



Mycophenolate Mofetil after Rituximab for Childhood-Onset Complicated Frequently-Relapsing or Steroid-Dependent Nephrotic Syndrome

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**Mycophenolate mofetil after rituximab for childhood-onset complicated
frequently-relapsing or steroid-dependent nephrotic syndrome**

Running title: MMF after rituximab for FRNS/SDNS

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Mycophenolate mofetil, rituximab, childhood-onset, complicated frequently-relapsing/steroid-dependent nephrotic syndrome, clinical trial

ABSTRACT

Background. Rituximab is the standard therapy for childhood-onset complicated frequently-relapsing or steroid-dependent nephrotic syndrome (FRNS/SDNS). However, most patients redevelop FRNS/SDNS following peripheral B cell recovery.

Methods. We conducted a multicenter, randomized, double-blind, placebo-controlled trial to examine whether mycophenolate mofetil (MMF) administration after rituximab can prevent treatment failure (FRNS, SDNS, steroid resistance, or use of immunosuppressive agents or rituximab). Thirty-nine patients (per group) were treated with rituximab, followed by either MMF or placebo until Day 505 (treatment period). The primary outcome was time to treatment failure (TTF) throughout the treatment and follow-up periods (until Day 505 for the last enrolled patient).

Results. TTFs were clinically but not statistically significantly longer among patients given MMF after rituximab than among patients receiving rituximab monotherapy (median: 784.0 vs. 472.5 days, hazard ratio (HR): 0.593, 95% confidence interval (CI): 0.336–1.049, log-rank test: $P=0.0694$). Because most patients in the MMF group presented with treatment failure after MMF discontinuation, we performed a post-hoc analysis limited to the treatment period and found that MMF after rituximab prolonged the TTF and decreased the risk of treatment failure by 80% (HR: 0.202, 95% CI: 0.081–0.503). Moreover, MMF after rituximab reduced the relapse rate and daily steroid dose during the treatment period by 74% and 57%, respectively. The frequency and severity of adverse events were similar in both groups.

Conclusions. During MMF administration, MMF after rituximab may sufficiently prevent the development of treatment failure and is well tolerated, although the relapse-preventing effect disappears after MMF discontinuation.

INTRODUCTION

Idiopathic nephrotic syndrome is the most common chronic glomerular disease in children, occurring in two of 100,000 Caucasian children per year.¹ Furthermore, children in Asian populations develop this syndrome at higher frequencies than Caucasians. Indeed, the frequency in a Japanese population was reported to be 6.49 per 100,000 children per year.² Eighty to ninety percent of patients with childhood idiopathic nephrotic syndrome achieve complete remission with steroid therapy (steroid-sensitive nephrotic syndrome, SSNS).³ However, 50%–60% of children with SSNS develop frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome (FRNS/SDNS) with a wide range of steroid-related side effects, such as growth retardation, obesity, diabetes mellitus, cataracts, glaucoma, hypertension, osteoporosis, and femoral head necrosis.⁴ Several guidelines recommend cyclophosphamide, chlorambucil, levamisole, cyclosporine (CyA), tacrolimus, or mycophenolate mofetil (MMF) as corticosteroid-sparing agents for children with FRNS/SDNS.^{5, 6} However, at least 10%–20% of children who receive these immunosuppressive agents still develop frequent relapses or steroid dependence during or after treatment. Additionally, some patients with steroid-resistant nephrotic syndrome (SRNS) develop frequent steroid-sensitive relapses or steroid dependence after achieving complete remission with immunosuppressive therapies, including calcineurin inhibitors.⁷ We defined these conditions as complicated FRNS/SDNS, which require long-term steroid treatment, even though patients experience serious drug-induced side effects.⁸⁻¹⁰

The Research Group of Childhood-onset Refractory Nephrotic Syndrome (RCRNS) in Japan conducted a multicenter, randomized, double-blind, placebo-

controlled, parallel-group trial (RCRNS01) to evaluate the efficacy and safety of rituximab in patients with childhood-onset complicated FRNS/SDNS. The results showed that rituximab is effective and safe, at least for 1 year, in children with these conditions (Clinical Trials Registry ID: UMIN000001405).¹⁰ Rituximab is currently administered as standard therapy for patients with complicated FRNS/SDNS in many countries, although its use remains off-label outside Japan.^{11, 12}

However, almost all patients with complicated FRNS/SDNS treated with rituximab experience relapses following recovery from rituximab-induced peripheral B cell depletion.¹³⁻¹⁵ Therefore, further modification of rituximab treatment strategies, including the use of repeated courses of rituximab and/or adjunct immunosuppressive therapies, may be necessary to maintain long-term remission. Because the safety of long-term B cell depletion caused by repeated administration of rituximab in children with developing immune systems is unknown, we believe a new maintenance therapy for the prevention of relapse following rituximab therapy is urgently needed.

