RITURNS PROTOCOL version 1.2 (FINAL VERSION)

Trial Title: Randomized Clinical Trial to Compare Efficacy and Safety of Rituximab to That of Calcineurin Inhibitor in Children with Steroid Dependent Nephrotic Syndrome.

Trial Code: RITURNS (rituximab for relapse prevention in nephrotic syndrome)

Protocol Number: Pednephro RCT/PM/NRSMCH-54
Name and address of the Principal investigator:
Biswanath Basu
Div. of Pediatric Nephrology
Department of Pediatric Medicine
Nilratan Sircar Medical College & Hospital,
38, AJC Bose Road, Kolkata 700014, INDIA

Name and address of the Co-investigator:
Prof. Franz Schaefer
Universitäts-Kinderklinik
Im Neuenheimer Feld 150
69120 Heidelberg, Germany

Name and address of the Co-investigator:
Birendranath Roy
Department of Pediatric Medicine
Nilratan Sircar Medical College & Hospital,
38, AJC Bose Road, Kolkata 700014, INDIA

Name and address of the Co-investigator:
TKS Mahapatra
Department of Pediatric Medicine
Nilratan Sircar Medical College & Hospital,
38, AJC Bose Road, Kolkata 700014, INDIA

Name and address of the responsible Biometrician:
Anja Sander
Institute of Medical Biometry and Informatics
University of Heidelberg
Im Neuenheimer Feld 130.3
D-69120 Heidelberg

Name and address of the representative of the Biometrician:
Stella Preußler
Institute of Medical Biometry and Informatics
University of Heidelberg
Im Neuenheimer Feld 130.3
D-69120 Heidelberg

Study Site:
Division of Pediatric Nephrology, Department of Pediatrics, Nilratan Sircar Medical College & Hospital, Kolkata, India.

Study Funding:
Nilratan Sircar Medical College & Hospital, Kolkata, India.
TABLE OF CONTENTS

1 INTRODUCTION .................................................................5
   1.1 Brief Background .......................................................5
   1.2 Study Rationale & Risk-benefit Assessment .......................5
2 STUDY OBJECTIVE & HYPOTHESIS ........................................7
   2.1 Objective of the Trial ..................................................7
   2.2 Hypotheses ...............................................................7
3 STUDY ENDPOINTS .............................................................7
   3.1 Primary endpoint .......................................................7
   3.2 Secondary endpoints ..................................................7
4 STUDY SITE ........................................................................9
5 STUDY FUNDING ................................................................10
6 APPROVALS & LEGAL ASPECTS ............................................10
   6.1 GCP Statement ..........................................................10
   6.2 Ethics approval & study registration .................................10
   6.3 Informed consent .........................................................10
   6.4 Confidentiality ...........................................................10
7 STUDY SUBJECTS ...............................................................11
   7.1 Inclusion Criteria ........................................................11
   7.2 Exclusion Criteria .........................................................11
8 STUDY DESIGN & DESCRIPTION ............................................12
   8.1 Study Design .............................................................12
   8.2 Patient Recruitment & Randomization ...............................12
   8.3 Blinding .................................................................12
9 STUDY INTERVENTIONS .....................................................13
   9.1 Tacrolimus ...............................................................13
   9.2 Co-intervention with Tacrolimus ......................................13
   9.3 Rituximab ...............................................................13
   9.4 Co-intervention with Rituximab .......................................13
   9.5 Targeting trough level of Tacrolimus ...............................13
10 STUDY VISITS & ASSESSMENT ...........................................15
   10.1 Baseline Assessment at Enrolment .................................15
10.2 Follow up visit and drug compliance ........................................15
10.3 Duration of study .................................................................15
11 STANDARD CASE MANAGEMENT .............................................16
  11.1 Relapse Management ...........................................................16
  11.1.1 Tacrolimus Group ............................................................16
  11.1.2 Rituximab Group .............................................................16
  11.2 Other co-interventions .........................................................16
12 SAFETY DATA .................................................................17
  12.1 Adverse event definition .....................................................17
  12.2 Severity of adverse events ................................................17
  12.3 Study termination ............................................................18
  12.4 Relationship and outcome of adverse events .........................18
  12.5 Monitoring by data-safety and monitoring board ....................20
13 DATA MANAGEMENT & QUALITY ASSURANCE .........................21
14 BIOSTATISTICAL CONSIDERATIONS ......................................21
  14.1 Sample size calculation .....................................................21
  14.2 Statistical analysis ............................................................21
15. TIME SCHEDULE OF ACTIVITIES ..........................................23
16 STANDARD DEFINITIONS FOR NEPHROTIC SYNDROME ............24
17 REFERENCES.................................................................25
18 STUDY FLOW CHART .........................................................27
19 CASE RECORD FORM .........................................................28
  19.1 Baseline characteristics of the patients ..................................28
  19.2 Follow up data ...............................................................29
20 RITUXIMAB ORDER FORM ....................................................30
1 INTRODUCTION

1.1 Brief Background
The vast majority of children with idiopathic nephrotic syndrome respond well to corticosteroid treatment. However, as many as 70% experience at least one relapse, and 30% develop a more complicated course with frequent relapses (FRNS) with or without steroid dependency (SDNS). Extended steroid exposure in these children often results in long-term complications. The management of patients with SDNS is challenging and expensive. Relapses may lead to serious complications, e.g. related to anasarca, hypertension, life threatening infections (peritonitis, pneumonia, meningitis), thrombosis and malnutrition. Repeated courses or even continuous steroid treatment lead to considerable medication related toxicity and morbidity.

