Rituximab for relapsing nephrotic syndrome in adults; A Randomized Clinical Trial

Yoshitaka Isaka, M.D., Ph.D.[1)*], Yusuke Sakaguchi, M.D., Ph.D.[1)], Maki Shinzawa, M.D., Ph.D.[1)], Shoichi Maruyama, M.D., Ph.D.[2)*], Mika Sakaguchi, M.D., Ph.D.[3)], Hiroki Hayashi, M.D., Ph.D.[4)], Yusuke Kaida, M.D., Ph.D.[5)], Shin Goto, M.D., Ph.D.[6)], Tatsuo Tsukamoto, M.D., Ph.D.[7)], Akito Maeshima, M.D., Ph.D.[8)*], Yoichiro Ikeda, M.D., Ph.D.[9)], Norihiko Sakai, M.D., Ph.D.[10)*], Naoki Sawa, M.D. [11)], Kengo Furuichi, M.D., Ph.D.[12)*], Kunihiro Yamagata, M.D., Ph.D.[13)*], Takehiko Wada, M.D., Ph.D.[14)], Yugo Shibagaki, M.D., Ph.D.[15)*], Keiju Hiromura, M.D., Ph.D.[16)*]

1) Department of Nephrology, The University of Osaka Graduate School of Medicine, Suita, Japan
2) Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan
3) Division of Nephrology, Department of Internal Medicine, Kindai University Faculty of Medicine, Osaka-Sayama, Japan
4) Department of Nephrology, Fujita Health University School of Medicine, Toyoake, Japan
5) Division of Nephrology, Department of Medicine, Kurume University School of Medicine, Kurume, Japan
6) Division of Clinical Nephrology and Rheumatology, Kidney Research Center, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
7) Department of Nephrology and Dialysis, Medical Research Institute Kitano Hospital, PIIF Tazuke-Kofukai, Osaka, Japan
8) Department of Nephrology and Hypertension, Saitama Medical Center, Saitama Medical University, Kawagoe, Japan
9) Division of Nephrology and Endocrinology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
10) Department of Nephrology and Rheumatology, Kanazawa University Hospital, Kanazawa, Japan
11) Nephrology Center, Toranomon Hospital Kajigaya, Kajigaya, Japan
12) Department of Nephrology, Kanazawa Medical University, Kanazawa, Japan
13) Department of Nephrology, Institute of Medicine, University of Tsukuba, Tsukuba, Japan
14) Department of Nephrology, Toranomon Hospital, Tokyo, Japan
15) Division of Nephrology and Hypertension, Department of Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan

16) Department of Nephrology and Rheumatology, Gunma University Graduate School of Medicine, Maebashi, Japan
*; full professor

Correspondence to: Yoshitaka Isaka, M.D., Ph.D.
Department of Nephrology, The University of Osaka Graduate School of Medicine
2-2-D11, Yamada-oka, Suita, Osaka, 565-0871, Japan
E-mail: isaka@kid.med.osaka-u.ac.jp
Phone: +81-6-6879-3857; Fax: +81-6-6879-3230

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Abstract

IMPORTANCE The effects of rituximab on relapse of nephrotic syndrome in patients with adult-onset frequently relapsing nephrotic syndrome (FRNS) or steroid-dependent nephrotic syndrome (SDNS) remain uncertain.

OBJECTIVE To evaluate the effects of rituximab for patients with FRNS or SDNS.

DESIGN, SETTING, and PARTICIPANTS A multicenter, double-blind, randomized, placebo-controlled trial conducted at 13 centers in Japan. Adults with FRNS or SDNS who had urine protein <0.3 g/gCr were enrolled between September 1, 2020 and June 28, 2022. Final follow-up occurred on March 15, 2024.

INTERVENTIONS Patients were randomized to receive either intravenous rituximab (375 mg/m^2) or placebo at weeks 1, 2, and 25. Patients were followed up for 49 weeks

MAIN OUTCOMES AND MEASURES The primary outcome was the proportion of patients who were relapse-free from nephrotic syndrome at 49 week follow-up. Relapse was defined as urinary protein of ≥1 g/gCr on two consecutive measurements.

RESULTS Of 72 randomized participants, 66 (92%) received the study drug at least once (mean ages: 49.1 and 46.8 in the rituximab and placebo groups, respectively). The relapse-free rate at week 49 was 87.4% (95% confidence interval [CI]: 69.8-95.1) in the rituximab group and 38.0% (95%CI: 22.1-53.8) in the placebo group (P < 0.0001, one-sided

log-rank test). The median relapse-free time was 49.0 weeks in the rituximab group and 30.8 weeks in the placebo group. A stratified Cox model showed a hazard ratio for relapse of 0.16 (95% CI: 0.05‐0.46) in the rituximab group compared to the placebo group. The most common side effect was infusion reaction (13 [40.6%] in the rituximab group and 1 [2.9%] in the placebo group). Adverse events of ≥Grade 3 (Common Terminology Criteria for Adverse Events v5.0) occurred in 15.6% and 5.9% of patients in the rituximab and placebo groups, respectively.

CONCLUSION AND RELEVANCE These results support use of rituximab to prevent relapse in adults with FRNS or SDNS.

TRIAL REGISTRATION Japan Registry of Clinical Trials: jRCT2051200045, the University Hospital Medical Information Network Clinical Trials Registry: UMIN000041475

Key Points

Question

Does rituximab, compared to placebo, prevent relapse in adult patients with frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome?

Findings

In this randomized clinical trial that included 66 adults with either frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome, rituximab treatment significantly improved the relapse-free rate at week 49 compared to placebo (87.4% vs 38.0%). The stratified hazard ratio for relapse was 0.16 in the rituximab group compared to the placebo group.

MEANING

In adult patients with frequently relapsing nephrotic syndrome or steroid-dependent nephrotic syndrome, these findings support rituximab for reducing the relapse rate of nephrotic syndrome.

