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

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► **To cite this version:**

Karine Dahan, Hanna Debiec, Emmanuelle Plaisier, Marine Cachanado, Alexandra Rousseau, et al.. Rituximab for Severe Membranous Nephropathy: A 6-Month Trial with Extended Follow-Up. *Journal of the American Society of Nephrology*, 2017, 28 (1), pp.348-358. 10.1681/ASN.2016040449 . hal-01490466

HAL Id: hal-01490466

<https://hal.sorbonne-universite.fr/hal-01490466>

Submitted on 15 Mar 2017

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1 **Rituximab for severe membranous nephropathy: a six-month trial with**
2 **extended follow-up**

3

4 **Running title:** Rituximab for membranous nephropathy

5

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28 Word Count (manuscript without abstract, methods, acknowledgement and references): 3188
29 words

30 Word count (abstract): 250

31 Content: 4 tables, 3 figures and Supplemental material

32 This work was presented in part at the meeting of the American Society of Nephrology in
33 San Diego, CA Nov 3-8 th, 2015

34

35 **ABSTRACT (250 words)**

36 There are no randomized trials of rituximab in primary membranous nephropathy (PMN). We
37 did a multicentre, randomized controlled trial at 31 French hospitals (NCT01508468). Patients
38 with biopsy proven PMN and nephrotic syndrome after 6 months of Non Immunosuppressive
39 Antiproteinuric Treatment (NIAT) were randomly assigned to 6-month therapy with NIAT
40 and 375 mg/m² intravenous rituximab on days 1 and 8, or NIAT alone. After 6 months,
41 patients continued observational follow-up. Median time to last follow-up was 17.0 (IQR=
42 [12.5; 24.0]) and 17.0 [13.0; 23.0] months in the NIAT-rituximab and NIAT groups,
43 respectively. Primary outcome was a combined endpoint of complete or partial remission of
44 proteinuria at 6 months. Of 80 patients enrolled, 77 were randomized and 75 received the
45 assigned intervention (37 were given NIAT-rituximab and 38 NIAT alone). At month 6, 13
46 (35.1%, 95% CI 19.7; 50.5) patients in the NIAT-rituximab group and 8 (21.1%, 95% CI 8.1;
47 34.0) in the NIAT group achieved remission (p=0.2055). Rates of PLA2R-Ab depletion were
48 14/25 (56%) and 1/23 (4.3%) at month 3 (p=0.0001), and 13/26 (50%) and 3/25 (12%) at
49 month 6 (p=0.0035), in the NIAT-rituximab and NIAT groups, respectively. Eight SAEs
50 occurred in each group. During the observational phase, remission rate before change of
51 assigned treatment was 24/37 (64.9%) and 13/38 (34.2%) in the NIAT-rituximab and NIAT
52 groups, respectively (p= 0.0079). A positive effect of rituximab on proteinuria remission was
53 delayed after 6 months. PLA2R-Ab levels are early markers of rituximab effect. Addition of
54 rituximab to NIAT has no impact on safety.

55

56 **INTRODUCTION (3188 /3000 words)**

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

59 Thickening of glomerular capillary walls results from subepithelial formation of immune
60 deposits containing IgG, the membrane attack complex of complement, which is the major
61 mediator of proteinuria, and antigens. Primary forms of MN, improperly called primary
62 membranous nephropathy, represent 70% to 80% of all cases. A major breakthrough was the
63 identification of the podocyte antigen PLA2R as the target of circulating antibodies in about
64 70% of PMN, which confirmed that the disease was auto-immune in nature.²

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

77 Both the evidence that B cells play a key role in the pathogenesis of PMN and drug
78 toxicity led to target B-cells with rituximab.¹⁷ Rituximab induced remission of nephrotic
79 syndrome in 60% to 80 % of the patients with long-lasting proteinuria despite blockade of the
80 renin-angiotensin system¹⁸⁻²¹ and in patients who had previously failed other treatments.
81 Reduction of PLA2R-Ab titre preceded remission of proteinuria by several months which
82 suggested a causal relationship.^{22, 23} A previous study showed that a B-cell driven approach