MMF is an immunosuppressant that selectively blocks *de novo* purine synthesis, suppresses T cell and B cell proliferation and antibody production, and has been used as immunosuppressive therapy after organ transplantation and for the treatment of various autoimmune diseases.¹⁶⁻¹⁹ Furthermore, MMF is considered effective in childhood-onset nephrotic syndrome and is currently used as an off-label drug in many countries.²⁰⁻²⁹ Our group conducted a pilot study and reported that maintenance therapy with MMF after rituximab in children with complicated SDNS significantly prolonged the relapse-free period compared with rituximab monotherapy.³⁰ Therefore, MMF is a promising drug for maintenance therapy following the administration of rituximab. However, a prospective randomized controlled trial is necessary to confirm the efficacy

and safety of MMF after rituximab therapy in children with complicated FRNS/SDNS.³¹

Therefore, we conducted a multicenter, randomized, double-blind, placebo-controlled, parallel-group trial (JSKDC07, Clinical Trials Registry ID: UMIN000014347, jRCTs051180081) to address this issue. The results of this trial are awaited by the Cochrane Collaboration and others.³²⁻³⁵

METHODS

Study design and participants

This multicenter, randomized, double-blind, placebo-controlled, parallel-group trial included patients enrolled from 27 pediatric nephrology centers in Japan. Definitions of nephrotic syndrome and related parameters pertaining to this study are shown in Table 1.^{6, 10} Major inclusion and exclusion criteria were as follows (See the full criteria in JSKDC07 Study Protocol ver. 3.1 in the Supplemental Material):

Inclusion criteria:

1. Idiopathic nephrotic syndrome (diagnostic criteria for idiopathic nephrotic syndrome at the time of initial diagnosis were based on criteria of the International Study of Kidney Disease in Children).
2. Age at the time of onset of idiopathic nephrotic syndrome (time of initial onset) was less than 18 years, and age at the time of assignment to one of the two groups in this study was not less than 2 years.
3. Met the criteria for complicated FRNS/SDNS (any of the requirements from 1) to 3) shown below) within 2 years before the last relapse date before the study.
 - a) Diagnosed with frequently-relapsing or steroid-dependent nephrotic syndrome and subsequently diagnosed with either of these conditions following the discontinuation of treatment with immunosuppressive drugs (e.g., cyclosporin, cyclophosphamide, or mizoribine).
 - b) Diagnosed with frequently-relapsing or steroid-dependent nephrotic syndrome and subsequently diagnosed with either of these conditions during treatment with immunosuppressive drugs (e.g., cyclosporin, cyclophosphamide, or mizoribine).

- c) Diagnosed with steroid-resistant nephrotic syndrome and achieved remission with immunosuppressive drug therapy (cyclosporin monotherapy or combination therapy with methylprednisolone) but diagnosed with frequently-relapsing or steroid-dependent nephrotic syndrome during or after the completion of treatment with immunosuppressive drugs.

Exclusion criteria:

1. Patients diagnosed with nephritic-nephrotic syndrome, such as IgA nephropathy, prior to assignment or those with suspected secondary nephrotic syndrome.
2. Patients previously treated with monoclonal antibodies other than rituximab.
3. Patients with infections.

Randomization and masking

At the data center, patients were randomly assigned to either the rituximab followed by MMF (MMF group) or rituximab followed by placebo (Placebo group) groups at an approximate ratio of 1:1 by dynamic allocation with the following allocation adjustment factors at the time of enrollment: institution, age, treatment history (presence or absence of immunosuppressive drug administration at the relapse immediately prior to assignment/presence or absence of steroid administration at the relapse immediately prior to assignment), interval between the last three relapses, and presence or absence of a history of SRNS. Patients, their guardians, treating investigators, and individuals who assessed outcomes and analyzed data were masked to assignments. To maintain masking, except for the independent data and safety monitoring committee, all treating and other investigators remained blinded to assignments until all data had been fixed after study completion. However,

investigators could urgently request the disclosure of a patient's allocation code under the following conditions:

- (1) The participant experienced a serious adverse event that led to death or was life-threatening.
- (2) The participant experienced another serious adverse event, and the information was essential for determining the relevant treatment.
- (3) Treatment failure (FRNS, SDNS, or SRNS).
- (4) The participant became pregnant and discontinued the investigational drug.

Procedures

Treatment and follow-up periods

In this trial, four weeks were defined as one month. The treatment period was defined as the time from the date of the first dose of rituximab (Day 1) to the date of completion of investigational drug (MMF or placebo) administration (Day 505). The follow-up period was defined as the time from Day 506 to the last scheduled treatment date of the last enrolled patient. During the follow-up period, investigators followed up all participants according to the specified schedule and conducted a follow-up survey using routine clinical data.

