Supplement 1: Study protocol of the RTXFIRPedINS Trial

List of amendments

Amendment n°1 (16/09/2021)

- Changed “Trial Sites (Coordinators)”: changed from “The Children's Hospital of Zhejiang University School of Medicine, Zhejiang: Maojianhua (PI); Shanghai Children's Hospital, Shanghai: Huang Wenyan (PI) and Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai: Li Yufeng (PI) to “Shandong Provincial Hospital, Shandong: Sun Shuzheng (PI); and the First Affiliated Hospital of Sun Yat-sen University, Guangzhou: Jiang Xiaoyun (PI)”.

Amendment n°2 (03/12/2021)

- Addition of a “Trial Sites (Coordinators)”: Xuzhou Children’ Hospital, Jiangsu: Zhang Ruifeng (PI).

Amendment n°3 (05/05/2023)

- Addition of a historical control: to compare the differences in the effects on relapse-free survival rate (at 12-month and 6-month follow up) between the rituximab infusion and steroid therapy without rituximab, a historical control will be selected from a previous trial. The historical control group included 33 patients receiving prednisolone therapy.
- Addition of a “Secondary outcomes: The proportion of patients diagnosed with FRNS/SDNS within 12 months.”

Title: The efficacy and safety of rituximab in the first episode of paediatric idiopathic nephrotic syndrome: An open-label, single-arm, multicentre clinical trial

Trial Registration ID: NCT04783675
May 5th 2023, Version: 1.4

RTXFIRPedINS Research Group

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Shandong Provincial Hospital, Shandong: Shuzheng Sun (PI), Jing Wang (coordinator);
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Clinical Research Institute, Shanghai Jiao Tong University School of Medicine, Shanghai: Biyun Qian, Li Xie.

INTRODUCTION

Idiopathic nephrotic syndrome (INS) is one of the most common paediatric glomerular diseases[1]. Its incidence is the highest in Asians, 7.14 per 100,000 children per year[2]. The pathogenesis is thought to involve immune dysregulation, systemic circulating factors, or inherited structural abnormalities of the podocyte[3].

The updated International Paediatric Nephrology Association (IPNA) 2020[4] and Kidney Disease Improving Global Outcome (KDIGO) 2020[5] guidelines recommend that children with the first episode of INS be treated with corticosteroids for a total of 8-12 weeks. Approximately 85% of patients experience complete remission of proteinuria within 4-6 weeks following guideline-recommended corticosteroids, and have steroid-sensitive NS (SSNS). However, the 1-year relapse-free survival rate is only approximately 30%[6], and the risk for all relapses is 66.2%-87.4%[7]. Half of these children will become frequently relapsing nephrotic syndrome or steroid-dependent NS (FRNS/SDNS)[8], and they may experience serious side effects from further steroid treatment or other immunosuppressive drugs. Therefore, prevention of relapse is an important objective of therapy.

Rituximab (RTX) is a monoclonal antibody against the cluster of differentiation antigen 20 (CD20) on B cells, and may be a valuable additional agent for the treatment of children with FRNS/SDNS. Recently, the Cochrane Database of Systematic Reviews[9] reported that in children with FRNS/SDNS, RTX used alone or with other immunosuppressive therapies probably reduces the number of children who relapse at 3, 6 and 12 months. Although the risk of infections may not be increased, but infusion reactions may be more common.

Whether the benefits of RTX extend to the first relapse is unknown. The Efficacy and Safety of Rituximab in the First Episode of Paediatric Idiopathic Nephrotic Syndrome (RTXFIRPedINS) trial is testing the hypothesis that RTX, in addition to guideline-recommended corticosteroids, safely increases the 1-year relapse-free survival rate in children with the first episode of SSNS.

Objectives

Primary outcomes:

The primary objective is to assess whether RTX added to guideline-recommended corticosteroids is effective for maintaining remission through one year after infusion for children with the first episode of SSNS.

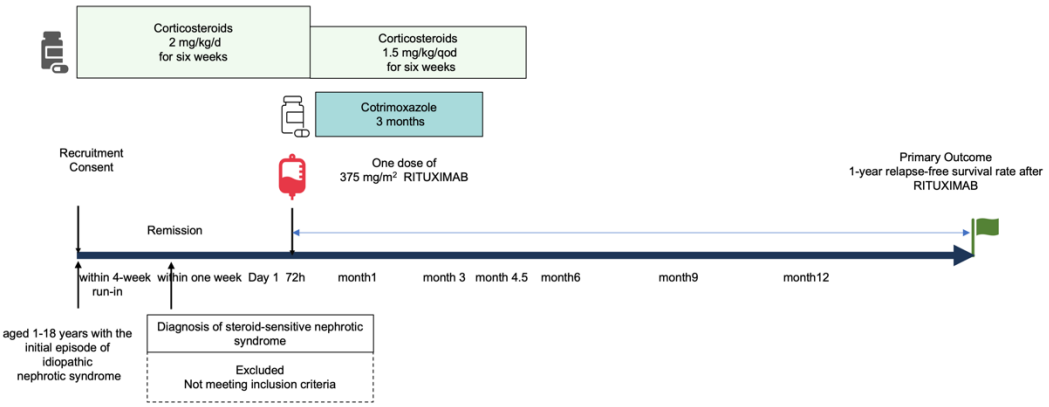
Key secondary outcomes:

The number of days from the infusion of RTX to the occurrence of the first relapse, the 6-month relapse-free survival rate, , the proportion of patients diagnosed with FRNS/SDNS within 12 months, B-cell recovery time and treatment-related adverse events.

Exploratory endpoints include changes in immunological factors to be studied as predictors of response and relapse.

Design

RTXFIRPedINS is an open-label, single-arm, multicentre clinical trial that recruited 44 patients from eight hospitals in China. Figure 1 shows the overall-design of the study.



Trial Sites:

The trial will be conducted in eight academic hospitals in China. A list of all participating centers can be

found on https://clinicaltrials.gov

Trial participants

The trial participants will be patients aged 1-18 years with the first episode of SSNS. Patients will be required to achieve complete remission of proteinuria within four weeks following the guideline-recommended corticosteroids. Furthermore, patients should not have recently received immunosuppressive agents or live vaccines. The additional inclusion and exclusion criteria are listed in Table 1.

Table 1. Inclusion and exclusion criteria of the RTXFIRPedINS trial

Eligibility criteria
1. Children between 1 and 18 years with Steroid-Sensitive Nephrotic Syndrome (nephrotic-range proteinuria and either hypoalbuminemia or edema when albumin level is not available) 2. Estimated glomerular filtration rate (eGFR) ≥90 ml/min per 1.73 m2 at study entry 3. Remission at study entry 4. CD20 positive cells in peripheral blood ≥1% total lymphocytes 5. No immunosuppressive agents have been used within 3 months of enrolment, except for the use of corticosteroid to treat nephrotic syndrome 6. Provision of consent by a legal representative using a document approved by the institutional review board after receiving an adequate explanation of this clinical trial. For children ages 8-18, written assent is required using age-appropriate and background-appropriate documents
Exclusion criteria
1. Diagnosis of secondary NS 2. Patients showing one of the following abnormal clinical laboratories values: leukopenia (white blood cell count ≤$3.0*10^9$/L); moderate and severe anemia (hemoglobin <9.0g/dL); thrombocytopenia (platelet count <$100*10^{12}$/ L); positivity of autoimmunity tests (ANA, Anti DNA antibody, ANCA) or reduced C3 levels; Alanine aminotransferase or aspartate aminotransferase > 2.5× upper limit of normal value 3. Presence of severe or chronic infections within 6 months before assignment: tuberculosis or in whom tuberculosis is suspected; Epstein-Barr virus or CMV virus; hepatitis B or hepatitis C or hepatitis B virus carrier, human immunodeficiency virus or other active viral infections 4. Live vaccination within last month 5. Patients with poorly controlled hypertension 6. Patients with severe brain, heart, liver, and other important organs, as well as blood and endocrine system diseases 7. Presence or history of autoimmune diseases, primary immunodeficiency, or tumor 8. Patients with a known allergy to Rituximab and its excipients 9. Assessed to be unfit for participation by the investigators (patients highly likely to be lost to follow-up or provide inaccurate data, for example, patients with alcohol or other substance misuse disorders, and patients with psychological disorders)

Enrolment

All patients affected by INS at the nephrology units of registered hospitals will undergo evaluation for recruitment. A preliminary interview will be conducted to verify eligibility criteria. A study coordinator will explain the project and provide informational materials. Eligible participants will receive steroid treatment after blood sample collection. For children with HBs-Ab <20 mIU/mL, it is recommended to receive hepatitis B vaccination before commencing corticosteroid treatment. They will then enter a 4-week run-in period, during which instructions on urine collection and dipstick readings will be carefully reviewed, and compliance will be assessed until complete remission is achieved (urine protein/creatinine ratio ≤0.2 mg/mg or negative or trace dipstick on three or more consecutive occasions in first morning samples).

Once all inclusion/exclusion criteria are confirmed, secondary registration will be carried out. Written informed consent from the parent or guardian and the child's assent (Supplementary material) will be obtained prior to performing any study-related procedures.

Study period

Investigators will conduct observations and examinations in accordance with the prescribed schedule (Table 2).

Table 2 Clinical trial schedule

	Screening period	Observation period		Follow-up period						
Visit	**V0**	**V1**		**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**Relapse**
Day	**Within 28 days**	**Rituximab administration period**		**28±7**	**91±7**	**133±7**	**182±7**	**273±7**	**365±7**	**Within 3 days**
		Before RTX	**Within 72 hours after RTX**							
Obtaining informed consent	√	√								
Background survey	√									
Medical examination	√	√	√	√	√	√	√	√	√	√
Height/weight/Blood pressure/Pulse	√	√	√	√	√	√	√	√	√	√
Urinalysis	√	√	√	√	√	√	√	√	√	√
Complete blood count and biochemistry (kidney function, liver function, lipid status-cholesterol and triglycerides, albumin)	√	√	√	√	√	√	√	√	√	√
Lymphocyte subset testing	√	√	√	√	√	√	√	√	√	√
Immunoglobulin examination	√	√	√	√	√	√	√	√	√	√
Peripheral blood B Cell, T Cell and Myeloid Cell subsets	√**Before corticosteroids**	√	√	√	√		√	√	√	√
Chest X-ray	√									√
Echocardiography and electrocardiogram	√									√

Any adverse report yes/no (if yes –describe in full detail)		√	√	√	√	√	√	√	√	√
Use of any other drugs	√	√	√	√	√	√	√	√	√	√

The study period will begin on the date consent is obtained and end on the date of completion of the observation period. Dipsticks for proteinuria determination will be evaluated daily. Study visits are scheduled for the screening period as follows: RTX administration; 1, 3, 4.5, 6, 9, and 12 months after RTX administration; and at the time of relapse. Each visit will include the collection of information regarding potential endpoints, adverse events, and concomitant therapies. Vital signs will be recorded. Urinalysis, complete blood count, biochemistry, immunoglobulins, and lymphocyte subpopulations will be evaluated according to the standard laboratory practice. A study coordinator maintains ongoing contact to minimise dropouts. Follow-up visits will be performed at the same institution or by local nephrologists if travel is impossible.

