention in Nephrotic Syndrome (RITURNS) trial, we demonstrated superior efficacy of single-course rituximab over maintenance tacrolimus in preventing relapses in children with steroid dependent nephrotic syndrome (SDNS) during a 1-year observation. Here we present the long-term outcomes of all 117 trial completers, who were followed up for another 2 years.

Methods: Relapsing patients in the rituximab arm received a second course of rituximab, either with ($n = 44$) or without mycophenolate mofetil (MMF) cotreatment ($n = 15$). In the tacrolimus arm, second line rituximab monotherapy was initiated after relapses ($n = 32$) or electively ($n = 24$).

Results: All 12-month relapse-free patients in the rituximab arm relapsed in the second postexposure year, resulting in similar median relapse-free survival times in the 2 trial arms (62 vs. 59 weeks). Second line rituximab in the tacrolimus arm was less effective than first-line therapy in patients switched to rituximab following a relapse (relapse-free survival 55 vs. 63 weeks, $P < 0.01$). B-cell counts 6 months post-rituximab predicted relapse risk both for first and second line therapy. MMF cotreatment yielded much improved 2-year relapse-free survival as compared to rituximab monotherapy (67% vs. 9%, $P < 0.0001$). Higher grade 2 adverse event rates were observed post-rituximab versus on tacrolimus (0.87 vs. 0.53 per year).

Conclusion: The superior therapeutic effect of rituximab in SDNS vanishes during the second year post-exposure. Rituximab appears to yield longer remission when applied as first line as compared to second line therapy. Maintenance MMF following rituximab induces long-term disease remission.

Kidney Int Rep (2023) **8,** 1575–1584; https://doi.org/10.1016/j.ekir.2023.05.022
KEYWORDS: childhood nephrotic syndrome; rituximab; steroid dependent nephrotic syndrome; tacrolimus

Although most children with idiopathic nephrotic syndrome respond well to glucocorticoid treatment, approximately 40% develop a complicated course with frequent relapses or even steroid dependency.[1] Calcineurin inhibitors are an established first-line steroid-sparing therapy for patients with SDNS, whereas B-lymphocyte depleting therapy is mostly used as a rescue for calcineurin inhibitor-resistant cases.[1–9]

A single course of rituximab reliably retains disease remission for 6 to 12 months and the side effect profile observed to date is benign. The excellent efficacy and safety profile of rituximab raises the question of whether it could be used as a first-line alternative to calcineurin inhibitor therapy.[3–10]

In the randomized controlled RITURNS trial, we recently demonstrated superior efficacy of a single course of rituximab over maintenance tacrolimus therapy in children with SDNS, with 90% versus 63% relapse-free survival and a favorable side effect profile for rituximab during a 12-month observation period.[2] The 117 completers of this single-center trial were followed-up with for another 2 years after the end of the randomized study period and received further treatment according to standardized protocols during this extended follow-up period. This provided the

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Received 6 February 2023; revised 18 May 2023; accepted 22 May 2023; published online 29 May 2023

opportunity to analyze several important aspects of B-lymphocyte depleting therapy in SDNS, such as the following: (i) the eventual duration of disease remission after a single course of rituximab and during maintenance tacrolimus treatment, (ii) the efficacy of second-line versus first-line rituximab therapy, (iii) the added efficacy of MMF comedication over rituximab monotherapy, and (iv) the predictive value of B-lymphocyte counts obtained 6 months following rituximab administration. In addition, we analyzed the long-term safety of rituximab and tacrolimus and assessed the long-term impact of different treatment protocols on steroid exposure and related obesity.

METHODS

Study Design and Setting

In this study, we analyzed the 3-year outcomes of 117 children who were followed-up with in the RITURNS trial, a prospective, single-center, open-label, 2-parallel-arm, phase 3 randomized clinical trial to test the efficacy of single-course rituximab compared with maintenance tacrolimus in maintaining disease remission during 1 year among children with SDNS (ClinicalTrials.gov: NCT02438982; Clinical Trial Registry of India: CTRI/2014/01/004355).[2] All patients were followed-up with for another 24 months after the end of the 12-month trial phase, resulting in a total observation period of 36 months (STROBE checklist in online supplement). Audiovisual consents and assents were initially taken for the original RITURNS trial with a 12-month trial period. Separate informed consents were taken for the follow-up study after completion of the RITURNS trial.

The study was approved by the Institutional Review Board of NRS Medical College and Hospital, Kolkata, India, and was performed in accordance with the Declaration of Helsinki at Pediatric Nephrology center of this institute.

Inclusion and Exclusion Criteria

Eligibility criteria were as in the RITURNS trial along with the availability of long-term data.[2] Inclusion requirements comprised, among others, an estimated glomerular filtration rate >80 ml/min per 1.73 m^2, current proteinuria remission, and no previous exposure to a steroid-sparing agent. Exclusion criteria were congenital nephrotic syndrome, secondary causes of nephrotic-syndrome (known etiology e.g., lupus erythematosus, IgA nephropathy, amyloidosis; known chronic infections such as tuberculosis, HIV, hepatitis B or C; and known malignancy). All patients had undergone kidney biopsy with light and immunofluorescence microscopy before enrolment.