Study treatments

Rituximab and the investigational drugs were administered (Figure 1). After rituximab was administered at a dose of 375 mg/m² (maximum dose: 500 mg) once weekly for four weeks, on Days 1, 8, 15, and 22, MMF was administered at a dose of 1,000–1,200 mg/m²/day (maximum 2 g/day) twice daily after breakfast and dinner for 17 months (from Day 29 until Day 505). In the placebo group, rituximab was provided in the

same manner, and placebo was administered instead of MMF. When the treating physician thought it's safer, the investigational drug could have been started at half the dose and increased to the defined dose in the absence of adverse reactions within 3 months. If patients could not receive the full dose because of adverse events, investigators determined the dose reduction. To prevent infusion reactions, patients received premedication with methylprednisolone, acetaminophen, and d-chlorpheniramine maleate approximately 30 min prior to the administration of each dose of rituximab.¹⁰

Prednisolone treatment for relapse at screening and during the study period

Participants taking prednisolone at the relapse immediately before enrollment in this study were obligated to take 60 mg/m² prednisolone orally in three daily divided doses (maximum 80 mg/day or 60 mg/day, depending on the institutional policy) for 4 weeks. Participants not taking prednisolone at the relapse immediately before enrollment in this study were obligated to take the same dose of prednisolone until 3 days after complete remission was achieved. Following this full-dose treatment period and confirmation of complete remission, patients received 60 mg/m² prednisolone in the morning on alternate days (maximum 80 mg/day or 60 mg/day) for 2 weeks and then 30 mg/m² on alternate days (maximum 40 mg/day or 30 mg/day) for 2 weeks, followed by 15 mg/m² on alternate days (maximum 20 mg/day or 15 mg/day) for 2 weeks. When patients experienced relapses during the study period, they received 60 mg/m² prednisolone in three daily divided doses (maximum 60 mg/day) until 3 days after complete remission was achieved. They were then administered 60 mg/m² prednisolone in the morning on alternate days (maximum 60 mg/day) for 2 weeks and 30 mg/m² on alternate days (maximum 30 mg/day) for the next 2 weeks, followed by 15 mg/m² on

alternate days (maximum 15 mg/day) for 2 weeks.

Concomitant drugs and combination therapy

If patients were on calcineurin inhibitor therapy (CyA or tacrolimus) at screening, tapering of the drug began at day 86, with discontinuation by day 169. The doses were sequentially reduced every 28 days from day 86 onward and discontinued on approximately day 169. If patients were taking any other immunosuppressive agents (e.g., MMF, mizoribine, azathioprine, cyclophosphamide, or chlorambucil), these drugs were discontinued by the beginning of rituximab administration (Day 1) (Figure 1). Trimethoprim-sulfamethoxazole was administered from the beginning of rituximab therapy (Day 1) until the date on which peripheral B cell recovery (≥ 5 cells/ μ L) was confirmed to prevent *Pneumocystis jirovecii* infection.

Combination therapy with the following drugs/treatments was prohibited during the treatment period.

1. Commercially available rituximab.
2. Immunosuppressive drugs or alkylating agents with an immunosuppressive effect, except under the following conditions:
 - a) CyA, tacrolimus, cyclophosphamide, mizoribine, MMF, or chlorambucil use was continued from before the start of the clinical trial.
 - b) Treatment failure was determined.
3. Live vaccines were administered.

Discontinuation of investigational drug administration

Investigators could discontinue the administration of investigational drugs under any of the following conditions:

- (1) Treatment failure (FRNS, SDNS, or SRNS) was observed during the treatment period.

- (2) A prohibited drug (see above) was used as treatment for nephrotic syndrome.
- (3) The participant or legal representative requested discontinuation of the investigational drug.
- (4) The investigators determined that continuing administration of the investigational drug was not feasible for any other reason, such as the occurrence of adverse events.
- (5) The participant became pregnant.

Visit schedule

During the treatment period, investigators carried out observations, examinations, and surveys in accordance with the prescribed schedule (see the trial protocol in the Supplementary Appendix). Briefly, the rituximab dosing start date was defined as Day 1 and Visit 1. Study visits occurred every week during the rituximab administration period [Visit 1 (Day 1)–Visit 4 (Day 22)], every month during the first 6 months of the investigational drug administration period [Visit 5 (Day 29)–Visit 10 (Day 169)], and every 2 months thereafter [Visit 11 (Day 225)–Visit 16 (Day 505)]. Between Visit 6 (Day 57) and Visit 10 (Day 169), changes “within ± 14 days” were acceptable. At Visit 11 (Day 225) and beyond, changes “within ± 28 days” were acceptable. Urine and blood samples were collected at each visit.

Treatment during the follow-up period

When possible, patients in remission at the end of the treatment period were followed without treatment until relapse. Decisions regarding treatment for relapses during the follow-up period were made by the investigators. Investigators provided treatments that were considered optimal for the individual patients, including initiation of a new immunosuppressive drug. Treatments provided during the follow-up period (drug name and treatment duration) were reported in the follow-up report.