Introduction

Primary nephrotic syndrome, including minimal change nephrotic syndrome (MCNS), has an incidence of approximately 0.2–0.8 adults/100,000 person-years[1], and is characterized by severe proteinuria and hypoalbuminemia. Corticosteroids attain remission in approximately 90% of adults with MCNS, but approximately half of these patients relapse during corticosteroid tapering.[1] Long-term corticosteroid use causes complications including osteoporosis and infections and negatively affects quality of life and life expectancy.[2] The Kidney Disease: Improving Global Outcomes (KDIGO) 2021 glomerular disease guideline recommends using cyclophosphamide, rituximab, calcineurin inhibitors or mycophenolic acid analogues to treat frequently relapsing nephrotic syndrome (FRNS) or steroid-dependent nephrotic syndrome (SDNS).[3]

Rituximab, an anti-CD20 monoclonal antibody, depletes B cells by binding to CD20. Rituximab is approved in Japan for childhood-onset FRNS/SDNS[4], but not for adult-onset cases. In the U.S. Food and Drug Administration, rituximab is approved for non-Hodgkin's lymphoma, chronic lymphocytic leukemia, rheumatoid arthritis, vasculitis, and pemphigus vulgaris, but not for nephrotic syndrome. In observational studies, rituximab was associated with remission of FRNS/SDNS, ranging from 65–100%.[5-7] In these observational studies, relapses decreased by approximately 5-fold in the year after rituximab administration, with median corticosteroid dose reduced from 0.27 to 0 mg/kg.[7] However, to our knowledge, clinical trials of rituximab to treat adult-onset FRNS/SDNS have not been reported. The primary aim of this randomized clinical trial was to evaluate whether rituximab, compared to placebo, attained remission of adult-onset FRNS/SDNS. In exploratory analyses, patients who relapsed in the rituximab or placebo group were treated with corticosteroids followed by rituximab in an open-label fashion, to evaluate rates of persistent remission with rituximab

134 therapy following remission induction with corticosteroids. An additional aim was to evaluate

135 associations of B-cell depletion with infection rates in each study group.

Methods

Study Design and Participants

A multicenter, double-blind, randomized, placebo-controlled trial was conducted at 13 medical centers in Bunkyo, Kahoku, Kanazawa, Kawagoe, Kawasaki, Kurume, Nagoya, Niigata, Osaka, Osaka-sayama, Suita, Toyoake, and Tsukuba, Japan. The protocol is in Supplement 1 and the statistical analysis plan is in Supplement 2. This study was approved by the Ethical Review Committee of Osaka University Hospital (Approval No. 209004-A) and each institution, and was conducted in accordance with the Declaration of Helsinki.[8] Written informed consent was obtained from all enrolled patients. Eligible participants were enrolled between September 1, 2020, and June 28, 2022. Final follow-up was completed on March 15, 2024. The trial was designed and reported according to the CONSORT (Consolidated Standards of Reporting Trials).

Inclusion Criteria

Eligible patients were those aged ≥18 years with previously diagnosed FRNS (patients with nephrotic syndrome that relapse more than twice within a six-month period) or SDNS (patients with nephrotic syndrome for whom corticosteroids cannot be discontinued due to relapse two or more times after reduction or discontinuation of corticosteroids) and with urinary protein <0.3 g/gCr on ≥2 urine tests after initiation of corticosteroid for the latest relapse. Additional inclusion criteria were peripheral blood CD20 positive cells >5 cells/μL, preserved major organ function (liver, heart, lungs, and peripheral blood counts) except kidneys, life expectancy ≥12 months, and willingness for a 1 or 2 night hospital stay from initial study drug administration. Preserved major organ function was defined as: aspartate

aminotransferase and alanine aminotransferase ≤3 × upper limit of institutional normal and total bilirubin ≤2.0 mg/dL; neutrophil count ≥1.5 × 10^3/μL, platelet count ≥1.0 × 10^5/μL, hemoglobin ≥8.0 g/dL; and adequate cardiac and pulmonary function as judged by investigators.

Exclusion Criteria

Exclusion criteria were secondary nephrotic syndrome, prior rituximab use, eGFR < 44 ml/min/1.73 m^2, active infection, active cancer, autoimmune diseases, comorbidities such as central nervous system disorders, alcohol misuse, substance use disorders, mental disorders, psychiatric symptoms, and comorbidities that could affect safety and efficacy of the therapy. Patients who were pregnant or who had undergone organ transplant were excluded.

Randomization

Patients were randomized 1:1 to rituximab or placebo using a stratified permuted block design with a block size of four. Randomization was stratified by the presence or absence of concomitant immunosuppressive medication at the time of the last relapse and by presence of FRNS or SDNS.

Rituximab Intervention during the double-blind period (Phase 1)

Patients randomized to the intervention and placebo groups received rituximab 375 mg/m^2/dose or placebo at weeks 1, 2, and 25 (Figure S1). Patients taking corticosteroids at enrollment kept the same dosage for the first four weeks after the first administration of the study drug and then tapered every 4 weeks until discontinued (Figure S2). After

corticosteroid discontinuation, other immunosuppressants that had been administered prior to randomization were reduced or discontinued at the discretion of treating physicians, with at least 4 weeks between dose reductions.

This study was conducted in a double-blind manner, and the placebo was indistinguishable from rituximab in appearance. The clinical research coordinators, who were also blinded to treatment allocation, confirmed both the administration of the study drug and infusion reactions.

Post-relapse treatment period (Phase 2)

Patients who experienced relapse (urinary protein ≥1 g/gCr) after study drug initiation discontinued the double-blind period (Phase 1) at the time of relapse, and transitioned to the 49-week post-relapse treatment period (Phase 2) if they achieved remission (urine protein levels <0.3 g/gCr on ≥2 consecutive tests at least one day apart) with corticosteroids therapy at the discretion of the treating physicians. Open-label rituximab (375 mg/m^2/dose) was administered at weeks 1, 2, and 25, using the same protocol in the double-blind period (Phase 1).

