Rituximab for Severe Membranous Nephropathy: A 6-Month Trial with Extended Follow-Up

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ABSTRACT (250 words)

There are no randomized trials of rituximab in primary membranous nephropathy (PMN). We did a multicentre, randomized controlled trial at 31 French hospitals (NCT01508468). Patients with biopsy proven PMN and nephrotic syndrome after 6 months of Non Immunosuppressive Antiproteinuric Treatment (NIAT) were randomly assigned to 6-month therapy with NIAT and 375 mg/m^2 intravenous rituximab on days 1 and 8, or NIAT alone. After 6 months, patients continued observational follow-up. Median time to last follow-up was 17.0 (IQR= [12.5; 24.0]) and 17.0 [13.0; 23.0] months in the NIAT-rituximab and NIAT groups, respectively. Primary outcome was a combined endpoint of complete or partial remission of proteinuria at 6 months. Of 80 patients enrolled, 77 were randomized and 75 received the assigned intervention (37 were given NIAT-rituximab and 38 NIAT alone). At month 6, 13 (35.1%, 95% CI 19.7; 50.5) patients in the NIAT-rituximab group and 8 (21.1%, 95% CI 8.1; 34.0) in the NIAT group achieved remission (p=0.2055). Rates of PLA2R-Ab depletion were 14/25 (56%) and 1/23 (4.3%) at month 3 (p=0.0001), and 13/26 (50%) and 3/25 (12%) at month 6 (p=0.0035), in the NIAT-rituximab and NIAT groups, respectively. Eight SAEs occurred in each group. During the observational phase, remission rate before change of assigned treatment was 24/37 (64.9%) and 13/38 (34.2%) in the NIAT-rituximab and NIAT groups, respectively (p= 0.0079). A positive effect of rituximab on proteinuria remission was delayed after 6 months. PLA2R-Ab levels are early markers of rituximab effect. Addition of rituximab to NIAT has no impact on safety.

INTRODUCTION (3188 /3000 words)

Membranous nephropathy (MN) accounts for about 20% of cases of nephrotic syndrome in the adult and is the leading glomerulopathy recurring after kidney transplantation.[1]

Thickening of glomerular capillary walls results from subepithelial formation of immune deposits containing IgG, the membrane attack complex of complement, which is the major mediator of proteinuria, and antigens. Primary forms of MN, improperly called primary membranous nephropathy, represent 70% to 80% of all cases. A major breakthrough was the identification of the podocyte antigen PLA2R as the target of circulating antibodies in about 70% of PMN, which confirmed that the disease was auto-immune in nature.[2]

The optimal treatment of patients with PMN is still a matter of debate.[3,4] Thirty to 40% of affected patients will undergo spontaneous, usually partial remission, usually within one year from disease onset, whereas about one third will progress to end-stage kidney disease.[5, 6] Treatments with corticosteroids and alkylating agents significantly increase the rates of remission and slow renal function loss in patients with persistent nephrotic syndrome.[7-11] Calcineurin inhibitors induce remission in a majority of patients, but relapse rates exceed 50% and renal toxicity is a concern.[10, 12-14] The latest Kidney Disease Improving Global Outcomes (KDIGO) guidelines restricted the indication of alkylating agents to patients at high risk of progression, and considered calcineurin inhibitors as an alternative therapy.[15] In patients with even more restricted indications of alkylating agents, the rate of serious adverse events (SAE), particularly malignancy, was higher in patients who received long-term immunosupression than in those with supportive therapy.[16]

Both the evidence that B cells play a key role in the pathogenesis of PMN and drug toxicity led to target B-cells with rituximab.[17] Rituximab induced remission of nephrotic syndrome in 60% to 80 % of the patients with long-lasting proteinuria despite blockade of the renin-angiotensin system[18-21] and in patients who had previously failed other treatments. Reduction of PLA2R-Ab titre preceded remission of proteinuria by several months which suggested a causal relationship.[22, 23] A previous study showed that a B-cell driven approach

with only one or two infusions of Rituximab 375 mg/m² per week could allow reducing cost in comparison with the standard 4 weekly infusions[24]

Because of the lack of randomized controlled trial (RCT) using rituximab and of high rate of spontaneous remission, the present trial was designed in order to evaluate the efficacy of rituximab given to all patients at a standard dose (375 mg/m^2) in 2 infusions added to supportive therapy compared to supportive therapy alone, in patients with persistent nephrotic syndrome.

RESULTS

Between January 17, 2012, and July 3, 2014, eighty patients were enrolled in the RCT phase (Figure 1). Three patients withdrew their consent before randomization. Thirty-nine patients were assigned to NIAT-rituximab and 38 to NIAT only. Thirty-seven patients in the NIAT-rituximab group and 38 in the NIAT group received the assigned treatment. Baseline characteristics in the two groups were similar (Table 1).

Primary end point. The 6-month trial failed to achieve the primary end point. Thirteen patients (35.1%, 95% CI- 19.7; 50.5) in the NIAT-rituximab group and 8 patients (21.1 %, 95% CI- 8.1; 34.0) in the NIAT group achieved proteinuria remission at month 6 following randomization (p= 0.2055), (Table 2; OR 2.0 95% CI 0.7-5.7). Results were not sensitive to missing data replacement.

Secondary end points. Protein/creatinine ratio decreased similarly in both groups at months 3 and 6 (Table 2, Figure 2A). Percent increase of serum albumin was significantly higher at months 3 and 6 in the NIAT-rituximab group (Figure 2B). Serum creatinine and eGFR by MDRD formula, serum triglycerides, total and LDL cholesterol, body weight, and need for diuretic therapy did not differ at months 3 and 6 between the 2 groups (Table 2, Supplemental Table S1).

PLA2R-Ab was detected in 27 (73%) and 28 (74%) patients at baseline in the NIAT-rituximab and the NIAT groups, respectively (Table 1). As early as month 3, rate of PLA2R-Ab positive patients (31 % vs. 83 %, p<0.0001) and PLA2R-Ab titer (0.0 IQR [0.0; 49.1] vs. 54.6 IQR [16.5; 278.4] RU/ml, p=0.0005) were lower in the NIAT-rituximab group than in the NIAT alone (Table 2). No further decrease in the rate of PLA2R-Ab positive patients was observed between months 3 and 6, and the difference between PLA2R-Ab titer at month 3 and month 6 was 0.0 [0.0; 19.8] in the NIAT-rituximab group. No change in the rate of PLA2R-Ab positive patients and in PLA2R-Ab titer occurred between baseline, and months 3 and 6 in the NIAT group (Table 2).

In the subgroup of patients who were initially positive for PLA2R-Ab, a significant decrease of the titer of PLA2R-Ab was observed at month 3 (0.0 [0.0; 60.5] RU/ml, p<0.0001) and month 6 (8.3 [0.0; 73.5], p=0.0004) compared to baseline (102.5 [36.1 ; 672.5]) in the NIAT-rituximab group, and only at month 6 (62.9 [16.6 ; 449.3] vs 199.5 [24.2 ; 491.4] RU/ml at baseline, p=0.0168) in the NIAT alone. Percent decrease of PLA2R-Ab titer was significantly higher in the NIAT-rituximab group at month 3 and month 6 (Figure 2C).

Complete immunological remission (full PLA2R-Ab depletion) was observed in the NIAT-rituximab group in 14/25 (56 %) and 13/26 (50 %) patients at months 3 and 6, respectively, as compared with 1/23 (4 %, p=0.0001) and 3/25 (12 %, p=0.0035) patients, respectively, in the NIAT group (Table 2). Of the 14 rituximab-treated patients that were antibody depleted at months 3, 6 (43 %) patients subsequently achieved the primary end point, compared with only 2 of the 11 patients (18%) without antibody depletion. PLA2R-Ab level <275 RU/mL at baseline was associated with the primary end point (OR 4.3, 95% CI 1.1- 17.3, p=0.0424), and this was independent from treatment group, age, gender, baseline proteinuria, serum albumin and creatinine (Supplemental Table S2).

PLA2R-Ab was also measured at a very early time point (Day 8). Rates of PLA2R-Ab positivity, and PLA2R-Ab titer in the whole population and in the subset of patients who were positive at baseline were similar in both treatment groups (Table 2). Among the 8 patients of the NIAT-rituximab group who were PLA2R positive at baseline and achieved remission at 6 months, 2 were antibody depleted at day 8, and 2 had a marked reduction by 78% and 42% of antibody titer, respectively.