Outcomes

The primary endpoint was defined as the time to treatment failure (development of frequent relapses, steroid dependence or resistance, or use of immunosuppressive agents or rituximab) throughout the study period, including the treatment and follow-up periods (until Day 505 for the last enrolled patient). The secondary endpoints were the relapse rate during the treatment period, time to relapse, time to FRNS, time to SDNS, time to SRNS throughout the treatment and follow-up periods, daily steroid dose during the treatment period, and peripheral B cell depletion period. Adverse events were recorded throughout the treatment period and assessed using CTCAE.

Statistical Considerations

Sample size

The primary aim of this study was to examine the superiority of MMF administration (MMF group) compared with placebo (Placebo group) after rituximab therapy for preventing treatment failure throughout the treatment and follow-up periods (until Day 505 for the last enrolled patient). Events in the time to treatment failure analysis were defined as the development of frequent relapses, steroid dependence or resistance (exacerbation of disease), or use of immunosuppressive agents or rituximab (concomitant drug use), whichever occurred first. Cases without events were terminated at the end of the entire study. However, if the study participant and/or the guardian wished to discontinue the trial, it was terminated at the time.

On the basis of our previous study,¹⁰ we predicted a 1-year event rate of 40% in the placebo group and expected treatment with MMF to decrease that value to 20%.

Thirty-seven patients in each group were necessary to have 80% power for a log-rank test with a 5% significance level under the assumptions of a proportional hazard model, 3-year enrollment period, and 1.5-year follow-up period. To allow for withdrawal of consent after participation in the study or loss to follow-up, we set the total sample size to 80 participants. The power calculation was performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

Analyses

The full analysis set was defined as a population consisting of all enrolled subjects for whom study treatment was started and efficacy endpoints were measured.

The primary outcome, time to treatment failure, was summarized using the Kaplan–Meier method and the median times were reported within each treatment group. The distributions of the times to treatment failures were compared using the log-rank test. Hazard ratios (HRs) with 95% confidence intervals (CIs) were estimated using a Cox proportional hazard model.

Secondary endpoints, including the time to relapse, time to SRNS, and B cell depletion period, were analyzed in the same manner as the primary endpoint. For time to relapse, an analysis adjusting peripheral blood B cell depletion as a time varying covariate was also performed. For time to FRNS and time to SDNS, analyses that treated the diagnosis of steroid resistance as a competing risk were performed, and the results were compared using the Fine-Gray test. Relapse rates during the treatment period were compared by permutation testing, in which P-values were calculated based on the null distribution of the rate ratio constructed by generating 20,000 random assignments. Daily steroid doses during the treatment period were compared with the Wilcoxon rank-sum test.

The following sensitivity analyses were conducted: an analysis based on the per-protocol set; a stratified analysis on the full analysis set by stratified log-rank test, in which the stratification factor was a history of rituximab treatment; an analysis including patients who were excluded from the full analysis set. Additionally, subgroup analyses were conducted in patients who were taking cyclosporine at relapse immediately before assignment and in those who were not.

After data fixation, separately post-hoc exploratory analyses limited to the treatment period (until Day 505) and limited to the follow-up period (after Day 506) for time to treatment failure, time to relapse, time to FRNS, and time to SDNS were performed to evaluate the efficacy of MMF during its administration and after its discontinuation.

The two-sided significance level was set at 5%. Multiplicity adjustments were not performed in the secondary endpoint analyses, sensitivity analyses, or post-hoc analyses.

All adverse events that occurred during the treatment period were analyzed. Patients who discontinued study treatment were also followed for adverse events. Intergroup comparisons were made using Fisher's exact test as required. The incidence of infections that required treatment was calculated and compared between the groups using the proportional mean model and described as the mean cumulative function.³⁶

Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA).

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Data sharing policy

The trial protocol is available as the Supplemental Material. Data that support the findings from this study are available from the corresponding author [K.I.] upon reasonable request.

RESULTS

Patients

Patients were enrolled from February 2015 to May 2018. The first patient provided consent on June 4, 2015. On December 14, 2016, the independent data and safety monitoring committee conducted a planned interim safety analysis and approved the continuation of the trial because no safety issues were identified. Day 505 for the last enrolled patient was October 16, 2019. Of 137 patients assessed for eligibility, 86 were randomized: 43 to the MMF group and 43 to the placebo group. Seventy-eight patients (39 per group) received the intervention and were included in the full analysis set (Figure 2).

Baseline features of patients were similar between the two groups (Table 2). The predominant histological type in each group was minor glomerular abnormalities. All patients were treated with steroids and/or immunosuppressants at the relapse immediately before assignment.

