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Original Article

ORIGINAL ARTICLE

A European multicentre and open-label controlled randomized trial to evaluate the efficacy of Sequential treatment with TAcrolimus–Rituximab versus steroids plus cyclophosphamide in patients with primary MEmbranous Nephropathy: the STARMEN study

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Abstract

Background: Patients with primary membranous nephropathy (MN) and persistent nephrotic syndrome have a high risk of progression to end-stage renal disease. The Ponticelli protocol (steroids with alkylating agents) is the most effective immunosuppressive therapy for this condition, but it has severe adverse effects. Tacrolimus and rituximab have demonstrated efficacy for remission of nephrotic syndrome in MN with a safer profile. However, the published evidence is largely based on small or short-term observational studies, historical cohorts, comparisons with conservative therapy or clinical trials without appropriate control groups, and there is no head-to-head comparison with the Ponticelli protocol.

Methods: The STARMEN randomized clinical trial will compare the efficacy of sequential tacrolimus–rituximab therapy with a modified Ponticelli protocol (steroids plus cyclophosphamide). The trial will also evaluate the role of antibodies against the M-type phospholipase A_2 receptor (anti-PLA_2R) and other antibodies as markers of response to treatment and long-term prognosis.

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Results: The trial has already started with 23 patients having been enrolled as of 1 April 2015, an estimated 21.7% of the estimated sample.

Key words: biomarker, immunosuppression, membranous nephropathy, nephrotic syndrome, randomized controlled trial

Introduction

Primary membranous nephropathy (MN) is the commonest cause of primary nephrotic syndrome in adults [1]. Up to 30–50% of high-risk patients will progress to end-stage renal disease (ESRD) within 5–10 years [2–6]. Antibodies against the M-type phospholipase A_2 receptor (anti-PLA_2R) are present in >70% of cases and have a pathogenic role [7–9]. The reduction in serum levels of anti-PLA_2R during and after therapy could be an early marker of response to therapy and of sustained remission at long-term follow-up [10].

Kidney Disease: Improving Global Outcomes guidelines recommend cyclical treatment with corticosteroids and alkylating agents (cyclophosphamide or chlorambucil, the 'Ponticelli protocol') for 6 months for patients with persistent nephrotic syndrome after 6–12 months of conservative therapy or with a decreased baseline renal function [11]. This recommendation is based on a higher efficacy than supportive therapy to induce remissions and to avoid long-term deterioration of renal function [12–15]. The percentage of remissions after 1 year of treatment is 50–60%. However, the number and severity of side effects are important drawbacks of these therapies.

Calcineurin inhibitors (CNI) (cyclosporine or tacrolimus) are well tolerated and induce a significantly higher number of remissions (70–80% at 1 year) than supportive therapy, especially in patients with relatively preserved renal function [16–18]. In retrospective analyses, cyclosporine was associated with higher remission rates (85 versus 55%) but also with higher relapse rates (41 versus 29%) than cytotoxic drugs [17]. Thus, the main limitation of CNI in MN is the high relapse rate after CNI discontinuation (up to 50–60% of patients). In a recent multicentre Spanish cohort study, monotherapy with tacrolimus induced remission of nephrotic syndrome in >80% of patients with MN, with few adverse events [19]. Thus, in MN patients with relatively preserved renal function, tacrolimus may be a therapeutic alternative that provides a high remission rate without severe side effects. However, the frequency of partial versus complete remission could indicate a higher risk of relapse after tacrolimus withdrawal. An extended treatment with CNI could be a logical alternative but may be associated with higher risk of nephrotoxicity.

Very few studies have directly compared CNI with corticosteroids–alkylating agents in the treatment of MN. A Chinese randomized controlled trial (RCT) showed a significantly higher remission rate at 6 months among patients treated with tacrolimus compared with cyclophosphamide [20]. A recent multicentre study from the UK showed that patients treated with corticosteroids and chlorambucil had better renal outcomes than patients treated with corticosteroids plus cyclosporine, or supportive therapy [21]. However, these patients had a reduced and declining renal function, a condition where CNI-associated nephrotoxicity is more relevant.