Twenty patients had undetectable PLA2R-Ab at baseline (10 in the NIAT-rituximab group and 10 in the NIAT group). However, 3 patients in the NIAT group later developed PLA2R-Ab, and were considered as having PLA2R-related PMN. At baseline, no statistical difference in age, protein/creatinine ratio, serum albumin and eGFR was seen according to presence (n=55 patients) or absence (n=20) of PLA2R-Ab. The effect of rituximab on proteinuria remission did not differ according to the serological status at baseline. Two PLA2R-Ab negative patients were positive for THSD7A-Ab. The first patient (1/100 dilution at baseline) received NIAT-rituximab and achieved remission at month 6 with sustained antibody depletion from month 3. The second patient was treated with NIAT alone, achieved partial remission at month 6, but relapsed at one year. In this patient, THSD7A-Ab remained detectable at low level (1/10) at any time.

CD19+ B-cells remained below normal range (100 to 500/mm^3) throughout the observation period in the NIAT-rituximab group. Median CD19+ B-cell count was 11/mm^3 (IQR=(2.0; 22.0)) at month 3 and 61/mm^3(IQR=(34.0; 100)) at month 6 (Table 2). Among PLA2R-Ab positive patients at baseline, there was no difference in CD19 count between patients who were PLA2R-Ab depleted and those who were not, at month 3 (p=0.7587, N=23 patients) and month 6 (p=0.8862, N=24 patients), respectively.

Post-hoc composite end point. In a post-hoc analysis that includes reduction of proteinuria > 50% and an increase of serum albumin level > 30% at month 6, fifteen patients (41 %) in

157 the NIAT-rituximab group and 5 patients (13 %) in the NIAT group achieved the composite
158 end point at month 6 following randomization (OR 0.22, 95% CI 0.07-0.70, p=0.0073) (Table
159 2).

160 *Post-RCT observational period*. Median duration from inclusion to last follow-up was 17.0
161 (IQR= [12.5; 24.0]) and 17.0 [13.0; 23.0] months in the NIAT-rituximab and NIAT groups,
162 respectively. The rate of KDIGO remission occurring without modification of initial
163 immunosuppressive treatment (NIAT-rituximab) or introduction of an immunosuppressive
164 treatment (NIAT) was 24/37 (64.9 %) and 13/38 (34.2%) in the NIAT-rituximab and NIAT
165 groups, respectively (OR 3.5, 95% CI 1.7-9.2; p=0.009), (Table 3). Numbers of complete
166 remission were 7/37 and 1/38 in the NIAT-rituximab and NIAT groups, respectively
167 (p=0.0284). Median time to remission was 7.0 IQR= [5.5; 10.5] months (n=24) and 7.0 [4.0;
168 13.0] months (n=13) in the NIAT-rituximab and NIAT groups, respectively.
169 Protein/creatinine ratio was lower in the NIAT-rituximab group (2194.8 [1309.8; 5310.0]
170 mg/g) than in the NIAT group (4701.1; [2027.8; 8265.3], p=0.0232), while serum albumin
171 level was higher (32 [26; 35] vs 27 [20; 30] g/L, p=0.0337). Serum creatinine and eGFR by
172 MDRD formula did not differ between the 2 groups (Table 3). In multivariate analyses,
173 KDIGO remission was associated with PLA2R-Ab level <275 RU/mL at baseline (OR 3.5,
174 95% CI 1.1-10.7; p=0.0296), and this was independent from treatment group, age, gender,
175 baseline proteinuria, serum albumin and creatinine (Supplemental Table S3). KDIGO
176 remission was also associated with composite end point at month 6 (OR 30.1, 95% CI 3.9-
177 262.8; p= 0.0012), regardless of treatment group. In the NIAT-rituximab group, CD19 counts
178 at months 3 and 6 were not associated with remission.

179 *Severe adverse events*. Number of SAEs was comparable in both groups (Table 4). Only
180 one SAE was related to NIAT-rituximab treatment in a patient who developed prostatitis with
181 favourable outcome. In the rituximab group, no leukopenia was observed. Patients received a

premedication protocol with 100 mg of solumedrol, 1 g of paracetamol, and 5 mg of dexchlorpheniramine; no allergic reactions were observed.

DISCUSSION

In the present randomized study, we analysed the effect of rituximab combined with NIAT in patients with PMN and severe nephrotic syndrome which had resisted maximally tolerated anti-proteinuric therapy. The RCT showed that compared to NIAT alone, addition of two infusions of rituximab to NIAT decreased PLA2R-Ab as early as month 3 and was associated with a higher percent increase of serum albumin at month 3 and 6. However, the effect of this combined treatment on the rate of proteinuria remission (primary end point) was not observed during the RCT but was delayed to the post-RCT observational period (median time to remission, 7 months). The trial thus provides new biomarkers of early treatment response.

We compared NIAT-rituximab to NIAT because there was no evidence-based proof of the efficacy of rituximab in PMN even if several non-randomized studies suggested that rituximab was efficient[18-21]; the possibility of bias linked to a high rate of late spontaneous remissions as confirmed in the present study, called for a randomized trial. With this aim in mind, an ideal trial would have been a prolonged trial for more than one year. However, we considered unethical to maintain the patients on NIAT for more than 6 months. Since no major complication occurred in the NIAT group, the risk taken was acceptable although after 6 months, PLA2R-Ab positive patients had a markedly higher antibody titre in the NIAT group than in the NIAT- rituximab group, and it is uncertain whether a delay by one year in the NIAT group would impact any future response to immunosuppressive agents or to rituximab therapy. Alternatively, we could have set the end point at 12 months with pre-specified measures in the most aggressive forms for the patients in the NIAT group. However, this protocol would have assessed a global treatment strategy (NIAT +/- retreatment, and

NIAT+ rituximab +/- retreatment) and not only the efficacy of rituximab added to supportive therapy. We therefore opted for a pragmatic approach with a first 6-month period of RCT followed by an observational phase.

This RCT failed to reach the primary end point. The lack of effect of NIAT-rituximab on the rate of proteinuria remission at 6 months has several explanations: i) the high rate of remission (21%) in the NIAT group, ii) the lower rate of remission (35%) in the NIAT-rituximab group than we expected because sample size was calculated from initial studies on rituximab,[17, 18, 25] which probably overestimated the rate of remission in the NIAT-rituximab group and led to a lack of power; iii) the short duration of the RCT phase, and iv) the fact that proteinuria is a delayed marker of treatment effect.[20, 21, 23]

However, we did observe an effect of rituximab on serum albumin variation from baseline (increase) and PLA2R-Ab levels as early as month 3, which was confirmed at month 6. The increase from baseline of serum albumin contrasting with persisting high-level proteinuria at month 6 in the NIAT-rituximab group might be related to decreased tubular reabsorption of albumin when serum albumin increases, [26] and/or increased albumin anabolic rate in the liver resulting in increasing protein load to the glomerulus, which would offset the improving glomerular sieving function.[27] We thus considered a post-hoc composite end point with the aim to define an early clinical criterion of response to rituximab, which associated a reduction of proteinuria > 50% and an increase of serum albumin level > 30% at month 6. A significantly higher number of patients treated with NIAT-rituximab compared with NIAT reached this composite end point at month 6. Moreover, remission defined on composite end point at month 6 was associated with proteinuria remission occurring at any time before any change of initially assigned treatment. This composite end point might therefore better reflect early renal outcome although it should be validated in further studies.

We continued to follow the patients during a post-RCT observational phase. The suspected beneficial clinical effect of rituximab at month 6 was confirmed by the data of the observational phase which were recorded before any potential modification of treatment assigned at randomization. Proteinuria remission rate was substantially higher in the NIAT-rituximab group than in the NIAT alone group (64.9 vs. 37.5%), with proteinuria dropping to a much lower level in the NIAT-rituximab group.In the patients treated with NIAT-rituximab, proteinuria remission rate and median time to remission (7 months) during the follow-up study were similar to those reported in previous non randomized series.[20, 21] Remission rate was comparable to the one achieved with the Ponticelli's protocol in the same time frame (50% at 6 months,[8] 32% within one year,[9]) considering that in those studies, patients were enrolled without a run-in period. It was lower than in patients treated with cyclosporine[13] and tacrolimus[14] who had a remission rate of 75% after 26 weeks and 58% after 6 months, respectively, but baseline serum albumin was higher by >0.5 g/dl than in our study and these drugs are known to have an effect on glomerular hemodynamics with a high risk of relapse at discontinuation and to be associated with a clinically relevant nephrotoxicity. The ongoing Membranous Nephropathy Trial of Rituximab (MENTOR, ClinicalTrials.gov number, NCT01180036) will hopefully show whether rituximab is superior to cyclosporine in term of proteinuria remission over a 24-month period. The high rate of spontaneous remission that may occur more than a year after disease onset in our study and the relatively low rate of remission with NIAT-rituximab, as with other immunosuppressive treatments, indicate that we have not yet reached an optimal treatment in patients with persisting nephrotic syndrome. It is difficult to extrapolate what would be the remission rate with rituximab only since all patients with persisting nephrotic syndrome are treated with NIAT according to KDIGO recommendations.