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Intervention

RTX will be infused intravenously at a dose of 375 mg/1.73 m^2 (maximum dose:500 mg) within one week of achieving complete remission. Every 100 mg of RTX will be diluted in 100 mL of normal saline and infused at a rate of 25 mL/hour for the first 30 minutes. Thereafter, the rate will be doubled every 30 min to a maximum of 100 mL/hour.

Interventions will be administered in an inpatient setting at the nephrology unit of the registered hospitals. Interventions will be discontinued if the investigator determines that continuation continuing would result in a significant safety risk.

Related medications

The initial treatment of NS is daily prednisone/prednisolone 2 mg/kg/d or 60 mg/m^2/d (maximum 60 mg/d) for six weeks, and then 1.5 mg/kg/d or 40 mg/m^2 (maximum 40 mg/d) on alternate days for another six weeks.

To prevent infusion reactions, 30 min before the RTX infusion, the patient should be administered antipyretic and analgesic drugs, such as acetaminophen/ibuprofen, once at a regular dose. Anti-allergy medications, cetirizine hydrochloride/cyproheptadine/loratadine, should be administered–once at a regular dose. Before RTX infusion, oral corticosteroids will be switched to methylprednisolone 1.6 mg/kg intravenously once.

To prevent *Pneumocystis carinii*, trimethoprim-sulfamethoxazole (SMZ) will be administered for 3 months from the beginning of the RTX treatment (day 1), the prophylactic dose of TMP will be 3 mg/kg on alternate days, and the maximum dose of SMZ will be 960 mg on alternate days.

In cases of relapse, if there is an infection, the coinfection will be controlled first. If the patients cannot achieve complete remission within 7 days, they will be treated with prednisone/prednisolone at a daily dose of 60 mg/m^2 until complete remission is achieved for at least three days, and then the dose will be reduced to 40 mg/m^2 on alternate days for at least four weeks. The treatment for subsequent recurrences will be based on the clinical guidelines.

Outcome definitions

Efficacy outcomes

The primary outcome for the evaluation of the effect of RTX added to guideline-recommended corticosteroids is the 1-year relapse (recurrence of nephrotic-range proteinuria, urine protein/creatinine ratio ≥2 mg/mg or dipstick ≥ 3+ on three consecutive days in the first morning samples)-free survival rate in children with the first episode of SSNS. Secondary outcomes are as follows: the number of days from the infusion of RTX to the occurrence of the first relapse, the 6-month relapse-free survival rate, and the time to the first detection of CD19+ cells above 1% of total CD45+ lymphocytes after CD19+ cell depletion. Using fluorescence-activated cell sorting, the effect of RTX on peripheral blood B cells, T-cell and myeloid cell subsets will be studied as biomarkers of response and relapse, before and after infusion of RTX within 72 h, 1, 3, 6, and 12 months, and when a relapse occurs.

Safety outcomes

Only serious adverse events of interest will be recorded. The number of participants with treatment-related adverse events will be assessed using CTCAE v5.0 which is a binary variable (1/0). The variable is set as "1" if any adverse events occur, including infusion-related reactions (within 24 hours of infusion); symptoms including fever, chills and rash, flushing, angioedema, nausea, urticaria/rash, fatigue, headache, throat irritation, rhinitis, vomiting, hypotension and bronchospasm; infection (upper respiratory tract infection, hepatitis B virus reactivation, herpes zoster infection, pneumocystis pneumonia, sepsis, etc.); persistent hypogammaglobulinemia, leucopenia and neutropenia; encephalopathy, fatal pulmonary fibrosis, ulcerative colitis, Crohn’s disease and fulminant myocarditis.

Statistical methods

The 1-year relapse-free survival rate and 95% confidence interval will be assessed. Relapse-free survival is defined as the date from the injection of RTX until the first relapse or the last follow-up, whichever occurs first. The Kaplan–Meier method will be used to assess relapse-free survival. Potential risk factors assumed to affect the time to the first relapse will be assessed using a Cox proportional hazards regression model. Secondary endpoints, including time to relapse, 6-month relapse-free survival rate, B-cell depletion period, and treatment-related adverse events, will be analysed. Peripheral blood B cells, T-cell and myeloid cell subsets will be explored.

Sample size

The sample size is based on the expected rate of the primary efficacy endpoint and the anticipated size of the effect of RTX treatment. According to previous literature, the 1-year relapse-free survival rate is approximately 30% in children with the first episode of SSNS after steorid treatment[6]. Based on this previous study[6], we estimated that a sample size of 44, under the assumption of a 10% dropout rate, would provide 80% power to detect a 20% increase in the relapse-free rate between the traditional method and RTX treatment at a two-sided alpha level of 0.05.

Patient and public involvement

No patients were involved in the study's design.

Acknowledgment

We thank patients and their relatives, physicians, nurses and clinical research staff of the participating centres for their involvement in this study.

Publication of research results

We aim to publish the results of this trial in a scientific journal or present them at academic conferences. Personal information will be carefully concealed when we present the results.

Research funds

Key Development Program of Children's Hospital of Fudan University
Shanghai Kidney Development and Pediatric Kidney Disease Research Center
Clinical Research Plan of Shanghai Hospital Development Center

Competing interests

HX has received a grant from Shanghai Henlius Biotech, Inc [EK00000710].

Patient Information and Informed Consent Form
for Parent/guardian

Dear sir/madame：

Your child is being invited to take part in a research study： An open-label, single-arm, multicentre clinical study to evaluate the efficacy and safety of rituximab in the first episode of paediatric idiopathic nephrotic syndrome (RTXFIRPedINS).

Before you decide, it is important for you to understand why the research is being carried out and what it will involve. Please take time to read the following information carefully. This document explains the purpose of this study, what it means to participate in it, and what your child can expect. Ask your child's study doctor if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to have your child take part in this study.

This is a multicentre clinical study on the nephrotic syndrome in children led by professor Xu hong from the Children's Hospital of Fudan University.

1. What is the purpose of the study?

Idiopathic nephrotic syndrome (INS) is one of the most common paediatric glomerular diseases. Although 80%–90% of children achieve complete remission after initial steroid therapy (steroid-sensitive nephrotic syndrome; SSNS); however, relapses are common after the reduction or discontinuation of corticosteroids. Half of these children will experience frequent relapses (FRNS) or become steroid-dependent (SDNS). They may experience serious side effects from further steroid treatment or other immunosuppressive drugs (including mycophenolate mofetil, calcineurin inhibitors, etc.). In addition, up to 15%–25% of childhood relapses persist into adulthood, and some may develop late-onset steroid resistance (SRNS) with a higher risk of progression to kidney failure and relapse after kidney transplantation. Long-term side effects of steroids and non-corticosteroid immunosuppressive medications are common and may include: poor growth, obesity, hypertension, cataracts, glaucoma, psychological disturbances, osteoporosis, nephrotoxicity, diabetes mellitus, dyslipidaemia, gonadal toxicity, and carcinogenicity, which significantly affect the prognosis and quality of life. In recent years, many high-quality clinical studies have shown that the use of anti-CD20 monoclonal antibody-rituximab (RTX) in FRNS/SNDS can effectively reduce relapse and promote better growth catch-up, even when steroids and other immunosuppressants are discontinued. The 2020 Kidney Disease Improving Global Outcome (KDIGO) guidelines are recommended to be used for children with FRNS/SDNS. Our centre is the first in China to report the efficacy and safety of RTX for treating FRNS/SDNS. The primary objective of our study is to assess whether RTX is effective and safe for children to maintain remission when initiated at the first episode of SSNS with the guideline-recommended corticosteroids. Exploratory endpoints include changes in immunologic factors, to be studied as predictors of response to RTX and relapse of INS.

2. What will happen in the study?

Forty-four subjects will take part in the study from eight hospitals. This study will use competitive enrolment, and the follow-up period will be one year.

You and your child should attend all study visits as scheduled.

Screening

The study will begin, after signing this informed consent form, with a screening visit. The purpose of the screening visit is to find out if your child meets the requirements to participate in this study.

What needs to be done during the screening visit: 1. Routine pre-enrolment examinations: demographic information, medical history collection, physical examination, vital signs (blood pressure and heart rate), height, and weight. 2. Laboratory tests: complete blood count, C-reactive protein, urine and urine sediment, urine protein/creatinine, 24-hour urine protein (child >3 years old), urinary microprotein concentrations, serum creatinine, blood urea nitrogen, albumin, alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides, serum electrolyte levels (sodium, potassium, chloride, calcium, and phosphorus), blood gas analysis, EBV/CMV DNA and antibodies, HBsAg, HBsAb, HBeAg, HbcAb, HCV, syphilis, HIV, tuberculosis, antinuclear antibodies, cluster of differentiation, immunoglobulin, pre-steroid blood samples. 3. Ophthalmological evaluation, urinary system ultrasound, electrocardiogram, echocardiography, and chest X-ray.

Nephrotic syndrome is treated with a standard course of steroids. If complete remission is achieved within 4 weeks, steroid-sensitive nephrotic syndrome is diagnosed. The study doctor will determine if your child is eligible to continue in the study. Medical history collection, physical examination, vital signs (blood pressure, heart rate), height, weight, urine routine, urine sediment, urine protein/creatinine, complete blood count, C-reactive protein, liver and kidney function, cluster of differentiation, immunoglobulin, and blood samples will be repeated before administration of RTX. To prevent infection, trimethoprim-sulfamethoxazole (SMZ) will be used for 3 months, starting with RTX treatment initiation.

Later, if your child is not required in the study, the study doctor will explain the reason for it and

discuss other treatment options with you.

Treatment Visit

Your child will have to follow the prescribed schedule for routine observations and examinations.

At month 1, medical history collection, physical examination, vital signs (blood pressure and heart rate), height, weight, urine and urine sediment, urine protein/creatinine, complete blood count, C-reactive protein, liver and kidney function, cluster of differentiation, immunoglobulin, and blood, and urine specimens will be collected.

At month 3, medical history collection, physical examination, vital signs (blood pressure and heart rate), height, weight, urine and urine sediment, urine protein/creatinine, complete blood count, C-reactive protein, liver and kidney function, cluster of differentiation, immunoglobulin, blood and urine specimens will be collected. Additionally, the ophthalmological evaluation will also be conducted.

At months 4.5, medical history collection, physical examination, vital signs (blood pressure and heart rate), height, weight, urine routine, urine sediment, urine protein/creatinine, complete blood count, C-reactive protein, liver and kidney function, immunoglobulin, and blood and urine samples will be collected.

At months 6, 9, and 12, medical history collection, physical examination, vital signs (blood pressure and heart rate), height, weight, urine routine, urine sediment, urine protein/creatinine, complete blood count, C-reactive protein, liver and kidney function, cluster of differentiation, immunoglobulin, and blood and urine samples will be collected.