Primary Outcome

The primary outcome was the relapse-free rate at 49 weeks. Proteinuria was measured at weeks 1, 2, 9, 17, 25, 33, 41, and 49, and at discontinuation or suspected relapse. Since a 1-week window was permitted for urinary testing at week 49, the maximum follow-up duration could be 50 weeks. Relapse was defined as two persistent episodes of urinary protein ≥1 g/gCr. The second test was performed within 2 weeks, leaving at least 1 day between the tests. The relapse date was defined as the first day on which urinary

protein was ≥1 g/gCr. In cases of suspected relapse, if a new treatment, including dose escalation of concomitant medications, was initiated before a second urine test, the participant was considered to have relapsed.

Secondary outcomes

Secondary outcomes were the rate of relapse-free survival at week 25; time from initiation of the investigational drug to relapse; change in corticosteroid dose at weeks 25 and 49; the percentage of patients who discontinued corticosteroids at weeks 25 and 49; change in immunosuppressant dose at weeks 25 and 49; the percentage of patients who discontinued immunosuppressants at weeks 25 and 49; and change in renal function parameters (urine protein, serum albumin, total cholesterol, serum creatinine) from baseline to weeks 25 and 49.

Exploratory outcomes

Exploratory outcomes included peripheral blood B cells and T cells, human anti-drug antibodies, and immunoglobulin levels. B-cell repopulation was defined as a B-cell count ($CD19^+$ or $CD20^+$) of ≥5 cells/μL after administration of rituximab.

Adverse event outcomes

The incidence of Grade 3 or higher adverse events, adverse side effects, serious adverse events, and adverse events leading to death or discontinuation were assessed after the first administration of the study drug. Adverse event grading was based on the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Grade 3 events were defined as severe or medically significant but not immediately life-threatening (e.g., requiring

hospitalization or prolongation of hospitalization). Grade 4 events were defined as life-threatening, and Grade 5 events as death.

Exploratory Outcomes during the post-relapse treatment period (Phase 2)

All pre-specified outcomes as in the double-blind period (Phase 1) were also pre-specified outcomes in the post-relapse treatment period (Phase 2). Additionally, the cumulative relapse curve, the median recurrence period, and the relapse-free rates at 25 and 49 weeks were estimated according to the randomized group in the double-blind period (phase 1) using the Kaplan-Meier method.

Power considerations

Based on previous studies evaluating the efficacy of corticosteroids in patients with FRNS[9] and rituximab in patients with SDNS[9], the relapse-free rates at one year after the administration of rituximab and placebo were anticipated to be 80% and 45%, respectively. With a one-tailed significance level of 2.5% and 80% power, the sample size required for the primary outcome analysis based on the log-rank test was estimated to be 19 for each group. To attain a conservative sample size and to obtain sufficient data for safety assessment, we also considered a method using binomial distribution, which indicated a minimum of 29 patients per group. Assuming that 10% of randomized patients would not receive the study drug due to dropout before administration, the planned target sample size was 32 patients per group. Thirty two participants per group provided statistical power of 96.1% using the log-rank test and a one-sided P value of 2.5%.

Statistical Analyses

Primary outcome

The primary analysis was conducted in patients who received at least one dose of the study drug during the double-blind period (Phase 1) to evaluate the direct pharmacological effect of rituximab (Full analysis set [FAS]). The Kaplan-Meier analysis and the log-rank test were used to compare the relapse-free rates between groups during the double-blind period (Phase 1).

Secondary outcomes

In a secondary analysis of the primary outcome, a stratified Cox model was used to estimate hazard ratio (HR) for relapse with stratification based on presence or absence of concomitant immunosuppressant at the latest relapse before enrollment, FRNS or SDNS, and histological type of nephrotic syndrome.

The primary analysis was repeated in the per-protocol subset (PPS) which included patients in FAS who adhered to the protocol during the double-blind period (Phase 1). Patients were excluded from the PPS if the double-blind was compromised.

Exploratory subgroup analyses were performed according to 1) concomitant use of immunosuppressive drugs at the latest relapse before enrolment; 2) FRNS or SDNS; and 3) histological types of nephrotic syndrome.

Secondary outcomes including changes in corticosteroid and immunosuppressant dosage and in renal function parameters were compared between groups using unpaired t-tests (5% two-sided). The number of patients who discontinued corticosteroid and immunosuppressants was compared using Fisher's exact test (5% two-sided). Because no adjustments were made for multiple comparisons, these secondary outcomes should be considered exploratory.

Exploratory outcomes

Fisher's exact test was used to evaluate the association of B-cell levels, presence of anti-drug antibody, and immunoglobulin levels with the occurrence of adverse events or infections (5% two-sided). The cumulative incidence curve was estimated from the date of B-cell depletion to repopulation using the Kaplan-Meier method in cases where B-cell depletion was observed.

Safety analysis was performed in safety analysis set (SAS), which included patients receiving at least one dose of the study drug during the double-blind period (Phase 1). Statistical analysis was performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

Study population and characteristics

Between September 1, 2020 and June 28, 2022, 72 patients were randomized to receive rituximab (36 patients) or placebo (36 patients). Six patients (rituximab group: 4, placebo group: 2) withdrew before receiving the investigational drugs, leaving 66 (rituximab 32, placebo 34) who received the investigational drugs during the double-blind period (Full analysis set [FAS]) (Figure 1). The characteristics of these patients are shown in Table 1. Thirty-six patients (rituximab 24, placebo 12) completed the double-blind period without relapse, whereas 25 relapsed (rituximab 4, placebo 21). Five patients dropped out due to an adverse event (rituximab 0, placebo 1) or physician judgement (rituximab 4, placebo 0): of the latter, two were removed from the study for treatment of adrenal insufficiency, and two were removed from the study for inability to taper corticosteroid doses per protocol. Dropout rates were 12.5% and 3.0% in the rituximab and placebo groups, respectively. Median (range) follow-up periods were 48.0 weeks (3-50) and 30.5 weeks (1-50) in the rituximab and placebo groups, respectively.