Rituximab is a monoclonal antibody that depletes CD20+ B cells and is used in autoimmune diseases and B cell neoplasia [22]. Several non-controlled studies have reported that rituximab induces complete remission in 15–20% of primary MN patients and partial remission in 35–40% [23–25]. Variable doses of rituximab and different baseline prognostic factors could explain in part the variability in results [26]. Optimal dose and frequency and long-term adverse events of rituximab are issues that need to be better established. Furthermore, a prospective RCT comparing rituximab with supportive treatment or with the Ponticelli protocol is not available. Rituximab has also been reported to prevent nephrotic syndrome relapse after CNI withdrawal in a series of primary MN patients who had a good response to CNI but exhibited a clear CNI dependence thereafter [27]. The efficacy of rituximab may be compromised by the severity of proteinuria that may result in urinary losses of rituximab [28] and by the development of anti-rituximab antibodies following repeated infusions [29]. In this regard, previous treatment with CNI may be hypothesized to result in enhanced efficacy of rituximab by reducing urinary losses and the need for multiple rituximab courses. Recent observational studies using sequential tacrolimus–rituximab therapy have supported this hypothesis by showing a high number of remissions and a low rate of relapses, with good tolerance [27]. However, no RCT has compared sequential tacrolimus–rituximab with the Ponticelli protocol recommended by KDIGO guidelines.

Based on this unmet need, the STARMEN RCT (available at http://www.clinicaltrials.gov, NCT01955187) will compare the efficacy and safety of sequential treatment with tacrolimus–rituximab versus steroids plus cyclophosphamide in nephrotic patients with primary MN [30]. The key outcomes will be remission of nephrotic syndrome, long-term renal survival and safety. The STARMEN study will be the first head-to-head trial comparing the Ponticelli protocol with newer therapeutic agents and will provide high-quality clinical evidence levels on which to base recommendations regarding the relative roles of sequential tacrolimus–rituximab and conventional immune suppression in the treatment of primary MN.

STARMEN will also address the role of anti-PLA_2R antibodies in the early assessment of the response to therapy using different therapeutic regimens. Most studies have shown a good correlation between activity of disease and levels of anti-PLA_2R [7–10, 31]. In a recent retrospective cohort, the disappearance of anti-PLA_2R during immunosuppressive treatment predicted good long-term outcome, but persistent detection of anti-PLA_2R was associated with a low probability of persistent remission during follow-up [31]. These studies suggest that antibody-guided immunotherapy may help to personalize the therapeutic regimen. However, given the use of different techniques for determination of anti-PLA_2R levels and the retrospective design of published studies, firm conclusions cannot be drawn about the role of anti-PLA_2R as an early biomarker of response to therapy and of long-term prognosis. An RCT will provide high-quality evidence on the role of anti-PLA_2R to guide therapy and to establish a prognosis. Novel autoantibodies have been recently described in patients with primary MN. Circulating antibodies directed against podocyte cytoplasmic antigens (aldose reductase, SOD2, α-enolase) or against thrombospondin type-1 domain-containing 7A could define subsets of patients with positive anti-PLA_2R or persistently negative anti-PLA_2R, respectively, and perhaps with a different response to therapy [32, 33]. These autoantibodies and their clinical long-term relevance may also be prospectively validated in this RCT.

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Objectives of the study

We will prospectively evaluate the long-term efficacy and safety of sequential tacrolimus–rituximab therapy compared with steroids–cyclophosphamide therapy in patients with primary MN.

The *principal objective* is to evaluate at 24 months whether sequential therapy with tacrolimus–rituximab is superior to cyclical treatment (corticosteroids and cyclophosphamide) to achieve a complete or partial remission with stable renal function.