The present trial has several strengths. First, it is the first RCT in PMN patients with a monitoring of PLA2R-Ab, detected in 71 % of the patients as in previous studies[28], and of THSD7A-Ab.[29] Rituximab associated with NIAT reduced median PLA2R-Ab titre as early as month 3 and induced complete immunological remission in 56% and 50% of the patients at months 3 and 6, respectively. Multivariate analyses showed that PLA2R-Ab <275RU/mL at baseline was the only factor associated with remission occurring at month 6 (primary end point) and during the post-RCT observational phase without modification of treatment assigned at randomization, regardless of treatment group and other adjustment variables. Our results also suggest that THSD7A-Ab may be useful for the monitoring of MN patients. On the other hand, our univariate and multivariate analyses failed to identify classical predictors of long-term outcome and proteinuria remission such as proteinuria, serum creatinine and eGFR, serum albumin, age, and gender. A possible reason is that we studied a relatively homogeneous population after a 6-month run-in period of maximally tolerated conservative therapy. This discrepant observation gives even more importance to PLA2R-Ab as a predictor of proteinuria remission, in agreement with the auto-immune nature of the disease. The finding that PLA2R-Ab positivity and titer tended to decrease as early as 8 days in the NIAT-rituximab group was somewhat unexpected given the half-life of immunoglobulins of about 3 weeks, but confirmed previous observations by Hoxha et al.[30] This suggests that PLA2R-Ab might be a very early biomarker of rituximab efficacy although this has to be confirmed in further prospective studies.

Second, this trial shows that B-cell counts in rituximab treated subjects do not predict proteinuria remission and confirms that PLA2R-Ab depletion rather than $CD20^+$ depletion, achieved in all patients, matters for prediction of rituximab response.[23]It does not tell us, however, whether the absence of immunological remission at 3 months is due to lack of efficacy of rituximab or insufficient dosage, and whether patients without antibody depletion

at 6 months should be re-infused or shifted to a new-generation anti-CD20 antibody or to another immunosuppressant.

Third, we made the important observation that two infusions of rituximab were not associated with an increased risk of SAEs, which differs from all the other current immunosuppressive therapies for PMN.

The present trial has certain limitations. First, 11 patients among the 24 patients who entered remission in the NIAT-rituximab group reached the primary end point lately during the post-RCT observational study, as compared to only 4 in the NIAT group. However, the observational nature of the data does not provide similar strength of evidence than those provided by the randomized period. Second, one cannot exclude that some patients without circulating PLA2R-Ab at treatment onset had still PLA2R-related PMN. This question could be addressed by analysis of kidney biopsies,[31] which was not possible in this multicentric trial. Third, the trial was not blinded. However, data assessors were blinded to treatment allocation and SAEs were monitored by an independent organization. Fourth, the trial was too short to determine whether the relapse rate was influenced by immunosuppressive treatment. Most of the remissions were partial. Since relapses of nephrotic syndrome and disease progression are more frequent in patients with partial remission, long-term studies with rituximab should be advocated.

In conclusion, this trial shows that serum albumin and PLA2R-Ab levels are early markers of NIAT-rituximab efficacy, while the effect on proteinuria remission appears after 6 months. Addition of rituximab to NIAT has no impact on safety. This first RCT is a further step toward the use of rituximab as first-line therapy in severe forms of PMN. It also suggests that criteria for definition of remission should include serum albumin and PLA2R-Ab levels, particularly in trials where rapid responses on drug efficacy and surrogate criteria are needed.

CONCISE METHODS

Study design

Patients were enrolled at 31 hospital nephrology units throughout France in the present multicentre, open-labelled, randomized, controlled trial. After a run-in period of 6 months during which patients were treated with maximal tolerated dose of Non Immunosuppressive Antiproteinuric Treatment (NIAT, angiotensin-converting enzyme inhibitors and/or angiotensin-2 receptor blockers, diuretics and statin), patients were randomly assigned to 6-month therapy with NIAT plus rituximab or NIAT alone (Figure 3). The NIAT group was aimed to determine the percentage of non immunosuppressant-induced remissions, which was known to be high during the first 12 months.[6] We deliberately opted for a short trial of 6 months to avoid any loss of a chance of receiving an immunosuppressive treatment in patients who only received a supportive treatment. After the end of the randomized phase, patients were followed up to 24 months during a post-RCT observational phase. The study was approved by an institutional review board in Paris, France (Comité de Protection des Personnes Ile-de-France XI).

Patients

Eligible patients were 18 years of age or older, had a biopsy proven diagnosis established less than 2 years before inclusion, a urinary protein excretion or a urinary protein/creatinine ratio greater than, or equal to, 3.5 g/day or 3500 mg/g, respectively, and a serum albumin lower than 30 g/l for at least 6 months despite maximal tolerated dose of Non Immunosuppressive Antiproteinuric Treatment (NIAT, angiotensin-converting enzyme inhibitors and/or angiotensin-2 receptor blockers, diuretics and statin). Proteinuria was measured repeatedly before inclusion and treatment assignment to confirm persistence of full-blown nephrotic syndrome. The estimated GFR by MDRD formula had to be above 45 ml/min/1.73m^2.

Exclusion criteria were secondary MN, pregnancy, breast-feeding, immunosuppressive treatment in the three preceding months, and active infectious disease. Hepatitis B serology included Hbs antigen, and Hbs and Hbc antibodies. Patients with active hepatitis B and those with past hepatitis B infection without anti-Hbs antibodies were excluded. Four patients had previously received chemotherapy according to Ponticelli's protocol: one in the NIAT-rituximab group had chemotherapy completed 13 months before inclusion; three in the NIAT group had chemotherapy completed 8 months, 2.5 and 6 years, respectively, before inclusion. After 12 months, we deleted the time limit for the kidney biopsy and we decreased the eGFR threshold down to 30 ml/min/1.73m² to improve recruitment. Sixty-nine patients had a renal biopsy taken less than 2 years before inclusion. In the 5 remaining patients, the renal biopsy was taken 25, 26, 28, 41, 78 months, respectively, before inclusion. Seven patients had an eGFR ≤ 45 and > 30 ml/min/1.73 m². All patients gave written informed consent.

Procedures and follow-up

Patients received NIAT in association with 375 mg/m² of intravenous rituximab on days 1 and 8 following randomization, or NIAT alone (Figure 3). We selected this dosing schedule on the basis of previous reports of rituximab's ability to induce proteinuria remission and CD19 depletion.[18, 24] At the end of the 6-months randomized phase, referring physicians were free of re-infusing rituximab or shifting immunosuppressant, and introducing an immunosuppressant, in patients of the NIAT-rituximab and NIAT groups, respectively, and patients were followed up to 24 months during an observational period. The same antiproteinuric drugs were allowed before and after randomization.

Study visits occurred at baseline, at weeks 1 and 2, and at months 3 and 6 during the RCT. At each study visit, clinical data and medications were recorded. Blood and urine samples were collected at baseline, months 3 and 6 for serum creatinine, serum albumin and

proteinuria over creatinine ratio or proteinuria excretion per day. PLA2R-Ab was measured at baseline, day 8, and months 3 and 6. CD19+ B-lymphocyte counts were measured at months 3 and 6 in the NIAT-rituximab group.

During the post-RCT observational phase, visits occurred according to the habits of the clinician in charge. Proteinuria, serum albumin, serum creatinine, and immunosuppressive treatment modifications, were collected.

Data were collected in each of the 31 hospital nephrology units in a paper case report form and entered in a database located at URCEST, an external and independent organization.