Efficacy analyses

Primary analysis

Analysis of the primary endpoint was performed in the full analysis set. The cumulative treatment failure-free survival curves for both groups are shown in Figure 3. Events occurred in 23 patients in the MMF group [six during the treatment period (four exacerbation of disease: FRNS, SDNS, or steroid resistance and two concomitant drug use: use of immunosuppressive agents or rituximab) and 17 during the follow-up period (five exacerbation of disease and 12 concomitant drug use)]. In the placebo group, 25 patients experienced events [21 during the treatment period (19 exacerbation of disease

and two concomitant drug use) and four during the follow-up period (one exacerbation of disease and three concomitant drug use)]. In both groups, there were few events until Day 169 after assignment. In the MMF group, the cumulative treatment failure-free probability was 97.4% at Day 337 and 84.6% at Day 505 (end of the treatment period), whereas in the placebo group, the cumulative treatment failure-free probability began to decrease after Day 253, reaching 73.7% at Day 337 and 44.7% at Day 505. Therefore, the times to treatment failures throughout the study period (the treatment period and follow-up period) were longer in the MMF group than in the placebo group, although the difference was not statistically significant (median: 784.0 vs. 472.5 days, HR: 0.593, 95% CI: 0.336–1.049, log-rank test: $P=0.0694$) (Table 3a).

Secondary endpoint analyses

Analyses of secondary endpoints were performed in the full analysis set. Regarding secondary endpoints, the relapse rate during the treatment period in the MMF group was lower than in the placebo group [Mean $\pm$ standard deviation (SD): 0.43 ± 0.90 vs. 1.99 ± 2.73 per person-years; HR: 0.257, 95% CI: 0.084–0.480] (Table 4). The times to relapse throughout the study period in the MMF group were longer than in the placebo group although not statistically significantly (median: 654.0 vs. 320.0 days, HR: 0.618, 95% CI: 0.367–1.041) (Figure 4a, Table 5a). The times to relapse adjusting peripheral blood B cell depletion as a time varying covariate showed that the HR for the MMF group was 0.671 (95% CI: 0.395–1.140), which was consistent with the results of the comparison between the groups without adjusting peripheral blood B cell depletion. The times to frequent relapse throughout the study period were longer in the MMF group compared with the placebo group (median: 853.0 vs. 452.0 days, HR: 0.561, 95% CI: 0.316–0.999) (Figure 4b, Table 6a). The times to steroid dependence throughout the

study period in the MMF group were longer than in the placebo group although not statistically significantly (median: 853.0 vs. 452.0 days, HR: 0.602, 95% CI: 0.326–1.077) (Figure 4c, Table 7a). One and two patients developed steroid-resistant relapse in the MMF group and placebo group, respectively (HR: 0.454, 95% CI: 0.044–4.699). The daily steroid dose after assignment during the treatment period was lower in the MMF group than in the placebo group (mean $\pm$ SD: 4.45 $\pm$ 3.52 vs. 10.45 $\pm$ 12.49 mg/m²/day, Wilcoxon test: P=0.0004).

Sensitivity analyses

Sensitivity analyses were carried out in the per-protocol set. Five patients were censored on Day 505 (because of the administration of the study drug beyond Day 505), and two were censored on Day 225 (because of low adherence between Day 225 and Day 505), although the subjects remained unchanged. The results from the per-protocol set analysis were similar to those from the full analysis set (median times to failure: 784.0 vs. 452.0 days, HR: 0.552, 95% CI: 0.304–1.002) (Table 8).

Eleven patients in the MMF group and 10 in the placebo group had a history of rituximab treatment before enrollment. In a stratified log-rank test with histories of rituximab treatment as the stratification factor, the times to treatment failures throughout the study period in the MMF group were longer than in the placebo group (HR: 0.547, 95% CI: 0.306–0.975) (Table 9).

We performed a sensitivity analysis by including eight patients who were excluded from the full analysis set because the study treatment was not started. Four patients in the MMF group and three in the placebo group (relapse before the 1st rituximab) were assumed to experience treatment failure at day 0, and one patient in the placebo group (withdrew consent) was assumed to be censored at day 0. The results

were similar to the primary results (median: 782.0 vs. 449.0 days, HR: 0.658, 95% CI: 0.386-1.121).

We also analyzed the effect of MMF in patients who were taking cyclosporine at relapse immediately before assignment and in those who were not. MMF treatment prolonged the times to treatment failures in both groups [HR: 0.474 (95% CI: 0.231-0.971) and 0.723 (95% CI: 0.270-1.934), respectively].

Post-hoc analyses limited to the treatment period

Many patients in the MMF group experienced an event after stopping MMF (during the follow-up period). This prompted us to perform post-hoc analyses limited to the treatment period (until Day 505). The post-hoc analyses were performed using the full analysis set. During the treatment period, events occurred in 6 patients in the MMF group and 21 patients in the placebo group. Therefore, the times to treatment failures until Day 505 in the MMF group were longer than in the placebo group (median: not reached vs. 493.0 days, HR: 0.202, 95% CI: 0.081–0.503) (Figure 3, Table 3b). The times to relapse until Day 505 in the MMF group were longer than in the placebo group (median: not reached vs. 320.0 days, HR: 0.280, 95% CI: 0.138–0.568) (Figure 4a, Table 5b). The times to frequent relapse until Day 505 were longer in the MMF group compared with the placebo group (not reached vs. 493.0 days, HR: 0.202, 95% CI: 0.084–0.483) (Figure 4b, Table 6b). The times to steroid dependence until Day 505 were longer in the MMF group than in the placebo group (median: not reached vs. 506.0 days, HR: 0.220, 95% CI: 0.091–0.528) (Figure 4c, Table 7b).