1.2 Study Rationale & Risk-benefit Assessment
The goal of treatment in SDNS is to reduce the frequency of relapses, the cumulative dose of corticosteroids, and the incidence of serious complications. In order to minimize the side effects of steroid therapy, different steroid sparing agents such as cyclophosphamide, calcineurin inhibitors (CNI, cyclosporin A and tacrolimus), levamisole, and mycophenolate mofetil (MMF) have been used in SDNS. CNI are usually considered the steroid sparing drug class of first choice, and more effective than MMF and levamisole. However, CNIs are potentially nephrotoxic, neurotoxic, and diabetogenic, cause disturbing cosmetic side effects and require regular drug monitoring. Several case reports and prospective studies have suggested that Rituximab might be a safe and effective therapeutic alternative to achieve and maintain remission in this population. Single courses of rituximab infusion have demonstrated efficacy in inducing remission of proteinuria for 6 to 12 months and the side effect profile observed to date is benign. Nonetheless, as of to date rituximab is considered a 2nd line treatment reserved for cases which are incompletely controlled by, show intolerance to, or have required CNI for long periods. No controlled trial has directly compared the first-line use of Rituximab head-to-head with a CNI. In this randomized controlled trial, we will compare the efficacy and safety of Rituximab to that of Tacrolimus in children with SDNS. If superior efficacy and/or safety of
rituximab relative to Tacrolimus is found, this will be important novel information which may change treatment practice in children with SDNS.
2 STUDY OBJECTIVE & HYPOTHESIS

2.1 Objective of the Trial
The aim of the RITURNS study is to evaluate the efficacy and safety of single course of rituximab infusion compared to maintenance tacrolimus treatment in children with steroid dependent nephrotic syndrome (SDNS).

2.2 Hypotheses
A single course of Rituximab (2 to 4, weekly intravenous infusions of rituximab 375mg/m²) will result in higher rate of relapse-free survival during a 12-month period than daily oral Tacrolimus (Tablet form, 0.2mg/kg/day, aiming for drug trough level 5-7 ng/ml.).

3 STUDY ENDPOINTS

3.1 Primary endpoint
The primary endpoint is the 12-month relapse-free survival

3.2 Secondary endpoints
1. 6-month relapse-free survival
2. Occurrence of 2 or more than two relapses within 6 months
3. Occurrence of 4 or more relapses in 12 months during study period (= definition of frequent relapse).
4. Frequent relapse: composite endpoint consisting of the two endpoints defined under 2 and 3.
5. More than one episode of life threatening infection or severe relapse (anasarca, hypovolemia or thrombosis) requiring hospital admission within 6 months and 12 months, respectively.
6. Impairment of renal function (eGFR <30 ml/min per 1.73 m² or loss of eGFR by >= 30% with regard to baseline) within 6 months and 12 months, respectively.
7. Treatment failure (yes/no) defined as composite endpoint consisting of the three endpoints as defined above under 4, 5 and 6 referring to month 12.
8. Number and severity of adverse events.
9. eGFR at 3, 6, 9 and 12 months, respectively.
10. Amount of cumulative prednisolone requirement (mg/kg/yr) over 12 months
11. Number of relapses within months 0-12, 0-6 and 7-12.
12. Number of different steroid toxicity events (new onset) within month 0-12
13. Time to first relapse
14. Fraction of patients off steroids at month 12
15. Abnormal values in biochemical tests and haematology assessments.
16. Height sds at 6 and 12 months.
17. Absolute and relative change in height sds from baseline to 12 months.
18. BMI sds at 6 and 12 months.
19. Absolute and relative change in BMI sds from baseline to 12 months.
20. B-cell count.
4 STUDY SITE
Division of Pediatric Nephrology, Department of Pediatrics, NRS Medical College & Hospital, Kolkata, India. This tertiary care Government Medical colleges of Eastern India cater not only the whole of Eastern India but also the neighboring countries of Bangladesh, Bhutan and Nepal (approx total population of 0.8 billion). The pediatric Nephrology unit of NRS Medical College & Hospital, Kolkata is the sole dedicated Pediatric Nephrology Division at Government Sector in Eastern India. An average of 140-180 children are seen per week in the nephrotic syndrome outpatient clinic; approx. 20-30% of these are new (incident) cases.

5 STUDY FUNDING
NRS Medical College & Hospital, Kolkata, India has agreed for study funding and supply of all drugs related to this study. The funding source shall not be involved in collection, analysis, interpretation of data, writing of the report, or the decision to submit the paper for publication.
6 APPROVALS & LEGAL ASPECTS

6.1 GCP Statement
The study will be conducted in compliance with Good Clinical Practices and the Declaration of Helsinki, and in accordance with applicable legal and regulatory requirements.

6.2 Ethics approval & study registration
The original study protocol was approved in December 2013 (NMC/10047) and the amended study protocol in November 2014 (NMC/6896) by the Ethics Committee (Institutional Review Board) of Nil Ratan Sircar Medical College, Kolkata, India. The trial is also approved by Drug Controller General of India. The trial is registered in “Clinicaltrial.gov” (NCT02438982) and in “CTRI (Clinical trial registry of India)” (CTRI/2014/01/004355).