Outcomes

The primary end point was the percentage of patients with complete or partial remission of nephrotic syndrome at 6 months of follow-up. Remission was defined accordingly to 2012 KDIGO[15] as 1) complete in case of urinary protein excretion less than 500 mg per day or 500 mg/g creatinine; 2) partial in case of urinary protein excretion < 3.5 g per day or 3500 mg/g creatinine and ≥ 500 mg/g creatinine with at least 50% reduction compared to baseline. Secondary end points were rate of proteinuria, serum albumin, serum creatinine, PLA2R-Ab levels and SAEs. PLA2R-Ab was measured by using a quantitative ELISA (EuroImmune AG, Lübeck, Germany); anti-thrombospondin domain 7A antibodies (THSD7A-Ab) were assessed by an immunofluorescence test (EuroImmune). Antibody depletion was defined as complete disappearance of antibodies in PLA2R-Ab positive patients. Because albumin level may be an earlier marker of response than end points defined by proteinuria only,[23, 27] we also considered a post-hoc composite end point defined as reduction of proteinuria > 50% and increase of serum albumin level > 30% at month 6 follow-up.

Adverse events and unexpected changes in clinical or laboratory parameters were reported in patient case report forms and monitored up to complete resolution. All SAEs were monitored by URCEST and reported to the sponsor.

During the observational phase, remission defined according to KDIGO and other variables were recorded before potential modification of treatment assigned at randomization, i.e. before any amendment of initial immunosuppressive treatment in the NIAT-rituximab group or addition of an immunosuppressive treatment in the NIAT group. Follow-up was too short to record relapses.

Statistical Analyses

Based on previous studies,[17, 18, 25] rituximab was effective in decreasing proteinuria as early as 3 months[21, 30] and achieving remission at 20 weeks[17] to one year[18, 25] in 60% to 80% of patients with PMN and nephrotic syndrome persisting after 6 months of supportive therapy. The trial was designed to establish whether rituximab was superior in term of efficacy as assessed by the number of remissions. Assuming a remission rate of 20% in the NIAT group, the inclusion of 80 patients would provide 80% power at two-sided α of 0.05 to detect a 30% absolute increase in the remission rate (50%) and under assumption of 10% exclusion or dropout rates (Fisher exact test).

Baseline characteristics of the study population were expressed as frequency and percentage for qualitative variables and as median and interquartile range (IQR) for continuous variables. Remission rates were expressed as frequency and percentage and its 95% confidence interval. All PLA2R-Ab titres not achieving the 14 RU/ml detection threshold of the method were spiked at 0. PLA2R-Ab titre was considered as a continuous variable, as a binary variable (absence/presence), or at baseline only, as a categorical variable according to tertiles (<22.5, lowest; 22.5-275.5, middle; ≥275.5, highest, RU/ml). Because at

univariate analysis, tertiles 1 and 2 did not show any statistically significant difference, a binary variable was created (highest versus middle/lowest). Quantitative variables were compared by a Student's t-test or a Wilcoxon rank-sum test, and categorical variables were compared by a Pearson's Chi-square test or a Fisher's exact test.

Sensitivity analyses were performed to check the impact of replacement methods of missing values with missing data considered as 1) success (remission) in the NIAT group and as failure (no remission) in the NIAT-rituximab group; 2) failure in the NIAT group and as success in the NIAT-rituximab group. Additional analyses were performed with missing data being replaced by last available data (proteinuria at 3 months) and with available data under the hypothesis of data missing completely at random (MCAR).

Additional logistic regression analyses were performed to identify potential prognostic factors of remission. Following variables of interest were analysed in univariate and multivariate analysis: treatment, age, gender, proteinuria, serum creatinine, serum albumin at baseline, and presence of PLA2R-Ab at baseline. In other exploratory analyses, mean percent changes from baseline of proteinuria, serum albumin and PLA2R-Ab levels at months 3 and 6 were plotted and compared using Wilcoxon matched-pair signed rank test.

Statistical analyses were performed blinded to treatment allocation, based on intention-to-treat, including all patients who received at least one dose of treatment and without consent withdrawal. Safety population was defined as patients who received at least one dose of treatment.

All tests were two-sided and p values <0.05 were considered to indicate statistical significance, except when Bonferroni correction was applied and mentioned. SAS V.9.3 software (SAS Institute, Cary, North Carolina, USA) was used for statistical analyses.

Findings from the trial are described in accordance with Consolidated Standards of Reporting Trials (CONSORT) guidelines (www.consort-statement.org). The trial was registered as GEMRITUX Clinical Trials.gov number, NCT01508468.

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Disclosures

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REFERENCES

1. Ponticelli C, Glassock RJ. Glomerular diseases: Membranous nephropathy- A modern view. *Clin J Am Soc Nephrol* 9: 609-616, 2014
2. Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ: M-type phospholipase A2 receptor as target antigen in idiopathic MN. *N Engl J Med* 361: 11-21, 2009
3. Hofstra JM, Fervenza FC, Wetzels JF: Treatment of idiopathic membranous nephropathy. *Nat Rev Nephrol* 9: 443-458, 2013
4. Cravedi P, Remuzzi G, Ruggenenti P: Rituximab in primary membranous nephropathy, first-line therapy, why not? *Nepron Clin Prat* 128:261-269, 2014
5. Schieppati A, Mosconi L, Perna A, Mecca G, Bertani T, Garattini S, Remuzzi G: Prognosis of untreated patients with idiopathic membranous nephropathy. *N Engl J Med* 329: 85-89, 1993

476 6. Polanco N, Gutiérrez E, Covarsí A, Ariza F, Carreño A, Vigil A, Baltar J, Fernández-
477 Fresnedo G, Martín C, Pons S, Lorenzo D, Bernis C, Arrizabalaga P, Fernández-Juárez G,
478 Barrio V, Sierra M, Castellanos I, Espinosa M, Rivera F, Oliet A, Fernández-Vega F,
479 Praga M; Grupo de Estudio de las Enfermedades Glomerulares de la Sociedad Española de
480 Nefrología : Spontaneous remission of nephrotic syndrome in idiopathic membranous
481 nephropathy. *J Am Soc Nephrol* 21:697-704, 2010
482 7. Ponticelli C, Zucchelli P, Passerini P, Cagnoli L, Cesana B, Pozzi C, PasqualiS, Imbasciati
483 E, Grassi C, Redaelli B: A randomized trial of methylprednisolone and chlorambucil in
484 idiopathic membranous nephropathy. *N Engl J Med* 320:8-13, 1989
485 8. Ponticelli C, Altieri P, Scolari F, Passerini P, Roccatello D, Cesana B, Melis P, Valzorio B,
486 Sasdelli M, Pasquali S, Pozzi C, Piccoli G, Lupo A, Segagni S, Antonucci F, Dugo M,
487 Minari M, Scalia A, Pedrini L, Pisano G, Grassi C, Farina M, Bellazzi R : A randomized
488 study comparing methylprednisolone plus chlorambucil versus methylprednisolone plus
489 cyclophosphamide in idiopathic membranous nephropathy. *J Am Soc Nephrol* 9:444-450,
490 1998
491 9. Jha V, Ganguli A, Saha TK, Kohli HS, Sud K, Gupta KL, Joshi K, Sakhuja V : A
492 randomized, controlled trial of steroids and cyclophosphamide in adults with nephrotic
493 syndrome caused by idiopathic membranous nephropathy. *J Am Soc Nephrol* 18:1899-
494 1904, 2007
495 10. Howman A, Chapman TL, Langdon MM, Ferguson C, Adu D, Feehally J, Gaskin GJ,
496 Jayne DR, O'Donoghue D, Boulton-Jones M, Mathieson PW : Immunosuppression for
497 progressive membranous nephropathy: a UK randomized controlled trial. *Lancet* 381:744-
498 751, 2013

11. Chen Y, Schieppati A, Cai G, Chen X, Zamora J, Giuliano GA, Braun N, Perna A: Immunosuppression for membranous nephropathy: a systematic review and meta-analysis of 36 clinical trials. *Clin J Am Soc Nephrol* 8:787-796, 2013
12. Cattran DC, Greenwood C, Ritchie S, Bernstein K, Churchill DN, Clark WF, Morrin PA, Lavoie S : A controlled trial of cyclosporine in patients with progressive membranous nephropathy. Canadian Glomerulonephritis Study Group.*Kidney Int* 47:1130-1135, 1995
13. Cattran DC, Appel GB, Hebert LA, Hunsicker LG, Pohl MA, Hoy WE, Maxwell DR, Kunis CL; North America Nephrotic Syndrome Study Group : Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. *Kidney Int* 59:1484-1490, 2001
14. Praga M, Barrio V, Juárez GF, Luño J, Grupo Españolde Estudio de la Nefropatía Membranosa: Tacrolimus monotherapy in membranous nephropathy: a randomized controlled trial. *Kidney Int* 71: 924-930, 2007
15. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int* Suppl.2: 186-197, 2012
16. Van den Brand JA, van Dijk PR, Hofstra JM, Wetzels JF: Long-term outcomes in idiopathic membranous nephropathy using a restrictive treatment strategy. *J Am Soc Nephrol* 25: 150-158, 2014
17. Remuzzi G, Chiurchiu C, Abbate M, Brusegan V, Bontempelli M, Ruggenenti P: Rituximab for idiopathic membranous nephropathy. *Lancet* 360: 923-924, 2002
18. Fervenza FC, Cosio FG, Erickson SB, Specks U, Herzenberg AM, Dillon JJ, Leung N, Cohen IM, Wochos DN, Bergstralh E, Hladunewich M, Cattran DC: Rituximab treatment of idiopathic membranous nephropathy. *Kidney Int* 73: 117-125, 2008

