Supplementary appendix

Patients, inclusion and non-inclusion criteria of the GEMRITUX study[23]

Eligible patients were 18 years of age or older and had a biopsy proven PMN. Patients were enrolled from January 2012 to July 2014 in 31 French centers if they had 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 full dose of Non Immunosuppressive, Antiproteinuric Treatment (NIAT) (angiotensin-converting enzyme inhibitors and/or angiotensin-2 receptor blockers, diuretics and statin). The estimated GFR by MDRD formula had to be above 30 ml/min/1.73m^2. Exclusion criteria were secondary MN, pregnancy, breast-feeding, immunosuppressive treatment in the three preceding months, and active infectious disease. Only one patient among the 25 of this study had received 2 infusions of rituximab, 375 mg/m² at one-week interval more than 6 months before its inclusion in January 2012 in the NIAT-Rituximab group. At that time, CD19 % was 12.9% (all patients with PMN, 13 +/-1) and CD19 absolute count was 278/mm^3 (all patients, 239 +/-25). All patients provided written informed consent.

Treatment and monitoring of the GEMRITUX study[23]

Eligible patients were randomly assigned, in a 1:1 ratio, to receive NIAT for 6 months or NIAT in association with 375 mg/m² of intravenous rituximab on days 1 and 8 following randomization. Study visits were scheduled at enrolment, days 1 and 8, months 3 and 6 after randomization. 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. Anti-PLA2R and anti-THSD7A antibodies, and lymphocyte populations were assessed at baseline, day 8, and months 3 and 6.

Clinical and immunological remission

Clinical remission was defined accordingly to 2012 KDIGO (KDIGO clinical practice guideline for glomerulonephritis. *Kidney Int* Suppl.2: 186-197, 2012) 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. Antibody depletion was defined as complete disappearance of antibodies in PLA2R-Ab positive patients.

Supplementary methods: flow cytometry analysis

Blood subsets (CD3+, CD4+, CD8+ T lymphocytes, CD19+ B lymphocytes and CD3-CD56+ NK cells) counts (cells/µl) were established from fresh blood samples using CYTO-STAT tetraCHROME kits with Flowcount fluorescents beads and tetra CXP software with a FC500 cytometer according to manufacturer's instructions. Peripheral blood mononuclear cell (PBMC) were analyzed using multicolor FC and mAbs directly conjugated either to Fluorescein isothiocyanate (FITC), Phycoerythrin (PE), Phycoerythrin-Texas Red (ECD), Allophycocyanin (APC), phycoerythrin-Cyanyn 7 (PE-Cya7), APC -Alexafluor 700 (APCa700) or APC -Alexafluor 750 (APC-a750) were used for: CD3-APCa750, CD4-ECD, CD8-APCa700, CD16-PC7, CD19-ECD, CD21PE, CD28-FITC, CD38 PC7, CD56-APC, CD69-APC, CD127-PC7, CD152-PE, CD197-PE, DR-FITC, TCR-γδ FITC all from Beckman Coulter. IgD-FITC, CD25-PE, CD27-APC, CD45RA-APC, CD45RA-FITC and CD73-FITC were from BD Biosciences, CD62L-PC7 and Helios-FITC were from eBioscience. CD25-APC, CD39-FITC and GITR-PE were from Miltenyi Biotech. LAP-PE was from R&D Systems. Intra-nuclear Helios and Foxp3 labeling was performed after CD3, CD4, CD8, CD127 and CD25 membrane staining using APC anti-human Foxp3 kit (PCH101 clone, eBioscience) using fixed and permeabilized cells using Intracellular Fixation and Permeabilization Buffer Set (eBioscience) and according to manufacturer's instructions. Cells acquisition and analysis were performed using a Navios Cytometer and data were analyzed with Kaluza software (Beckman Coulter).

Supplementary tables

Supplementary Table 1. Demographic and biological data

Patient Number	Age (year)	Gender (M/F)	Proteinuria (g/day)	Serum albumin (g/dL)	Serum creatinine (mg/dL)
1	59	M	14.4	1.2	1.90
2	64	F	6.4	2.6	1.02
3	30	M	6.5	1.4	1.4
4	63	M	9.03	2.0	2.7
5	74	M	9.44	2.9	2.80
6	40	M	15.9	1.7	1.23
7	64	M	4.26	2.1	1.15
8	59	M	9.20	1.9	1.14
9	36	M	3.17	1.7	0.83
10	42	M	7.68	1.8	0.77
11	27	M	3.80	2.1	0.80
12	47	F	4.04	2.2	0.88
13	52	M	3.28	2.2	1.54
14	33	F	4.4	1.9	0.68
15	32	M	4.7	1.8	1.56
16	67	M	6.10	1.9	1.38
17	63	M	7.64	2.4	1.37
18	51	M	8.24	1.7	1.29
19	46	M	11.0	2.5	0.94
20	61	F	7.59	2.0	2.07