6.3 Informed consent
Informed written and audiovisual consent from the parents (and assent of the child if >7yr old) will be obtained before enrolment, after provision of detailed oral and written information concerning the context of the study, potential benefit to the child and comprehensive safety aspects. The informed consent process will be in accordance with International Council for Harmonization and Good Clinical Practice, the Declaration of Helsinki and local regulatory requirements. Parent information sheet containing background information, safety of the study and possible benefit will be provided prior to obtaining consent for the study.

6.4 Confidentiality
The investigator will ensure that the subjects’ anonymity is maintained. On the case report forms or other documents, participants will not be identified by their names, but by their assigned identification number. If participant names are included on copies of documents submitted to the principal investigator, the names will be obliterated and the assigned subject numbers added to the documents.
7 STUDY SUBJECTS

7.1 Inclusion Criteria

- Children between 3 and 16 years with SDNS
- Minimal Change disease/ FSGS/MesPGN as per Kidney Biopsy report.
- Estimated glomerular filtration rate(eGFR) >80 ml/min per 1.73 m² at study entry.
- Remission at study entry (Urine albumin nil or trace (or proteinuria <4 mg/m²/h) for 3 consecutive early morning specimens).
- Not received any steroid sparing agent previously.
- Parents willing to give informed written and audiovisual consent.
- Ability to swallow tablet

7.2 Exclusion Criteria

- Known etiology (e.g., lupus erythematosus, IgA nephropathy, amyloidosis, malignancy, other secondary forms of NS)
- Patients with severe leucopenia (leucocytes <3.0× 1000 cells/mm³), severe anemia (haemoglobin <8.9 g/dl), thrombocytopenia (platelet <100.0 × 1000 cells/mm³) or deranged liver function tests (AST or ALT to >50 IU/L ) at enrolment.
- Known active chronic infection (tuberculosis, HIV, hepatitis B or C)
- Live vaccination within last one month.
8 STUDY DESIGN & DESCRIPTION

8.1 Study Design
This is a prospective, single-center, open-label, two-parallel-arm randomized controlled phase III study to examine the efficacy and safety of Rituximab vs. Tacrolimus in patients with SDNS.

8.2 Patient Recruitment & Randomisation
Subsequent cases of SDNS presenting at our center and fulfilling the inclusion criteria, are screened and recruited for the study. After informed consent is given, randomization will be done within one week. Randomization will be performed 1:1 using stratified block randomization with varying block sizes and including sex, age (≤ 7 vs. > 7 years), and renal histology (MCD vs. FSGS) as stratification factors. To achieve comparable intervention groups and to minimize a potential selection bias, patients will be allocated in a concealed fashion by means of randomization. The trial will be open-label with no masking of patients or study staff to the treatment allocation.

8.3 Blinding
The following reasons justify the lack of participant and investigator blinding: Additional administration of placebo tablets and placebo infusion to ensure blinding to treatment allocation is not acceptable in this vulnerable population of children. However, due to the objective assessment of the primary outcome bias is decreased. Bias by potential influential factors will be addressed by inclusion as covariates into the statistical analysis.
9 STUDY INTERVENTIONS

9.1 Tacrolimus
Oral (Tablet form) 0.2mg/kg/day (in two divided doses) starting dose and continued over 12 months. Target tacrolimus trough level: 5-7 ng/ml.

9.2 Co-intervention with Tacrolimus
**Prednisolone** With the start of tacrolimus therapy, patients who are on daily prednisolone therapy will receive 1.5 mg/kg on alternate days (maximum 40 mg) prednisolone; those who are already on alternate day therapy will remain on their current dose. The initial dose shall be maintained for 4 weeks following randomization, then reduced by 0.25 mg/kg every 2–4 weeks and continued at 0.2–0.3 mg/kg/48 hours until 6 months of relapse free survival.

9.3 Rituximab
Two infusions will be administered intravenously at a 7-day interval at standard dose (rituximab 375mg/m², maximum 500mg). Circulating B cells will be measured 24 hours after rituximab administration. If B cell count exceeds 5/mm³, it will be measured again after 1 week. If count is still >5/mm³, two more doses of rituximab will be administered. (Rituximab order form in annex).

9.4 Co-intervention with Rituximab
**Prednisolone** Prednisolone will be continued at alternate-day doses for 4 weeks (1.5 mg/kg (max.40 mg) per 48 hours in patients on daily prednisolone at time of randomization; pre-randomization dose in those already on alternate-day dosing). At 4 weeks, prednisolone will be discontinued.

9.5 Targeting trough level of Tacrolimus
For tacrolimus trough-level monitoring, a blood sample will be drawn 12 h after the last dose (i.e., immediately before the next dose). First trough level will be measured 2 weeks after beginning of therapy or before in the presence of acute nephrotoxicity. Dose adjustments, by 20–25%, will be performed every 7–10 days until a level of 5–7
ng/ml is achieved; lower level will be accepted if patient is in remission. During relapse, trough level shall be documented.
10 STUDY VISITS & ASSESSMENT

10.1 Baseline Assessment at Enrolment

Retrospective clinical information will be obtained from the case records and clinic files. This will include information regarding age of onset of disease, disease type, duration of total disease etc; and treatment received, number of relapses, cumulative steroid dose, detailed anthropometry and investigations during the last 12 months. The patients shall be clinically screened for significant infection. The information will be entered into the patient data sheet. Clinical examination shall be done and data shall be recorded.