19. Bomback AS, Derebail VK, McGregor JG, Kshirsagar AV, Falk RJ, Nachman PH: Rituximab therapy for membranous nephropathy: a systematic review. *Clin J Am Soc Nephrol* 4: 734-744, 2009

20. Fervenza FC, Abraham RS, Erickson SB, Irazabal MV, Eirin A, Specks U, Nachman PH, Bergstralh EJ, Leung N, Cosio FG, Hogan MC, Dillon JJ, Hickson LJ, Li X, Cattran DC; Mayo Nephrology Collaborative Group: Rituximab therapy in idiopathic membranous nephropathy: a 2-year study. *Clin J Am Soc Nephrol* 5: 2188-2198, 2010

21. Ruggenenti P, Cravedi P, Chianca A, Perna A, Ruggiero B, Gaspari F, Rambaldi ,A, Marasà M, Remuzzi G:Rituximab in idiopathic membranous nephropathy. *J Am Soc Nephrol* 23: 1416-1425, 2012

22. Beck LH Jr, Fervenza FC, Beck DM, Bonegio RG, Malik FA, Erickson SB, Cosio FG, Cattran DC, Salant DJ : Anti-PLA_2R autoantibodies and response to rituximab treatment in membranous nephropathy. *J Am Soc Nephrol* 22: 1543-1550, 2011

23. Ruggenenti P, Debiec H, Ruggiero B, Chianca A, Pellé T, Gaspari F, Suardi F, Gagliardini E, Orisio S, Benigni A, Ronco P, Remuzzi G: Anti-PLA2R antibody titer predicts post-rituximab outcome of membranous nephropathy. *J Am SocNephrol* 26:2545-2558, 2015

24. Cravedi P, Ruggenenti P, Sghirlanzoni MC, Remuzzi G: Titrating rituximab to circulating B cells to optimize lymphocytolytic therapy in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol* 2:932-937, 2007

25. Ruggenenti P, Chiurchiu C, Brusegan V, Abbate M, Perna A, Filippi C, Remuzzi G : Rituximab in idiopathic membranous nephropathy: a one-year prospective study. *J Am Soc Nephrol* 14: 1851-1857, 2003

26. Wagner MC, Campos-Bilderback SB, Chowdhury M, Flores B, Lai X, Myslinski J, Pandit S, Sandoval RM, Wean SE, Wei Y, Satlin LM, Wiggins RC, Witzmann FA, Molitoris BA:

547 Proximal tubules have the capacity to regulate uptake of albumin. *J Am Soc Nephrol*
548 27:482-494, 2016
549 27. Ruggenenti P, Mise N, Pisoni R, Arnoldi F, Pezzotta A, Perna A, Cattaneo D, Remuzzi G:
550 Diverse effects of increasing lisinopril doses on lipid abnormalities in chronic
551 nephropathies. *Circulation* 107: 586-592, 2003
552 28. Du Y, Li J, He F, Lv Y, Liu W, Wu P, Huang J, Wei S, Gao H : The diagnosis accuracy of
553 PLA2R-AB in the diagnosis of idiopathic membranous nephropathy: a meta-analysis.
554 *PLoS One* 9: e104936, 2014
555 29. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, Dolla G,
556 Hoxha E, Helmchen U, Dabert-Gay AS, Debayle D, Merchant M, Klein J, Salant DJ, Stahl
557 RA, Lambeau G : Thrombospondin type-1 domain-containing 7A in idiopathic
558 membranous nephropathy. *N Engl J Med* 371: 2277-2287, 2014
559 30. Hoxha E, Harendza S, Zahner G, Panzer U, Steinmetz O, Fechner K, Helmchen U, Stahl
560 RA : An immunofluorescence test for phospholipase A_2-receptor antibodies and its clinical
561 usefulness in patients with membranous glomerulonephritis. *Nephrol Dial Transplant*
562 26:2526-2532, 2011
563 31. Debiec H, Ronco P: PLA2R autoantibodies and PLA2R glomerular deposits in
564 membranous nephropathy. *N Engl J Med* 364: 689-690, 2011

TABLES

Table 1. Baseline characteristics

	NIAT-rituximab Group (N =37)	**NIAT Group (N=38)**	**Total (N=75)**
Age — yr	53.0 (42.0 ; 63.0)	58.5 (43.0; 64.0)	56 (42.0; 64.0)
Male sex — no. (%)	28 (75.7)	24 (63.2)	52 (69.3)
Weight — kg	76.0 (70.0 ; 85.0)	76.5 (67.0; 85.0)	76.0 (67.0; 85.0)
Blood pressure — mmHg			
Systolic	124 (110 ; 140)	125 (117; 140)	125 (115; 140)
Diastolic	77 (68 ; 82)	76 (70 ; 81)	76 (70 ; 81)
Creatinine— µmol/L	98.1 (73.4 ; 122.9)	91.1 (74.3; 122.0)	93.8 (76.9; 122.9)
eGFR — ml/min/1.73 m²	66.7 (55.4 ; 82.5)	72.7 (58.1; 88.6)	68.6 (55.4 ; 88.6)
Protein/creatinine ratio — mg/g	7680.0 (4584.3 ; 10399.0)	7195.1 (5363.1; 8965.1)	7363.2 (4702.9 ; 9735.0)
Albumin level — g/L	22 (18; 25)	22 (20; 26)	22 (19; 26)
Median time since biopsy-proven diagnosis — months	8 (6; 13)	8 (6; 11)	8 (6 ; 13)
PLA2R-Ab positive patients (ELISA) — no. (%)	27 (73.0)	28 (73.7)	55 (73.3)
PLA2R-Ab titre (ELISA) — RU/ml*	40.5 (0.0; 275.5)	43.3 (0.0; 457.5)	40.5 (0.0 ; 440.9)
Diuretics — no. (%)	32 (86.5)	32 (84.2)	64 (85.3)

ACE inhibitors and/or ARB — no. (%)			
ACE inhibitors	16 (43.2)	14 (38.9)	30 (41.1)
ARB	12 (32.4)	8(22.2)	20 (27.4)
ACE inhibitor and ARB	9 (24.3)	14(38.9)	23 (31.51)
Statins — no. (%)	31 (83.8)	26 (68.4)	57 (76.0)

Data are n (%), median (IQR) * Median and IQR of PLA2R-Ab titre in all patients with and without PLA2R-Ab. ACE, angiotensin converting enzyme; ARB, angiotensin-2 receptor blocker; ELISA, enzyme-linked immunosorbent assay; eGFR, estimated glomerular filtration rate (calculated according to the Modification of the Diet in Renal Disease equation); NIAT Non Immunosuppressive, Antiproteinuric Treatment.