Procedures and Treatment

Standard definitions were used for frequently relapsing nephrotic syndrome and steroid dependent nephrotic syndrome (Supplementary Table S1). Remission was defined as urine protein-to-creatinine ratio <0.2 mg/mg, serum albumin >2.5 g/dl, and no edema (or urine albumin nil or trace for 3 consecutive early morning specimens). Relapse was defined as urine albumin 3+ or 4+ for 3 consecutive early morning specimens (or urine protein-to-creatinine ratio >2 mg/mg), after previous remission.

In all relapses, remission was induced using standard oral prednisone therapy (60 mg/m^2 per day) until protein excretion was normalized for 3 consecutive days. Rituximab treatment courses comprised 2 rituximab infusions (@375 mg/m^2 maximum 500 mg) administered within a 7-day interval after attainment of remission. Tacrolimus was administered at an initial dose of 0.2 mg/kg/d which was subsequently adapted to achieve trough levels of 5 to 7 ng/ml, along with tapering doses of alternate day prednisolone.

Secondary treatment in the patients of the rituximab arm who developed a first relapse depended on the time when the relapse occurred relative to the initial rituximab treatment course. Those patients who relapsed within the 12-month randomized trial period ($n = 6$) received a second course of rituximab (2 infusions @375 mg/m^2, maximum 500mg) and MMF (1200 mg/m^2 per day orally in 2 divided doses) was started after completion of the trial period (i.e., in week 53 after randomization) (early relapsers). Those patients who relapsed between weeks 52 and 80 after the first rituximab course received a second course of rituximab immediately combined with maintenance MMF therapy (intermediate relapsers). The patients who relapsed beyond week 80 received a second course of rituximab only, without maintenance MMF (late relapsers) (see Figure 1 for overview).

The patients in the tacrolimus arm were administered a course of rituximab at the time of the first relapse after induction of remission by standard steroid therapy and tacrolimus was discontinued within 15 days of the second rituximab infusion. No prednisone was administered during this transition. In 24 patients, parents opted for elective rituximab therapy at the end of the trial period while the patient was still in remission. In case of re-relapse after rituximab monotherapy, a second course of rituximab was administered and MMF maintenance therapy was added.

Data regarding the number of relapses, side effects, cumulative steroid dose, circulating B-cell count (number/mm^3) measured via flow cytometry,