10.2 Follow up visit and drug compliance

Study visits will be scheduled at enrolment, then weekly for the first month, then at 3rd month and then 3 monthly till end of the study or during relapse and if there is any specific need after enrolment. Complete blood count, kidney function, liver enzymes, serum electrolytes, plasma proteins, serum cholesterol, serum albumin and B lymphocyte count/ tacrolimus trough level as applicable) will be obtained during protocol visits and in between period if needed. Circulating B-cell count (number/mm³) will be measured at enrolment, then every fortnight for the first month, then at 3rd month and then 3 monthly till end of the study or during relapse and if there is any specific need after enrolment.

At each visit, the drug will be handed over to the parent/guardian in an amount sufficient to last the interim duration; ten extra doses would be provided to ensure compliance even if follow up is delayed for some reason. At the follow up visit, empty packs of drug provided in previous visit would be collected and pill count done to check compliance to intervention. At discharge from the Nephrology Unit, each patient will receive a clinical diary, to be filled with dipstick proteinuria levels and current treatment. Adherence will also be recorded by the patients’ diary.

10.3 Duration of study

Each study subject after enrolment is followed up for 12 months following start of study treatment.
11 STANDARD CASE MANAGEMENT

11.1 Relapse Management

11.1.1 Tacrolimus Group
Relapses will be treated by reinstitution of daily therapy with prednisolone (2 mg/kg/day, maximum 60 mg) until remission, followed by alternate-day dosing (1.5 mg/kg, maximum 40 mg)) for four weeks, and then stopped if relapse occurs after complete discontinuation of prednisolone, or tapered if relapse occurred while patient was still on alternate day prednisolone.

11.1.2 Rituximab Group
Relapses will be treated by reinstitution of daily therapy with prednisolone (2 mg/kg/day, maximum 60 mg)) until remission, followed by alternate-day dosing (1.5 mg/kg, maximum 40 mg)) for four weeks, and then stopped.

11.2 Other co-interventions
Hypertension, defined as blood pressure >95th percentile (for age, height and sex) in those previously normotensive, shall be treated at the discretion of the investigator. Children shall be treated with oral calcium when on oral prednisolone. Other medications shall be used at the discretion of the treating physician. The investigator will record all concomitant medications taken by the subject during the study from the date of informed consent, in the appropriate section of the case report form.
12 SAFETY DATA

We will collect any untoward medical occurrence in the form of signs, symptoms, abnormal laboratory findings, or diseases that emerges or worsens relative to baseline (i.e. present at the initial study visit).

12.1 Adverse event definition

Adverse events include suspected adverse drug reactions, other medical experiences, regardless of their relationship with the investigative drugs, such as injury, surgery, accidents, extensions of symptoms or apparently unrelated illnesses, and significant abnormalities in clinical laboratory values, or physical examination findings. Those medical conditions related to the disease under study whose changes during the study are consistent with natural disease progression, or which are attributable to a lack of clinical efficacy of the study interventions, are not considered as adverse events, however, those will be recorded in the case report forms. All other medical conditions that are present at baseline will not be considered as adverse events unless a worsening will occur.

Data on adverse events will be obtained at scheduled or unscheduled study visits, based on information spontaneously provided by the subject and/or through questioning of the participant.

12.2 Severity of adverse events

Adverse events will be graded according to the Common Terminology Criteria for Adverse Events, version 3. A serious adverse event is defined as an adverse event that at any dose results in inpatient care or hospital admission or significant disability or incapacity. Any serious adverse event requires expedited reporting to the sponsor safety department, regardless of its relationship to the study intervention. Hospital admissions for study procedures, or for normal disease management (relapse management) are not to be considered as serious adverse events according to this criterion. Also medically important conditions, which may not be immediately life threatening or result in death or hospitalization, but are clearly of major clinical significance, have to be considered as serious adverse events. The investigator will comply with any applicable requirements related to the reporting of serious adverse
events involving his/her subjects to the Independent Ethics Committee (IEC) that approved the study and to the appropriate regulatory authority.

12.3 Study termination

Subjects will be informed that they have the right to withdraw from the study at any time, without prejudice to their medical care, and that they are not obliged to state their reasons. Follow-up will be considered complete when the participant has completed all study procedures and assessments up to the Month-12 visit. The investigator may withdraw a subject at any time if this is considered to be in the subject’s best interest. Study termination will be mandatory in the following situations: development of steroid resistance disease requiring other steroid-sparing agents, pregnancy, significant worsening of renal function, onset of malignancy, serious hypersensitivity or allergic reaction, any other serious adverse event, serious inter-current illness, administrative reasons, or investigator’s or subject’s or parents’/legal tutor’s request.

12.4 Relationship and outcome of adverse events

The investigator will evaluate each adverse event that occurred after administration of the investigational drug regarding the relationship with the administration of the investigational drug:

**Definitely related:** There is a reasonable possibility that the event may have been caused by the investigational drug. A certain event has a strong temporal relationship and an alternative cause is unlikely.

**Probable:** An adverse event that has a reasonable possibility that the event is likely to have been caused by the investigational drug. The adverse event has a timely relationship and follows a known pattern of response, but a potential alternative cause may be present.

**Possible:** An adverse event that has a reasonable possibility that the event may have been caused by the investigational drug. The adverse event has a timely relationship to the investigational drug; however, the pattern of response is untypical, and an alternative cause seems more likely, or there is significant uncertainty about the cause of the event.
\textit{Unlikely}: Only a remote connection exists between the investigational drug and the reported adverse event. Other conditions including concurrent illness, progression or expression of the disease state or reaction of the concomitant medication appear to explain the reported adverse event.