The secondary objectives are to evaluate the following:

(i) The percentage of patients that achieve a complete and partial remission with stable renal function at 12 and 18 months.
(ii) The number and time to nephrotic syndrome relapses at 12, 18 and 24 months.
(iii) The time to remission.
(iv) The percentage of patients with preserved renal function [estimated glomerular filtration rate (eGFR) ≥45 mL/min/1.73 m^2] at 12, 18 and 24 months.
(v) The number of patients with limited response at 12, 18 and 24 months.
(vi) The number of patients with ≥50% increase of serum creatinine (SCr) from baseline at the end of the follow-up.
(vii) The number and severity of side effects during the study.
(viii) Serum anti-PLA_2R levels at 6, 12 and 24 months post-treatment compared with baseline. Optionally, anti-PLA_2R can be obtained at 3, 9 and 18 months.
(ix) The number of immune cells (CD4+ and CD8 + T cells and CD19+ B cells) after 12 and 24 months of treatment compared with baseline.

An additional aim is to characterize known and novel clinical, laboratory and histologic factors that predict response to treatment, relapse and renal outcomes.

Materials and methods

Study design

This is an open label, randomized and active controlled trial (Phase III study) with three stages: screening and recruitment of patients, treatment period (6 months for corticosteroids and cyclophosphamide group, and 9 months for tacrolimus–rituximab) and a post-treatment follow-up period of 24 months from initial treatment.

Population

Patients with biopsy-proven idiopathic or primary MN with nephrotic proteinuria and normal or slightly decreased renal function will be enrolled.

Inclusion criteria

(i) Patients older than 18 years that provide written informed consent.
(ii) Biopsy-proven primary MN within 2 years of enrolment. Patients with nephrotic syndrome relapse after remission (either spontaneous or induced by immunosuppression) can be included without a new renal biopsy if they meet all the other inclusion/exclusion criteria.
(iii) Estimated GFR ≥45 mL/min/1.73 m^2 in at least two measurements performed within the 2 weeks prior to randomization.
(iv) Nephrotic-range proteinuria (>4 g/day and remaining >50% of the baseline value) plus hypoalbuminemia (<3 g/dL) during at least a 3-month period before screening. These values must be met in at least two measurements performed within the 2 weeks prior to randomization. Patients showing severe or disabling symptoms related to the nephrotic syndrome or severe hypoalbuminemia (<2 g/dL) can be included before the completion of this 6-month observation period, at the investigator's discretion.
(v) Treatment with an angiotensin-converting enzime inhibitor (ACEI) or angiotensin-receptor blocker (ARB) for at least 2 months before screening [unless intolerance to ACEI/ARB, contraindications to their use or a low blood pressure (BP) that could induce side effects, at the investigator's discretion] with a controlled BP for at least last 3 months (target <140/90 mmHg).
(vi) Negative urine pregnancy test for potentially fertile females.

Exclusion criteria

(i) Diagnosis of secondary causes of MN: diagnosis of Type 1 or 2 diabetes mellitus, cancer, systemic infections, systemic autoimmune diseases (e.g. systemic lupus erythematosus), amyloidosis, or any other acute or chronic inflammatory disease.
(ii) Moderate or severe liver disease [aspartate amino-transferase (AST) and alanine amino-transferase (ALT) >2.5× upper range limit and total bilirubin >1.5× upper range limit].
(iii) Patients who are taking part in any other investigational study and/or are receiving or have received treatment with another investigational drug or intervention (within 1 month prior to the study).
(iv) Suspected or known hypersensitivity, allergy and/or immunogenic reaction history of any interventional drug or any of their ingredients (including excipients).
(v) Previous treatment with corticosteroids or any other immunosuppressive agent in the 6-month period before screening.
(vi) Previous treatment with rituximab or any other biological agent in the 2-year period before screening.
(vii) Patients who were non-responders to previous immunosuppressant drugs.
(viii) Women showing a positive pregnancy test or during lactation period or plans to become pregnant.
(ix) Inability or unwillingness of individual or legal guardian/representative to give written informed consent.
(x) Any other medical unstable, uncontrolled or severe condition or any other relevant laboratory test finding which, at the investigator's own discretion, could increase the associated risk of the patient's participation in the study.
(xi) Current drug or alcohol use or dependence that would interfere with adherence to study requirements.