Table 2: Efficacy outcome variables

	NIAT-rituximab group (N=37)	NIAT group (N=38)	P Value
Remission (complete and partial remission*)	13 (35.1 [19.7; 50.5])	8 (21.1 [8.1; 34.0])	0.2055
Protein/creatinine ratio — mg/g			
Baseline	7680.0 (4584.3 ; 10399.0)	7195.1 (5363.1; 8965.1)	
3 months	4814.4 (3205.5; 7398.6)	4832.1 (2424.9; 7911.9)	0.9418#
6 months	3531.2 (1796.6 ; 6469.4)	5265.8 (2500.1; 7690.7)	0.1784#
Serum albumin level - g/L			
Baseline	22 (18; 25)	22 (20 ; 26)	
3 months	27 (21 ; 31)	23 (19 ; 27)	0.0991#
6 months	30 (26 ; 34)	24 (20 ; 29)	0.0288#
Serum creatinine - µmol/L			
Baseline	98.1 (82.2 ; 122.9)	91.1 (74.3; 122.0)	
3 months	94.6 (78.7 ; 114.0)	100.8 (81.3 ; 115.8)	0.8795#
6 months	94.6 (75.1 ; 130.8)	97.2 (76.0 ; 126.4)	0.6705#
eGFR — ml/min/1.73 m²			

Baseline	66.7 (55.4 ; 82.5)	72.7 (58.1; 88.6)	
3 months	66.7 (57.2 ; 87.1)	68.9 (45.7 ; 89.7)	0.9463[#]
6 months	65.6 (51.0 ; 89.0)	72.5 (52.4 ; 89.7)	0.7463[#]
PLA2R-Ab pos. patients-(ELISA)			
Baseline	27 (73.0)	28 (73.7)	
Day 8	18 (52.9)	17 (68.0)	0.2446[§]
3 months	11 (31.4)	25 (83.3)	**<0.0001**[§]
6 months	13 (36.1)	24 (75.0)	**0.0013**[§]
PLA2R-Ab depleted pts			
3 months	14/25 (56.0)	1/23 (4.3)	**0.0001**[#]
6 months	13/26 (50.0)	3/25 (12.0)	**0.0035**[#]
PLA2R-Ab titre - (all pts) - RU/ml			
Baseline	40.5 (0.0 ; 275.5)	43.3 (0.0 ; 457.5)	
Day 8	27.1 (0.0 ; 126.1)	65.5 (0.0 ; 345.5)	0.2354[§]
3 months	0.0 (0.0 ; 49.1)	54.6 (16.5 ; 278.4)	**0.0005**[§]
6 months	0.0 (0.0 ; 34.0)	45.7 (7.6 ; 262.2)	**0.0023**[§]
PLA2R-Ab titre - (positive pts)[**] - RU/ml			
Baseline	102.5 (36.1 ; 672.5)	199.5 (24.2 ; 491.4)	
Day 8	63.2 (12.9 ; 382.0)	163.5 (34.7 ; 438.5)	0.4054[§]

3 months	0.0 (0.0 ; 60.5)	77.5 (30.3 ; 325.9)	**0.0033**§
6 months	8.3 (0.0 ; 73.5)	62.9 (16.6 ; 449.3)	**0.0102**§
Post-hoc composite end point at 6 months	15 (40.5 [24.7; 56.4])	5 (13.2 [2.4; 23.9])	**0.0073**
CD19 (*/mm³)****			
3 months	11 (2.0; 22.0)	NA	
6 months	61 (34.0; 100)	NA	

Data are n (%) or n (% and 95% CI) or medians (interquartile range).

* Complete and partial remission was defined according to 2012 KDIGO criteria based on proteinuria; composite end point was defined as reduction of proteinuria > 50% and increase of serum albumin > 30%. ** Patients with at least one positive detection of PLA2R-Ab at any time. eGFR, estimated glomerular filtration rate (calculated according to the Modification of the Diet in Renal Disease equation); NA, not available; NIAT Non Immunosuppressive, Antiproteinuric Treatment; pos, positive; pts, patients. *** Normal range (100 to 500/mm^3). # P value < 0.025 indicates statistical significance (Bonferroni correction). § P value < 0.017 indicates statistical significance (Bonferroni correction).

Table 3. Results of Efficacy Analysis at Last Follow-up

	NIAT-rituximab group (N=37)	NIAT group (N=38)	P Value
Remission (complete and partial remission*)	24 (64.9 [49.5; 80.2])	13(34.2 [19.1; 49.3])	0.0079
Protein/creatinine ratio — mg/g	2194.8 (1309.8; 5310.0)	4701.1(2027.8; 8265.3)	0.0232
Serum albumin level — g/L	32 (26 ; 35)	27 (20 ; 30)	0.0337
Serum creatinine — µmol/L	101 (87 ; 135)	97.2 (78.5 ; 133.5)	0.5032
eGFR — ml/min/1.73 m²	61.1 (48.7 ; 83.4)	73.1(50.4; 90.5)	0.4785

Data are n (% and 95% CI) or medians (interquartile range). Data were recorded before any potential modification of treatment assigned at randomization (modification of initial immunosuppressive treatment in the NIAT-rituximab group, addition of any immunosuppressive treatment in the NIAT group). * Complete and partial remission was defined according to 2012 KDIGO criteria based on proteinuria.

Table 4. Severe Adverse Events According to Treatment Group.

	NIAT-rituximab Group (N=37)	NIAT group (N=38)	P-value
Number of events			0.8663
0	31	33	
1	5	4	
≥2	1	1	
Event details			
Acute renal failure**	0	2	
Infection			
Prostatitis	1	0	
Pleural effusion**	0	1	
Cardiac and vascular disorders			
Myocardial infarction	1	1	
Critical limb ischemia	0	1	
Mesenteric Ischemia*	1	0	
Carotid endarteriectomy*	1	0	
Aorto Iliac femoral bypass*	1		
Cancer**	0	1	
Others			
Oedema	1	1	
Pain and fever	1	0	
Diarrhea	1	0	
Asthma	0	1	

Data are n. *These SAEs occurred in the same patient. **These SAEs occurred in the same patient.

FIGURES

Figure 1: Trial profile

Premature discontinuation occurred in 5 patients within 3 months after inclusion: (1) 2 remissions at day 1 or inclusion; (2) 1 NIAT for less than 6 months; (3) 1 lost of follow-up, 1 diagnosed with a pulmonary neoplasia. (4) 3 treatment shifts between 3 and 6 months: two received rituximab or steroids because of deterioration of clinical condition, 1 was referred to another centre.

NIAT, Non Immunosuppressive, Antiproteinuric Treatment.

Figure 2: Percent changes in proteinuria, serum albumin and PLA2R-Ab with time

Mean±SEM percent changes from baseline in proteinuria (A), serum albumin (B), and anti-PLA2R-Ab (C) levels. Please note that Figure 3C shows percent changes of PLA2R antibodies in the subset of patients who had PLA2R-Ab at baseline. **P*<0.017, ***P*<0.001, ****P*<0.0001 (Bonferroni correction was applied; p value < 0.017 indicate statistical significance). NIAT (blue line), Non Immunosuppressive, Antiproteinuric Treatment; NIAT-rituximab (red line).

Figure 3: Study design

NIAT, Non Immunosuppressive Antiproteinuric Treatment; R, rituximab.

Figure 1

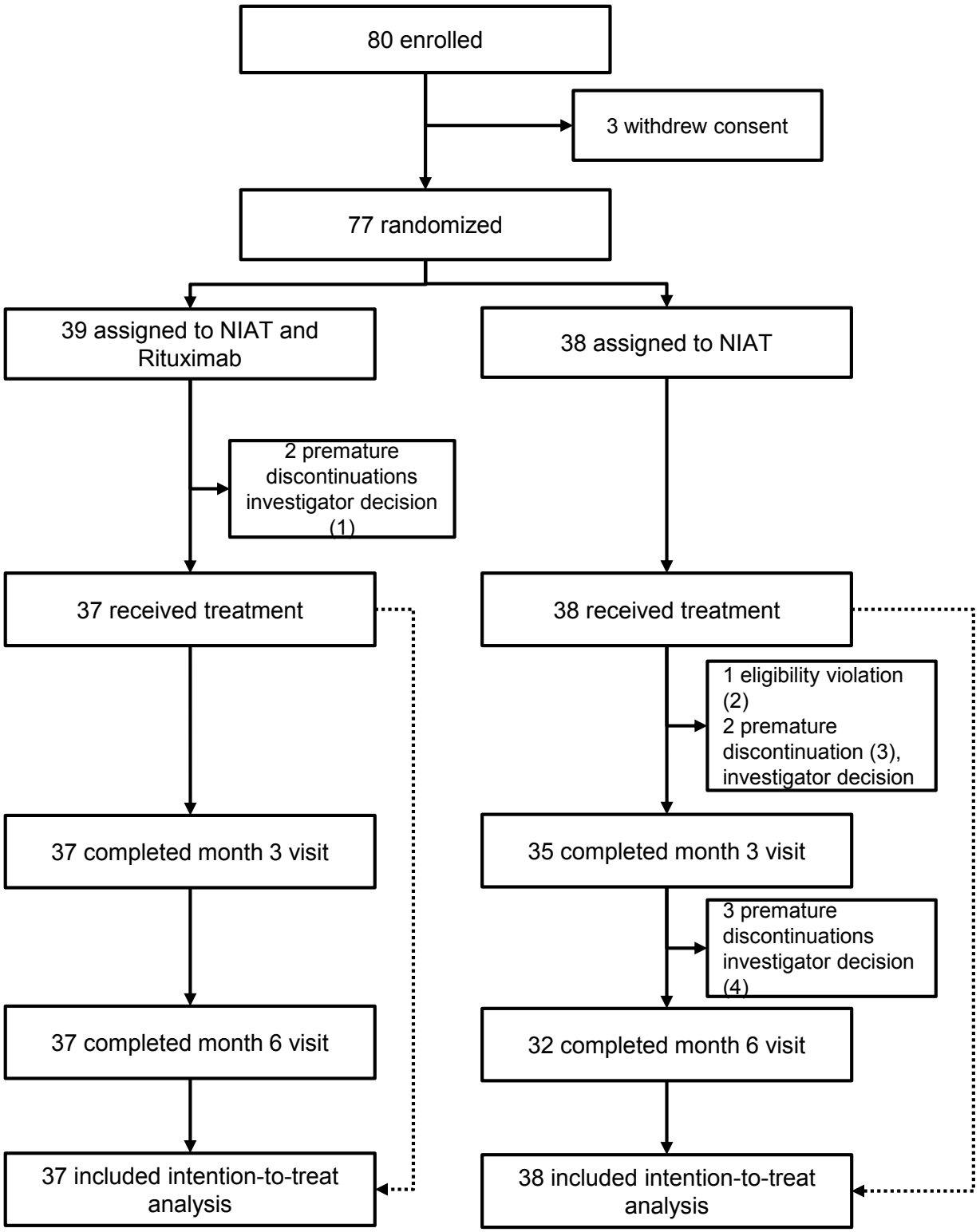
80 enrolled
3 withdrew consent
77 randomized
39 assigned to NIAT and Rituximab
38 assigned to NIAT
2 premature discontinuations investigator decision (1)
37 received treatment
38 received treatment
1 eligibility violation (2)
2 premature discontinuation (3), investigator decision
37 completed month 3 visit
35 completed month 3 visit
3 premature discontinuations investigator decision (4)
37 completed month 6 visit
32 completed month 6 visit
37 included intention-to-treat analysis
38 included intention-to-treat analysis