\textit{Not related}: An adverse event that does not follow a reasonable temporal sequence related to the investigational drug and is likely to have been produced by the subject’s clinical state, other modes of therapy or other known etiology.

\textit{Not assessable}: There is insufficient or incomplete evidence to make a clinical judgment of the causal relationship.

All subjects who have reportable adverse events, whether considered associated with the use of the study medication or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up until resolution or normalization of changed laboratory parameters or until it has changed to a stable condition.

The outcome of an adverse event at the time of the last observation will be classified as:

\textit{Recovered/resolved}: All signs and symptoms of an adverse event disappeared without any sequels at the time of the last interrogation.

\textit{Recovering/resolving}: The intensity of signs and symptoms has been diminishing and/or their clinical pattern has been changing up to the time of the last interrogation in a way typical for its resolution.

\textit{Not recovered/not resolved}: Signs and symptoms of an adverse event are mostly unchanged or worsened at the time of the last interrogation.

\textit{Recovered/resolved with sequel}: Actual signs and symptoms of an AE disappeared but there are sequels related to the AE.

\textit{Fatal}: Resulting in death. If there are more than one adverse event only the adverse event leading to death will be characterized as ‘fatal’.

\textit{Unknown} The outcome is unknown or implausible and the information cannot be supplemented or verified.

The term “countermeasures” refers to the specific actions taken to treat or alleviate adverse events or to avoid their sequels. Following categories will be used to
categorize the countermeasures to adverse events:

*None:* No action taken.

*Drug treatment:* Newly-prescribed medication or change in dose of a medication.

*Others:* Other countermeasures, e.g. an operative procedure.

### 12.5 Monitoring by data-safety and monitoring board

No interim analyses are planned. The data-safety and monitoring board will decide if the study is safe when 50% of the participants are enrolled (without comparisons). Independent DSMB will monitor the study progress at regular interval (3 monthly). Annual report of the study progress shall be submitted by principal investigator to both institute ethics committee and the national drug regulator. The sponsor may temporarily or permanently discontinue the study for safety, ethical, compliance or other reasons. If the study is suspended or discontinued, the investigator will be responsible for promptly informing the Independent Ethics Committee.
13 DATA MANAGEMENT & QUALITY ASSURANCE
The investigators, or designees, will be responsible for recording study data in the case report form (CRF). The clinical and laboratory data shall be entered in electronic format. The CRF should be completed as soon as possible after the information is collected, preferably on the same day when a patient is seen for an examination, treatment, or any other study procedure. Double data entry will be done and the files will be compared. At monthly intervals, the data entries shall be checked for completeness and the files reviewed for errors.

14 BIOSTATISTICAL CONSIDERATIONS
This section describes the considerations underlying the choice of the sample size as well as the statistical methodology applied for the analysis of primary and secondary outcome variables. More details can be found in the statistical analysis plan which will be finalized prior to database closure and before performing any analyses.

14.1 Sample size calculation
The sample size calculation is based on the primary efficacy endpoint “12-month relapse-free survival”. A rather wide range of relapse-free survival rate for children receiving standard tacrolimus therapy (the control group) has been reported in previous literature. We decided to assume a relapse rate of 50% based on the study of Wang et al., which is the only published prospective study in a large pediatric cohort available in literature.\(^\text{12}\) Assuming that therapy with Rituximab shall result in 80% of patients being relapse-free after 12 months, 48 patients are required in each group to proof efficacy with a power of 90% and a two-tailed alpha level of 0.05 (calculations performed with https://www.sealedenvelope.com/power/binary-superiority).\(^\text{7}\) To account for a 10% drop-out rate, and major protocol violations and missed observations in further 10%, a total of 120 patients will be randomized.

14.2 Statistical analysis
Baseline characteristics will be compared among the two groups in descriptive way. The primary endpoint, the rate of relapse free survival after 12 months, will be compared using the chi-squared test with a two-sided significance level of 5%. Primary analysis set is the intention to treat set (ITT). In addition, as sensitivity
analysis, a per protocol analysis (PP) will be performed. The assignment of each patient to the ITT and PP set, respectively, will be defined within the statistical analysis plan before database closure. Quantitative variables will be compared with the use of Student’s t-test, paired t test or the Wilcoxon rank sum test as appropriate and categorical variables will be analyzed with the chi-squared test. Kaplan–Meier survival analysis will be performed and the time to first relapse compared between the groups using log-rank test. In addition, the possible influence of age, sex, disease duration, renal histology on time to first relapse is evaluated within a multivariate Cox regression analysis including treatment group. All patients who have received at least one dose of a study medication will be included in the safety analysis in the group they were treated with. The statistical analyses to be performed will be described in detail in a Statistical Analysis Plan (SAP) which will be finalized before database closure.