In case of relapse, medical history, physical examination, vital signs (blood pressure and heart rate), height, weight, complete blood count, C-reactive protein, urine and urine sediment, urine protein/creatinine, 24-hour urine protein (child >3 years-old), urinary microprotein concentrations, serum creatinine, blood urea nitrogen, albumin, alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides, serum electrolytes (sodium, potassium, chloride, calcium, and phosphorus), blood gas analysis, EBV/CMV DNA and antibodies, HBsAg, HBsAb, HBeAg, HbcAb, HCV, syphilis, HIV, tuberculosis, antinuclear antibodies, cluster of differentiation, immunoglobulin, and blood and urine specimens will be recorded. Ophthalmological evaluation, urinary system ultrasound, electrocardiogram, chest X-ray, and echocardiography will also be performed.

Follow-up visits are required to be conducted on the prescribed schedule. The doctor will measure your child's height, weight, and blood pressure at each visit, with urine and blood samples collection. Dipsticks for proteinuria determination will be evaluated daily. In case of relapse, you should contact us immediately. The treatment will progress according to the treatment instructions. Physical examination and urine and blood samples will be taken at each visit and relapse.

The amount of blood drawn each time will be about 10–20 mL, which is similar to the amount drawn in routine clinical treatments. Since this test needs to detect morning urine, liver, and kidney functions, you will have to refrain your child from eating and drinking plenty of water before visiting the doctor.

3. Are there other treatment options for my child?

The other treatment plan for this disease includes paying close attention to the urine protein levels; if there are one or more relapses within 1 year of treatment, steroids and non-corticosteroid immunosuppressive medications may be required following renal biopsy and other examinations, to reach long-term remission.

4. What issues should be paid attention to during the study？

Provide truthful information about your medical history and current medical condition. At each visit, you should convey to your child's study doctor any changes in your child's health or condition. You must also communicate with the child's study doctor immediately if there are any major changes in your child's health or condition between visits or if you have any concerns regarding the study. Notify the study doctor about any discomfort and other treatments your child has had during this study. Tell the doctor if your child has recently participated or is currently participating in other studies.

5. What are the side effects or risks of participation?

The drugs used in this trial are approved drugs that have been marketed in China and are also used in routine clinical treatment. The associated adverse reactions are similar to those observed in current clinical therapies and are detailed below:

Adverse consequences of glucocorticoid medication (prednisone, prednisolone or methylprednisolone) include: (1) Fluid and electrolyte disturbances: sodium retention, congestive heart failure in some sensitive patients, hypertension, fluid retention, potassium loss, hypokalaemic alkalosis; (2) Musculoskeletal system: steroid myopathy, muscle weakness, osteoporosis, aseptic necrosis, compressive vertebral fractures, pathological fractures, tendon ruptures, especially the Achilles tendon; (3) Gastrointestinal tract: peptic ulcer that may perforate or bleed, gastrointestinal bleeding, pancreatitis, esophagitis, intestinal perforation; (4) Skin and subcutaneous soft tissue: poor wound healing, petechiae

and ecchymosis, brittle skin, thin skin, acne; (5) Metabolism: negative nitrogen balance due to protein breakdown; (6) Nervous system: elevated intracranial pressure, pseudo-brain tumour, confusion; (7) Endocrine: menstrual disorders, triggers Cushing's symptoms, affects the pituitary-adrenal axis, impairs glucose tolerance, triggers latent diabetes, increases the demand for insulin and oral hypoglycaemic drugs in diabetic patients, and stunts growth (8) Allergic reactions: angioedema, severe anaphylaxis; (9) Eye: long-term use can cause subcapsular cataracts, glaucoma, and possible damage to the optic nerve, and increase the risks of secondary fungal or viral infections in the eyes. To prevent corneal perforation, glucocorticoids should not be used in children with herpes simplex and herpes zoster with ocular symptoms, increased intraocular pressure, or proptosis; (10) Cardiovascular symptoms: myocardial rupture after myocardial infarction, high doses may cause tachycardia; (11) Immune system: decreased ability to detect infection, the onset of underlying infections, opportunistic infections, may suppress skin test reactions.

The use of RTX, as an immunosuppressive agent, may be dangerous in the presence of an infection. We will conduct clinical screening for detecting serious or underlying infections. Your child will receive a careful physical examination. Complete blood count, urine and urine routine, liver and kidney function, immune function, infection indicators, electrocardiogram, and chest radiograph will be assessed. It is recommended to receive a hepatitis-B vaccination. Because RTX is a chimeric anti-CD20 monoclonal antibody, patients may experience allergic reactions, including nausea, rash, itching, fever, chills, throat irritation, swelling of the tongue or throat (angioedema), headache, rhinitis, cough and bronchi spasticity, and tachycardia, with or without drug-related hypertension or hypotension. We will provide pre-medications to minimize the occurrence of these side effects. ECG, blood pressure, and transcutaneous oxygen saturation will be monitored during intravenous infusion therapy. If respiratory symptoms (dyspnoea, bronchospasm, or hypoxemia), hypotension, or other side effects occur during intravenous infusion, ECG and blood pressure monitoring will continue until 24 hours after the end of the infusion. RTX may increase the risk of respiratory infections, and reactivate hepatitis B, varicella zoster, pneumocystis carinii pneumonia, and other infections. Other adverse reactions include neutropenia, leukopenia, and persistent hypogammaglobulinemia.

The adverse reactions of sulfamethoxazole include: allergic reactions, drug eruptions, photosensitivity, drug fever, and other serum sickness-like reactions; higher risks of haemolytic anaemia and haemoglobinuria in patients with glucose-6-phosphate dehydrogenase deficiency; crystalluria, haematuria, and casturia in the kidneys; gastrointestinal symptoms, such as nausea, vomiting, loss of appetite, diarrhoea; jaundice and liver dysfunction are mild symptoms which do not affect continued medication.

Risks of blood draw: This study requires phlebotomy, which is the same procedure as routine blood draw in hospitals without additional risks. A small number of people may experience temporary discomfort and/or bruising at the venipuncture point, which resolves spontaneously within a short period.

Other risks: There may also be some currently unforeseen risks, discomforts, drug interactions, or adverse reactions.

Principles for dealing with major adverse events: if the above adverse reactions caused by the drug are observed, the dose of the drug may need to be reduced or stopped, and symptomatic treatment will be initiated. If the adverse reactions subside, the therapeutic dose can be resumed according to the patient's condition. In this study, we will conduct a complete blood count, urine tests, and liver and kidney function tests during each visit, to achieve early detection and early treatment. We will try to minimize the impact of these reactions as much as possible on the premise of ensuring stability of the child's condition.

6. What are the possible benefits of participating in the study?

There can be no certainty that your child will benefit from the study medication. Your child's condition may improve. RTX may reduce relapse of nephrotic syndrome within one year. The information that the study provides to the study sponsor may help to better treat children with nephrotic syndrome and improve treatment protocols for children.

7. Do I receive payment or compensation?

You will not receive any compensation for participating in this study.

8. Will the study cost me anything?

Participation in this research project will not add to the patient's additional costs on regular monitoring.

9. What if my child is harmed in the study?

Please consult your child's study doctor for further information. If your child suffers an injury or has an adverse event from prescribed medication, please contact the doctor and your child will be treated in time.

10. How will my child's data be handled and used?

We will make every effort to protect your and your child's privacy to the extent permitted by law. Any public reporting of the results of this study will not disclose any personal information about you and your child. The data of this study may be consulted by the investigators, the Ethics Committee of the Children's hospital of Fudan University, and the State Drug Administration.

Your child's data, when forwarded to the study sponsor or other parties, will be represented by a code instead of your child's name. This will ensure that your child's identity is kept confidential. We call this encoded data. The documents linking the code with your child's name will be kept at the study doctor's site.

If any new information becomes available during the study which may affect your decision to continue your child's participation, you will be notified.

11. What will happen if I do not wish to continue with the study?

Participation in this research project is entirely up to you and your child. You and your child may refuse to participate in this study, or withdraw from the study at any time during the study, without affecting your relationship with your doctor or affecting your child's medical care.

The doctor may terminate the study if your child requires additional treatment, if he/she does not follow the study plan, if a trial-related injury occurs, or for other reasons.

12. Whom can I contact for further information?

If you have any questions or concerns about this study, you can consult the contact doctor: Dr. Liu Jialu, whose contact number is: 13816360839. If you have any questions about your child's right to participate in this study, please consult the Ethics Committee of Children's Hospital of Fudan University at 021-64931221 from 8.00 am to 11.30 am and 1.30 pm to 5.00 pm, Monday–Friday.

Informed Consent Form (Signature Page)

I have read and understood the information in this informed consent form. I have had the opportunity to ask questions and am satisfied with the answers to all of them. I have been given enough time and opportunity to ask for details about the study and to consider whether to participate in the study. I volunteered to have my child participate in this study. By signing this informed consent form, I do not waive any of my statutory rights.

I have been informed that I will receive a signed copy of this document.

Child's Full Name (print): _____

Parent/guardian (print)：_____

Signature: _____

Date: _____

Relationship with patient：_____

Contact number：_____

(Note: For children under the age of 8 years-old, who have a certain ability to understand and express, the legal representative and doctor are required to ask the child if he/she is willing to participate, after which his legal representative should sign the consent form. Children aged ≥8 years and adolescents will be required to provide their own signatures and consents along with the consents of their legal representatives.)

Researcher/person, taking the assent, declares:

I confirm that the details of this study, including the child's rights and possible benefits and risks of participation, have been explained to the parent/guardian. A copy of this assent form has been provided.

Name of researcher/person taking the assent (print): _____

Signature: _____

Date: _____

Contact number：_____

Patient Information and Informed Consent Form
(minors ≥ 8 years old)

1. What is the purpose of the study?

Idiopathic nephrotic syndrome (INS) is one of the most common paediatric glomerular diseases. Although 80%–90% of children achieve complete remission after initial steroid therapy (steroid-sensitive nephrotic syndrome; SSNS); however, relapses are common after the reduction or discontinuation of corticosteroids. Half of these children will experience frequent relapses (FRNS) or become steroid-dependent (SDNS). They may experience serious side effects from further steroid treatment or other immunosuppressive drugs (including mycophenolate mofetil, calcineurin inhibitors, etc.). In addition, up to 15%–25% of childhood relapses persist into adulthood, and some may develop late-onset steroid resistance (SRNS) with a higher risk of progression to kidney failure and relapse after kidney transplantation. Long-term side effects of steroids and non-corticosteroid immunosuppressive medications are common and may include: poor growth, obesity, hypertension, cataracts, glaucoma, psychological disturbances, osteoporosis, nephrotoxicity, diabetes mellitus, dyslipidaemia, gonadal toxicity, and carcinogenicity, which significantly affect the prognosis and quality of life. In recent years, many high-quality clinical studies have shown that the use of anti-CD20 monoclonal antibody-rituximab (RTX) in FRNS/SNDS can effectively reduce relapse and promote better growth catch-up, even when steroids and other immunosuppressants are discontinued. The 2020 Kidney Disease Improving Global Outcome (KDIGO) guidelines are recommended to be used for children with FRNS/SDNS. Our centre is the first in China to report the efficacy and safety of RTX for treating FRNS/SDNS. The primary objective of our study is to assess whether RTX is effective and safe for children to maintain remission when initiated at the first episode of SSNS with the guideline-recommended corticosteroids. Exploratory endpoints include changes in immunologic factors, to be studied as predictors of response to RTX and relapse of INS.

2．What will happen in the study?