Subject withdrawal criteria

The investigator may withdraw a patient from the study at any time if the investigator considers it necessary for any reason, including the following:

(i) Ineligibility (arising either during the study or retrospectively having been overlooked at screening).
(ii) Significant protocol deviation or violation.
(iii) Significant non-compliance or non-adherence with treatment regimen or study requirements.
(iv) An adverse event, especially when serious that requires discontinuation of the study medication or results in inability to continue to comply with study procedures.
(v) Any medical condition or disease progression, that requires discontinuation of the study medication or results

in inability to continue to comply with study procedures. These patients will be followed to analyse whether they reach end points.

(vi) Consent withdrawn. Patient's request to withdraw study at any time.

(vii) Pregnancy.

(viii) Lost to follow-up.

All subjects withdrawn from the study will be attempted to be included in the end-of-treatment visit in order to determine all efficacy and safety measurements and the patient outcome.

Definitions of end points

Primary end point: The proportion of patients reaching either complete or partial remission at 24 months of study treatment.

Secondary end points:

(i) The number of patients with an increase of SCr ≥50% at the end of follow-up (renal survival).

(ii) The proportion of patients with relapsing nephrotic syndrome among patients who previously underwent partial remission or complete remission.

(iii) The time to nephrotic syndrome relapse.

(iv) The number of patients with limited response at 12, 18 and 24 months of study treatment.

(v) The percentage of patients with preserved renal function (eGFR ≥45 mL/min/1.73 m^2) at the end of follow-up.

(vi) Serum anti-PLA_2R levels before treatment and at 12 and 24 months post-therapy.

(vii) The number of immune cells (CD4+ and CD8+ T cells, and CD19+ B cells) before treatment and at 12 and 24 months post-therapy.

(viii) Proportion of patients with drug-related adverse events during the study.

Figure 1 and Table 1 show a schematic diagram of the trial design, procedures, stages and data collection, and all trial periods.

Definitions of outcomes

(i) *Complete remission:* A reduction of proteinuria to ≤0.3 g/24 h plus stable renal function (eGFR ≥45 mL/min/1.73 m^2).

(ii) *Partial remission:* A reduction of proteinuria to 0.3–3.5 g/24 h and 50% lower than baseline with stable renal function (eGFR ≥45 mL/min/1.73 m^2).

(iii) *Limited response:* Proteinuria is reduced from baseline level >50% but remains >3.5 g/24 h.

(iv) *Non-response:* A reduction of proteinuria <50% from baseline level.

(v) *Renal survival:* At the end of the follow-up, sCr does not increase ≥50% of baseline SCr concentrations.

(vi) *Relapse:* Reappearance of proteinuria >3.5 g/24 h and at least 50% higher than the lowest post-treatment value in at least three consecutive visits in those who previously presented a partial or complete remission.

(vii) *Renal function:* This will be evaluated by means of SCr values and eGFR, calculated by the Modifications in Diet and Renal Disease four-variable equation (MDRD-4).