All analyses will employ SAS Version 9.3 or higher.
### 15. TIME SCHEDULE OF ACTIVITIES

<table>
<thead>
<tr>
<th>Milestone</th>
<th>Expected start months</th>
<th>Expected completion months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient enrolment, basic data collection, serum &amp; urine biochemistry</td>
<td>0</td>
<td>06</td>
</tr>
<tr>
<td>Patient follow up, data collection, serum &amp; urine biochemistry</td>
<td>07</td>
<td>18</td>
</tr>
<tr>
<td>Data analysis &amp; report writing</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>
16 STANDARD DEFINITIONS FOR NEPHROTIC SYNDROME\textsuperscript{14,15}

<table>
<thead>
<tr>
<th>Remission</th>
<th>Urine albumin nil or trace (or proteinuria &lt;4 mg/m\textsuperscript{2}/h) for 3 consecutive early morning specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapse</td>
<td>Urine albumin 3+ or 4+ (or proteinuria &gt;40 mg/m\textsuperscript{2}/h) for 3 consecutive early morning specimens, having been in remission previously</td>
</tr>
<tr>
<td>Frequent relapse</td>
<td>Two or more relapses in initial six months of initial response, or &gt;3 relapses in any 12 months</td>
</tr>
<tr>
<td>Steroid dependence</td>
<td>Two consecutive relapses when on alternate day steroids or within 14 days of its discontinuation</td>
</tr>
</tbody>
</table>

Interpretation of Albustix

Results of Albustix
- Negative
- Trace
- 30 mg/dL = +
- 100 mg/dL = ++
- 300 mg/dL = +++
- $\geq$ 2000 mg/dL = ++++
17 REFERENCES


18 STUDY FLOW CHART

Patient with SDNS

Screening
Inclusion criteria fulfilled?
Exclusion criteria Absent?
Informed consent of parents and assent of child if >7 years?

Screening failure

Treatment Randomization

Allocated to Tacrolimus arm

Allocated to Rituximab arm

Follow up for 12 months
## 19 CASE RECORD FORM

### 19.1 Baseline characteristics of the patients

<table>
<thead>
<tr>
<th>Patient’s code:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex:</td>
</tr>
<tr>
<td>Age (years):</td>
</tr>
<tr>
<td>Weight (Kg):</td>
</tr>
<tr>
<td>Height (cm):</td>
</tr>
<tr>
<td>BMI for age Z score:</td>
</tr>
<tr>
<td>Height for age Z score:</td>
</tr>
<tr>
<td>Duration of nephrotic syndrome (years):</td>
</tr>
<tr>
<td>No. of relapse episodes in previous year:</td>
</tr>
<tr>
<td>Cumulative steroid dose in previous year (mg/kg/yr):</td>
</tr>
<tr>
<td>Current prednisolone dose (mg/kg/alternate day):</td>
</tr>
<tr>
<td>Renal Histopathology:</td>
</tr>
<tr>
<td>Serum albumin (g/dl):</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl):</td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1.73 m²):</td>
</tr>
<tr>
<td>Urine dipstick for proteinuria:</td>
</tr>
<tr>
<td>Blood Count (TLC/DLC/Hb/Plt):</td>
</tr>
<tr>
<td>Serum Biochemistry (Na/K/Ca/AST/ALT/CPK/ALP/FBG):</td>
</tr>
<tr>
<td>B Lymphocyte count:</td>
</tr>
<tr>
<td>Clinical exam:</td>
</tr>
<tr>
<td>Any Steroid toxicity:</td>
</tr>
</tbody>
</table>
19.2 Follow up data

<table>
<thead>
<tr>
<th>Patient’s code:</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>1 month</th>
<th>3 month</th>
<th>6 month</th>
<th>9 month</th>
<th>12 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI for age Z score:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height for age Z score:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If on Oral Steroid Dose (Mg/kg alternate day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative steroid dose during study period (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>If on Tacrolimus Dose (mg/kg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tacrolimus Trough (T0) level (ng/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of Rituximab injection received:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B Lymphocyte count:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Relapse during this period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to first relapse (weeks)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>****</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine protein-creatinine ratio</td>
<td>****</td>
<td>********</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Count (TLC/DLC/Hb/Plt) (If done)</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na/K/Ca/AST/ALT/CPK/ALP/FBG (If done)</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
<td>****</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>****</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated GFR, ml/min/1.73 m²</td>
<td>****</td>
<td>****</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of any other drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical exam (if specific abnormal –describe below) (Hypertension-Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any adverse report yes/no (if yes –describe in full detail)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REMARKS:

ADVERSE EVENTS:
20 RITUXIMAB ORDER FORM

Code No. ________________________________     ______

1. Weight      kg; Height     cm; BSA      m²

2. Rituximab      (375 mg/ m²) in      ml normal saline (2 mg/ml)

   **Infuse as follows**

   ml/hour [0.5 ml/kg/hr for 30 minutes]
   ml/hour [1.0 ml/kg/hr for 30 minutes]
   ml/hour [1.5 ml/kg/hr for 30 minutes]

3. Premedications [administer 30 minutes prior to rituximab]
   
   Paracetamol  mg (15 mg/kg/dose po)
   Diphenhydramine  mg (0.5 mg/kg/dose PO)
   Hydrocortisone  mg (4 mg/kg/dose IV) (only @ first dose)

4. Monitor blood pressure and vitals q 15 minutes X 2, q 30 minutes X 2, then hourly

5. **Stop** infusions for moderate, severe reactions

6. Emergency medications for moderate/severe reactions

   Promethazine  mg (0.5 mg/kg/dose iv)
   Hydrocortisone  mg (4 mg/kg/dose iv)
   Epinephrine 1:1000  mg (0.01 mg/kg/dose iv)
RITURNS statistical analysis plan (SAP) for Final Analysis

Trial title: Randomized Clinical Trial to Compare Efficacy and Safety of Rituximab to That of Calcineurin Inhibitor in Children with Steroid Dependent Nephrotic Syndrome.