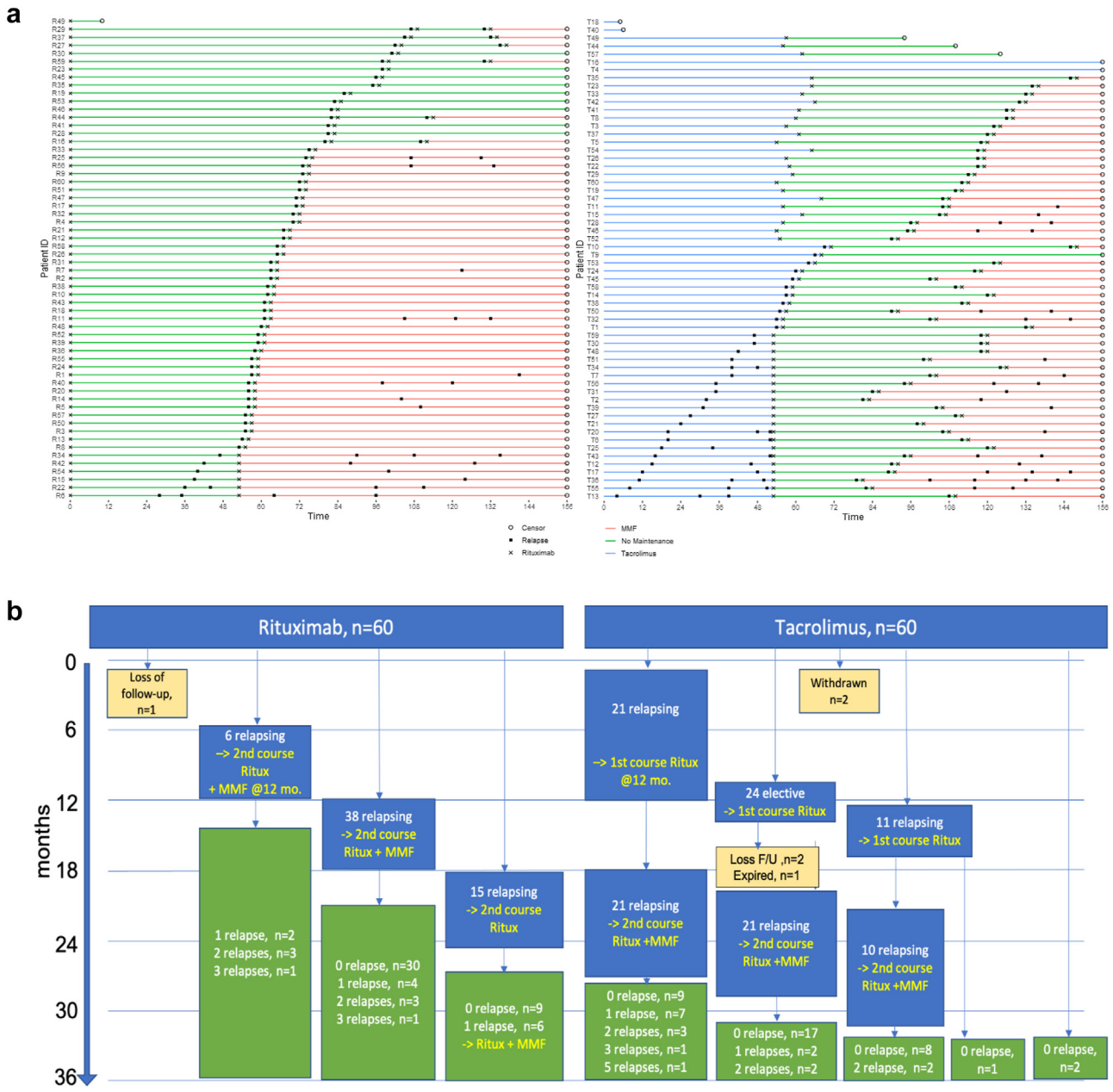

Figure 1. (a) Synopsis of disease activity and therapies applied in rituximab arm (left panel) and tacrolimus arm (right panel) during 3-year observation period. Full square: relapse treated with standard oral prednisone schema; cross: rituximab administration; open circle: censoring event. Blue, red, and green lines indicate times of tacrolimus, MMF, and no maintenance immunosuppressive therapy, respectively. Cases sorted by time to first relapse. (b) Flow chart of relapse activity and follow-up interventions during 3 years follow-up in the 2 trial arms. Blue boxes reflect timing of relapses and secondary interventions in subgroups with early (<12 months), intermediate (12–18 months) and late relapses (>18 months) following primary intervention or elective switching from tacrolimus to rituximab after completion of 12-month randomized trial period.

as well as hematological and biochemical parameters were noted during regular follow-up and relapse as necessary. In the rituximab arm, B-lymphocyte counts were measured during relapse and after 2 weeks, 3, 6, and 9 months following rituximab exposure.

Medication intake and proteinuria dipstick results were recorded daily in a patient diary. Adverse events were graded according to the Common Terminology Criteria for Adverse Events, version 3.[11]

Clinical and Biochemical Assessment

Clinical histories with the dates and treatments of all relapses as well as adverse events were recorded over the 36-month follow-up period after standardized screening of the patients' hospital records. Retrospective data regarding relapses and cumulative steroid dosage in the year before enrolment in the RITURNS trial were also available for analysis.

Proteinuria was assessed by measurement of a 24-hour urine sample or by assessing urine protein-to-creatinine

ratio. In addition, parameters such as blood counts, serum creatinine, serum albumin, and serum cholesterol values were obtained. Glomerular filtration rate was estimated using the modified Schwartz formula.[12]

Statistical Analysis

The cohorts were characterized using descriptive statistical methods. The cohort was described as a whole and separated by treatment groups. Time-to-event endpoints were visualized by Kaplan-Meier plots, and median event-free survival times with associated 95% confidence intervals (CIs), and log-rank *P*-values were calculated. Furthermore, multivariable Cox regression models and Prentice-Williams-Peterson gap time models were fitted to assess the impact of further variables on the time-to-event endpoints and recurrent relapses over time, respectively.[13] In Prentice-Williams-Peterson models, the times between recurrent events (''gap times'') are modeled by a stratified proportional hazards model, where events are analyzed in strata according to the number of respective previous events.

For the analysis of the primary end point (time to first relapse), all patients of the original RITURNS trial were included and analyzed as per their previous group allocation (patients of the tacrolimus arm who switched to rituximab without prior relapse were censored at the time of switching). A multivariable Cox regression model with respect to the time to first relapse included the following covariates: treatment group (rituximab vs. tacrolimus), gender, age, pretrial disease duration, and renal histopathology (minimal change disease vs. focal segmental glomerulosclerosis). Additional multivariable Cox models were calculated in the rituximab cohort to further evaluate the impact of the following variables: in a model for the time to first relapse, the B-cell count 6 months after rituximab exposure was included on top of the above-listed covariates; in a model for the time to re-relapse following the second rituximab course, MMF cotreatment, and the B-cell count 6 months post-rituximab reexposure were additionally included.

In a Prentice-Williams-Peterson gap time model for the time between relapses after the second rituximab course, the variables MMF cotreatment, time to second rituximab administration, gender, age, and renal histopathology were considered as covariates.

The group comparison with respect to 2-year relapse-free survival was analyzed by a 2-sample test for equality of proportions with Yates' continuity correction, where the associated 95% CI for the difference of the proportions is also given.

To assess the association of the B-lymphocyte count 6 months after the first rituximab course and the time to relapse after the first rituximab dose or the B-lymphocytes 6 months after the second rituximab course, a linear regression model was fitted, respectively. The regression lines are given in the associated scatter plots, and corresponding regression coefficients or Pearson's product-moment correlations with associated 95% CIs were calculated. We analyzed the receiver operating characteristic curve of the 6-month B-cell count as a predictor of relapses within 12 months and determined the cut-off value with the maximal Youden Index. Sensitivity and specificity with respect to this cut-off value are presented.