83 with only one or two infusions of Rituximab 375 mg/m² per week could allow reducing cost
84 in comparison with the standard 4 weekly infusions²⁴

85 Because of the lack of randomized controlled trial (RCT) using rituximab and of high rate
86 of spontaneous remission, the present trial was designed in order to evaluate the efficacy of
87 rituximab given to all patients at a standard dose (375 mg/m²) in 2 infusions added to
88 supportive therapy compared to supportive therapy alone, in patients with persistent nephrotic
89 syndrome.

90

91 **RESULTS**

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

97 *Primary end point.* The 6-month trial failed to achieve the primary end point. Thirteen
98 patients (35.1%, 95% CI- 19.7; 50.5) in the NIAT-rituximab group and 8 patients (21.1 %,
99 95% CI- 8.1; 34.0) in the NIAT group achieved proteinuria remission at month 6 following
100 randomization (p= 0.2055), (Table 2; OR 2.0 95% CI 0.7-5.7). Results were not sensitive to
101 missing data replacement.

102 *Secondary end points.* Protein/creatinine ratio decreased similarly in both groups at months
103 3 and 6 (Table 2, Figure 2A). Percent increase of serum albumin was significantly higher at
104 months 3 and 6 in the NIAT-rituximab group (Figure 2B). Serum creatinine and eGFR by
105 MDRD formula, serum triglycerides, total and LDL cholesterol, body weight, and need for
106 diuretic therapy did not differ at months 3 and 6 between the 2 groups (Table 2, Supplemental
107 Table S1).

108 PLA2R-Ab was detected in 27 (73%) and 28 (74%) patients at baseline in the NIAT-
109 rituximab and the NIAT groups, respectively (Table 1). As early as month 3, rate of PLA2R-
110 Ab positive patients (31 % vs. 83 %, $p<0.0001$) and PLA2R-Ab titer (0.0 IQR [0.0; 49.1] vs.
111 54.6 IQR [16.5; 278.4] RU/ml, $p=0.0005$) were lower in the NIAT-rituximab group than in
112 the NIAT alone (Table 2). No further decrease in the rate of PLA2R-Ab positive patients was
113 observed between months 3 and 6, and the difference between PLA2R-Ab titer at month 3
114 and month 6 was 0.0 [0.0; 19.8] in the NIAT-rituximab group. No change in the rate of
115 PLA2R-Ab positive patients and in PLA2R-Ab titer occurred between baseline, and months 3
116 and 6 in the NIAT group (Table 2).

117 In the subgroup of patients who were initially positive for PLA2R-Ab, a significant
118 decrease of the titer of PLA2R-Ab was observed at month 3 (0.0 [0.0; 60.5] RU/ml,
119 $p<0.0001$) and month 6 (8.3 [0.0; 73.5], $p=0.0004$) compared to baseline (102.5 [36.1 ;
120 672.5]) in the NIAT-rituximab group, and only at month 6 (62.9 [16.6 ; 449.3] vs 199.5 [24.2
121 ; 491.4] RU/ml at baseline, $p=0.0168$) in the NIAT alone. Percent decrease of PLA2R-Ab titer
122 was significantly higher in the NIAT-rituximab group at month 3 and month 6 (Figure 2C).

123 Complete immunological remission (full PLA2R-Ab depletion) was observed in the NIAT-
124 rituximab group in 14/25 (56 %) and 13/26 (50 %) patients at months 3 and 6, respectively, as
125 compared with 1/23 (4 %, $p=0.0001$) and 3/25 (12 %, $p=0.0035$) patients, respectively, in the
126 NIAT group (Table 2). Of the 14 rituximab-treated patients that were antibody depleted at
127 months 3, 6 (43 %) patients subsequently achieved the primary end point, compared with only
128 2 of the 11 patients (18%) without antibody depletion. PLA2R-Ab level <275 RU/mL at
129 baseline was associated with the primary end point (OR 4.3, 95% CI 1.1- 17.3, $p=0.0424$),
130 and this was independent from treatment group, age, gender, baseline proteinuria, serum
131 albumin and creatinine (Supplemental Table S2).