Trial code: RITURNS (rituximab for relapse prevention in nephrotic syndrome)
Protocol Number: Pednephro RCT/PM/NRSIMCH-54
Name and address of the responsible Biometrician:
   Anja Sander  
   Institute of Medical Biometry and Informatics  
   University of Heidelberg  
   Im Neuenheimer Feld 130.3  
   D-69120 Heidelberg

Name and address of the representative of the Biometrician:
   Stella Preußler  
   Institute of Medical Biometry and Informatics  
   University of Heidelberg  
   Im Neuenheimer Feld 130.3  
   D-69120 Heidelberg

Name and address of the Coordinating investigator:
   Biswanath Basu  
   Div. of Pediatric Nephrology  
   Department of Pediatric Medicine  
   Nilratan Sircar Medical College & Hospital,  
   38, AJC Bose Road, Kolkata 700014, INDIA
Objective of the Trial

The aim of the RITURNS study is to evaluate the efficacy and safety of single course rituximab infusion compared to maintenance tacrolimus in children with steroid dependent nephrotic syndrome (SDNS).

2. Study design

This is a prospective, mono-center, open-label, two-parallel-arm randomized controlled phase III study. Randomization was performed 1:1 using stratified block randomization with varying block sizes (4, 6, 8) and including sex, age (≤ 7 vs. > 7 years), and renal histology (MCD vs. FSGS) as stratification factors. Data on cumulative steroid dose and number of relapses in the pre-study year were collected retrospectively.

3. Analysis sets

Primary analysis set is the intention to treat set (ITT). All patients treated with at least one dose of study medication (Rituximab and Tacrolimus) are included and analysed in the group they were randomized to. In addition, as sensitivity analysis, a per protocol analysis (PP) will be performed. Hereby, only patients without major protocol violations are included (all inclusion and exclusion criteria are fulfilled and all medical procedures/visits have been carried out according to the protocol). The inclusion into the PP set in disputable cases will be clarified in discussion with the responsible investigator and fixed in a signed protocol prior to database closure.

All safety endpoints will be analysed based on the safety population which comprises all patients who were treated at least for one day and in the group as treated.

4. Definitions of endpoints to be analysed

Primary endpoint:

The primary endpoint is the 12-month relapse-free survival (yes/no). Missing values will be handled as described below in Chapter 5 Data handling.

Secondary endpoints:

1. 12-month relapse-free survival
2. Occurrence of 2 or more than two relapses within 6 months
3. Occurrence of 4 or more relapses in 12 months during study period (= definition of frequent relapse).
4. Frequent relapse: composite endpoint consisting of the two endpoints defined under 2 and 3.
5. More than one episode of life threatening infection or severe relapses (anasarca, hypovolemia or thrombosis) requiring hospital admission within 6 months and 12 months, respectively.
6. Impaired renal function (eGFR < 30 ml/min per 1.73 m$^2$ or loss of eGFR by $\geq$ 30% with regard to baseline) within 6 months and 12 months, respectively.
7. Treatment Failure (yes/no) defined as composite endpoint consisting of the three endpoints as defined above under 4, 5 and 6 referring to month 12.
8. Number and severity of adverse events.
9. eGFR at 3, 6, 9 and 12 months, respectively.
10. Amount of Cumulative prednisolone requirement (mg/kg/yr) over 12 months
11. Number of relapses within month 0-12, 0-6 and 7-12.
12. New onset of at least one event of steroid toxicity within 12 months (composite endpoint of Growth failure( $\geq$-2 Z score loss), Cushingoid appearance, Obesity ($\geq$2 Z score), Hypertension, and Patients ($\geq$5years) with school dropout)
13. Number of different steroid toxicity events (new onset) within month 0-12
14. Time to first relapse
15. Off steroids at month 12
16. Abnormal values in biochemical tests and haematology assessments.

5. Data handling

Z-score is calculated by WHO app “WHO anthro” and “WHO anthro-plus”, using height and weight of the patient at study entry and at 12 mo. Cumulative prednisolone dose is counted directly from the daily entry of drug intake amount, at patient’s nephrology diary. Histological diagnosis was done by renal biopsy. If renal biopsy was not done previously, it was done after taking consent but before randomization. So categorization was done at study entry. Although allowed, no patient with diagnosis MesPGN was included into this study.

Protocol deviations- Subjects in whom the intervention cannot be given for one year, or is interrupted for more than two weeks period of time; e.g. subjects who become very sick and have to be withdrawn from the study. All these patients will be accounted for in the intention to treat analysis.

For the primary analysis within the ITT set, missing values on the primary endpoint will be replaced using the method as proposed by Higgins (2008).
6. Statistical methods

The statistical methods are used to describe the recruitment, quality of data, homogeneity of treatment groups, and the evaluation of efficacy and safety of the treatments. For the confirmatory analysis of the primary endpoint original SAS output will be reported to ensure transparency. Despite the primary analysis of the primary endpoint all further analyses are descriptive.

6.1. Descriptive methods

Continuous variables will be described using number of non-missing values, mean, standard deviation, median, Q1, Q3, minimum and maximum. For binary or categorical variables absolute and relative frequencies will be provided. For comparisons between the groups descriptive p-values will be provided to show comparability of the groups in baseline characteristics and to provide a further descriptive measure.