The follow-up period will be one year. In the beginning, your doctor will do a physical examination, collect urine, draw blood for tests, and ask your parent/guardian about your medical history. Additionally, a urinary ultrasound, electrocardiogram, cardiac ultrasound, and chest radiography will be performed along with an ophthalmological evaluation; the whole process takes about 1 hour. At each visit during the study, blood will be drawn once with a needle, approximately 20 mL, and your urine sample will be taken for testing. Before the doctor confirms that you can use rituximab, you need to undergo a physical examination, urine retention test, and blood test, and ask your parents/guardians about your condition. Blood will be drawn once with a needle, about 10 ml each time, and then take a sample of your urine for testing.

During the trial, you will need to go back to the hospital 8 times. Because of the need for a doctor's examination, you cannot eat breakfast and should not drink plenty of water on the morning of your visit to the hospital.

3. Is there anything in the research that makes me feel bad, scared, or uncomfortable?

During the study, you may experience some side effects. These side effects may cause nausea, vomiting, leg pain, rash, difficulty in breathing, etc. These conditions may not happen to every child. But if these side effects occur, you need to tell your doctor who will help you immediately.

During the test, the following situations may also be encountered:

- Questions the doctor or nurse asks may make you feel tired or embarrassed.
- When the blood is drawn for sampling, the needle will go into your arm and you may feel pain.
- You may get red spots, bruises, or feel soreness in your arms.
- An infection may occur in your arm where the needle was inserted.

You may also have other feelings, and you must tell your parents or your doctor if you feel unwell or take any other medication while participating in the study. You or your parents can call your doctor at any time.

4. Will participating in the research project help me?

By participating in this study, you will have regular follow-ups that will help monitor the progress of your own disease. You may or may not feel better with time, we cannot guarantee it. However, your participation may help children with the same disease as you, in the future.

5. Do I have to participate in this study?

It is your decision whether to participate in this study. If you choose not to take part in this study, no one will blame you. Neither your doctor nor your parents can force you to participate in the study if you do not agree. If you agree now and change your mind later, you can stop participating in the research at any time. At any time during the study, if you wish to withdraw just notify your doctor or your parent/legal representative. If you drop out of this study, you will be asked to do some security checks before leaving the study centre. Even if you do not want to take part in this study, your doctor will still

take care of you.

5. How will my privacy be protected if I participate in this study?

We will make every effort to protect your privacy to the extent permitted by law. Any public reporting of the results of this study will not disclose any personal information about you. The data of this study may be consulted by the investigators, the Ethics Committee of the Children's hospital of Fudan University, and the State Drug Administration.

Your data, when forwarded to the study sponsor or other parties, will be represented by a code instead of your child's name. This will ensure that your identity is kept confidential. We call this encoded data. The documents linking the code with your name will be kept at the study doctor's site.

If any new information becomes available during the study which may affect your decision to continue your participation, you will be notified.

6.Who should I contact if I have questions?

If you have any questions or concerns about this study, you can consult the contact doctor: Liu Jialu, whose contact number is: 13816360839. If you have any questions about your right to participate in this study, please consult the Ethics Committee of Children's Hospital of Fudan University at 021-64931221 from 8.00 am to 11.30 am and 1.30 pm to 5.00 pm Monday – Friday.

Informed Consent Form (Signature Page)

The doctor explained this information to me in detail, and I also asked the doctor about the words that I did not understand. I understand that participation in this study is voluntary. After consideration, I am willing to participate in this study and cooperate with the doctor's diagnosis, treatment, and follow-up. If there is any discomfort, I will promptly notify my parents/guardians or my doctors.

I understand that I can choose to stop participating in this study at any time and the doctors will still help me with other treatments.

Child's Full Name (print): _____ Date of birth: _____

Signature: _____ Date: _____

Researcher/person, taking the assent, declares:

I confirm that the details of this study, including the child's rights and possible benefits and risks of participation, have been explained to the child. A copy of this assent form has been provided.

Name of researcher/person taking the assent (print): _____

Signature: _____

Date: _____ Contact number：_____

Patient Information and Informed Consent Form
(<8-year-old Minor Oral Notification Version)

Dear kids,

The foam in your urine and oedema are features of nephrotic syndrome, which is a very common kidney disease in children. Most children can be cured by taking steroids every day, but many of these children will get sick again within a year, and they will need to take medicine again, every day for several months. Long-term repeated use of medicine may cause weight gain, unhappiness, poor growth, and other side effects. There is a medicine called rituximab that can be used, with the help of doctors, to stop the need for other drugs earlier, and reduce the chance of getting sick again. This medicine was used for children who used to get repeatedly sick. Now, the doctor hopes to use this medicine earlier during treatment, so that you will not get sick again next year. If you get sick again, the doctors will help you get better also.

1. What do I need to do if I participate in the study?

If you try to use this medicine early, we will take care of you very carefully and do some tests on you, which is a routine clinical process. You can ask your parents and doctor to help you decide whether to participate in this study.

Your doctor will ask about your current treatments/drugs and may adjust some of them. You will need to come to the hospital with your mom, dad, or other family members to visit the doctor eight times during the study. If you want to participate in the study, we will also ask your parents for their approval.

2. Is there anything in the research that makes me feel bad, scared, or uncomfortable?

We hope the rituximab injection helps you feel better, but sometimes it can make you feel a little uncomfortable. Hence, if you feel sick, you have to tell your mom, dad, your doctor, or nurse. We will take good care of you so that your discomfort can get better quickly. During the test, you may experience uneasiness; for example, a blood draw, during which a doctor or nurse will use a needle to draw blood from your arm. It may become a little sore later. Your arm may develop a few red spots or bruises also.

3. Will participating in this research help me?

By participating in this study, you will have regular check-ups, and blood and urine tests that will help monitor the progress of your own disease. You may or may not get better with time, we cannot guarantee it. Your participation may help other children in the future with the same disease as you.

4. Is participation in this research voluntary?

No one will be offended whether you choose to participate or not. You may choose not to participate in this study, if you do not wish to. If you agree to take part in this study now and later do not want to, you can stop participating any time. Even if you do not want to participate in this study, your doctor will still take care of you

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Supplement 2: Statistical Analysis Plan of the RTXFIRPedINS Trial

List of amendments

Amendment n°1 (28/11/2021)

- Changed from "The Children's Hospital of Zhejiang University School of Medicine, Zhejiang: Maojianhua (PI); Shanghai Children's Hospital, Shanghai: Huang Wenyan (PI) and Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai: Li Yufeng (PI) to "Shandong Provincial Hospital, Shandong: Sun Shuzheng (PI); Xuzhou Children' Hospital, Jiangsu: Zhang Ruifeng (PI); and the First Affiliated Hospital of Sun Yat-sen University, Guangzhou: Jiang Xiaoyun (PI)".

Amendment n°2 (28/06/2023)

- Addition of a historical control: to compare the differences in the effects on relapse-free survival rate (at 12-month and 6-month follow up) between the rituximab infusion and steroid therapy without rituximab, a historical control will be selected from a previous trial. The historical control group included 33 patients receiving prednisolone therapy.
- Addition of a "Secondary outcomes: The proportion of patients diagnosed with FRNS/SDNS within 12 months."

The efficacy and safety of rituximab in the first episode of paediatric idiopathic nephrotic syndrome: An open-label, single-arm, multicentre clinical trial

Trial Registration ID: NCT04783675

Statistical Analysis Plan

The Data Management and Statistical Analysis Plan is directed to support the aims of the study

Version 3.0: June 28, 2023

Signed by:

Hong Xu (Principal Investigator)	Hong Xu
Weili Yan (Trial Senior Statistician)	Weili Yan
Yi Zhang (Trial Statistician)	Zhang Yi
Yalan Dou (Trial Statistician)	Yalan Dou
Qian Shen (Project Executor)	Qian Shen
Jialu Liu (Project Executor)	Jialu Liu.

Previous versions: Version 1.0: November 30, 2020; Version 2.0, November 28, 2021

Catalogue

1. Introduction

Idiopathic nephrotic syndrome (INS) is one of the most common glomerular diseases in children[1]. The 8-12 weeks treatment with prednisolone/prednisone (PDN) is considered the cornerstone for the initial episode[2,3]. Steroid-sensitive nephrotic syndrome (SSNS) accounts for over 80% of cases, but the 12-month relapse-free survival rate is only approximately 30%[4,5]. And a significant number of patients (up to 50%) may develop frequently relapsing or steroid-dependent nephrotic syndrome (FRNS/SDNS). Additionally, a considerable proportion (up to 42%) of children with SSNS continue to experience relapses into adulthood[6,7]. The frequency of relapse during the first 12-month is correlated with relapse rate into adulthood[2].

Although non-corticosteroid immunosuppressive medications like calcineurin inhibitors (CNI), cyclophosphamide, mycophenolate mofetil, and levamisole can extend periods of remission, they come with potential adverse effects, such as nephrotoxicity, hypertension, seizures, tremors, hirsutism, gum hyperplasia, diabetes mellitus, leukopenia, neutropenia, severe infections, alopecia, infertility, and hemorrhagic cystitis. Monitoring CNI levels is typically necessary to mitigate the risk of nephrotoxicity.

Rituximab, a chimeric anti-CD20 monoclonal antibody used for lymphoma, has shown promise as a maintenance remission treatment for FRNS/SDNS, surpassing the efficacy of other immunosuppressants. Its potential in pediatric NS was discovered incidentally in 2004 when a child with SDNS received rituximab for idiopathic thrombocytopenic purpura[8].

Previous studies have reported acceptable safety profiles for rituximab in pediatric nephrology, with infusion reactions being the most common side effect. The international Paediatric Nephrology Association (IPNA)[3] and Kidney Disease Improving Global Outcome (KDIGO)[2] guidelines have recommended rituximab for the treatment of FRNS/SDNS by since 2020.

Although rituximab effectively prolongs remission in FRNS/SDNS patients, prolonged exposure to corticosteroids or other immunosuppressants may have already caused side effects. The repeated disruption of immune mechanisms may negatively impact treatment outcomes and prognosis. Currently, although the 12-month relapse-free survival rate of SSNS is only 30%, and at least 50% progress to FRNS/SDNS, there is no consensus on the optimal non-corticosteroid agent, in combination with PDN, for children experiencing the initial episode of SSNS. We hypothesize that early use of rituximab, in addition to PDN, is effective in preventing 12-month relapse in children with the initial episode of SSNS, potentially improving long-term prognosis.

To date, there has been limited research that comprehensively observing variations of T cells, B cells, myeloid cells, NK cells, cytokines in peripheral blood mononuclear cells (PBMC), as well as serum and urine protein biomarker signatures in NS patients before and during rituximab treatment. Understanding the underlying mechanisms and exploring diagnostic and predictive biomarkers would greatly contribute to patient management.

2. Study Objective and Outcomes

2.1 Study Objective

The primary objective is to evaluate the effect of rituximab biosimilar in maintaining remission during the first 12 months of onset in pediatric SSNS patients who achieved corticosteroid-induced remission.

The secondary objectives include the safety profile and the underlying immunological mechanisms contributing to the effectiveness of the rituximab biosimilar treatment, as well as the relapse-free survival rate at 12 months compared to corticosteroid treatment based on historical controls.