In a linear regression analysis, the change in body mass index (BMI) measurements to standard deviation scores with respect to the relative time on tacrolimus exposure (continuous, 0–1), relative time on MMF (continuous, 0–1), and the number of rituximab doses (0, 1, 2, and 3) is investigated. Effect estimates with 95% CIs are presented.

All other secondary endpoints (number of relapses following rituximab retreatment, biochemical parameters, cumulative prednisolone dose, anthropometry, and therapeutic monitoring) are presented using descriptive statistical measures. All *P*-values should be interpreted in a descriptive manner.

RESULTS

The 3-year course of the total trial population is summarized in Figure 1a and b. The baseline patient characteristics for the original rituximab and tacrolimus trial cohorts are given in Table 1. The total number of relapses observed during the 3 years was 89 (0.5/patient per year) among the patients originally randomized to rituximab, and 139 (0.8/patient per year) among those randomized to tacrolimus.

First-Line Rituximab Versus Tacrolimus Therapy

Whereas only 6 of the 60 patients randomized to rituximab therapy relapsed in the first year, all remaining 53 patients (1 withdrew from the study during the initial trial period) followed-up with for 36 months developed relapses in the second treatment year (Figure 1a and b). Among the 60 patients in the tacrolimus cohort, 32 relapsed while on tacrolimus, 24 patients electively opted to switch to rituximab therapy on completion of the 12-month trial period (2 of whom were subsequently lost to follow-up and 1 expired unrelated to treatment), 2 patients remained relapse-free throughout the 3-year observation period and 2 withdrew from the study during the initial trial period.

Whereas relapses occurred later in the rituximab than in the tacrolimus treatment arm in the first year, more patients in the rituximab arm developed their

Table 1. Baseline characteristics stratified by treatment arm and *post hoc* analysis grouping

	Tacrolimus	Rituximab	Rituximab arm subgroups by time to relapse			Tacrolimus arm subgroups by modality of switch to rituximab	
Variables	$n = 60$	$n = 59$	Early relapse (week 28–52) $n = 6$	Intermediate relapse (week 53–80) $n = 38$	Late relapse (week 81–107) $n = 15$	Elective switch $n = 24$	Postrelapse switch $n = 32$
Age (yrs)	7.2 ± 2.8	7.1 ± 2.8	6.7 ± 2.8	7.2 ± 2.9	7.1 ± 2.8	6.9 ± 3.2	7.3 ± 2.6
Duration of disease (yrs)	2.5 ± 1.5	2.3 ± 1.7	3.6 ± 2.1	2.3 ± 1.6	2.0 ± 1.7	1.8 ± 1.4	3.0 ± 1.5
FSGS (%)	18 (30%)	17 (29%)	4 (67%)	10 (26%)	3 (20%)	7 (29%)	11 (34%)
Relapses per patient in pretrial year	3.9 ± 1.1	3.7 ± 1.3	4.8 ± 1.6	3.7 ± 1.2	3.1 ± 1.0	3.5 ± 1.0	4.1 ± 1.1
Cum. prednisolone dose in pretrial year (mg/kg/ yr)	246 ± 48	239 ± 53	298 ± 81	237± 48	223± 36	229 ± 47	260 ± 46
Height Z-score	−1.2 ± 0.64	−1.43 ± 0.75	−1.66 ± 0.40	−1.54 ± 0.85	−1.06 ± 0.40	−1.15 ± 0.81	−1.25 ± 0.50
BMI Z-score	2.2 ± 0.93	2.2 ± 1.1	2.75 ± 1.14	2.28 ± 1.04	1.93 ± 1.02	2.06 ± 1.04	2.28 ± 0.88
Serum albumin (g/dl)	4.3 ± 0.81	4.16 ± 0.73	3.43 ± 0.10	4.27 ± 0.71	4.18 ± 0.77	4.45 ± 0.80	4.19 ± 0.83
eGFR (ml/min per 1·73 m^2)	103 ± 11	100 ± 8.7	100 ± 9.5	99 ± 9	103 ± 7.3	106 ± 12.4	101 ± 9.31

BMI, body mass index; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis.

first relapse in year 2, resulting in no significant difference in relapse-free survival for the original trial arms for the entire period of observation (median [95% CI] time to relapse: 63 [60–71] vs. 59 [54–69] weeks, $P = 0.49$) (Figure 2).

By multivariable Cox regression analysis, the overall risk of developing a first relapse did not differ between the original trial arms (Hazard ratio [HR] 1.19, 95% CI: 0.76–1.87; $P = 0.45$). The pretrial disease duration (HR 1.42, 95% CI:1.17–1.72; $P < 0.001$) and patient age (HR 0.86, 95% CI: 0.76–0.97; $P = 0.016$), but not female sex (HR 0.78, 95% CI: 0.5–1.22; $P = 0.28$), or the histopathological diagnosis of focal segmental glomerulosclerosis (HR 0.74, 95% CI: 0.47–1.18; $P = 0.21$) significantly impacted the relapse risk.