Figure 2

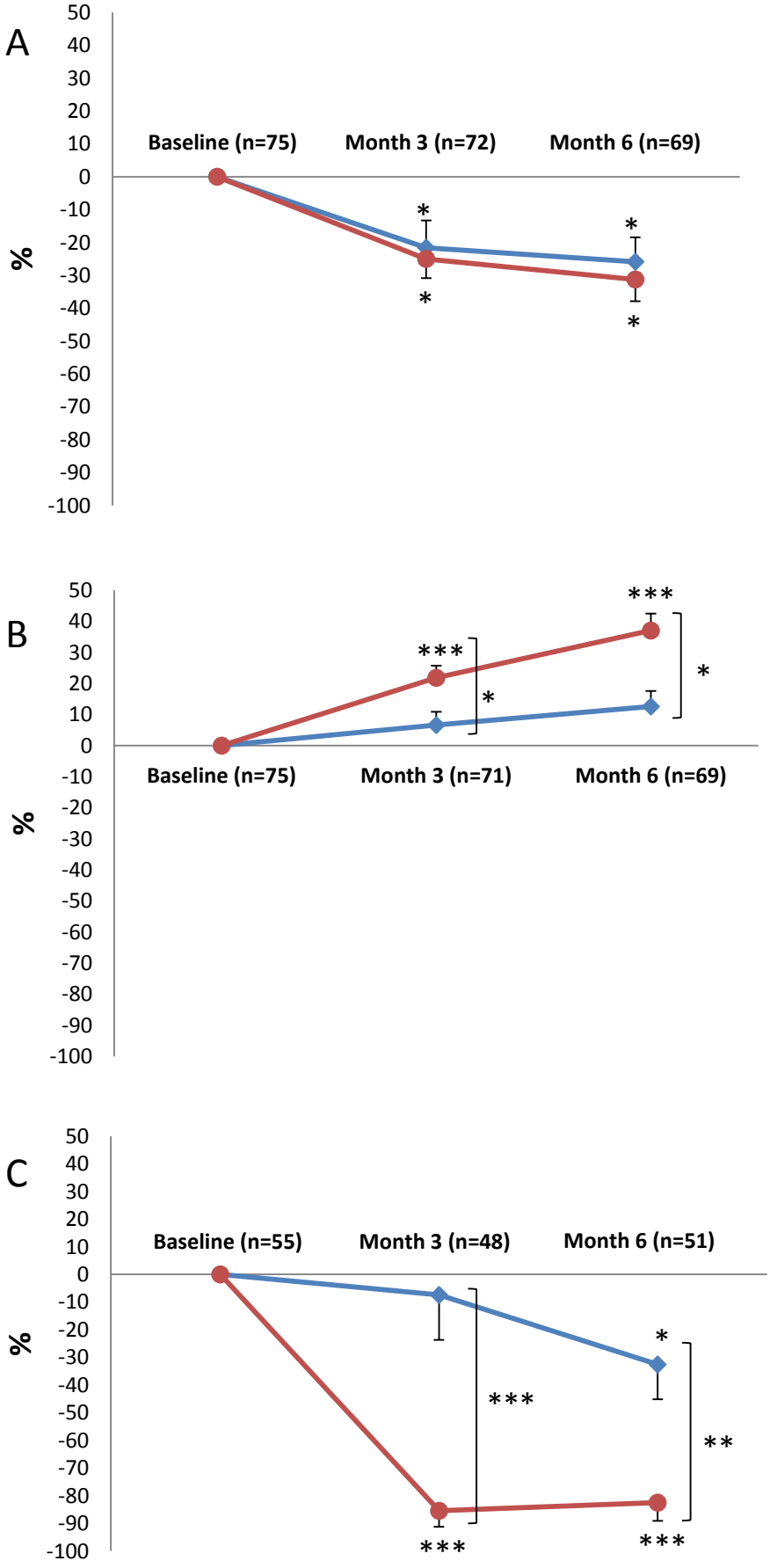
A
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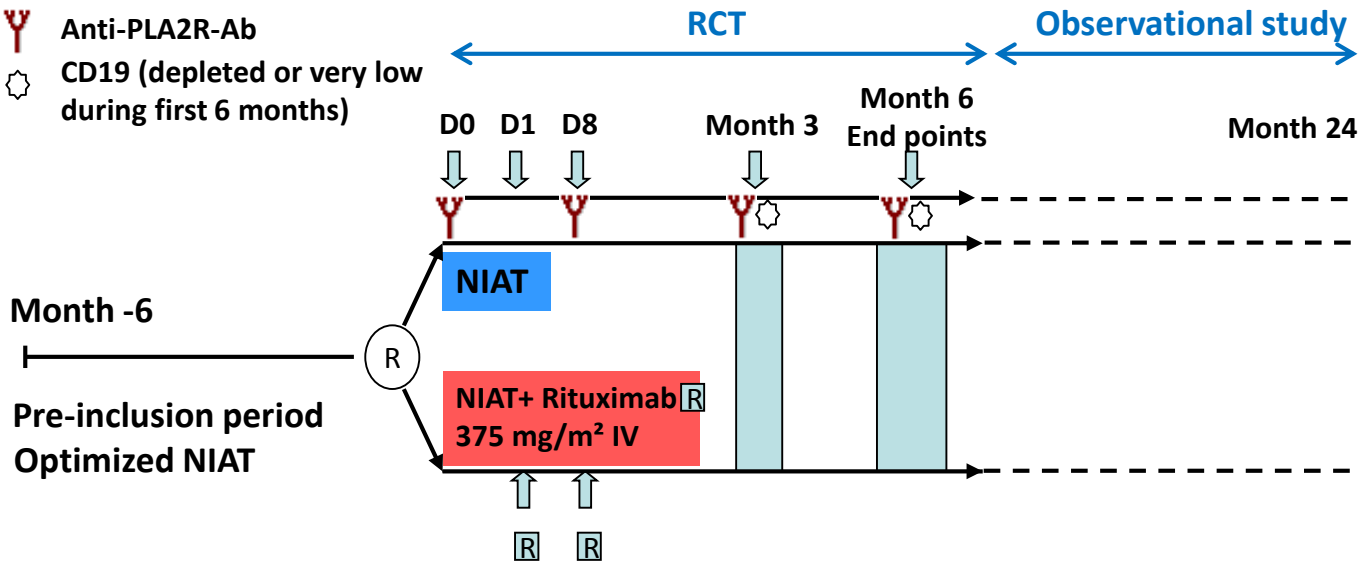
Figure 3