The study also aims to identify potential perturbations in immunological cell subsets as potential predictors of SSNS relapse.

2.2 Outcomes

2.2.1 Primary outcome

The primary outcome is the relapse-free survival rate at 12 months after rituximab biosimilar infusion in children with a first episode of SSNS.

- Description: Relapse is defined as the recurrence of nephrotic-range proteinuria, urine protein/creatinine ratio ≥2 mg/mg or dipstick ≥3+ on 3 consecutive days in the first morning samples. Dipsticks for proteinuria determination are evaluated daily.
- Time Frame: Within 12-month after rituximab biosimilar infusion.
- Type: time to event variable

2.2.2 Secondary outcomes

(1) The time from the infusion of rituximab biosimilar to the occurrence of the first relapse (day)
- Time Frame: Within 12 months after rituximab biosimilar infusion.
- Type: time to event variable

(2) The relapse-free survival rate at 6-month
- Time Frame: Within six months after rituximab biosimilar infusion.

- Type: time to event variable

(3) The time to the first detection of CD19+ cells above 1% of total CD45+ lymphocytes after CD19+ cell depletion

- Lymphocyte subset (including the percentage of total CD45+ lymphocytes and total CD19+ cells) will be measured by flow cytometry before steroid therapy, before rituximab biosimilar infusion and at 72 hours, 1 month, 3 months, 6 months, 9 months, 12 months after rituximab biosimilar infusion, and at the time of relapse.
- Time Frame: Within 12 months after rituximab biosimilar infusion.
- Type: continues variable

(4) Changes in absolute counts (and/or the percentage) of peripheral blood B cells

- Absolute counts (and/or the percentage) of peripheral blood B cells will be measured by fluorescence-activated cell sorting before steroid therapy, before rituximab biosimilar infusion and at 72 hours, 1 month, 3 months, 6 months, 9 months, 12 months after rituximab biosimilar infusion, and at the time of relapse. The surface markers of B cells are as follows.
- Type: repeatedly-measured continues variable

No.	Immune cell	Surface markers
1	**B CELLS**	
2	Transitional B	CD20+CD19+CD27−CD24hiCD38hi
3	Early transitional B	CD20+CD27−CD21low
4	Late transitional B	CD20+CD27−CD21+
5	Naïve B	CD20+CD27−IgM+IgDhi
6	Memory B	CD20+CD19+CD27+IgD-
7	Switched memory B	CD20+CD19+CD27+CD38lowIgM-IgD-
8	IgM memory B	CD20+CD27+IgMhiIgD±
9	IgG memory B	CD20+CD27+IgG+
10	Plasmablasts	CD20±CD19+CD38hiCD27hiCD24-
11	CD21low B	CD19+CD21lowCD38low

(5) Changes in absolute counts (and/or the percentage) of peripheral blood T cells

- Absolute counts (and/or the percentage) of peripheral blood T cells will be measured by fluorescence-activated cell sorting before steroids therapy, before rituximab biosimilar infusion and at 72 hours, 1 month, 3 months, 6 months, 9 months, 12 months after rituximab biosimilar infusion, and at the time of relapse. The surface markers of T cells are as follows.
- Type: repeatedly-measured continues variable

No.	Immune cell	Surface markers
1	**CD3+T**	CD3+
2	**CD4+T CELLS**	CD3+CD4+
3	Naïve T	CD3+CD4+CCR7+CD45RA+
4	Central memory T	CD3+CD4+CCR7+CD45RA−
5	Effector memory T	CD3+CD4+CCR7−CD45RA−
6	Treg	CD3+CD4+CD25hiCD127loFoxP3+
7	Tfh	CD3+CD4+CD45RA−CXCR5+
8	Th1	CD3+CD4+CD45RA−CXCR5−CXCR3+
9	Th2	CD45RA−CXCR5−CXCR3−CCR6−CCR4+
10	Th17	CD3+CD4+CD45RA−CXCR5−CCR6+
11	**CD8+T cells**	CD3+CD8+
12	Naïve T	CD3+CD8+CCR7+CD45RA+
13	Central memory T	CD3+CD8+CCR7+CD45RA−
14	Effector memory T	CD3+CD8+CCR7−CD45RA−
15	Revertant memory（TEMRA）	CD3+CD8+CCR7−CD45RA+

(6) Changes in absolute counts (and/or the percentage) of peripheral blood myeloid cells

- Absolute counts (and/or the percentage) of peripheral blood myeloid cells will be measured by fluorescence-activated cell sorting before steroids therapy, before rituximab biosimilar infusion and at 72 hours, 1 month, 3 months, 6 months, 9 months, 12 months after rituximab biosimilar infusion, and at the time of relapse. The surface markers of peripheral blood myeloid cells are as follows.
- Type: repeatedly-measured continues variable

No.	Immune cell	Surface markers

1	**non-B/T cells**	CD3-CD20-
2	**Dendritic cells**	CD14-HLADR+
3	plasmacytoid DCs	CD11c-CD123+
4	myeloid DCs/monocytic DCs	CD11c+CD123-
5	**Monocytes**	CD14+HLADR+/−
6	classical monocytes	CD16-HLADR+
7	non-classical monocytes	CD16+HLADR+
8	myeloid derived suppressor cells	CD16-HLADR-
	Other granulocytes and NKs	CD14-HLADR-
9	**Basophils**	CD56-CD123+
10	CD56briCD16neg NKs	CD3-CD20-CD14-CD56briCD16neg
11	CD56dimCD16neg NKs	CD3-CD20-CD14-CD56dimCD16neg
12	CD56dimCD16bri NKs	CD3-CD20-CD14-CD56dimCD16bri
13	CD56negCD16bri NKs	CD3-CD20-CD14-CD56negCD16bri

(7) Changes in concentration of cytokine profiling

- Using a 27-cytokine panel on a Luminex Technology platform and ELISA, changes in concentration of cytokine profiling will be measured before steroids therapy, before rituximab biosimilar infusion and at 72 hours, 1 month, 3 months, 6 months, 9 months, 12 months after rituximab biosimilar infusion, and at the time of relapse. The markers of cytokine profiling are as follows.
- Type: repeatedly-measured continues variable

Product's Name	**Commodity number**	**Methods**	**Cytokines**
Bio-Plex Pro Human Cytokine 27-plex	LX-M500KCAF0Y	Luminex	FGF basic, Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1β, IL-1rα, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17, IP-10, MCP-1(MCAF), MIP-1α, MIP-1β, PDGF-BB, RANTES, TNF-α, VEGF
Human Luminex Discovery Assay	LXSAHM-02	Luminex	IL-18, IL-23
Bio-Plex Pro™ TGF-β 3-plex Assay -171W4001M	171W4001M	ELISA	TGF-β1、TGF-β2、TGF-β3

(8) The proportion of patients diagnosed with FRNS/SDNS within 12 months.

- Description: FRNS: ≥2 relapses per 6 months or ≥4 relapses per 12 months. SDNS: relapses during therapy with prednisone or prednisolone (either at full dose or during tapering) or within 15 days of prednisone or prednisolone discontinuation.
- Time Frame: At 12-month after rituximab biosimilar infusion.
- Type: binomial variable

3.Study Design

3.1 Design

This is a multicenter, open-label, single-arm trial. Children who are experiencing their first episode SSNS and meet the eligibility criteria outlined in the protocol[9] will be invited to participate. In this single-arm trial, all enrolled patients will be treated with a single intravenous infusion of 375 mg/m^2 rituximab biosimilar (HANLIKANG) within one week after achieving corticosteroid-induced remission.

To compare the differences in the effects on relapse-free survival rate (at 12-month and 6-month follow up) and the proportion of patients diagnosed with FRNS/SDNS at 12-month follow up between the rituximab biosimilar infusion and steroid therapy without rituximab, a historical control will be selected from a previous trial[5]. The historical control group includes 33 patients receiving prednisolone therapy, and the inclusion and exclusion criteria are consistent with this single-arm trial. Full details of inclusion criteria and prednisolone therapy for historical control patients are described in the previous study[5].

3.2 Trial Sites (Coordinators):

The trial will be conducted in eight hospitals in China. Children's Hospital of Fudan University, Shanghai: Hong Xu (PI, lead investigator), Qian Shen (Co-PI, principal investigator), Jialu Liu (coordinator); Anhui Provincial Children's Hospital, Anhui: Fang Deng (PI), Shaohang Fang (coordinator); Children's Hospital affiliated to Zhengzhou University, Henan: Cuihua Liu (PI), Shufeng Zhang (coordinator); Wuhan Children's Hospital, Hubei: Xiaowen Wang (PI) , Daojing Wang (coordinator); Shandong Provincial Hospital, Shandong: Shuzheng Sun (PI), Jing Wang (coordinator); Xuzhou Children' Hospital, Jiangsu: Ruifeng Zhang (PI), Tingting Yuan (coordinator); Children's Hospital of Nanjing Medical University, Jiangsu: Aihua Zhang (PI) , Chunhua Zhu (coordinator); and the First Affiliated Hospital of Sun Yat-sen University, Guangdong: Xiaoyun Jiang (PI), Mengjie Jiang(coordinator).

3.3 Interventions

Since this is a single-arm study, all enrolled patients who meet eligibility criteria will be treated with rituximab infusion. Rituximab will be infused intravenously at a dose of 375 mg/1.73 m^2 (maximum dose: 500 mg) within 1 week of achieving complete remission. Every 100 mg of rituximab will be diluted in 100 mL of normal saline and infused at a rate of 25 mL/hour for the first 30 mins. Thereafter, the rate will be doubled every 30 mins to a maximum of 100 mL/hour. Interventions will be administered in an inpatient setting at the nephrology units of the registered hospitals. Details are included in the protocol[9].

3.4 Sample Size

This study is a single-arm study. The sample size is based on the expected rate of the primary treatment effect endpoint and the size of the effect of rituximab biosimilar treatment. According to previous literature[4], the 12-month relapse-free survival rate is approximately 30% in children with the first episode of SSNS after prednisone/prednisolone treatment. Based on which, we estimated that at a two-sided alpha level of 0.05, with an assumed dropout rate of 10%, a sample size of 44 would provide 80% power to detect a 20% increase in the relapse-free rate in patients receiving rituximab biosimilar treatment as compared with the traditional treatment.

4.Analysis Populations

4.1 Study population data sets

The following populations will be defined in the analysis. Primary analysis for the primary objective will be based on the intention-to-treatment population.

- **Intention-to-Treat population**
  Intention-to-treat (ITT) population will be defined as all patients who meet the eligibility criteria, are enrolled in the study, and receive the rituximab biosimilar intervention.
- **Safety population**
  This population will be defined as all patients who received the RITUXIMAB intervention.

4.2 Study Close Date

The data collection close date for this SAP is the date on when the last patient completed the follow-up 12-month. After 12 months, the patient will continue to receive routine clinical follow-up.

4.3 Data Cleaning

The data will then be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any changes will be made on the database.

4.4 Data Check-up

Once all data have been inputted and checked, the database will be locked and a data download request made. The data will be downloaded into SPSS and Stata formats for statistical analyses.