First-Line Versus Second-Line Rituximab Monotherapy

All but 4 patients in the original tacrolimus arm were switched to rituximab monotherapy either following the first relapse or electively after completion of the 12-month randomized trial period. This allowed us to compare the outcomes of first-line (original rituximab arm) and second-line rituximab therapy in SDNS. The median (95% CI) time to first relapse after rituximab exposure in patients with prior maintenance tacrolimus therapy was 55 (51–59) weeks, as compared to 63 (60–71) weeks after first-line rituximab therapy ($P = 0.0055$). Among the patients with second-line rituximab therapy, 32 were switched to rituximab following a relapse on tacrolimus whereas 24 were switched electively from tacrolimus to rituximab. The latter subgroup was characterized by a significantly shorter pretrial disease duration ($P = 0.005$) and a lower pretrial relapse frequency ($P < 0.05$) (Table 1). The electively switched patients tended to have a longer time to relapse following rituximab than the patients switched postrelapse ($P = 0.07$) (Figure 3). In comparison with the first-line rituximab treatment arm, patients who had relapsed on tacrolimus had a significantly shorter time to relapse following second-line rituximab therapy (52 [41–57] weeks, $P < 0.001$), whereas time to relapse in the elective switchers did not differ (59.5 [52-65] weeks, $P = 0.4$).

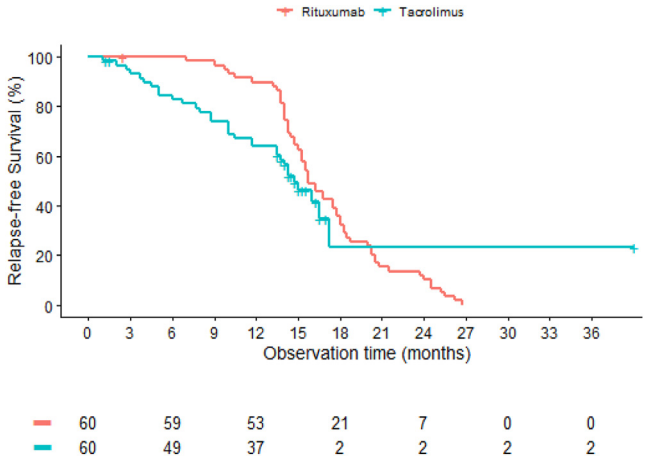

Figure 2. Long-term relapse-free survival of patients in the rituximab and tacrolimus trial arms. The table below the graph indicates the number of patients still at risk at the respective points in time.

Impact of MMF Maintenance Therapy on Post-Rituximab Relapse-Free Survival

To assess the impact of maintenance MMF medication following rituximab on relapse-free survival, the time to re-relapse was assessed in 44 patients from the original rituximab arm and 52 patients from the original tacrolimus arm who received a second course of rituximab followed by MMF maintenance therapy after relapsing after rituximab monotherapy. The cumulative risk of re-relapse while on MMF therapy was compared with the relapse risk without maintenance immunosuppression, for which the observation periods of all patients of the original rituximab arm and the 56 patients of the tacrolimus arm who were switched to

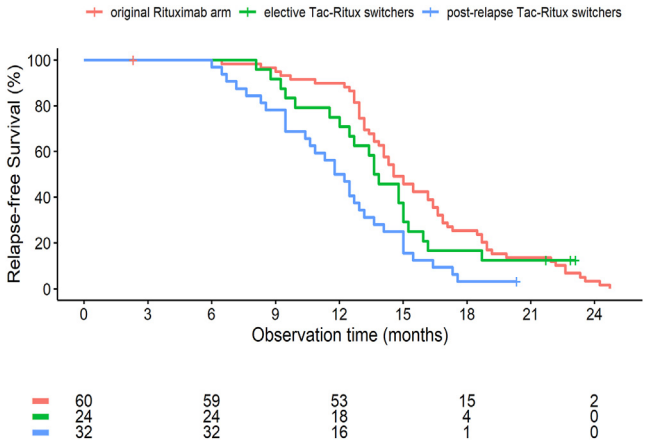

Figure 3. Time to first relapse after first-line versus second-line rituximab therapy. Red: all 60 patients of original rituximab arm; green: 24 patients of original tacrolimus arm electively switched to rituximab from tacrolimus; blue: 32 patients of original tacrolimus arm switched to rituximab after relapsing on tacrolimus. The table below the graph indicates the number of patients still at risk at the respective points in time.

rituximab monotherapy were combined. Whereas no difference in re-relapse rates was apparent in the first year of observation, the 2-year relapse-free survival was 67% with maintenance MMF therapy as compared to 9% for the periods without post-rituximab maintenance immunosuppression ($P < 0.0001$; difference of proportions: 58%; 95% CI 45%–71%).

The impact of MMF on re-relapse-free survival on rituximab reexposure was also analyzed selectively for the patients of the rituximab trial arm (Supplementary Table S2), who were prescribed MMF maintenance therapy if the first relapse had occurred within 18 months of rituximab administration. Whereas the comparison of patients with and without MMF maintenance showed an insignificant difference in relapse-free survival ($P = 0.21$, Supplementary Figure S1), multivariable Cox regression analysis disclosed a nearly 4-fold increased risk of developing a re-relapse in patients who received a second course of rituximab without MMF comedication versus patients who received MMF (HR 3.95; 95% CI 1.25–12.39; $P = 0.019$). Age, sex, pretrial disease duration, and histopathological diagnosis were not significantly associated with the re-relapse risk. A Prentice-Williams-Peterson gap time model analysis considering all relapses after the second rtuximab course confirmed a significant decrease in the risk of relapses by maintenance MMF therapy (Table 2).