The per protocol set (PPS) included 62 patients (rituximab 30, placebo 32) after excluding four from FAS (rituximab 2, placebo 2) due to ineligibility or compromised double-blinding.

Primary Outcome

The 49-week relapse-free rate in the double-blind period (Phase 1) was 87.4% (95% confidence interval [CI]: 69.8-95.1) in the rituximab group and 38.0% (95%CI: 22.1–53.8) in the placebo group ($P < 0.0001$, one-sided log-rank test).

Secondary Outcomes

In the secondary analysis, a stratified Cox model showed a HR for relapse of 0.16 (95% CI: 0.05‐0.46) in the rituximab group compared with the placebo group. The proportional hazards assumption was verified visually by the scaled Schoenfeld residuals against time. Similar results were observed in PPS: the relapse-free rate at 49 weeks was 86.5% (95% CI: 68.0‐94.7%) in the rituximab group and 37.2% (95% CI: 21.0‐53.5%) in the placebo group (P < 0.0001, one-sided log-rank test) (Figure 2).

The 25-week relapse-free rate in the double-blind period (Phase 1) was 87.4% (95% CI: 69.8‐95.1%) in the rituximab group and 61.8% (95% CI: 43.4‐75.7%) in the placebo group. Median (range) time to relapse was 49.0 weeks (range, 4‐50+) and 30.8 weeks (range, 2‐51+) in the rituximab group and placebo group, respectively (Table S1).

The proportion of patients who discontinued corticosteroids at 49 weeks was 71.9% and 36.4% in the rituximab and placebo groups, respectively (P = 0.0061) (Table S2). The mean (standard deviation [SD]) change in corticosteroid doses in the rituximab and placebo groups were -5.5 (3.4) and -4.8 (4.2) mg/day at Week 25, and -5.8 (4.0) and -5.3 (5.3) mg/day at Week 49, respectively (Table S2). The proportion of patients who discontinued cyclosporine at 49 weeks was 26.1% and 12.5% in the rituximab and placebo groups, respectively (Table S3). The mean (SD) change in cyclosporine doses in the rituximab and placebo groups were -4.0 (8.3) and -1.7 (3.8) mg/day at Week 25, and -9.1 (12.8) and -4.5 (7.2) mg/day at Week 49, respectively (Table S3).

The mean (SD) urine protein in the rituximab and placebo groups was 89 (153) and 225 (252) mg/L at Week 49, respectively (Table S4). The mean (SD) serum albumin in the rituximab and placebo groups was 4.3 (0.3) and 4.2 (0.3) g/dL at Week 49, respectively

(Table S4). The mean (SD) total cholesterol in the rituximab and placebo groups was 5.03 (0.82) and 4.87 (0.74) mmol/L at Week 49, respectively (Table S4). The mean (SD) serum creatinine in the rituximab and placebo groups was 65.2 (15.0) and 66.4 (23.7) umol/L at Week 49, respectively (Table S4).

Exploratory Outcomes

The mean (SD) peripheral blood $CD19^+$ B cell counts in the rituximab group decreased from Week 1 through Week 41 (89 [104] to 5 [5] per μL), and slightly increased at Week 49 (9 [9] per μL) (Table S5). Similarly, the mean (SD) peripheral blood $CD20^+$ B cell counts in the rituximab group decreased from Week 1 through Week 41 (95 [106] to 6 [4] per μL), and slightly increased at Week 49 (11 [10] per μL) (Table S5). Associations between B-cell repopulation and relapse could not be analyzed since all patients achieved B-cell repopulation (Table S6; Figure S3). Anti-drug antibody was observed 5 in 32 (15.6%) patients in the rituximab group and 1 in 34 (2.9%) patients in the placebo group. The mean (SD) IgG levels were not substantially altered from Week 1 to Week 49 (8.3 [2.0] to 10.4 [2.2] g/L) in the rituximab group (Phase 1) (Table S7).

In the rituximab group, infections occurred in 9 of 30 (30.0%) patients with B-cell depletion and none in 2 patients without depletion (P>0.99) (Table S8). In the placebo group, where no patients developed B-cell depletion, infections occurred in 6 of 34 (17.6%) patients. Anti-drug antibody was detected in 5 of 32 (15.6%) patients in rituximab group and 1 of 34 (2.9%) in the placebo group. While all of these patients developed adverse events, no significant association was found between anti-drug antibody positivity and adverse events in both groups (P > 0.99) (Table S9). In the rituximab group, infections occurred in 8 of 19 (42.1%) patients with low immunoglobulin levels and in 1 of 12 (8.3%) patients without low

immunoglobulin levels (P = 0.0497) (Table S10). In the placebo group, infections occurred in 4 of 22 (18.2%) patients with low immunoglobulin levels and in 2 of 12 (16.7%) patients without low immunoglobulin levels (P >0.99).

In the exploratory subgroup analysis, there was no clear evidence that the effect of rituximab on the primary outcome was modified by 1) concomitant use of immunosuppressive drugs at the latest relapse before enrolment; 2) FRNS or SDNS; or 3) histological types of nephrotic syndrome. (Table S11). Due to the small number of patients with histopathology other than MCNS, the efficacy of rituximab in these subgroups was not estimated.

Adverse event outcomes (Phase 1)

The most common side effect was infusion reaction (13 [40.6%] in the rituximab group and 1 [2.9%] in the placebo group). Adverse events of Grade 3 (CTCAE v5.0; severe or medically significant but not immediately life-threatening events) occurred in 15.6% (5/32) and 5.9% (2/34) of patients in the rituximab and placebo groups, respectively (Table 3). Grade 3 treatment-related side effects occurred in 0% (0/32) and 2.9% (1/34; skin rash after infusion of placebo) in the rituximab and placebo groups, respectively. Grade 3 infections as an adverse event occurred in 3.1% (1/32) and 0% (0/34) in the rituximab and placebo groups, respectively.