5. Statistical Analyses

5.1 Primary Outcome Analysis

5.1.1 Primary analysis

The primary analysis will be based on the ITT population as defined above. The primary outcome is a time to event outcome. The Kaplan-Meier method will be used to generate survival curves, and obtain estimation of 12-month relapse-free survival rate and median survival time with their 95% confidence intervals (CIs).

5.1.2 Secondary analysis

The difference in the 12-month relapse-free survival rate between the two treatments will be compared

using Cox proportional hazards regression model, and adjusted for age at onset of diseases (continuous variable), sex (categorical variable), the time of remission induced by corticosteroid (continuous variable), the hazard ratio (HR) and its 95% CI will be reported. If the proportional risk assumptions for performing Cox regression model are not met, the Kaplan-Meier method will be used to estimate the 12-month relapse-free survival rate for the two treatments, and a rate difference with 95%CI will be reported[10].

5.1.3 Subgroup analysis of the primary outcome

Subgroup analyses for the primary outcome will be performed using the same Kaplan-Meier method as the primary analysis above. We will stratify patients by age, gender and the time of remission induced by corticosteroid. Age will be divided into <4 years group and ≥4 years group. Gender will be divided into male and female. The time of remission induced by corticosteroid will be divided into <10 days group and ≥10 days group. Survival curves, the 12-month relapse-free survival rate at fixed time points, and median survival time with their 95% CIs will be reported by Kaplan-Meier method. The log-rank tests will be used to test the differences between subgroups.

5.2 Secondary Outcome Analysis

Secondary outcome analyses will be based on the ITT population unless specified. Categorical outcomes will be summarized by number (%) of patients with event. Continuous outcomes will be summarized as medians and ranges. Time to event outcomes will be analysed using Kaplan-Meier method, survival curves, the 12-month relapse-free survival rate at fixed time points, and median survival time with their 95% CIs will be reported.

For repeatedly-measured continuous variables (absolute and/or the percentage changes of peripheral immunological biomarkers at follow-up visits from the baseline, including counts of B cells, T cell and myeloid cell subsets and cytokines levels in peripheral blood), mean differences of the change of biomarkers across the eight follow-up visits will be tested using mixed-effects linear regression models, treating the change as the dependent variable, time-point of the follow-up visit (categorical variable: 1= baseline, 2= before rituximab biosimilar, 3=72 hours after rituximab biosimilar, 4=1 months after rituximab biosimilar, 5=3 months after rituximab biosimilar, 6=6 months after rituximab biosimilar, 7=9 months after rituximab biosimilar, and 8=3 months after the treatment) as the fixed effect, id number of subjects as the random effect, baseline values of immunological biomarkers (absolute counts and/or the percentage) , age (years), weight (kg) and height (m) at baseline, and sex (binary variable: 1=male, 2=female) as the covariates, and using a restricted maximum likelihood fit model. Adjusted means and standard errors at each visit will be compared by margin estimation. A P value for time visit <0.05 will be used to indicate a significant treatment effect for that time as compared with the baseline (before treatment), when the overall model P value is <0.05.

5.3 Predictors of rituximab response (Relapse vs. Nonrelapse)

To explore the potential relationship between serum levels of immune biomarkers and relapse, we will conduct an analysis of cytokines and chemokines in the patients' peripheral blood at various time points of the trial.

Changes of immunological biomarkers (counts of B cells, T cell and myeloid cell subsets and cytokines levels in peripheral blood) at follow-up visits from the baseline (before rituximab biosimilar infusion) will also be treated as repeatedly-measured continuous variables. To investigate immunological biomarkers associated with relapse of SSNS, treating the change of immunological biomarkers as the dependent variables, relapse of SSNS (binary variable: 1=yes and 0=no), time-point of the follow-up (categorical variable: 1= baseline, 2= before rituximab biosimilar, 3=72 hours after rituximab biosimilar, 4=1 months after rituximab biosimilar, 5=3 months after rituximab biosimilar, 6=6 months after rituximab biosimilar, 7=9 months after rituximab biosimilar, and 8=3 months after rituximab biosimilar, and interaction between relapse status and time as the fixed effects, id number of subjects as the random effect, and baseline of immunological biomarkers (absolute counts and/or the percentage), age(years), weight (kg) and height (m) at baseline, and sex (binary variable: 1=male, 2=female) as the covariate, multilevel mixed-effects linear regression models will be constructed respectively for each parameter using a restricted maximum likelihood method. Adjusted mean group differences of the change of biomarkers and standard errors at each visit will be analysed by margin estimation.

5.4 Handling of Missing Data

According to the reasons for the missing data, different data imputation methods will be used. Missing baseline covariates will be imputed using simple imputation methods in the covariate adjusted analysis based on the covariate distributions, should the missing values for a particular covariate be less than 5%. For a continuous variable, missing values will be imputed from random values from a permutated normal

distribution with mean and SD calculated from the available sample. For a categorical variable, missing values will be imputed from random values from a uniform distribution with probabilities P1, P2, …, and Pk from the sample. For randomly missing data, multiple imputations (MI) will be used to handle the missing data in analyses. We do not assume the condition that missing of covariate variable at baseline would be over 5%.

5.5 Analysis of other secondary outcomes

Other statistical methods may be used if deemed necessary.

6.General Considerations for Data Analyses

R software, version 4.1.2 (R Foundation for Statistical Computing) will be used to perform all data analyses and generate majority of data displays. STATA (version 17.0) may also be used for some data analyses and generating statistical graphs. All analyses for the primary and secondary outcome variables will be primarily based on the ITT population. Two-sided test and P value will be reported. Regression models for repeated measurement will be used for outcomes that are repeatedly measured, and group differences at the prespecified time point will be computed and reported. Only the primary outcome, the relapse-free survival rate at 12 months after rituximab biosimilar infusion, the relapse-free survival rate at 6 months and the proportion of patients diagnosed with FRNS/SDNS within 12 months will be compared with the historical control group, because the cytokine and immunity biomarkers are not available in the control group.

6.1 Data Summaries (baseline characteristics)

Descriptive statistics will summarize the baseline characteristics (see "7. Study variable list") for all treated subjects. Continuous variables will be presented as means and standard deviations or medians and ranges. Categorical variables will be summarized as the absolute frequency and percentage of subjects (%). All baseline presentations will identify subjects with missing measurements.

The differences in continuous variables between the two treatments (rituximab biosimilar treatment vs. corticosteroid treatment) and two group subjects (relapse vs. nonrelapse) will be analyzed using the or the t-test or Mann-Whitney U test, and differences in categorical variables will be analyzed using the Chi-square test or Fisher's exact test.

6.2 Graphical / Table Displays

Mean values for some continuous outcomes will be plotted. The Kaplan-Meier method will be used to generate survival curves. P values will be calculated with the use of the log-rank test in the subgroup analyses for the primary outcome. Hazard ratio and 95% CIs will be calculated with the use of Cox proportional-hazards model. The proportional-hazards assumption of progression-free survival will be examined.

7. Study variable list

Variable	Variable interpretation	Variable Type
center	Name of center	Text
center_no	Number of center	Text
name	Initials of subject	Text
id	Study ID of subject	Number
date_start	Date of enrollment	Date
date_end	Date of end	Date
investigator	Initials of investigator	Text
arm	Intervention allocation	Categorical
date_birth	Date of birth	Date
gender	Male / Female	Binary
Height	Height at baseline in meters	Continuous
weight	Body weight at baseline in kilograms	Continuous
Body mass index	Weight in kilograms divided by the square of the height in meters at baseline	Continuous
The time of remission induced by corticosteroid	The days of remission induced by corticosteroid	Continuous
Days from remission to	Days from remission to rituximab	Continuous

rituximab infusion	infusion	
Days from corticosteroids to rituximab infusion	Days from corticosteroids to rituximab infusion	Continuous
Serum albumin	Serum albumin (g/dL)	Continuous
Serum total protein	Serum total protein (g/dL)	Continuous
Serum creatinine	Serum creatinine (mg/dL)	Continuous
Estimated glomerular filtration rate	Estimated glomerular filtration rate (mL/min/1.73 m^2)	Continuous
Adverse event	Number of adverse events	Continuous
The duration for corticosteroids	The duration for corticosteroids (weeks)	Continuous
Perturbations in immunological cell subsets	Absolute counts and/or the percentage of T cells, B cells, myeloid cells, NK cells in peripheral blood mononuclear cells	Continuous
Cytokines	Cytokines (concentration）	Continuous

8.Reference

1. Banh TH, Hussain-Shamsy N, Patel V, et al. Ethnic Differences in Incidence and Outcomes of Childhood Nephrotic Syndrome. Clin J Am Soc Nephrol 2016;11(10):1760-1768. DOI: 10.2215/CJN.00380116.
2. Kidney Disease: Improving Global Outcomes Glomerular Diseases Work G. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney Int 2021;100(4S):S1-S276. DOI: 10.1016/j.kint.2021.05.021.
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5. Tang X, Shen Q, Rao J, et al. Duration of initial prednisolone therapy for first episode of childhood nephrotic syndrome based on time to response. Front Pediatr 2022;10:1043285. DOI: 10.3389/fped.2022.1043285.
6. Korsgaard T, Andersen RF, Joshi S, Hagstrom S, Rittig S. Childhood onset steroid-sensitive nephrotic syndrome continues into adulthood. Pediatr Nephrol 2019;34(4):641-648. DOI: 10.1007/s00467-018-4119-8.
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8. Benz K, Dotsch J, Rascher W, Stachel D. Change of the course of steroid-dependent nephrotic syndrome after rituximab therapy. Pediatr Nephrol 2004;19(7):794-7. DOI: 10.1007/s00467-004-1434-z.
9. Liu J, Shen Q, Xie L, et al. Protocol for an open-label, single-arm, multicentre clinical study to evaluate the efficacy and safety of rituximab in the first episode of paediatric idiopathic nephrotic syndrome. BMJ Open 2022;12(10):e064216. DOI: 10.1136/bmjopen-2022-064216.
10. Stang A. Kenneth J. Rothman: Epidemiology. An introduction. European Journal of Epidemiology 2012;27(10):827-829. DOI: 10.1007/s10654-012-9732-4.

Supplement 3:

Supplementary Figures

Figure S1. Overall relapse-free survival in subgroups.

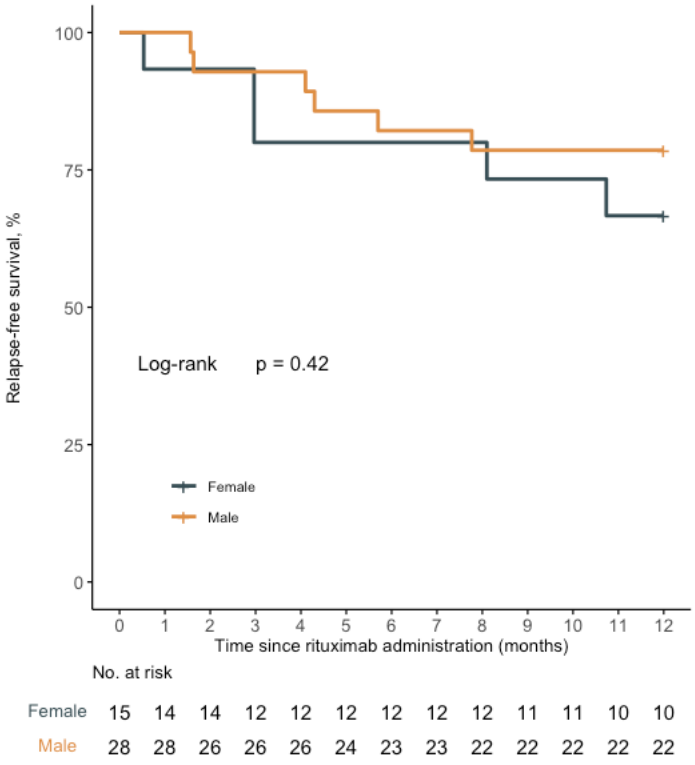
Figure S2. Characteristics of patients with relapse and non-relapse.