Recovery of peripheral B cell count

The median peripheral B cell depletion (< 5 cells/ μ l) period was 162.0 (95% CI:

53.0–195.0) days in the MMF group and 165.0 (95% CI: 112.0–217.0) days in the placebo group. There was no statistically significant difference between the groups (HR: 1.114, 95% CI: 0.709–1.752). There were no clinically nor statistically significant differences in the recovery of peripheral B cell counts between the two groups during the treatment period (Figure 5, Table 10). Additionally, peripheral B cell counts at the onset of the first relapse after assignment were similar in both groups [MMF group (N=9): 148.7/ μ l (0–2,608.79) vs. placebo group (N=22): 185.19/ μ l (0–1,381.8)].

Safety profile

In the MMF group, 30 infusion reactions occurred among 20 cases (51.3%), and 150 adverse events (other than infusion reactions) occurred among 31 cases (79.5%). In the placebo group, 20 infusion reactions occurred among 14 cases (35.9%), and 139 adverse events (other than infusion reactions) occurred among 30 cases (76.9%). Most adverse events were classified as grade 1 or 2, and the incidence of adverse events was not statistically significantly different between the two groups. No deaths were reported in this study. The most common grade 3–4 adverse event (other than infusion reaction) in the MMF group was neutropenia (Table 11). In the MMF group, eight serious adverse events occurred among seven cases, whereas in the placebo group, 13 occurred (one was an infusion reaction) among 10 cases. Six serious adverse events were reported as adverse reactions to the investigational drug in both groups, all of which resolved or improved. The incidence of infections that required treatment was higher in the MMF group than in the placebo group [1.59 infections (95% CI: 0.91–2.27) vs. 0.82 infections (95% CI: 0.48–1.16); mean ratio 1.94, 95% CI: 1.07–3.52]. However, only two infections in the MMF group and one in the placebo group were reported as serious adverse events,

all of which resolved.

DISCUSSION

This was the first multicenter, randomized, double-blind, placebo-controlled trial to test the efficacy and safety of MMF administration after rituximab for childhood-onset complicated FRNS/SDNS.

The present trial compared rituximab treatment of childhood-onset complicated FRNS/SDNS succeeded by MMF or placebo until Day 505, in each case followed by cessation of study drug. The times to treatment failure (frequent relapse, steroid dependence or resistance, or use of immunosuppressive agents or rituximab) over the entire study period, including 505 days of study drug treatment and a median of 208 days of post-treatment follow-up, were not statistically significantly longer among patients given MMF after rituximab than among patients receiving rituximab monotherapy (median: 784.0 vs. 472.5 days, HR: 0.593, 95% CI: 0.336–1.049, log-rank test: $P=0.0694$). However, the post-hoc analysis limited to the treatment period showed that MMF following rituximab reduced the risk of treatment failure by 80% (HR: 0.202, 95% CI: 0.081–0.503) during MMF administration. Moreover, secondary endpoint analyses revealed that MMF following rituximab decreased the relapse rate and daily steroid dose during MMF administration by 74% (HR: 0.257, 95% CI: 0.084–0.480) and 57% (mean $\pm$ SD: 4.45 ± 3.52 vs. 10.45 ± 12.49 mg/m²/day, $P=0.0004$), respectively. Regarding the safety profile, MMF following rituximab was well tolerated. These findings suggest that MMF following rituximab is clinically effective and safe for patients with childhood-onset complicated FRNS/SDNS although its benefit remains to be statistically proven.

Previous studies on the efficacy of MMF in pediatric patients with FRNS/SDNS described the clinical course after MMF withdrawal and showed that in a fair number of cases, long-term remission persisted, or only infrequent relapses were observed, even

following MMF withdrawal.^{21, 26, 27} Therefore, in the present trial, analysis of the primary outcome included the follow-up period following MMF discontinuation. The 1-year treatment failure rates estimated at the time of study design were 20% in the MMF group and 40% in the placebo group. In actuality, the 1-year treatment failure rates were 2.6% in the MMF group and 34.2% in the placebo group, demonstrating better-than-expected differences between the groups. However, because the effect of MMF disappeared after its discontinuation, the assumption of proportional hazards, inherent in our analytic approach and sample size justification was not satisfied by the data. Thus, many relapse cases occurred in the MMF group after the treatment period, which led to treatment failure mainly due to concomitant drug use before the development of FRNS, SDNS, or SRNS (Tables 3c, 5c, 6c and 7c). Consequently, the cumulative treatment failure-free probability in the MMF group decreased to a level similar to that in the placebo group toward the end of follow-up period. As a result, the effect of MMF was not statistically significant in the present trial (Figure 3). Therefore, MMF following rituximab may be sufficiently effective to prevent relapse during treatment, although it does not cure complicated FRNS/SDNS.