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

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

149 CD19+ B-cells remained below normal range (100 to 500/mm³) throughout the
150 observation period in the NIAT-rituximab group. Median CD19+ B-cell count was 11/mm³
151 (IQR=(2.0; 22.0)) at month 3 and 61/mm³(IQR=(34.0; 100)) at month 6 (Table 2). Among
152 PLA2R-Ab positive patients at baseline, there was no difference in CD19 count between
153 patients who were PLA2R-Ab depleted and those who were not, at month 3 (p=0.7587, N=23
154 patients) and month 6 (p=0.8862, N=24 patients), respectively.

155 *Post-hoc composite end point.* In a post-hoc analysis that includes reduction of proteinuria
156 > 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

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

184

185 **DISCUSSION**

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

194 We compared NIAT-rituximab to NIAT because there was no evidence-based proof of the
195 efficacy of rituximab in PMN even if several non-randomized studies suggested that
196 rituximab was efficient¹⁸⁻²¹; the possibility of bias linked to a high rate of late spontaneous
197 remissions as confirmed in the present study, called for a randomized trial. With this aim in
198 mind, an ideal trial would have been a prolonged trial for more than one year. However, we
199 considered unethical to maintain the patients on NIAT for more than 6 months. Since no
200 major complication occurred in the NIAT group, the risk taken was acceptable although after
201 6 months, PLA2R-Ab positive patients had a markedly higher antibody titre in the NIAT
202 group than in the NIAT- rituximab group, and it is uncertain whether a delay by one year in
203 the NIAT group would impact any future response to immunosuppressive agents or to
204 rituximab therapy. Alternatively, we could have set the end point at 12 months with pre-
205 specified measures in the most aggressive forms for the patients in the NIAT group. However,
206 this protocol would have assessed a global treatment strategy (NIAT +/- retreatment, and

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

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

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

231 We continued to follow the patients during a post-RCT observational phase. The suspected
232 beneficial clinical effect of rituximab at month 6 was confirmed by the data of the
233 observational phase which were recorded before any potential modification of treatment
234 assigned at randomization. Proteinuria remission rate was substantially higher in the NIAT-
235 rituximab group than in the NIAT alone group (64.9 vs. 37.5%), with proteinuria dropping to
236 a much lower level in the NIAT-rituximab group. In the patients treated with NIAT-rituximab,
237 proteinuria remission rate and median time to remission (7 months) during the follow-up
238 study were similar to those reported in previous non randomized series.^{20, 21}
239 Remission rate was comparable to the one achieved with the Ponticelli's protocol in the same
240 time frame (50% at 6 months,⁸ 32% within one year,⁹) considering that in those studies,
241 patients were enrolled without a run-in period. It was lower than in patients treated with
242 cyclosporine¹³ and tacrolimus¹⁴ who had a remission rate of 75% after 26 weeks and 58%
243 after 6 months, respectively, but baseline serum albumin was higher by >0.5 g/dl than in our
244 study and these drugs are known to have an effect on glomerular hemodynamics with a high
245 risk of relapse at discontinuation and to be associated with a clinically relevant
246 nephrotoxicity. The ongoing Membranous Nephropathy Trial of Rituximab (MENTOR,
247 ClinicalTrials.gov number, NCT01180036) will hopefully show whether rituximab is superior
248 to cyclosporine in term of proteinuria remission over a 24-month period. **The high rate of**
249 **spontaneous remission that may occur more than a year after disease onset in our study and**
250 **the relatively low rate of remission with NIAT-rituximab, as with other immunosuppressive**
251 **treatments, indicate that we have not yet reached an optimal treatment in patients with**
252 **persisting nephrotic syndrome. It is difficult to extrapolate what would be the remission rate**
253 **with rituximab only since all patients with persisting nephrotic syndrome are treated with**
254 **NIAT according to KDIGO recommendations.**