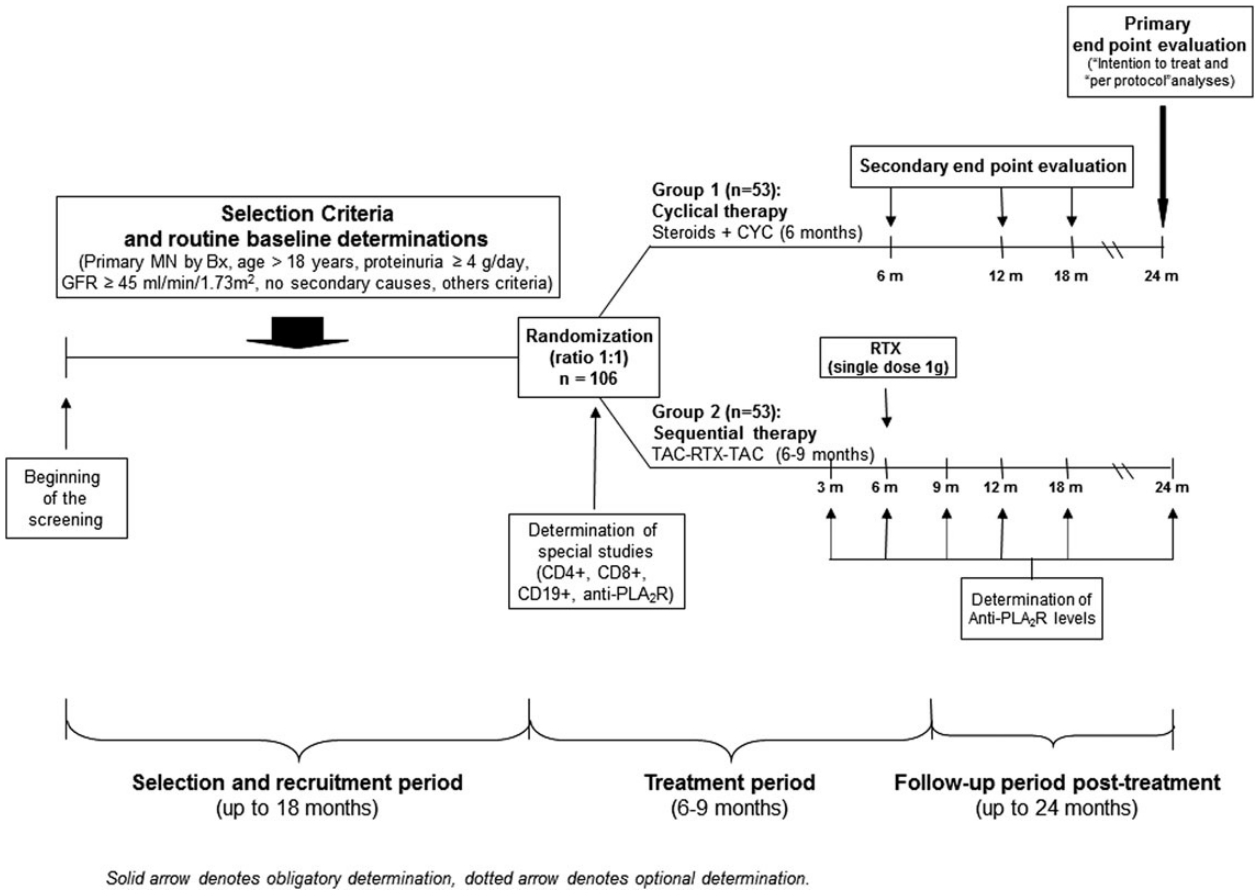

Fig. 1. Schematic overview of the STARMEN study. CYC, cyclophosphamide; TAC, tacrolimus; RTX, rituximab.

Table 1. Scheme of the activities that will take place at each contact with the participant after randomization

	Months since randomization												
Activity	0	1	3	6	7	8	9	10	12	15	18	21	24
Study procedures													
Informed consent	X												
Medical history	X												
Demography data	X												
Physical examination	X	X	X	X			X		X	X	X	X	X
Intervention													
Steroids + CF		X	X	X									
Tacrolimus + RTX		X[a]	X	X[b]	X[b]	X[b]	X[b]	X	X	X			
Blood tests													
Haematology	X	X	X	X	X	X	X	X	X	X	X	X	X
Biochemistry	X	X	X	X	X	X	X	X	X	X	X	X	X
Hormones	X								X				X
Immunology (CD8+, CD4+, CD19+ cells)	X		X[c]	X			X[c]		X		X[c]		X
Anti-PLA_2R antibodies	X		X[c]	X			X[c]		X		X[c]		X
Sample for biobank	X			X					X				X
Urine test													
Sediment and labstix	X			X			X		X	X	X		X
Proteinuria 24 h	X	X	X	X	X	X	X	X	X	X	X	X	X
UACR/UPCR	X	X	X	X	X	X	X	X	X	X	X	X	X
Sample for biobank	X			X					X				X
Evaluation													
Complete/partial remission				X			X		X	X	X	X	X
Limited/no response				X			X		X	X	X	X	X
Renal survival							X		X	X	X	X	X
Relapse							X		X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X