Figure S3. RTX treatment affects levels of B cells in patients with relapse and non-relapse.

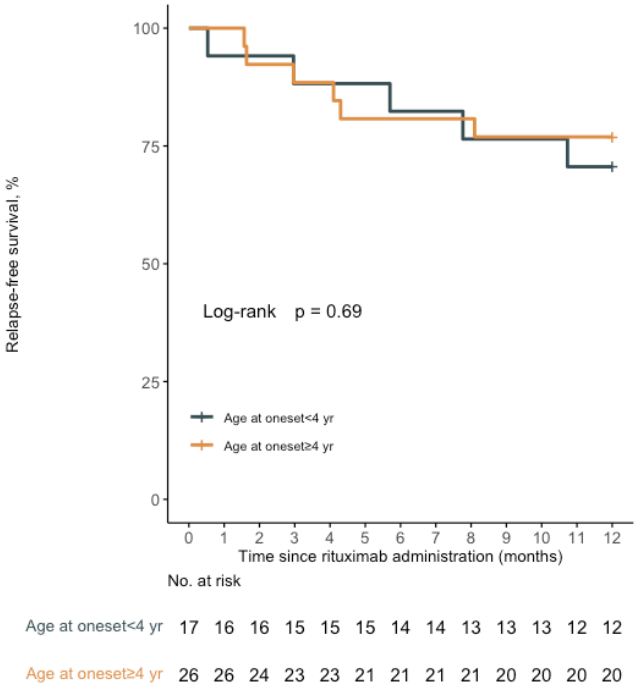
Figure S4. RTX treatment affects levels of IgG in patients with relapse and non-relapse.

Figure S1. Overall relapse-free survival in subgroups.

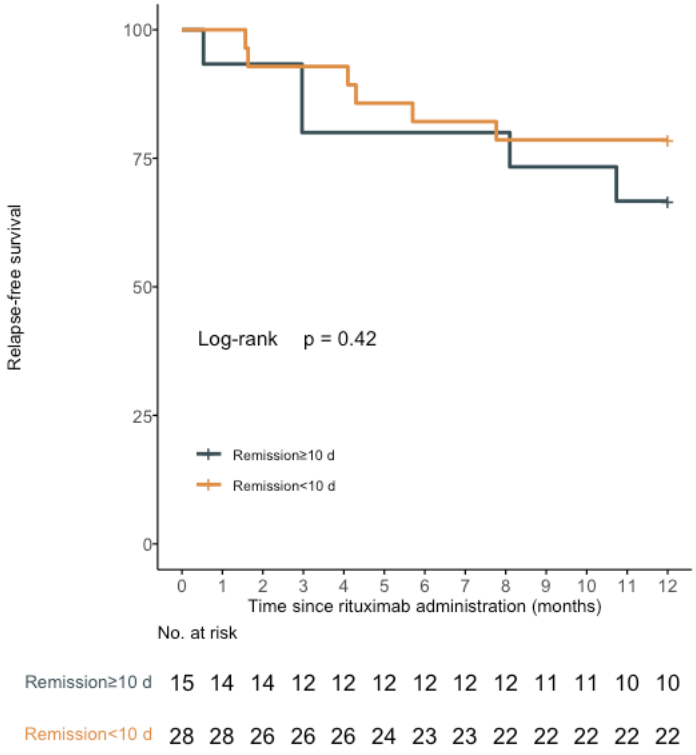
A Overall relapse-free survival in subgroups by sex



B Overall relapse-free survival in subgroups by age group



C Overall relapse-free survival in subgroups by time of remission induced by corticosteroid



No. at risk

Remission≥10 d	15	14	14	12	12	12	12	12	12	11	11	10	10
Remission<10 d	28	28	26	26	26	24	23	23	22	22	22	22	22

Figure S2. Characteristics of patients with relapse and non-relapse.

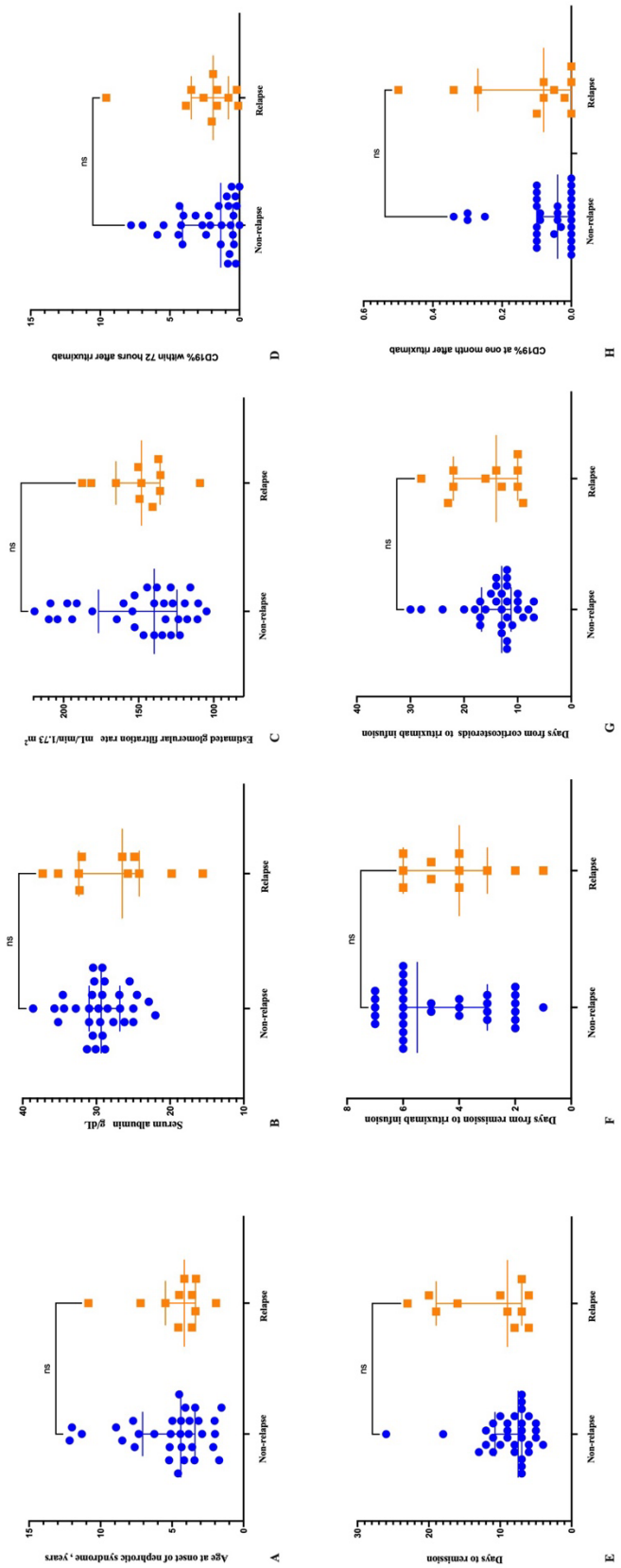


Figure S3. RTX treatment affects levels of B cells in patients with relapse and non-relapse.