Recently, Chan et al. conducted a large scale, international, multicenter, retrospective study to determine the optimal rituximab regimen and maintenance immunosuppression therapy in children with complicated FRNS/SDNS and concluded that the effect of maintenance immunosuppression on sustaining remission was most prominent in the low-dose (375 mg/m²) rituximab group but not apparent with higher doses (medium dose: 750 mg/m², high dose: 1,125–1,500 mg/m²).³⁴ However, the present trial clearly showed the opposite result, whereby MMF maintenance therapy after high-dose (four doses: 1,500 mg/m²) rituximab resulted in longer remission than

rituximab monotherapy. Moreover, preliminary results of follow-up work from the RETURNS study, which was a randomized controlled trial that examined the effects of rituximab for early-stage SDNS without previous immunosuppressive therapy, showed that children on MMF maintenance therapy after the second course of rituximab (two doses=medium dose, n=44) experienced longer periods of remission than those administered rituximab monotherapy (n=15) (80% relapse-free survival: 84 vs. 30 weeks).³⁷ The latter two studies strongly suggest that MMF maintenance therapy following medium-to-high dose rituximab also has a remission-sustaining effect. Selection and reporting bias owing to the uncontrolled and retrospective nature of the former study may account for the difference in conclusions between the above studies.

There were no differences between the two groups regarding the peripheral B cell depletion period, recovery of peripheral B cell counts, or peripheral B cell counts at the onset of the first relapse after assignment during the treatment period. Colucci et al. reported that recovery of the switched memory B cell compartment might be a strong predictor of nephrotic syndrome relapse after rituximab treatment, suggesting this B cell subset may have a pivotal role in the pathogenesis of nephrotic syndrome.³⁸ Recently, Bashford-Rogers et al. examined the effects of immunosuppressive drugs on the B cell receptor repertoire in immune-mediated diseases. Rituximab reduced the number of circulating B cells, but persisting cells were largely isotype-switched and clonally expanded. In contrast, MMF reduced both the number of isotype-switched B cells and clonality, with relative preservation of both IgM clones that underwent somatic hypermutation and those that did not compared with switched clones.³⁹ These findings may provide a rationale for the ability of MMF to prevent nephrotic syndrome relapse after rituximab therapy, as revealed in the present trial, although the number of switched

memory B cells and clonality were not assessed.

In the present trial, patients in the placebo group received the exact treatment as those in the rituximab group in our previous trial (RCRNS01).¹⁰ However, the times to relapse in the placebo group in the present trial were longer than in the rituximab group in the RCRNS01 trial (median: 320.0 days, 95% CI: 266.0–460.0 days vs. 267.0 days, 95% CI: 223.0–374.0 days). Furthermore, in the RCRNS01 trial, all patients relapsed 19 months after rituximab administration, whereas approximately 20% of patients in the placebo group in the present trial maintained long-term remission without treatment. In the present trial, 25.6% (20/78) of patients were not treated with immunosuppressive agents just before assignment, whereas this parameter was only 8.3% (4/48) in the RCRNS01 trial, suggesting disease activity was slightly lower in patients in the present trial compared with the RCRNS01 trial. These findings indicate that in children with FRNS/SDNS administered rituximab monotherapy, lower disease activity is associated with higher long-term remission rates. Recently, Ravani et al. reported that a single dose of rituximab induced long-term remission (4 years) without any other treatment in 53% (8/15) of children with early-stage very low-dose steroid-dependent nephrotic syndrome, supporting the above possibilities.⁴⁰ In Japan, a multicenter, double-blind, placebo-controlled, randomized trial examining the efficacy and safety of rituximab for childhood-onset early-stage uncomplicated (without a history of treatment with immunosuppressive agents) FRNS/SDNS (JSKDC10) is in progress.⁴¹ A long-term follow-up study of participants in JSKDC10 might further support the above hypothesis.

In the present trial, most adverse events were mild, and the incidence of adverse events was not statistically significantly different between the two groups. No deaths were reported. However, the incidence of infections that required treatment was higher

in the MMF group than in the placebo group. These findings indicate that MMF administered after rituximab therapy was well tolerated, although attention should be paid to infections.

The present trial had several limitations. As mentioned above, the effects of MMF maintenance therapy following rituximab on the B cell receptor repertoire were not examined. The long-term efficacy and safety of MMF maintenance therapy after rituximab remain unclear because the MMF treatment period was relatively short (until Day 505) in the present trial.

In conclusion, MMF maintenance therapy after rituximab may be an option for sustaining remission in children with complicated FRNS/SDNS although its benefit remains to be statistically proven. Further studies are necessary to clarify the effects of combined therapy with rituximab and MMF on the B cell receptor repertoire and examine the efficacy and safety of long-term MMF maintenance therapy after rituximab in children with complicated FRNS/SDNS. In addition, further studies are needed to clarify the pathogenesis of idiopathic nephrotic syndrome to develop curative therapies rather than those that sustain remission.