Rituximab Pharmacodynamic Monitoring

In the rituximab trial arm, the blood B-cell count decreased to <5/μl in all patients following 2 doses of rituximab and gradually recovered within 6 to 52 weeks, with large individual variability.

The B-lymphocyte count 6 months postdosing was inversely correlated with the time to the first relapse (linear regression coefficient beta = -3.18 [-4.82, -1.53] months per 100 B cells, $P < 0.001$; Figure 4a). The 6 patients who developed relapses within 12 months had largely recovered B-cell counts at 6 months. A 6-month B-cell count >270/ul was predictive of a relapse within 12 months of dosing at a sensitivity of 100% and a specificity of 96%.

In contrast, the intermediate and late relapsers could not be predicted from the 6-month B-cell count. When the B-cell counts were included in the multivariable Cox regression model, the risk for the first relapse in the rituximab group was increased at an HR of 2.13 (95% CI: 1.17–3.88) per 100 B-lymphocytes per uL ($P = 0.013$), whereas age, sex, prior disease duration, and histopathological diagnosis were not predictive.

The B-cell counts observed 6 months postdosing were closely correlated for the first and the second course of rituximab ($r = 0.92$ [0.87, 0.96], $P < 0.001$) (Figure 4b).

In the patients who received a second dose of rituximab following a relapse, the re-relapse risk was also associated with the 6-month post-rituximab B-cell count according to multivariate Cox regression (Supplementary Table S3), increasing 4.5-fold per 100 B-cells/ul (HR 4.49; 95% CI 1.87–10.8) ($P = 0.0008$).

At a group level, there were no significant differences of baseline, nadir, or 6-month posttreatment B-cell counts between the first and second rituximab administration in the rituximab trial arm and the first rituximab exposure in the previous tacrolimus trial arm

Table 2. Prentice-Williams-Peterson gap time model, modeling times between relapses after the second dose of rituximab via the following covariates: time to second rituximab administration, gender, age, MMF comedication, renal histopathology

Variables	Coefficient	SE	HR	CI 2.5 %	CI 97.5 %	P
MMF (reference = no MMF)	−1.655	0.7411	0.1911	0.04471	0.8167	0.03
Sex (reference = male)	−0.5184	0.3742	0.5955	0.286	1.24	0.2
Age (yr)	0.05825	0.07145	1.06	0.9215	1.219	0.4
Non-FSGS histopathology (reference = FSGS)	−0.7179	0.3977	0.4878	0.2237	1.063	0.07
Time to second rituximab administration (wks)	−0.03129	0.01763	0.9692	0.9363	1.003	0.08

FSGS, focal segmental glomerulosclerosis; MMF, mycophenolate mofetil.

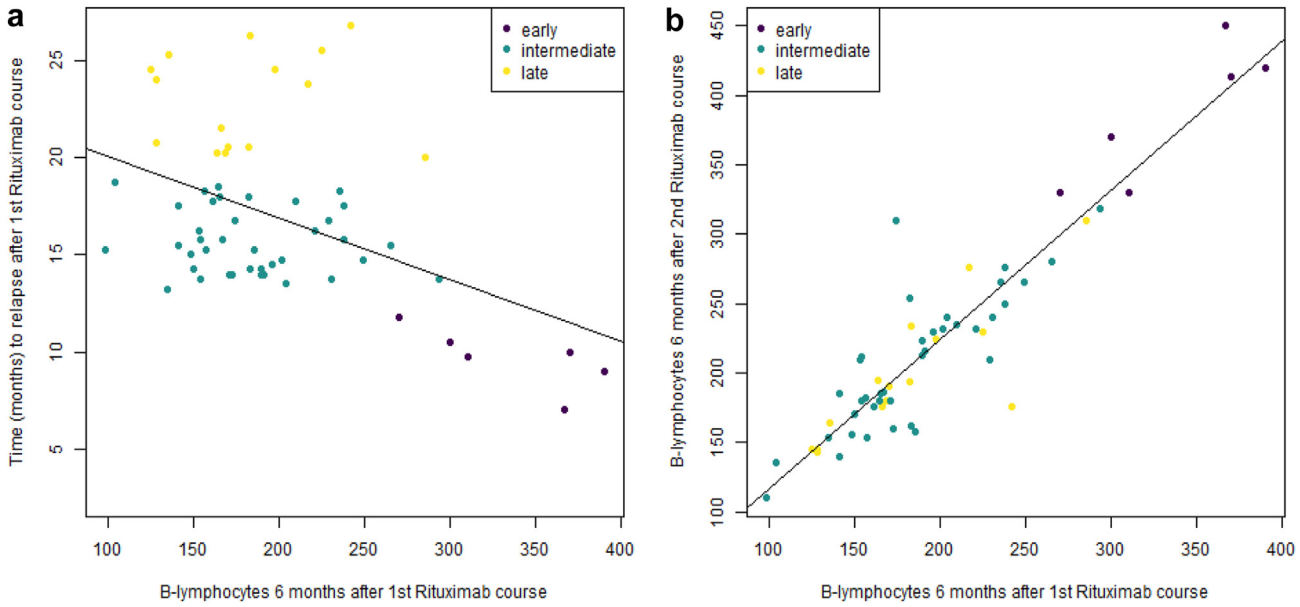

Figure 4. Post-rituximab B-lymphocyte recovery. (a) Left panel: relationship of 6-month B-lymphocyte count and time to first relapse; (b) Right panel: association of 6-month post-rituximab B-lymphocyte counts following first and second course of rituximab. Early, intermediate, and late relapsers are represented by blue, green, and yellow symbols.