255 The present trial has several strengths. First, it is the first RCT in PMN patients with a
256 monitoring of PLA2R-Ab, detected in 71 % of the patients as in previous studies²⁸, and of
257 THSD7A-Ab.²⁹ Rituximab associated with NIAT reduced median PLA2R-Ab titre as early as
258 month 3 and induced complete immunological remission in 56% and 50% of the patients at
259 months 3 and 6, respectively. Multivariate analyses showed that PLA2R-Ab <275RU/mL at
260 baseline was the only factor associated with remission occurring at month 6 (primary end
261 point) and during the post-RCT observational phase without modification of treatment
262 assigned at randomization, regardless of treatment group and other adjustment variables. Our
263 results also suggest that THSD7A-Ab may be useful for the monitoring of MN patients. On
264 the other hand, our univariate and multivariate analyses failed to identify classical predictors
265 of long-term outcome and proteinuria remission such as proteinuria, serum creatinine and
266 eGFR, serum albumin, age, and gender. A possible reason is that we studied a relatively
267 homogeneous population after a 6-month run-in period of maximally tolerated conservative
268 therapy. This discrepant observation gives even more importance to PLA2R-Ab as a predictor
269 of proteinuria remission, in agreement with the auto-immune nature of the disease. The
270 finding that PLA2R-Ab positivity and titer tended to decrease as early as 8 days in the NIAT-
271 rituximab group was somewhat unexpected given the half-life of immunoglobulins of about 3
272 weeks, but confirmed previous observations by Hoxha et al.³⁰ This suggests that PLA2R-Ab
273 might be a very early biomarker of rituximab efficacy although this has to be confirmed in
274 further prospective studies.

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

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

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

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

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

304

305 **CONCISE METHODS**

306 **Study design**

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

320

321 **Patients**

322 Eligible patients were 18 years of age or older, had a biopsy proven diagnosis established less
323 than 2 years before inclusion, a urinary protein excretion or a urinary protein/creatinine ratio
324 greater than, or equal to, 3.5 g/day or 3500 mg/g, respectively, and a serum albumin lower
325 than 30 g/l for at least 6 months despite maximal tolerated dose of Non Immunosuppressive
326 Antiproteinuric Treatment (NIAT, angiotensin-converting enzyme inhibitors and/or
327 angiotensin-2 receptor blockers, diuretics and statin). Proteinuria was measured repeatedly
328 before inclusion and treatment assignment to confirm persistence of full-blown nephrotic
329 syndrome. The estimated GFR by MDRD formula had to be above 45 ml/min/1.73m².

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

342

343 **Procedures and follow-up**

344 Patients received NIAT in association with 375 mg/m² of intravenous rituximab on days 1 and
345 8 following randomization, or NIAT alone (Figure 3). We selected this dosing schedule on the
346 basis of previous reports of rituximab's ability to induce proteinuria remission and CD19
347 depletion.^{18, 24} At the end of the 6-months randomized phase, referring physicians were free of
348 re-infusing rituximab or shifting immunosuppressant, and introducing an immunosuppressant,
349 in patients of the NIAT-rituximab and NIAT groups, respectively, and patients were followed
350 up to 24 months during an observational period. The same antiproteinuric drugs were allowed
351 before and after randomization.