CF, cyclophosphamide; RTX, rituximab; UACR, urinary albumin–creatinine ratio; UPCR, urinary proteinuria–creatinine ratio.
[a]Start of treatment with tacrolimus at initial dosages of 0.05 mg/kg/day based on serum drug concentrations of 5–7 ng/mL.
[b]Start of treatment with rituximab: cycle of 1 g IV (single dose) and decrease tacrolimus dosages at 25% per month, starting at the end of Month 6, resulting in a complete withdrawal at the end of Month 9.
[c]These determinations will be optional (Months 3, 9 and 18).

Any patient reaching a complete remission or partial remission will be considered a successful treatment.

Randomization

This study will be randomized, with an equal allocation ratio (1:1) to intervention with tacrolimus–rituximab or steroids plus cyclophosphamide. We will use a random number-producing algorithm by central computer systems for simple randomization. Subject numbers will be assigned sequentially as each subject enters the study.

Study procedures and baseline determinations

In each centre, potential patients will be identified from Nephrology Departments or renal biopsy records. If these patients meet the selection criteria, they will be proposed to participate in this trial after giving complete information about their disease, options of treatment, potential outcomes, risk and benefits of several therapies, and trial process, including the number of visits, clinical and laboratory determinations, and time of follow-up. Maximum duration allowed between screening and randomization will be 18 months.

The following screening procedures will be recorded in electronic Case Report Form (eCRF):

Demographics (date of birth, gender, race, smoking and drinking habits, and type of anticonception when applicable)

Medical history (any history of disease or surgical interventions)

Concomitant medication (all over-the-counter or prescription medication, vitamins and/or herbal supplements)

Physical examination (height, weight, oral temperature, resting pulse, BP and respiratory rate measurements will be measured after the participant has sat for at least 5 min)

Electrocardiogram

Laboratory tests (obligatory at baseline in all potential participants, Table 1).

Blood tests: Red blood cell count, haemoglobin, haematocrit, white blood cell, platelet count, prothrombin time, activated partial thromboplastin time, International Normalized ratio, fibrinogen, glucose, urea, creatinine, uric acid, ALT, AST, gamma-glutamyl-transpeptidase, partial and total bilirubin, alkaline phosphatase, total proteins, albumin, calcium, phosphorus, sodium, potassium, total cholesterol, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, triglycerides, thyroid-stimulating hormone, intact parathyroid hormone, 25-hydroxy-vitamin D and beta-human chorionic gonadotropin in case of doubt of urine pregnancy test.

Urine tests: Sediment, proteinuria in 24 h, creatinine in 24 h, volume in 24 h and pregnancy test when appropriate.

The following special analyses will be determined at baseline in all selected participants: serum anti-PLA_2R antibodies (ELISA, Euroimmun AG, Luebeck, Germany), the number of T cells CD4+, CD8+, CD4+/CD8+ cell ratio, and CD19+ cells. Each participating centre will collect blood samples for these determinations at

Months 0, 6, 12 and 24, and optionally at Months 3, 9 and 18. Blood samples for these special determinations shall be kept in each participating centre at −80°C. These samples will be sent to and processed in the Research Laboratory of Renal and Vascular Pathology, of the Nephrology Department at the Hospital Universitario Fundación Jiménez Díaz (Dr Jesus Egido), in Madrid (Spain).