(Supplementary Figure S2). The degree of both the initial depletion and the recovery within 6 months was similar for the 3 intervention groups.

Adverse Events

Almost all patients experienced at least 1 grade 1 adverse event (AE), whereas about 50% and 15% of patients developed at least 1 grade 2 or grade 3 AE respectively. Whereas the incidence of grade 1 AE was highest with rituximab monotherapy (1.85 AE per patient year), grade 2 AE occurred more frequently with tacrolimus monotherapy (0.87 AE per patient year) (Table 3). Most grade 1 AEs with rituximab were acute infusion reactions; these were mostly mild and transient. Most grade 2 AEs in all treatment cohorts were related to infections. Hypogammaglobulinemia was not documented in any infectious event. No treatment related death or rituximab related late AEs occurred during the 36 months follow up.

Table 3. Adverse events according to drug exposure times

Adverse events	Tacrolimus monotherapy	Rituximab monotherapy	MMF post-rituximab
No. of patients exposed	60	116	96
Total exposure time (patient mo)	813	1780	1542
Grade 1	54 pts (90%) 107 AE (1.60/y)	116 pts (100%) 274 AE (1.85/y)	78 pts (81%) 217 AE (1.69/y)
Grade 2	36 pts (60%) 59 AE (0.87/y)	48 pts (41%) 79 AE (0.53/y)	32 pts (33%) 68 AE (0.53/y)
Grade 3	9 pts (15%) 9 AE (0.13/y)	14 pts (12%) 14 AE (0.09/y)	12 pts (12%) 12 AE (0.09/y)

AE, adverse events.

Cumulative Prednisolone Dose and Obesity

The mean (SD) cumulative prednisolone dose in the rituximab arm decreased from 239 (53) mg/kg/yr in the pretrial year to 70 (45) mg/kg/yr in the 36 months on rituximab with or without MMF therapy. Steroid exposure was similar in patients with and without MMF comedication (44.5 [36.1] vs. 45.5 [27.9] mg/kg/yr). In the original tacrolimus arm, the prednisolone dose decreased from 246 (48) mg/kg/yr pretrial ($n = 60$) to 165 (72) mg/kg/yr ($n = 26$).

The mean (SD) BMI z-score decreased from 2.24 (1.05) before the trial to 1.63 (0.79) at month 12 and completely normalized by month 36 (0.09 [1.00]) in the original rituximab trial arm. In the original tacrolimus arm, BMI z-score was 2.20 (0.93) and 0.68 (0.71) at 0 and 36 months, respectively.

In a linear model of the change in BMI z-score in the entire RITURNS cohort throughout the 3 years follow-up, longer tacrolimus exposure (1.8 [0.83, 2.7], $P < 0.0005$) and the need for 3 rituximab doses (compared to no rituximab dose 1.86 [0.39, 3.34], $P < 0.05$) were associated with a smaller reduction of BMI z-score.

DISCUSSION

The primary objective of this extended follow-up assessment of the RITURNS cohort was to document the long-term efficacy of a single course of rituximab versus maintenance tacrolimus immunosuppression beyond 1 year. The proportion of patients with a first relapse steadily increased in the tacrolimus arm; only 2 patients remained relapse-free during the entire 3-year

observation period. In the rituximab arm, gradual B-cell recovery led to a sharp increase of first relapses in the second year. All rituximab patients had experienced a relapse by the end of year 2 and the median relapse-free survival time did not differ between the original trial arms. These findings provide evidence that permanent disease remission does not occur following a single course of rituximab in children with SDNS and the superior efficacy over tacrolimus maintenance therapy vanishes 12 to 18 months after administration. Our findings are in keeping with the results of previous studies, including a follow-up study of a placebo-controlled randomized controlled trial for complicated frequently relapsing nephrotic syndrome/SDNS from Japan where 94% of patients developed relapses during a median observation period of 5 years, 86% required readministration of immunosuppressive agents, and 43% received further courses of rituximab.[9,14] In contrast, in 2 smaller trials in Italian children with high dose or very low dose SDNS, 6 of 15 and 8 of 15 patients with SDNS achieved long-term remission after rituximab.[15,16] These variable results may point to ethnic or geographic differences in disease activity and/or rituximab responsiveness. It is well known that the incidence of nephrotic syndrome is higher and treatment response to immunosuppressants generally poorer among South Asian children.[17,18]

Notably, the B-cell count 6-months postdosing was found to predict the risk of an early relapse both after the first and the second rituximab administration and intraindividual comparisons of B-cell counts disclosed a high level of consistency of B-cell repletion. Therefore, future personalized treatment protocols may involve cell count-guided personalized redosing of B-cell depleting agents.[19-21]

The standardized switching of patients in the tacrolimus arm to rituximab provided the opportunity to compare the efficacy of rituximab when used as standard second-line rescue therapy in case of breakthrough relapses on tacrolimus with first-line administration at the time of SDNS diagnosis. Post-rituximab relapses occurred significantly earlier in the 32 patients switched after a relapse on tacrolimus than in the patients who had been randomized to rituximab as first-line therapy in the original trial, with median relapse-free survival times of 52 versus 63 weeks. In contrast, relapse-free time following second-line rituximab did not differ from first-line rituximab therapy in patients who were switched electively from tacrolimus to rituximab, a subgroup that was also characterized by slightly lower pretrial disease activity. Notably, the slightly lower efficacy of rituximab when administered following relapses on calcineurin inhibitor treatment was apparently not because of differences in B-cell counts before or after rituximab administration (Supplementary Figure S3).