352 Study visits occurred at baseline, at weeks 1 and 2, and at months 3 and 6 during the RCT.

353 At each study visit, clinical data and medications were recorded. Blood and urine samples
354 were collected at baseline, months 3 and 6 for serum creatinine, serum albumin and

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

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

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

363

364 **Outcomes**

365 The primary end point was the percentage of patients with complete or partial remission of
366 nephrotic syndrome at 6 months of follow-up. Remission was defined accordingly to 2012
367 KDIGO¹⁵ as 1) complete in case of urinary protein excretion less than 500 mg per day or 500
368 mg/g creatinine; 2) partial in case of urinary protein excretion < 3.5 g per day or 3500 mg/g
369 creatinine and \geq 500 mg/g creatinine with at least 50% reduction compared to baseline.
370 Secondary end points were rate of proteinuria, serum albumin, serum creatinine, PLA2R-Ab
371 levels and SAEs. PLA2R-Ab was measured by using a quantitative ELISA (EuroImmune AG,
372 Lübeck, Germany); anti-thrombospondin domain 7A antibodies (THSD7A-Ab) were assessed
373 by an immunofluorescence test (EuroImmune). Antibody depletion was defined as complete
374 disappearance of antibodies in PLA2R-Ab positive patients. Because albumin level may be an
375 earlier marker of response than end points defined by proteinuria only,^{23, 27} we also considered
376 a post-hoc composite end point defined as reduction of proteinuria > 50% and increase of
377 serum albumin level > 30% at month 6 follow-up.

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

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

386

387 **Statistical Analyses**

388 Based on previous studies,^{17, 18, 25} rituximab was effective in decreasing proteinuria as early as
389 3 months^{21, 30} and achieving remission at 20 weeks¹⁷ to one year^{18, 25} in 60% to 80% of
390 patients with PMN and nephrotic syndrome persisting after 6 months of supportive therapy.
391 The trial was designed to establish whether rituximab was superior in term of efficacy as
392 assessed by the number of remissions. Assuming a remission rate of 20% in the NIAT group,
393 the inclusion of 80 patients would provide 80% power at two-sided α of 0.05 to detect a 30%
394 absolute increase in the remission rate (50%) and under assumption of 10% exclusion or
395 dropout rates (Fisher exact test).

396 Baseline characteristics of the study population were expressed as frequency and
397 percentage for qualitative variables and as median and interquartile range (IQR) for
398 continuous variables. Remission rates were expressed as frequency and percentage and its
399 95% confidence interval. All PLA2R-Ab titres not achieving the 14 RU/ml detection
400 threshold of the method were spiked at 0. PLA2R-Ab titre was considered as a continuous
401 variable, as a binary variable (absence/presence), or at baseline only, as a categorical variable
402 according to tertiles (<22.5, lowest; 22.5-275.5, middle; \geq 275.5, highest, RU/ml). Because at

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

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

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

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

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

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

429

430 **Acknowledgments**

431 This study was funded by grants from the Programme Hospitalier de Recherche Clinique,
432 French Ministry of Health (AOM10089); from the European Research Council ERC-2012-
433 ADG_20120314 (Grant Agreement 322947); from Agence Nationale pour la Recherche
434 Programme Blanc SVSE1 (2012) Decision ANR-12-BSE1-0002-01; from Fondation pour la
435 Recherche Médicale Equipe FRM 2012 grant; and from 7th Framework Programme of the
436 European Community Contract 2012-305608 (European Consortium for High-Throughput
437 Research in Rare Kidney Diseases). The sponsor of this study was Assistance Publique-
438 Hôpitaux de Paris (Département de la Recherche Clinique et du Développement, Clinical
439 Research and Development Department). Rituximab was given by Hoffmann–La Roche.
440 None of the funding source played a role in the writing of the manuscript or the decision to
441 submit it for publication.

442 We thank Nora Semai, Jennifer Piro, Julie Claudepierre, Benjamin Laverdant, Matthieu Le
443 lay, Anne Seguin, Dominique Damas and Elodie Drouet from the Clinical Research Unit,
444 INSERM CIC P 0901, Saint Antoine University Hospital (Assistance Publique-Hôpitaux de
445 Paris, Université Pierre et Marie Curie, Paris) for trial monitoring and handling, preparation,
446 and submission of all required research ethics and regulatory documents. We thank
447 Emmanuel Roux for monitoring and handling the biobank at Tenon hospital, Paris (Prof.
448 Isabelle Brocheriou) and Christine Vial (UMR_S1155) for help in the preparation of the
449 manuscript.