AUTHOR CONTRIBUTIONS

K. Iijima and M. Sako conceptualized the study. K. Iijima, M. Sako, K. Nozu, K. Nakanishi, K. Ishikura, and S. I. designed the study. K. Iijima, M. Sako, K. Nozu, A. K., and H. N. managed the study. S. T., R. H., T. S., Y. Ohwada, T. N., T. Y., H. M., Y. S., R. T., H. K., Y. A., T. M., N. K., Y. G., Y. I., T. K., K. K., N. F., Y. Ohtsuka, T. Okamoto., T. Y., E. T., M. Shimizu, and T. H. carried out the trial and collected data. All authors were members of the writing group and agreed on the content of the report, reviewed drafts,

and approved the final version. M. O. (biostatistician) was responsible for statistical analyses.

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Supplemental Table of Contents

Multicenter, randomized, double-blind, placebo-controlled, parallel group trial of mycophenolate mofetil after rituximab for childhood-onset complicated frequently relapsing or steroid-dependent nephrotic syndrome (JSKDC07) Study Protocol

Outline

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- Study design (scheme)
- Objective
- Subjects (inclusion and exclusion criteria)
- Study treatment
- Target sample size and scheduled period

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- Idiopathic nephrotic syndrome in children and its treatment
- Childhood-onset, complicated, frequently relapsing/steroid-dependent nephrotic syndrome
- Treatment of childhood-onset, complicated, frequently relapsing/steroid-dependent nephrotic syndrome
- Efforts by the Japanese Study Group of Kidney Disease in Children (JSKDC)

Objective

Study design

- Study period and target sample size
- Rationale for the study design

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- Inclusion criteria
- Exclusion criteria

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- Clinical trial discontinuation or suspension

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Publication rules

Clinical trial registration

Research funds and conflict of interest

- Research funds
- Conflict of interest

References

Japanese Study Group of Kidney Disease in Children
JSKDC07 study team

Study director

Clinical trial steering committee

Protocol committee

Independent data and safety monitoring committee

Biostatistician

Investigators

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FIGURE LEGENDS

Figure 1. Study design. MMF: Mycophenolate mofetil, MZB: Mizoribine, CyA: Cyclosporine, Tac: Tacrolimus. After rituximab was administered at a dose of 375 mg/m² (maximum dose: 500 mg) once weekly for four weeks, on Days 1, 8, 15, and 22, MMF was administered at a dose of 1,000–1,200 mg/m²/day (maximum 2 g/day) twice daily after breakfast and dinner for 17 months (from Day 29 until Day 505). In the placebo group, rituximab was provided in the same manner, and placebo was administered instead of MMF. The follow-up period was defined as the time from Day 506 to the last scheduled treatment date of the last enrolled patient. During the follow-up period, investigators followed up all participants according to the specified schedule and conducted a follow-up survey using routine clinical data.

Figure 2. Flow diagram.

Figure 3. Kaplan–Meier curves for treatment failure (frequent relapses, steroid dependence or resistance, or use of immunosuppressive agents or rituximab)-free survival. MMF: Mycophenolate mofetil. The times to treatment failure were not statistically significantly longer throughout the study period (the treatment period and follow-up period) among patients given MMF after rituximab than among patients receiving rituximab monotherapy (hazard ratio (HR): 0.593, P=0.0694) (See also Table 3a). However, during the treatment period, rituximab followed by MMF decreased the development of treatment failure by 80% compared with rituximab monotherapy (HR: 0.202) (See also Table 3b).

Figure 4. Kaplan–Meier curves or cumulative incidence-free function for secondary outcomes. (a) Kaplan–Meier curves for relapse-free survival, (b) Cumulative incidence-free function for frequent relapses, (c) Cumulative incidence-free function for steroid-dependent relapses. MMF: Mycophenolate mofetil. Times to relapse and those to each of component outcomes of treatment failure such as frequent relapses and steroid dependent relapses were approximately 40% longer (although not statistically significantly) among patients given MMF after rituximab than among patients receiving rituximab monotherapy throughout the study (combined treatment and follow-up) period [hazard ratio (HR): (a) 0.618, (b) 0.561, (c) 0.602] (See also Table 5a, 6a, and 7a). However, during the treatment period, MMF after rituximab decreased the occurrence of relapse, frequent relapses, and steroid-dependent relapses by 70%–80% compared with rituximab monotherapy [(a) HR: 0.280, (b) HR: 0.202, (c) HR: 0.220] (See also Table 5b, 6b, and 7b).

Figure 5. Peripheral B cell counts. Peripheral CD19-positive cell counts were monitored until Day 505. Day 1 was the first day of rituximab administration. Boxes represent the quartile range for each grouping and time period, straight lines (beard) are connected from the top and bottom sides of the boxes to outliers within 1.5 times the quartile range width, and red crosses and blue open circles represent outlying observations in the MMF group and placebo group, respectively.