MMF maintenance comedication was introduced in the patients from the original rituximab cohort who received a second course of rituximab after relapsing within 18 months of the first course, and in all patients from the previous tacrolimus cohort who relapsed after being switched to rituximab. The time to re-relapse on rituximab-MMF cotreatment was compared with the relapse-free survival observed for all episodes of rituximab monotherapy combined. This comparison clearly supports an added benefit of MMF in maintaining long-term remission. The results of this mixed analysis were confirmed by selective assessment of the patients from the rituximab arm with post-rituximab relapses occurring within 18 months (who received MMF comedication) or later (who received rituximab monotherapy because of their less active disease). Even when assigned to a higher-risk patient group in this cohort, MMF maintenance therapy yielded superior long-term remission rates and independently reduced relapse risk by a factor of 4. These findings confirm and extend the results of previously published controlled and uncontrolled studies.[3,22-24]

It should be emphasized that all patients benefited from the steroid-sparing protocols applied during the trial and the follow-up period. Because of a reduction of the average relapse rate from 3.8 to 0.7 episodes per patient per year, the overall steroid exposure was further reduced during the second and third year of observation. Obesity, present in most children at time of enrolment and partially improved at year 1, largely resolved during the extension period. However, extended tacrolimus exposure time and the need for 3 rituximab pulses versus no doses were associated with smaller BMI reductions, most likely reflecting higher residual disease activity and cumulative steroid dosing.

Our extended follow-up of a large group of patients receiving tacrolimus, rituximab monotherapy and rituximab followed by MMF allowed to monitor adverse drug effects over a total exposure time of 360 patient years. This analysis confirmed our previous finding in the original trial analysis that although both treatment approaches are generally well tolerated and no catastrophic events were observed, tacrolimus therapy is associated with higher rates of grade 2 AEs, mainly infections, than rituximab therapy, whereas rituximab occasionally causes acute infusion reactions yielding a higher incidence of grade 1 AEs.

We recognize several limitations of this extension study. The treatments applied during the follow-up period were standardized and performed prospectively but were uncontrolled. Serum IgG levels were not routinely measured, and rituximab autoantibody

measurements and B-lymphocyte subtyping was not routinely performed. Furthermore, because the efficacy and safety of the drugs may vary depending on patient ethnicity and geographic location,[17,18] the findings of this single-center trial in a South Asian population may not be fully generalizable to other ethnic groups and regions.

In conclusion, the RITURNS trial has generated important randomized and nonrandomized evidence for the utility of rituximab in childhood SDNS. The observed lack of long-term superiority of a single course of rituximab over tacrolimus maintenance therapy, the shorter protection conferred by second-line rituximab following relapses on tacrolimus and the favorable extended results achieved with MMF maintenance therapy reported here add important pieces of evidence. Our results provide a rationale for further clinical research, including randomized comparisons of treatment protocols and the monitoring of more safety indicators such as immunoglobulinemia, to be performed in ethnically and geographically diverse populations. Ongoing clinical trials such as RITURNS-II, which compares repeated rituximab monotherapy with single dosing followed by MMF maintenance therapy, are expected to deliver further insight into the most efficacious, safe, and cost effective use of B-cell depleting agents in childhood idiopathic nephrotic syndrome.[25]

DISCLOSURE

The authors have declared no conflicting interests.

ACKNOWLEDGMENTS

We are grateful for the continued supply of drugs to the government, and for dedicated support by the pediatric nephrology staff at Nilratan Sircar Hospital in the long-term follow up of the patients.

AUTHOR CONTRIBUTIONS

BB contributed to protocol preparation, study design, study execution, data collection, and preparation of manuscript. FS contributed to protocol preparation, study design, as well as preparation and critical revision of manuscript. AS, JM, and SE contributed to statistical analysis plan writing and data analysis, and preparation of manuscript. TKSM contributed to study design and execution, data collection, and preparation of manuscript. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIALS

Supplementary File (PDF)

Figure S1. Time from second course of rituximab to second relapse in patients of previous rituximab trial arm with and without MMF maintenance therapy.

Figure S2. B-cell recovery following depletion after administration of rituximab in the Tacrolimus arm with that in the first-line rituximab therapy arm.

Table S1. Definitions of nephrotic syndrome categories.

Table S2. Secondary endpoints by time to first relapse in patients of rituximab arm.

Table S3. Multivariate Cox regression analysis of re-relapse risk in children re-exposed to a second course of rituximab following a relapse after first exposure.

STROBE Statement.

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