Subsequent assessments

For each visit, we will check eligibility, end points/outcome measures, safety (general and specific safety assessments, e.g. specific laboratory tests), adverse event collection, dispensing of study drugs, compliance with study drugs, recording of concomitant medications, and laboratory tests to evaluate treatment response as defined in the schedule.

End of trial assessment

The end of trial will be the date of the last scheduled visit of the last participant.

The end of study visit form should include assessment of end points/outcome measures, general and specific safety assessments (e.g. specific laboratory tests), adverse event collection, assessment of compliance with study drugs and recording of concomitant medications.

Treatment protocol

First Arm: Cyclical corticosteroids/cyclophosphamide for 6 months.

Months 1, 3 and 5: 1 g IV methylprednisolone daily (Days 1–3), then oral methylprednisolone (0.5 mg/kg/day) for 27 days (Days 4–30).

Months 2, 4 and 6: Oral cyclophosphamide (2.0 mg/kg/day) for 30 days.

Second Arm: Sequential tacrolimus–rituximab

(i) Tacrolimus: Initial dose of 0.05 mg/kg/day oral, adjusted to achieve blood trough levels of 5–7 ng/mL for 6 months. Starting at the end of Month 6, tacrolimus dosage will be reduced by 25% per month, resulting in a complete withdrawal at the end of Month 9.
(ii) Rituximab: A single dose of 1 g IV will be given at Day 180, before the onset of tacrolimus dose reduction.

In active control patients showing a 50% SCr increase, possible confounding factors such as an excessive diuretic doses or non-renal volume loss will be carefully excluded before adjudicating an end point.

In the tacrolimus arm, tacrolimus doses will be reduced by 25% every 2 weeks in the presence of a 50% SCr increase. If the elevated SCr persists at >50% of baseline values 2–4 weeks after >75% reduction of tacrolimus doses, an end point will be adjudicated. Once the end point of a 50% increase in baseline SCr concentrations has been established in either group, the patient will be taken off the study and he will be treated according to the best local practice.

To minimize infusion reactions with rituximab, patients will receive premedication with methylprednisolone 100 mg intravenously. Other additional drugs will be permitted according to each country's usual protocols, for example, oral acetaminophen/paracetamol (1 g) and diphenhydramine hydrochloride (50 mg).

Both treatment groups will receive antibiotic prophylaxis with cotrimoxazole (trimethoprim/sulfamethoxazole 160/800 mg, orally) three times a week during periods of treatment.

Assessment of efficacy and safety

Variables of efficacy:

(i) The number of patients with complete remission or partial remission of nephrotic syndrome at 6, 12, 18 and 24 months.
(ii) The number of nephrotic syndrome relapses at 12, 18 and 24 months.
(iii) The number of patients maintaining stable renal function at 6, 12, 18 and 24 months.
(iv) The number of patients reaching ESRD at 6, 12, 18 and 24 months.

Variables of safety:

(i) The number and severity of side effects at 6, 12, 18 and 24 months.
(ii) The number of patients withdrawn from the study because of side effects or drug intolerance at 6, 12 and 18 months.

Statistical analysis

Data will be entered by the principal investigator at each centre or their nominated deputies onto a central secure database. Continuous variables with normal distribution will be reported as mean ± SD. Otherwise, they will be reported as median and quartiles. Categorical variables will be reported as frequency. Differences between the two groups in continuous variables will be analysed using the unpaired Student's *t*-test or Wilcoxon's rank sum test, as appropriate. Differences between categorical variables will be analysed with likelihood chi-square and Fisher's exact tests, as appropriate. In both groups, for proteinuria, SCr and eGFR, study of changes since randomization until Months 3, 6, 9, 12, 18 and 24 will be assessed by covariance analysis (ANCOVA). Differences between treatments will be estimated by fixed model.