450

451 **Disclosures**

452 Pierre Ronco is a paid adviser for Otsuka, BMS, Amicus, and Air Liquide, has received
453 lecture fees from Shire (Belgium), Servier, Chugai and the Mayo Clinic, and research grants
454 from Amgen and Otsuka. Karine Dahan has received grants from the French Ministry of
455 Health and Roche Pharmaceutical. Tabassome Simon is a paid adviser for, and has received
456 lecture fees from AstraZeneca, Astellas, Bayer, BMS, Lilly, MSD and Sanofi; she has
457 received research grants from Astra Zeneca, Daiichi-Sankyo, Eli-Lilly, GSK, MSD, Novartis
458 and Sanofi. Emmanuelle Plaisier is a paid adviser for, and has received lecture fees from
459 Gilead Sciences; she has received research grants from Amgen. Hoffmann-La Roche provided
460 Rituximab for the study.

461

462

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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— $\mu\text{mol/L}$	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/mm³). # 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 endarterectomy*	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

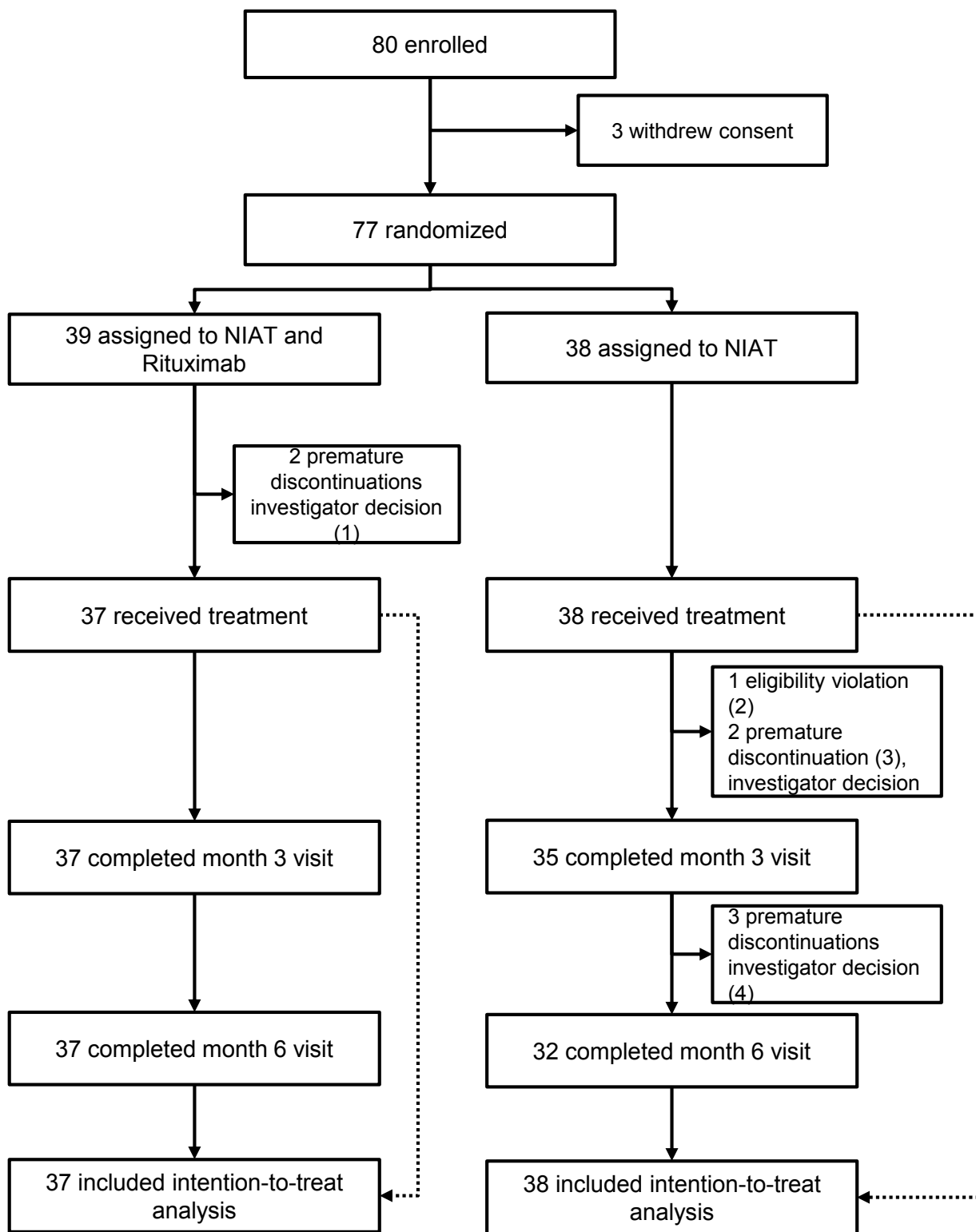


Figure 2

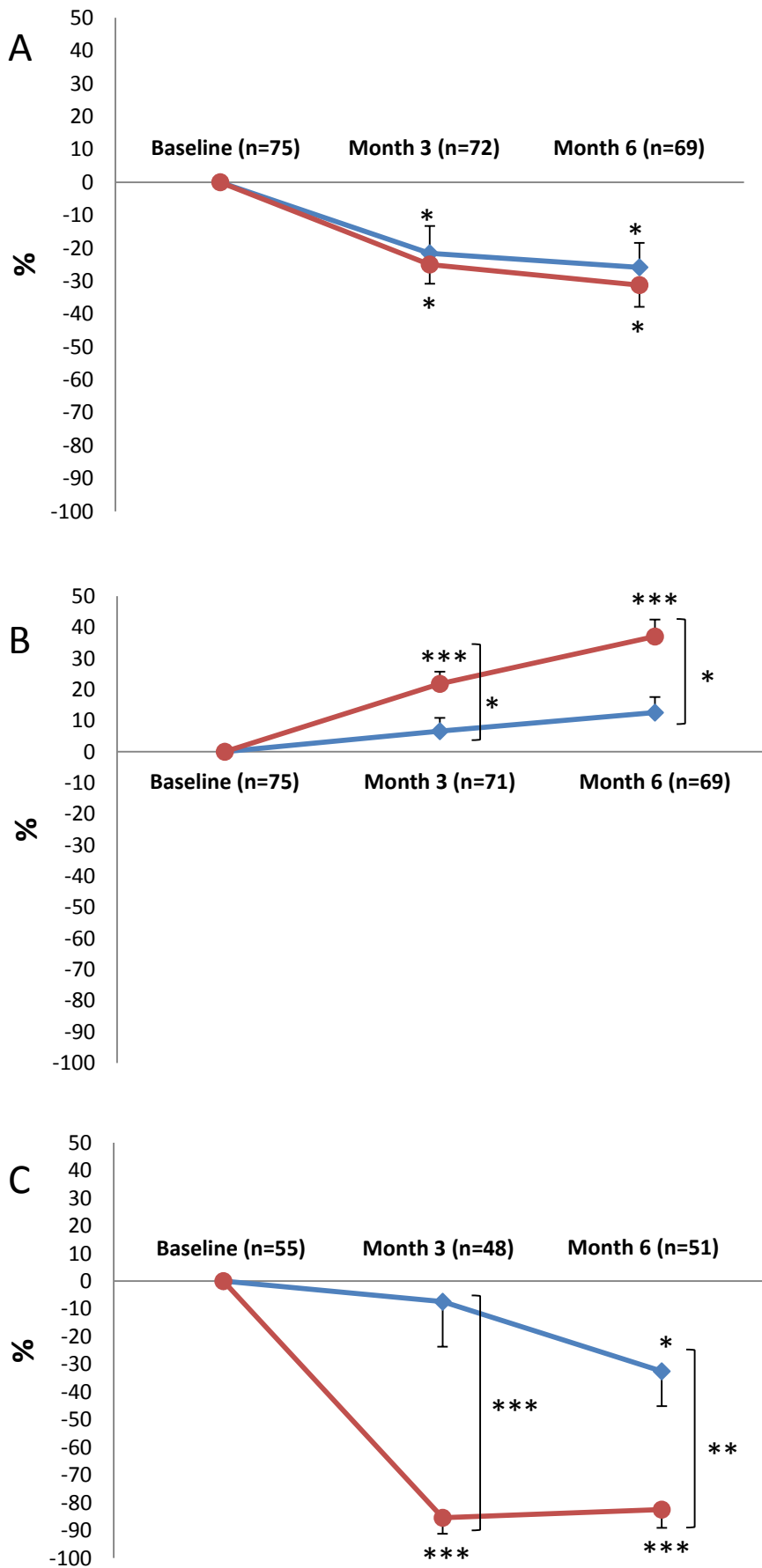
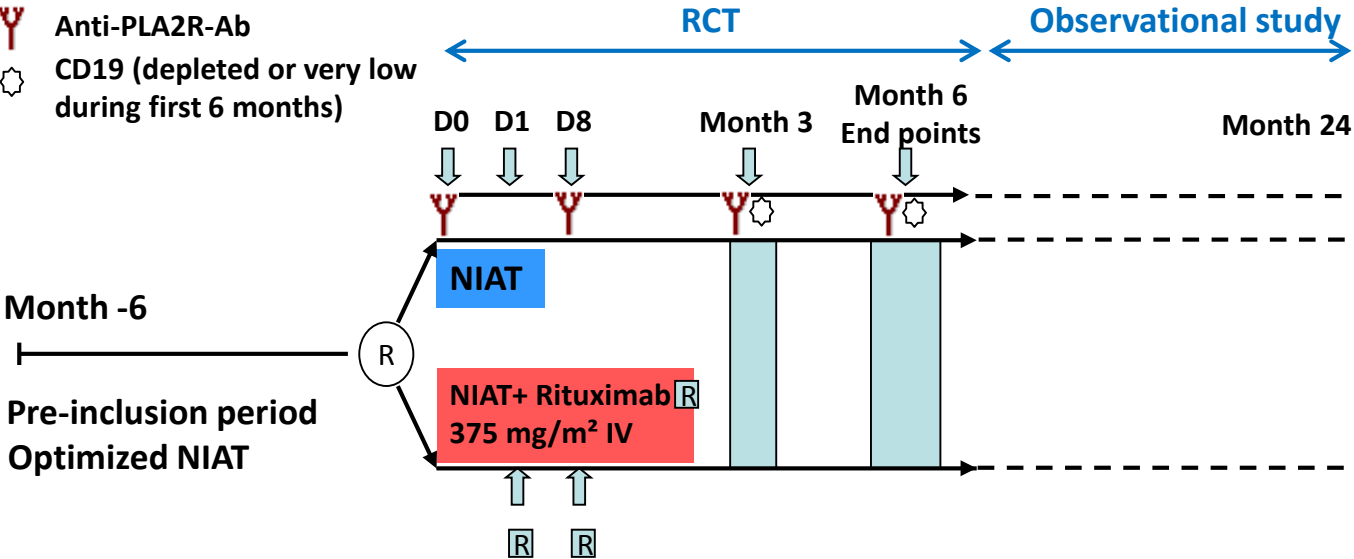


Figure 3



Supplemental material

1. **Clinical trial steering committee**
2. **Independent data and safety monitoring committee**
3. **Contributors**
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9. **CONSORT Statement**

1. Clinical trial steering committee

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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 1:100 were 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 and inter-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)

Characteristics	Complete or Partial remission (n=21/75)			
	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.

Characteristics	Complete or Partial remission (n=37/75)			
	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

9. CONSORT Statement



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.