Phase 2 exploratory outcomes

In the post-relapse treatment period (Phase 2), rituximab was administered to 3 of 4 patients in the rituximab group who relapsed during the double-blind period (Phase 1) (Table 2); the intervals between the last rituximab administration and the post-relapse

391 administration were 133, 161, and 288 days, respectively. Similarly, rituximab was
392 administered to 20 of 22 patients in the placebo group who relapsed during the double-blind
393 period (Phase 1) (Table 2). The baseline characteristics of these 23 patients (Retreatment
394 analysis set [RAS]) are shown in Table S12.
395 The overall relapse-free rate at week 25 was 95.7% (95% CI: 72.9-99.4): 66.7%
396 (95% CI: 5.4-94.5) in the rituximab-relapse group and 100.0% (95% CI: 100-100) in the
397 placebo-relapse group. The overall relapse-free rate at week 49 was 95.7% (95% CI: 72.9-
398 99.4): 66.7% (95% CI: 5.4-94.5) in the rituximab-relapse group and 100.0% (95% CI: 100-
399 100) in the placebo-relapse group ($P > 0.99$). (Figure S4). Three patients (13.0%)
400 experienced serious adverse events (Table S13).

401 Discussion

402 In this randomized trial of 66 adults with FRNS or SDNS, the primary outcome, the
403 49-week relapse-free rate, was significantly higher in the rituximab group compared with the
404 placebo group (87.4% vs. 38.0%). Although Grade 3 adverse events occurred more
405 frequently in the rituximab group, no rituximab-related side effects of ≥Grade 3 were
406 observed. These findings support the safe and efficacy of rituximab for preventing relapse of
407 FRNS/SDNS.

408 In a previous randomized trial including 48 childhood-onset FRNS/SDNS (mean age:
409 11.5–13.6), rituximab prolonged relapse-free periods compared with placebo during 1-year
410 follow-up (267 days vs 101 days).[4] However, 70.8% of patients in the rituximab group
411 relapsed within one year, perhaps because rituximab was administered weekly for four
412 doses during the first month, with no subsequent maintenance administration. In contrast, in
413 the current clinical trial, rituximab was repeatedly administered at weeks 1, 2, and 25, and 4
414 of 32 patients (12.5%) in the rituximab group relapsed during the 49-week follow-up. Among
415 20 patients in the placebo group who relapsed and subsequently received rituximab, none
416 relapsed during the 49-week post-relapse treatment period. Similarly, in a retrospective
417 study including 183 adult patients with podocytopathies, administration of at least one
418 additional rituximab dose within 12 months after the initial course for remission induction
419 was associated with higher relapse-free rates during 36 months of follow-up.[10] Moreover, in
420 a retrospective cohort of 250 pediatric patients with nephritis, repeated administration of
421 rituximab, generally given annually after confirming B cell repopulation, was associated with
422 lower relapse rates; however, 8.5% of patients exhibited moderate or severe
423 hypogammaglobulinemia.[11] Notably, in this study, serum IgG levels were not significantly
424 associated with the number of rituximab doses but positively correlated with age. In the

present study, including only adult patients, immunoglobulin levels in the rituximab group remained stable throughout the study period.

Limitations

This study has several limitations. First, the observation period was only up to six months after the last rituximab administration. Second, the sample size was small. Third, six randomized patients who did not receive the study drugs were excluded from the primary analysis and may have introduced bias. Fourth, only one block size was used, potentially preventing allocation concealment. Fifth, there were multiple secondary and exploratory outcomes without adjustment for multiple comparisons. Sixth, this study could not determine whether results were determined by depletion of B cells or another factor. Seventh, long-term efficacy of rituximab was not evaluated in this study.

Conclusion

These results support use of rituximab to prevent relapse in adults with FRNS or SDNS.

441 Figure legends

442

443 Figure 1. Eligibility, randomization, and flow of patients through the trial

444 The allocation to rituximab and placebo groups was made in a 1:1 ratio using a stratified
445 substitution block method (4 blocks) with the following allocation stratification factors: (1)
446 Concomitant use of immunosuppressive drugs at the time of relapse immediately prior to
447 enrollment and (2) Frequently recurrent nephrotic syndrome or steroid-dependent nephrotic
448 syndrome.

449

450 Figure 2. Relapse-free rate in the double-blind period (Phase 1) (%)

451 Regarding the relapse-free period, the log-rank test was used to compare the relapse-free
452 rates of the two groups during the double-blind period (Phase 1) from the date of the first
453 administration of rituximab or placebo. Dashed lines indicate censored observations. The
454 median (range) follow-up periods from the initiation of treatment for the rituximab and
455 placebo groups during the double-blinded period were 48.0 weeks (3-50) and 30.5 weeks
456 (1-50), respectively.

457

458 Authors' Contribution

459 YIs designed and managed the study and was responsible for the study concept. SM, MSs,
460 HH, YK, SG, TT, AM, Yik, NoS, NaS, KF and KY collected and interpreted data. TW, YS, and
461 KH evaluated the safety of this study. YS and MSh did statistical analysis. All authors were
462 members of the writing group and agreed on the content of the report, reviewed drafts, and
463 approved the final version.