Anti-PLA2R-Ab
CD19 (depleted or very low during first 6 months)
RCT
Observational study
D0
D1
D8
Month 3
Month 6
End points
Month 24
NIAT
Month -6
R
NIAT+ Rituximab R
375 mg/m² IV
Pre-inclusion period
Optimized NIAT
R
R

Supplemental material

1. Clinical trial steering committee

Karine Dahan, Pierre Ronco (Hôpital Tenon, AP-HP, France) ; Tabassome Simon, Alexandra Rousseau, Laura Wakselman (Hôpital Saint-Antoine, AP-HP, France)

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3. Contributors

Karine Dahan and Pierre Ronco were responsible for the study concept, designed the study and wrote the first draft of manuscript. Tabassome Simon participated to the study design, was in charge of the study management, and critically reviewed the manuscript. Laura Wakselman handled logistic and monitoring coordination of the study. Marine Cachanado did statistical analysis and critically reviewed the manuscript. Alexandra Rousseau handled data management and statistical analysis coordination, and participated to the study design and critically reviewed the manuscript. Emmanuelle Plaisier, Pierre-Antoine Michel, Fabrice Mihout, Bertrand Dussol, Marie Matignon, Christiane Mousson collected and interpreted data. Hanna Debiec measured PLA2R-Ab and THSD7A-ab levels. All authors were members

of the writing group and agreed on the content of the report, reviewed drafts, and approved the final version.

4. List of other investigators and members of the GEMRITUX Study Group who participated in the trial (in alphabetical order)

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5. Methods

Randomization

Once full eligibility was confirmed, patients were randomly assigned, in a 1:1 ratio, to receive NIAT plus rituximab or NIAT only for 6 months (Figure 1) by the investigator. Patients were assigned to groups centrally through computer-generated block randomisation (size 4) prepared by URCEST. Data assessors were blinded to treatment allocation and SAEs were monitored by an independent organization.

Role of the funding source

The funder was the French Ministry of health (PHRC, AOM10089), and the sponsor was Assistance Publique –Hôpitaux de Paris. Hoffmann-La Roche provided rituximab for the study. The funders of the study had no role in study design, data analysis, data interpretation or writing the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Anti-PLA2R autoantibody (PLA2R-Ab) evaluation

After sampling, all sera were immediately aliquoted, frozen and stored at –20° C. They were thawed only at the time of ELISA measurements. Previously unfrozen samples were never used for the tests. After thawing, all serum samples were tested for the presence of anti-PLA2R total IgG antibodies using the quantitative ELISA test commercialized by EuroImmune AG (Lübeck, Germany). In brief, sera diluted to 1:100were incubated with PLA2R already coated microplates and detected by incubation with antihuman IgG HRP conjugate. The final concentrations for each sample were calculated from the calibration curve extinction values plotted against the concentration for each calibrator. ELISA cut-off

values were established according to manufacturers' protocol and the results were considered as negative for<14 RU/ml and positive for ≥ 14 RU/ml. The coefficients of variation (CV) were assessed by using 3 selected serum samples covering the measuring range. The intra-assay and inter-assay CVs were based on 20 measurements for each serum in one set or on threefold replica in ten sets, respectively. In our laboratory, the calculated intra andinter-assay CVs are <4% and <9%, respectively. Up to five freeze/thaw cycles were found not to affect PLA2R-Ab binding by ELISA. All sera at the various time points were assessed in triplicates at the same time in the same ELISA run to allow optimal comparisons of antibody titre.

6. Table S1: Lipids, body weight and need for diuretics at baseline and during follow-up in the 2 treatment groups.

	NIAT-rituximab group (N=37)	**NIAT group (N=38)**	**P Value**
Triglycerides—mmol/L			
Baseline	1.9 [1.3 ; 3.0]	2.2 [1.6 ; 3.1]	
3 months	1.9 [1.1 ; 3.1]	2.1 [1.6 ; 3.0]	0.3315
6 months	1.9 [1.3 ; 2.5]	1.8 [1.4 ; 2.6]	0.7682
LDL cholesterol—mmol/L			
Baseline	4.4 [3.3 ; 5.9]	5.3 [3.4 ; 6.9]	
3 months	4.0 [3.4 ; 5.5]	4.9 [3.6 ; 7.2]	0.1835
6 months	3.5 [2.7 ; 4.5]	3.5 [2.9 ; 5.2]	0.6851
Total cholesterol—mmol/L			
Baseline	7.1 [5.5 ; 8.7]	7.5 [6.2 ; 9.5]	
3 months	6.6 [5.6 ; 8.2]	7.4 [5.8 ; 10.5]	0.1894
6 months	5.9 [4.9 ; 6.9]	6.2 [5.4 ; 7.0]	0.4752
Body weight--kg			
Baseline	76.0 (70.0 ; 85.0)	76.5 (67.0; 85.0)	
3 months	76.6 (72.0; 84.0)	76 (65.0;86.0)	0.8574
6 months	78.0 (72.0; 84.0)	77.4 (67.0; 85.0)	0.9490
Diuretics			
Baseline	32(86.5)	32 (84.2)	
3 months	31(83.8)	30 (78.9)	0.5910

6 months	31(83.8)	29(76.3)	0.4189

7. Table S2: Prognosis factors of KDIGO remission at 6 months (end of RCT)

	Complete or Partial remission (n=21/75)			
Characteristics	**Univariate**		**Multivariate**	
	Odds ratio (95% CI)	**P-value**	**Odds ratio (95% CI)**	**P-value**
Treatment (NIAT-rituximab vs. NIAT)	2.0 (0.7 ; 5.7)	0.1781	2.1 (0.7 ; 6.4)	0.2128
Age	1.0 (1.0 ; 1.0)	0.7861	1.0 (1.0 ; 1.1)	0.2845
Female gender	0.6 (0.2 ; 2.0)	0.4243	0.6 (0.2 ; 2.3)	0.4814
Proteinuria	1.0 (1.0 ; 1.0)	0.8046	1.0 (1.0 ; 1.0)	0.8358
Serum albumin	0.7 (0.2 ; 2.0)	0.4691	0.7 (0.2 ; 2.2)	0.4964
Serum creatinine	0.9 (0.8 ; 1.1)	0.3480	0.9 (0.8 ; 1.1)	0.1753
PLA2R-Ab at baseline < 275.5 RU/mL	4.1 (1.1 ; 15.7)	**0.0378**	4.3 (1.1 ; 17.3)	**0.0424**

8. Table S3: Prognosis factors of KDIGO remission without modification of treatment assigned at randomization.

	Complete or Partial remission (n=37/75)			
Characteristics	**Univariate**		**Multivariate**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Treatment (NIAT-Rituximab vs. NIAT)	3.5 (1.7-9.2)	**0.009**	4.1 (1.4 ; 12.2)	**0.0095**
Age	1.0 (1.0-1.0)	0.7914	1.0 (1.0 ; 1.1)	0.6377
Female gender	0.7 (0.3-1.9)	0.5007	1.0 (0.3 ; 3.1)	0.9906
Proteinuria	1.0 (1.0-1.0)	0.2508	1.0 (1.0 ; 1.0)	0.2758
Serum albumin	1.2 (0.5-3.1)	0.6856	1.3 (0.4 ; 3.9)	0.6262
Serum creatinine	1.0 (0.9-1.1)	0.8778	1.0 (0.9 ; 1.1)	0.5060
PLA2R-Ab at baseline < 275.5 RU/mL	3.8 (1.4-10.9)	**0.0110**	3.5 (1.1 ; 10.7)	**0.0296**

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	10, 11, Suppl page 3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	11
Participants	4a	Eligibility criteria for participants	11
	4b	Settings and locations where the data were collected	12
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	11, 12
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	12, 13
	6b	Any changes to trial outcomes after the trial commenced, with reasons	12
Sample size	7a	How sample size was determined	13
	7b	When applicable, explanation of any interim analyses and stopping guidelines	Not applicable
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	Suppl page 3
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	Suppl page 3
Allocation concealment	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Suppl page 3

mechanism			
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Suppl page 3
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	14, Suppl page 3
	11b	If relevant, description of the similarity of interventions	Not applicable
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	13, 14
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	14
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	4 and figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	4, 26, and figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	4
	14b	Why the trial ended or was stopped	
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1, pages 19 and 20
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	14, Tables 1 through 4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	4, 5
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	6, 7
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	7, Table 4
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	10
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	9, 10
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	10
Other information			
Registration	23	Registration number and name of trial registry	3, 14

Protocol	24	Where the full trial protocol can be accessed, if available	Will be made available
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	Suppl page 3

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.