Major end points in each arm of treatment will be determined as the proportion of events at the end of the follow-up. Primary end point (complete or partial remission) will be analysed at a one-tailed significance level of $P < 0.05$. Based on previous studies, we estimated that complete or partial remission will occur in 60% in the Ponticelli group and 85% in tacrolimus–rituximab group, a clinically relevant difference of 25% between experimental groups. Other outcomes will be assessed by a two-tailed significance level of $P < 0.05$. We will determine the probability of outcomes as time-to-event with the Kaplan–Meier curves, log-rank test and Cox's regression from baseline evaluation to last follow-up. Baseline factors associated with major outcomes will be determined with proportional hazard Cox's model. Results will be reported as hazard ratio with 95% confidence interval. All statistical analyses will be performed by a statistician who does not know the treatment groups, with Stata version 13.0 for Windows (Stata Corp., TX, USA).

Handling of missing data, withdrawals and subgroup analyses

All analyses will be done on an intention-to-treat basis, independently of numbers of non-compliers, withdrawals or lost to follow-up. Additionally, we will do analysis per protocol. Results will be reported with both analyses [34–37].

In case of a small number of missing data, we will use a method of multiple imputations using a mixed effects linear regression method, and we will perform a standard analysis for each imputation cycle. The final analysis will take into

consideration the variability across the imputation cycles. Results will be reported with missing data and with imputation method.

Based on known prognostic factors, subgroup analyses will be carried out. These results will be interpreted with caution and used to refine the primary hypothesis and specify to whom the intervention should be recommended. However, in general, these potential results only will be considered as hypothesis-generating to design new trials.

Sample size calculation

We will consider the proportion of patients with proteinuria remission (complete or partial) after treatment and at 2 years of follow-up. On the basis of previous studies, we assumed a remission of 60% for the steroids + cyclophosphamide group (p_0) and 85% for tacrolimus–rituximab (p_1), a difference between groups of 25%, a statistical power 80% and an alpha error 0.05 (one-tailed test).

Due to $p_1 > 0.80$ (group of tacrolimus–rituximab), we used the following formulae with Fleiss' correction for binary outcomes:

$$n_1 = \frac{\left[(z_{\alpha/2}\sqrt{(r+1)R(1-R)} + z_{\beta}\sqrt{p_0(1-p_0)+r(p_1)(1-p_1)}\right]^2}{r(p_1-p_0)^2}$$

$$n_0 = r \times n_1$$

$$R = \frac{p_1 + r(p_0)}{1+r}$$

Fleiss correction: $n1c = n1 + (r+1)/R(p_1 - p_0)$

where p_0 is the proportion of remission in the control group (methylprednisolone–cyclophosphamide), p_1 is the proportion of remission in experimental group (tacrolimus–rituximab), n_0 is the number of participants in methylprednisolone–cyclophosphamide group, n_1 is the number of participants in the tacrolimus–rituximab group, r is the ratio between groups (n_0/n_1) and R is the risk in total population. Values of z-alpha and z-beta, are respectively, 1.645 and 0.842.

With this method, we would need 47 patients per group and a total sample size of 94 patients. Furthermore, we assumed that 10% of patients will be withdrawals. We used the following correction: $N^* = N/(1 - R^*)$, where R^* is the assumed proportion of withdrawals patients (in this case, 10% of initial number).

After this correction, we will need 53 patients by group and 106 patients. These results were reproduced with the STATA command 'db nsize' and with command 'power two proportions' with correction for continuity. The estimated recruitment period at each participant centre is 18 months.

Interim analyses and stopping rules

An independent monitoring committee will ensure the safety of the participants and the integrity of the trial. This committee will perform the interim analyses in coordination with data analysts, suggesting if necessary, additional analysis. The reasons for terminating the trial will be safety or poor study performance (e.g. slow accrual, high losses to follow-up and poor quality control). Any evidence that compromises patient safety or any statistical rule could lead to a temporary suspension of enrollment of patients or any study intervention until the safety committee conducts a review of the case.

Current status of the trial

The trial has already started. The first patient was enrolled on 10 June 2014. As of 1 April 2015, 23 patients have been enrolled and randomized (21.7% of estimated sample).

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Conflict of interest statement

None declared.

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