464

465 Independent data and safety monitoring committee

466 Takehiko Wada (Toranomon Hospital), Yugo Shibagaki (St. Marianna University School
467 of Medicine), and Keiju Hiromura (Gunma University Graduate School of Medicine)

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469 Statistical analysis

470 Takeshi Shinohara (EPS Corporation), Eisuke Hida, Yusuke Sakaguchi and Maki Shinzawa
471 (The University of Osaka).

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473 Data sharing

474 Data from the A-TEAM study are not publicly available, since the data sharing for the
475 purpose of secondary data use is not described in the protocol of this study. Requests for
476 access to the data can be made by sending an email to isaka@kid.med.osaka-u.ac.jp
477 together with a research plan to the corresponding author. All requests will be reviewed and
478 require approval by the principal investigator

479

480 Declaration of interests

481 YI received research support from Zenyaku Kogyo, and honoraria from Chugai

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Table 1 Patient Characteristics at baseline in the double-blind period (Full analysis set)

Characteristic	Rituximab (N = 32)	Placebo (N = 34)

Sex, No. (%)		
Female	17 (53.1)	20 (58.8)
Male	15 (46.9)	14 (41.2)
Age (yr), Mean (SD)	49.1 (13.8)	46.8 (12.5)
Diagnosis of FRNS or SDNS, No. (%)		
FRNS	15 (46.9)	15 (44.1)
SDNS	17 (53.1)	19 (55.9)
Histopathology, No. (%)		
MCNS	27 (84.4)	30 (88.2)
FSGS	3 (9.4)	2 (5.9)
MN	1 (3.1)	2 (5.9)
Others	1 (3.1)	0
Urinary protein (mg/L), Mean (SD)	84.4 (98.0)	67.4 (75.5)
Serum albumin (g/dL) [*1] [*2], Mean (SD)	4.11 (0.29)	4.18 (0.33)
Total cholesterol (mmol/L) [*1] [*2], Mean (SD)]	5.61 (1.36)	5.68 (1.10)
Serum creatinine (μmol/L) [*1] [*2], Mean (SD)]	69.73 (15.51)	69.34 (17.87)
eGFR (mL/min/1.73 m^2) [*3], Mean (SD)	75.64 (17.74)	76.73 (21.14)
Prednisolone, No (%)	32 (100)	33 (97.1)
Dose at baseline (mg/day) [*4], median (IQR) [range]	10.00 (10.00-17.75) [4.0-40.0]	10.00 (7.00-20.00) [3.0-40.0]
Immunosuppressant use at relapse immediately before assignment[*5], No (%)	23 (71.9%)	24 (70.6%)
Immunosuppressant use on the day of first administration of study drug [*5], No (%)	23 (71.9)	24 (70.6)
Ciclosporin, No (%)	22 (68.8)	20 (58.8)
Dose at baseline (mg/day) [*4], median (IQR) [range]	100.0 (75.0-125.0) [50-150]	75.0 (50.0-100.0) [5-150]
Mizoribine, No. (%)	6 (18.8)	7 (20.6)
Dose at baseline (mg/day) [*4], median (IQR) [range]	150.0 (125.0-150.0) [100.0-150.0]	150.0 (100.0-150.0) [64.3-150.0]
Tacrolimus monohydrate, No (%)	0	2 (5.9)
Dose at baseline (mg/day) [*4], median (IQR) [range]	-	2.5 (2.0-3.0) [2-3]
Comorbidities[*6], No. (%)		
Dyslipidemia	13 (40.6)	9 (26.5)
Hypertension	11 (34.5)	12 (35.3)
Insomnia	5 (15.6)	9 (26.5)
Hyperlipidemia	4 (12.5)	6 (17.6)
Osteoporosis	5 (15.6)	4 (11.8)
Hyperuricemia	5 (15.6)	3 (8.8)
Iron deficiency anemia	3 (9.4)	4 (11.8)
Glaucoma	3 (9.4)	4 (11.8)
Steroid-induced osteoporosis	4 (12.5)	2 (5.9)
Diabetes	2 (6.3)	4 (11.8)

Abbreviations: eGFR, estimated glomerular filtration rate; FRNS, frequently relapsing nephrotic syndrome; FSGS, focal segmental glomerulosclerosis; IQR, interquartile range; MCNS, minimal change nephrotic syndrome; MN, membranous nephropathy; SD, standard deviation; SDNS, Steroid-dependent nephrotic syndrome.

Data are shown as n (%), mean (SD) or median (IQR) [range].

*1 Calculated based on values immediately before the first dose of rituximab or placebo.

*2 The reference values are as follows: Serum albumin: 3.6-5.3 g/dL, Total cholesterol: 120-248 mg/dL, Serum creatinine: 41-80 μmol/L/L for female and 53-106 μmol/L/L for male. The reference value for urinary protein follows the criteria diagnosis, remission and relapse of nephrotic syndrome.

*3 Calculated based on values during screening period.

*4 Dose immediately before first administration of rituximab or placebo. Dose of corticosteroid was maintained at the same dose from the day of the first rituximab or placebo until the fourth week and then the dose was tapered every four weeks. Immunosuppressants were not allowed to be changed, added, or increased in dosage after the start of screening. The method of reducing dosage and discontinuing were in accordance with the method of the institution.

*5 It does not include corticosteroids as immunosuppressants.

*6 Comorbidities occurring in 10% or more of the patients in either treatment group.

Table 2 Summary of remission and relapse rates in the double-blind and post-relapse rituximab treatment periods.

Outcome	Rituximab group	Placebo group
Double-blind period (Phase 1)	N=32	N=34
Remission	28/32 (88%)	12/34 (35%)
Relapse	4/32 (13%)	22/34 (65%)
Post-relapse rituximab treatment period (Phase 2)	N=4	N=22
Remission	2/4 (50%)	20/22 (91%)
Relapse	1/4 (25%)	0
Refused to participate	1/4 (25%)	2/22 (9%)

Table 3 Adverse events in the double-blind period (Safety analysis set)