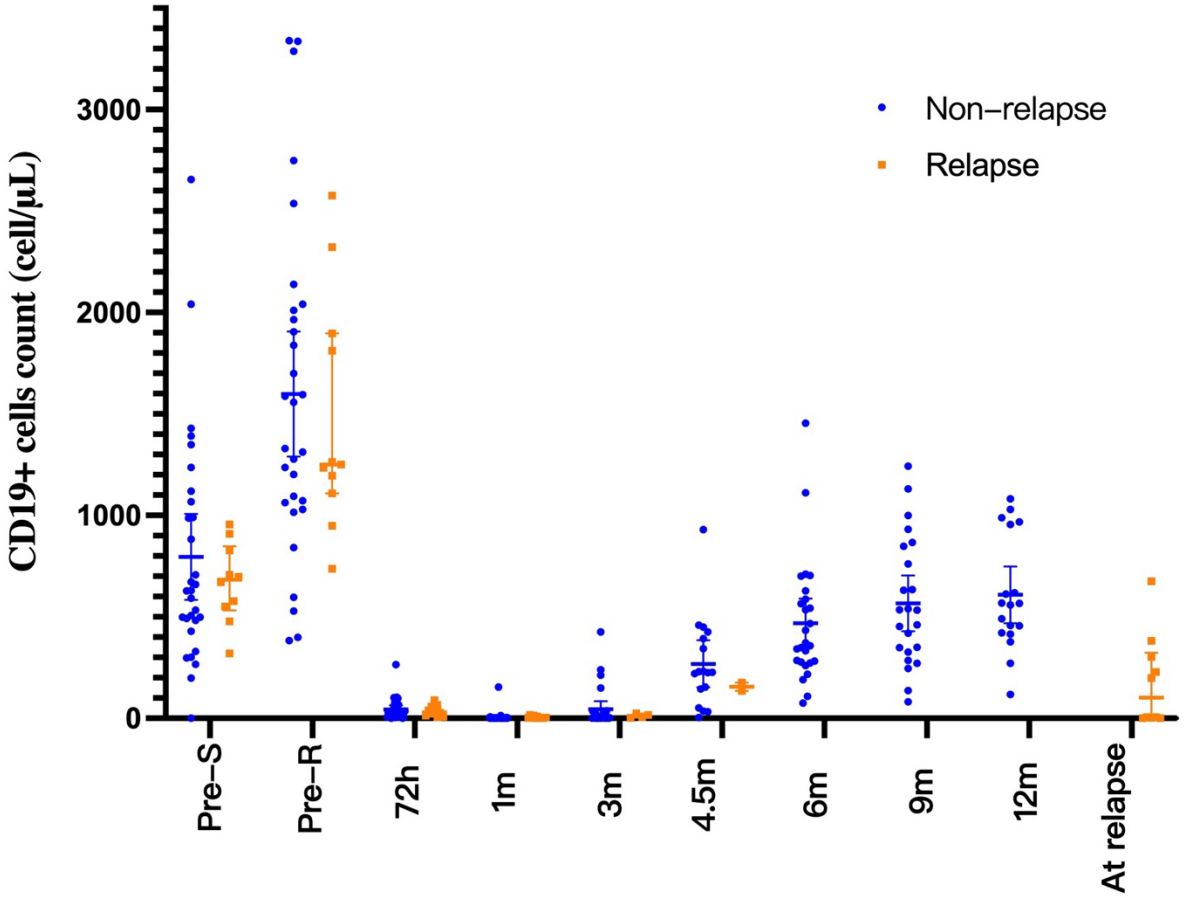
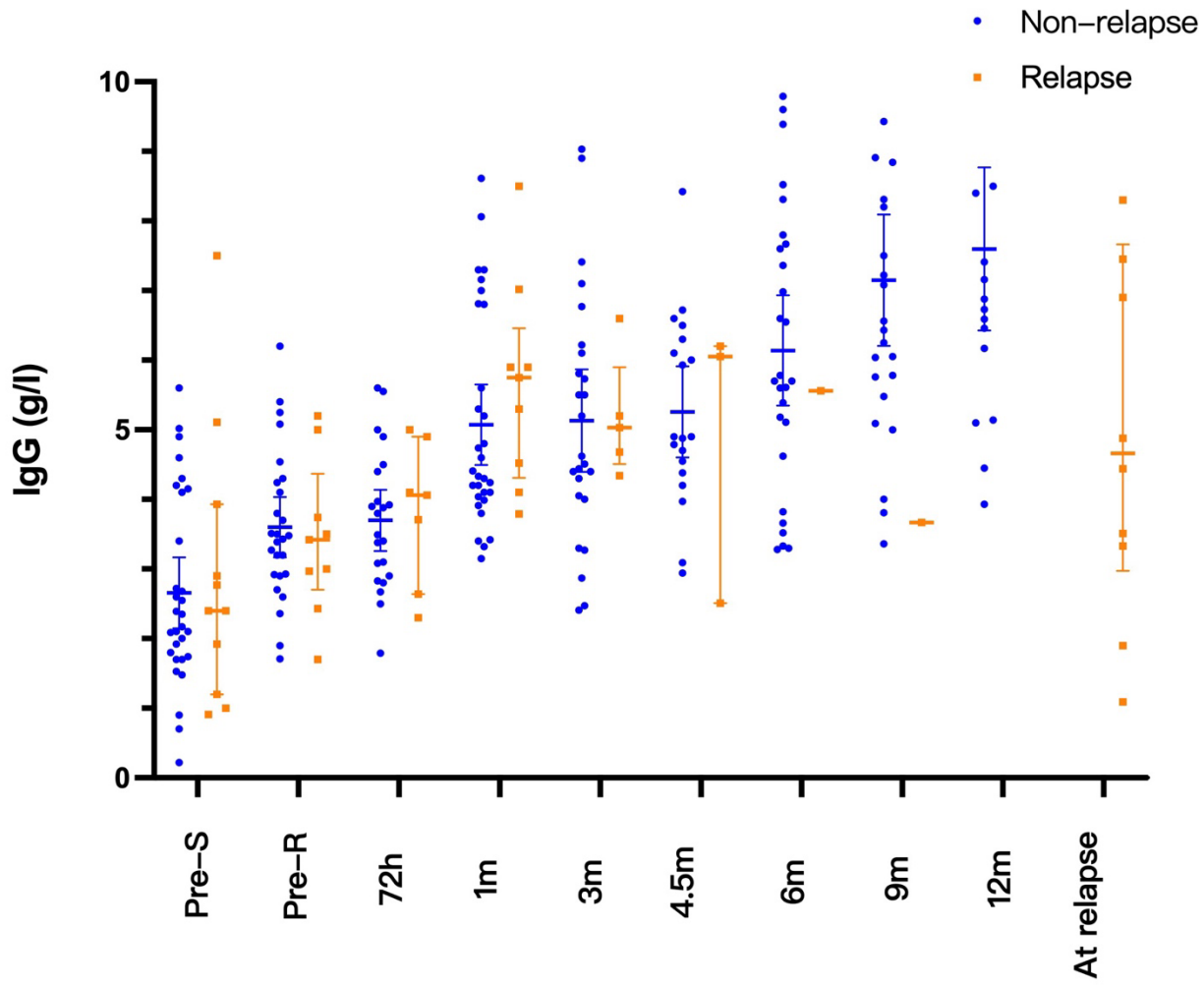


Figure S4. RTX treatment affects levels of IgG in patients with relapse and non-relapse.



Supplement 4: Data Sharing Statement
Data available: Yes
Data types: Deidentified participant data
How to access data: hxu@shmu.edu.cn
When available: With publication

Supporting Documents
Document types: None

Additional Information
Who can access the data: Data will be available to researchers with a clear research plan and hypothesis, with the appropriate team in place to undertake the work.
Types of analyses: For any appropriate purpose as detailed in a research plan.
Mechanisms of data availability: Requests for access to data from the RTXFIRPedINS trial should be addressed to the corresponding author at hxu@shmu.edu.cn. The individual participant data collected during the trial (including the data dictionary) will be available, after de-identification, when the article has been published with no end date. All proposals requesting data access will need to have a research plan and specify how the data will be used, and all proposals will need the approval of the trial co-investigator team (or individual(s) subsequently delegated this responsibility) before data release.

Supplement 5

TREND Statement Checklist

Paper Section/ Topic	Item No	Descriptor	Reported? ✓	Reported? Pg #
Title and Abstract				
Title and Abstract	1	• Information on how unit were allocated to interventions	√	1
		• Structured abstract recommended	√	3
		• Information on target population or study sample	√	3
Introduction				
Background	2	• Scientific background and explanation of rationale	√	5
		• Theories used in designing behavioral interventions	√	5
Methods				
Participants	3	• Eligibility criteria for participants, including criteria at different levels in recruitment/sampling plan (e.g., cities, clinics, subjects)	√	5–6
		• Method of recruitment (e.g., referral, self-selection), including the sampling method if a systematic sampling plan was implemented	√	5–6
		• Recruitment setting	√	5–6
		• Settings and locations where the data were collected	√	5–6
Interventions	4	• Details of the interventions intended for each study condition and how and when they were actually administered, specifically including:	√	7–8
		○ Content: what was given?	√	6
		○ Delivery method: how was the content given?	√	6
		○ Unit of delivery: how were the subjects grouped during delivery?	√	6
		○ Deliverer: who delivered the intervention?	√	5
		○ Setting: where was the intervention delivered?	√	5
		○ Exposure quantity and duration: how many sessions or episodes or events were intended to be delivered? How long were they intended to last?	√	6
		○ Time span: how long was it intended to take to deliver the intervention to each unit?	√	6
		○ Activities to increase compliance or adherence (e.g., incentives)	√	6
Objectives	5	• Specific objectives and hypotheses	√	5
Outcomes	6	• Clearly defined primary and secondary outcome measures	√	7–8
		• Methods used to collect data and any methods used to enhance the quality of measurements	√	7–8
		• Information on validated instruments such as psychometric and biometric properties	√	7–8
Sample Size	7	• How sample size was determined and, when applicable, explanation of any interim analyses and stopping rules	√	8
Assignment Method	8	• Unit of assignment (the unit being assigned to study condition, e.g., individual, group, community)	√	5
		• Method used to assign units to study conditions, including details of any restriction (e.g., blocking, stratification, minimization)	√	5
		• Inclusion of aspects employed to help minimize potential bias induced due to non-randomization (e.g., matching)	√	6,15

TREND Statement Checklist

Blinding (masking)	9	• Whether or not participants, those administering the interventions, and those assessing the outcomes were blinded to study condition assignment; if so, statement regarding how the blinding was accomplished and how it was assessed.	√	No, 6
Unit of Analysis	10	• Description of the smallest unit that is being analyzed to assess intervention effects (e.g., individual, group, or community)	√	8
		• If the unit of analysis differs from the unit of assignment, the analytical method used to account for this (e.g., adjusting the standard error estimates by the design effect or using multilevel analysis)		
Statistical Methods	11	• Statistical methods used to compare study groups for primary methods outcome(s), including complex methods of correlated data	√	8–9
		• Statistical methods used for additional analyses, such as a subgroup analyses and adjusted analysis	√	8–9
		• Methods for imputing missing data, if used	√	8
		• Statistical software or programs used	√	9
Results				
Participant flow	12	• Flow of participants through each stage of the study: enrollment, assignment, allocation, and intervention exposure, follow-up, analysis (a diagram is strongly recommended)	√	9
		○ Enrollment: the numbers of participants screened for eligibility, found to be eligible or not eligible, declined to be enrolled, and enrolled in the study	√	9
		○ Assignment: the numbers of participants assigned to a study condition	√	9
		○ Allocation and intervention exposure: the number of participants assigned to each study condition and the number of participants who received each intervention	√	9
		○ Follow-up: the number of participants who completed the follow-up or did not complete the follow-up (i.e., lost to follow-up), by study condition	√	9
		○ Analysis: the number of participants included in or excluded from the main analysis, by study condition	√	9
		• Description of protocol deviations from study as planned, along with reasons	√	10–11
Recruitment	13	• Dates defining the periods of recruitment and follow-up	√	9
Baseline Data	14	• Baseline demographic and clinical characteristics of participants in each study condition	√	9
		• Baseline characteristics for each study condition relevant to specific disease prevention research	√	9
		• Baseline comparisons of those lost to follow-up and those retained, overall and by study condition	√	9
		• Comparison between study population at baseline and target population of interest	√	9
Baseline equivalence	15	• Data on study group equivalence at baseline and statistical methods used to control for baseline differences	√	9

TREND Statement Checklist

Numbers analyzed	16	• Number of participants (denominator) included in each analysis for each study condition, particularly when the denominators change for different outcomes; statement of the results in absolute numbers when feasible	√	9
		• Indication of whether the analysis strategy was "intention to treat" or, if not, description of how non-compliers were treated in the analyses	√	9
Outcomes and estimation	17	• For each primary and secondary outcome, a summary of results for each estimation study condition, and the estimated effect size and a confidence interval to indicate the precision	√	9–11
		• Inclusion of null and negative findings	√	10, 11
		• Inclusion of results from testing pre-specified causal pathways through which the intervention was intended to operate, if any	√	10, 11
Ancillary analyses	18	• Summary of other analyses performed, including subgroup or restricted analyses, indicating which are pre-specified or exploratory	√	10–11
Adverse events	19	• Summary of all important adverse events or unintended effects in each study condition (including summary measures, effect size estimates, and confidence intervals)	√	11–12
DISCUSSION				
Interpretation	20	• Interpretation of the results, taking into account study hypotheses, sources of potential bias, imprecision of measures, multiplicative analyses, and other limitations or weaknesses of the study	√	12–15
		• Discussion of results taking into account the mechanism by which the intervention was intended to work (causal pathways) or alternative mechanisms or explanations	√	13
		• Discussion of the success of and barriers to implementing the intervention, fidelity of implementation	√	13–15
		• Discussion of research, programmatic, or policy implications	√	13–15
Generalizability	21	• Generalizability (external validity) of the trial findings, taking into account the study population, the characteristics of the intervention, length of follow-up, incentives, compliance rates, specific sites/settings involved in the study, and other contextual issues	√	14–15
Overall Evidence	22	• General interpretation of the results in the context of current evidence and current theory	√	15

From: Des Jarlais, D. C., Lyles, C., Crepaz, N., & the Trend Group (2004). Improving the reporting quality of nonrandomized evaluations of behavioral and public health interventions: The TREND statement. *American Journal of Public Health*, 94, 361-366. For more information, visit: http://www.cdc.gov/trendstatement/

√ 11

√ 10