6.2. Baseline characteristics

Both treatment groups will be characterized using descriptive methods based on the ITT set. Table 1 in the Appendix gives an overview on what to describe at baseline. Deviations from the inclusion and exclusion criteria will be listed individually.

6.3. Primary analysis

The primary endpoint analysis will be based on the ITT set following the intention-to-treat principle.

The null hypothesis of equal relapse free survival rates $p_{control}$ (Tacrolimus) and $p_{exp}$ (Rituximab) after 12 months is tested by a (two-sided) chi-squared test assessing the following test problem:

$$H_0: p_{control} = p_{exp} \quad \text{vs.} \quad H_1: p_{control} \neq p_{exp}$$

The two-sided significance level is set to $\alpha=0.05$.

6.5 Sensitivity analyses

In addition, as a sensitivity analysis, the relapse free survival rate will be compared using a logistic regression model including sex, age (continuous), disease duration (continuous), and renal histopathology (MCD vs. FSGS) as covariates.

Furthermore, the primary endpoint will be analysed additionally based on the PP set in the same manner. In addition, worst case and best case scenarios will be evaluated based on the ITT population (worst-case scenario: missing data in tacrolimus group will be considered as successes and missing data in the rituximab group will be considered failures; best case scenario vice versa).
6.6 Analysis of the secondary endpoints

Quantitative variables will be compared with the use of Student's unpaired t-test, paired t-test or the Wilcoxon rank sum test as appropriate and categorical variables will be analysed with the chi-squared test. 95% confidence intervals will be calculated. Kaplan Meier (KM) curves will be used to visualize time to first relapse and log rank test to compare both treatment groups. KM curves will also be created stratified by treatment group and renal diagnosis. In addition, time to first relapse will be compared using a Cox regression model including sex, age (continuous), disease duration (continuous), and renal histopathology (MCD vs. FSGS) as covariates.

All secondary endpoints will be analyzed based on the ITT set without imputation of missing values.

6.7 Safety and/or further analyses

The primary endpoint will be compared in patients with diagnoses MCD vs. FSGS. Absolute and relative changes in serum albumin, serum cholesterol, eGFR, Body mass Z score and height for age Z score from baseline to month 12 will be tabulated.

Number of relapses and cumulative steroid doses in the study year will be compared against the values of the pre-study year.

A CONSORT statement type flow chart will be used to depict all patients considered for selection, and patients actually selected for analysis.

All premature study discontinuations will be listed along with treatment group, time in study and reason for discontinuation.

7. Differences to trial protocol

Despite the amendment to the protocol, there were no differences to the trial protocol.

8. Interpretation of results

The sensitivity analyses will be considered when interpreting the results of the primary endpoint.

10. Software

All analyses will employ SAS Version 9.3 or higher.

11. References


Appendix
### A. Tables

Table 1. Baseline characteristics of patients by group.

<table>
<thead>
<tr>
<th></th>
<th>Tacrolimus ( n= X )</th>
<th>Rituximab ( n=Y )</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demography</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex-no.(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age(yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Anthropometry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height for age Z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body-mass index Z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disease history</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of disease(yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of relapse episodes per patient in pre-study year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Renal histology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal change-no.(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis-no.(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prednisolone therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative prednisolone dose in pre-study year (mg/kg/yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current prednisolone dose (mg/kg/d)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any Steroid toxicity pre-study-no.(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divided by type of toxicity – no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serum biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1·73 m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Efficacy of Tacrolimus and Rituximab.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Tacrolimus</th>
<th>Rituximab</th>
<th>Mean difference/ Risk ratio 95% CI (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of relapses per patient: mo. 0–12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute change in relapse rate from pre-study year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients with sustained remission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients with treatment failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of children off steroids at 12 mo.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative prednisolone dose (mg/kg/yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute change in cumulative prednisolone dose from pre-study year (mg/kg/yr)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisolone dose at 12 mo. (mg/kg/ad)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-mo. absolute change in prednisolone dose (mg/kg/ad)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum albumin at month 12 (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-mo. absolute change in serum albumin (g/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR at month 12 (ml/min/1·73 m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-mo. change in eGFR (ml/min/1·73 m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum cholesterol at month 12 (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-mo. absolute change in serum cholesterol (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI Z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-mo. change in BMI Z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height for age Z score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-mo. change in Height for age Z score</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ad: alternate day
Addendum to the final version of the SAP for Final Analysis

Trial title: Randomized Clinical Trial to Compare Efficacy and Safety of Rituximab to That of Calcineurin Inhibitor in Children With Steroid Dependent Nephrotic Syndrome.

Trial code: RITURNS (rituximab for relapse prevention in nephrotic syndrome)
Protocol Number: Pednephro RCT/PM/NRSMCH-54

Change in definition of two secondary endpoints and definition of further secondary endpoints.

Secondary endpoints:
1. 6-month relapse-free survival
2. New onset of at least one event of steroid toxicity within 12 months (composite endpoint of Growth failure (>2 Z score loss), Cushingoid appearance, Obesity (>2 Z score), Hypertension, and Patients (>5 years) with school dropout)
3. Height sds at 6 and 12 months
4. Absolute and relative change in height sds from baseline to 12 months
5. BMI sds at 6 and 12 months
6. Absolute and relative change in BMI sds from baseline to 12 months
7. B cell count