	Rituximab (N = 32)		Placebo (N = 34)	
	Treatment-related adverse events (Side effects)[*1]	All adverse events[*2]	Treatment-related adverse events (Side effects)[*1]	All adverse events[*2]
Adverse events[*3]	15 (46.9)	29 (90.6)	6 (17.6)	22 (64.7)
Death	0	0	0	0
Serious adverse events	1 (3.1)	4 (12.5)	1 (2.9)	2 (5.9)
≥Grade 3 adverse events[*4]	0	5 (15.6)	1 (2.9)	2 (5.9)
Infections and infestations	0	10 (31.3)	2 (5.9)	7 (20.6)
≥Grade 3 infections[*4]	0	1 (3.1)	0	0
Infection in ≥2patients				
Gastroenteritis	0	2 (6.3)	0	0
COVID-19	0	5 (15.6)	0	2 (5.9)
Neoplasm benign, malignant and unspecified	0	0	0	1 (2.9)
≥Grade 3 Neoplasm benign or malignant[*4]	0	0	0	0
Neoplasm benign or malignant in ≥2patients				
Adrenal insufficiency	0	3 (9.4)	0	0
≥Grade 3 Adrenal insufficiency[*4]	0	0	0	0
Steroid withdrawal syndrome	0	1 (3.1)	0	0
≥Grade 3 Steroid withdrawal syndrome[*4]	0	0	0	0
Adverse events in ≥10% of patients				
Oropharyngeal discomfort	6 (18.8)	6 (18.8)	1 (2.9)	1 (2.9)
COVID-19	0	5 (15.6)	0	2 (5.9)
Headache	0	5 (15.6)	0	2 (5.9)
Arthralgia	0	5 (15.6)	0	8 (23.5)
Malaise	0	4 (12.5)	0	2 (5.9)
Fever	0	4 (12.5)	0	3 (8.8)
Infusion reaction[*5]	13 (40.6)	-	1 (2.9)	-
Grade 1[*4]	11 (25.0)	-	1 (2.9)	-
Grade 2[*4]	5 (15.6)	-	1 (2.9)	-
Grade 3[*4]	0	-	1 (2.9)	-

The values indicate the number of patients (%).

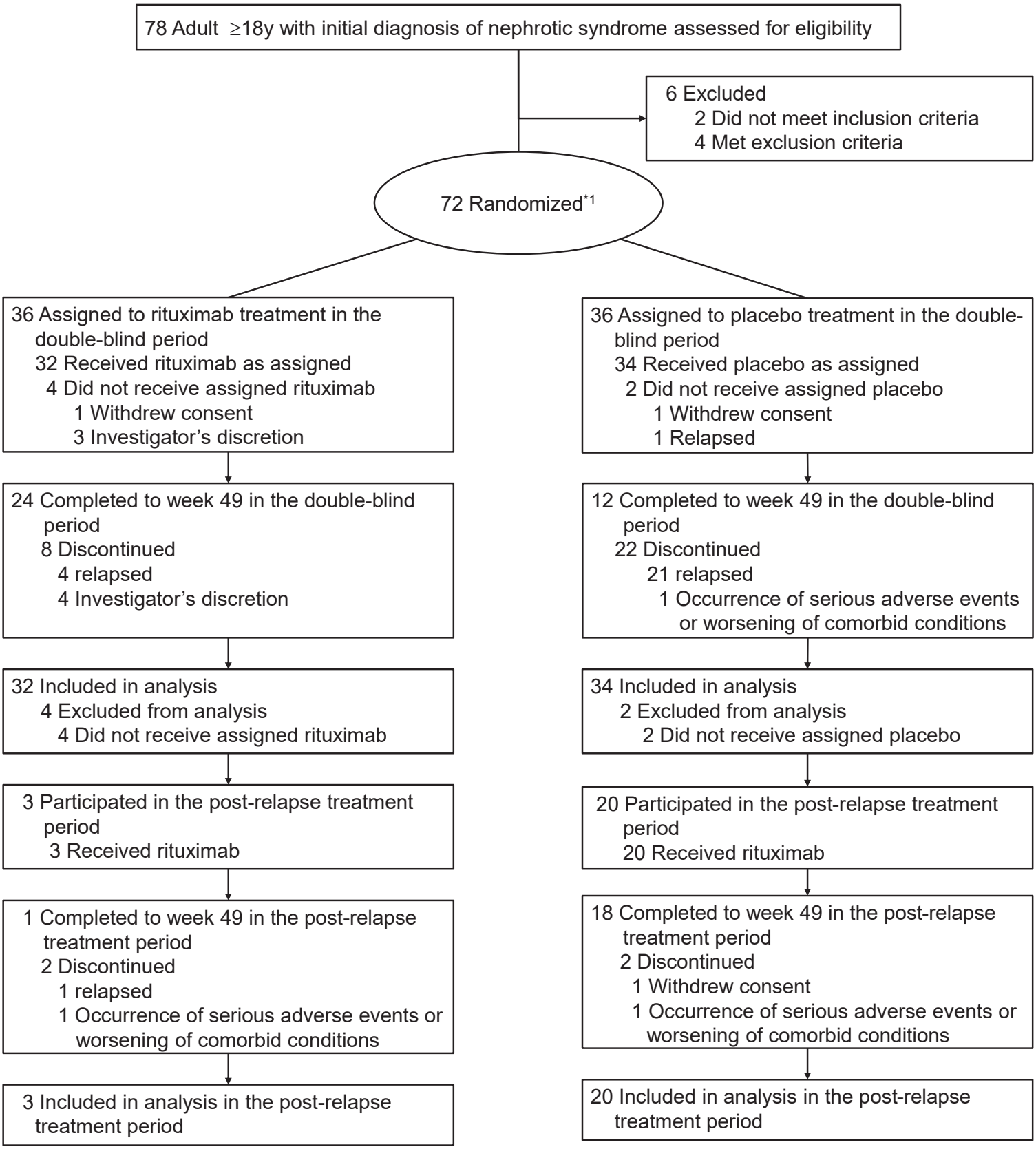
*1 Side effects were adverse events related to rituximab or placebo. Causality of events in relation to the study drug, including placebo, was judged by the treating physicians.

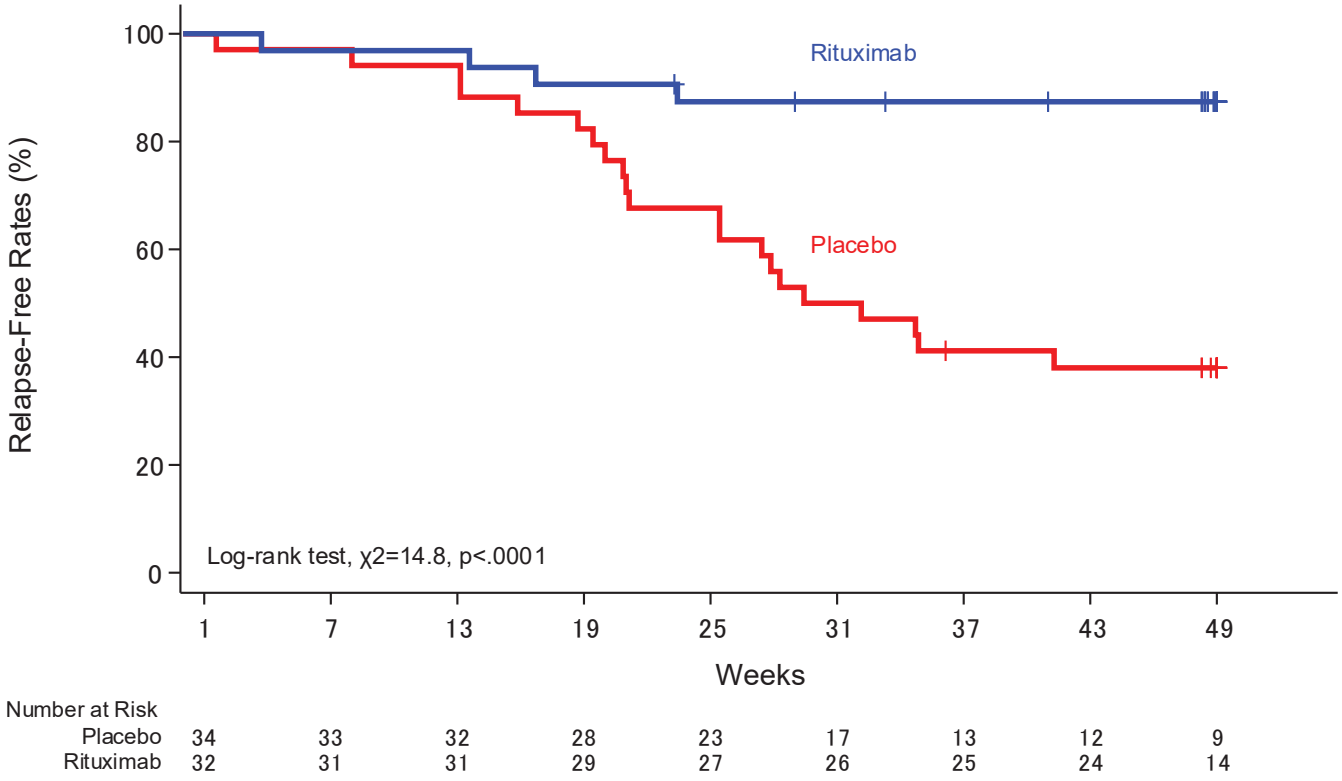
*2 Adverse events were any untoward or unintended sign, symptom, or disease, including abnormal laboratory test results, observed from the start of the first rituximab or placebo administration to the end of the last observation (the date of the end of the double-blind period or the date of discontinuation) in the double-blind period, regardless of whether it was related to rituximab or placebo.

*3 Adverse events were coded in the Medical Dictionary for Regulatory Activities (MedDRA)/J Ver. 24.0.

*4 The severity of adverse events was determined based on the Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Grade 3 corresponds to severe or medically significant but not immediately life-threatening events (e.g., hospitalization or prolongation of hospitalization indicated), Grade 4 to life-threatening consequences, and Grade 5 to death.

*5 Infusion reactions were defined as all hypersensitivity-like symptoms or allergy-like symptoms (including anaphylactoid reaction, lung disorder, cardiac disorder or abnormal vital signs) related to rituximab or placebo occurred or identified within 24 hours from start of rituximab or placebo administration.


78 Adult ≥18y with initial diagnosis of nephrotic syndrome assessed for eligibility
6 Excluded
2 Did not meet inclusion criteria
4 Met exclusion criteria
72 Randomized*1
36 Assigned to rituximab treatment in the double-blind period
32 Received rituximab as assigned
4 Did not receive assigned rituximab
1 Withdrew consent
3 Investigator's discretion
36 Assigned to placebo treatment in the double-blind period
34 Received placebo as assigned
2 Did not receive assigned placebo
1 Withdrew consent
1 Relapsed
24 Completed to week 49 in the double-blind period
8 Discontinued
4 relapsed
4 Investigator's discretion
12 Completed to week 49 in the double-blind period
22 Discontinued
21 relapsed
1 Occurrence of serious adverse events or worsening of comorbid conditions
32 Included in analysis
4 Excluded from analysis
4 Did not receive assigned rituximab
34 Included in analysis
2 Excluded from analysis
2 Did not receive assigned placebo
3 Participated in the post-relapse treatment period
3 Received rituximab
20 Participated in the post-relapse treatment period
20 Received rituximab
1 Completed to week 49 in the post-relapse treatment period
2 Discontinued
1 relapsed
1 Occurrence of serious adverse events or worsening of comorbid conditions
18 Completed to week 49 in the post-relapse treatment period
2 Discontinued
1 Withdrew consent
1 Occurrence of serious adverse events or worsening of comorbid conditions
3 Included in analysis in the post-relapse treatment period
20 Included in analysis in the post-relapse treatment period



Number at Risk									
Placebo	34	33	32	28	23	17	13	12	9
Rituximab	32	31	31	29	27	26	25	24	14