21	64	F	8.04	1.5	1.1
22	37	M	6.48	1.7	0.68
23	60	M	6.21	2.3	1.97
24	58	M	3.5	2.8	0.99
25	57	M	3.44	2.8	1.21

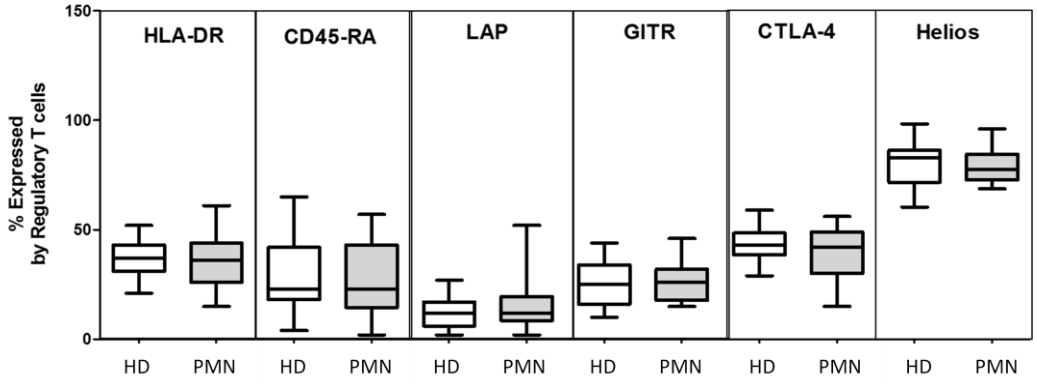
Supplementary Table 2. Lymphocyte subpopulations at baseline

	Primary MN (25)	Healthy Donors (27)
$CD3^+$ T cells		
Total (cells/mm^3)	1379±88	1214±80
%	76±1	73±1
$CD4^+$ T cells		
Total (cells/mm^3)	887±63	786±58
%	50±2	47±1
Naive ($CD45RA^+CCR7^+$)	46 ± 3	49 ± 2
CM ($CD45RA^-CCR7^+$) (%)	40 ± 3	42 ± 2
EM ($CD45RA^-CCR7^-$) (%)	10 ± 1*	6 ± 1
$HLA\text{-}DR^+$ **(%)**	8 ± 1	8 ± 1
$CD25^+$ (%)	47 ± 3	50 ± 2
CD69+ (%)	19 ± 3	18 ± 3
CD28+ (%)	94 ±2	95 ± 1
Treg ($CD4^+CD25^{hi}CD127^{-/lo}Foxp3^+$) cells		
Cells/mm^3	35±3.5	28±2.6
% ($CD4^+CD25^{hi}CD127^{-/lo}Foxp3^+$)/CD4+	3.2±0.2***	4.7±0.2
$CD8^+$ Tcells		
Total (cells/mm3)	440 ± 47	398 ± 34
%	25±2	54±1
Naive ($CD45RA^+CCR7^+$) (%)	40 ± 4	46 ± 3
CM ($CD45RA^-CCR7^+$) (%)	16 ± 2	14 ± 1
EM($CD45RA^-CCR7^-$) (%)	26 ± 4*	18 ± 1
tEM($CD45RA^+CCR7^-$) (%)	18 ± 2	22 ± 2
$HLA\text{-}DR^+$ (%)	30 ± 3	24 ± 3
$CD25^+$ (%)	13 ± 2	16 ± 2
$CD69^+$ (%)	15 ± 2	18 ± 3
$CD28^+$(%)	69 ± 4	69 ± 3
Ratio CD4/CD8	2.4 ± 0.2	2.1 ± 0.2
$CD19^+$ B cells		
Total (cells/mm3)	239 ± 25	218 ±26
%	13±1	13±1
Naive (IgD^+CD27^-) (%)	65 ± 3*	54 ± 4
NSM (IgD^+CD27^+) (%)	10 ± 1**	16 ± 2
SM (IgD^-CD27^+) (%)	20 ± 2*	27 ± 2
DN (IgD^-CD27^-) %	5.3±0.6**	3.6±0.3

Plasmablast ($IgD^{-}CD27^{+}CD38^{++}$) (%/SM)	5 ±1	4 ± 1
$CD21^{-}$ (%)	5 ± 1	5 ± 1
$CD3^{-}CD56^{+}$ NK cells		
Total (cells/mm3)	160 ± 31	180 ±19
%	8±1*	12±1
$CD16^{-/lo}CD56^{bright}$(%)	10 ± 1**	6 ± 1

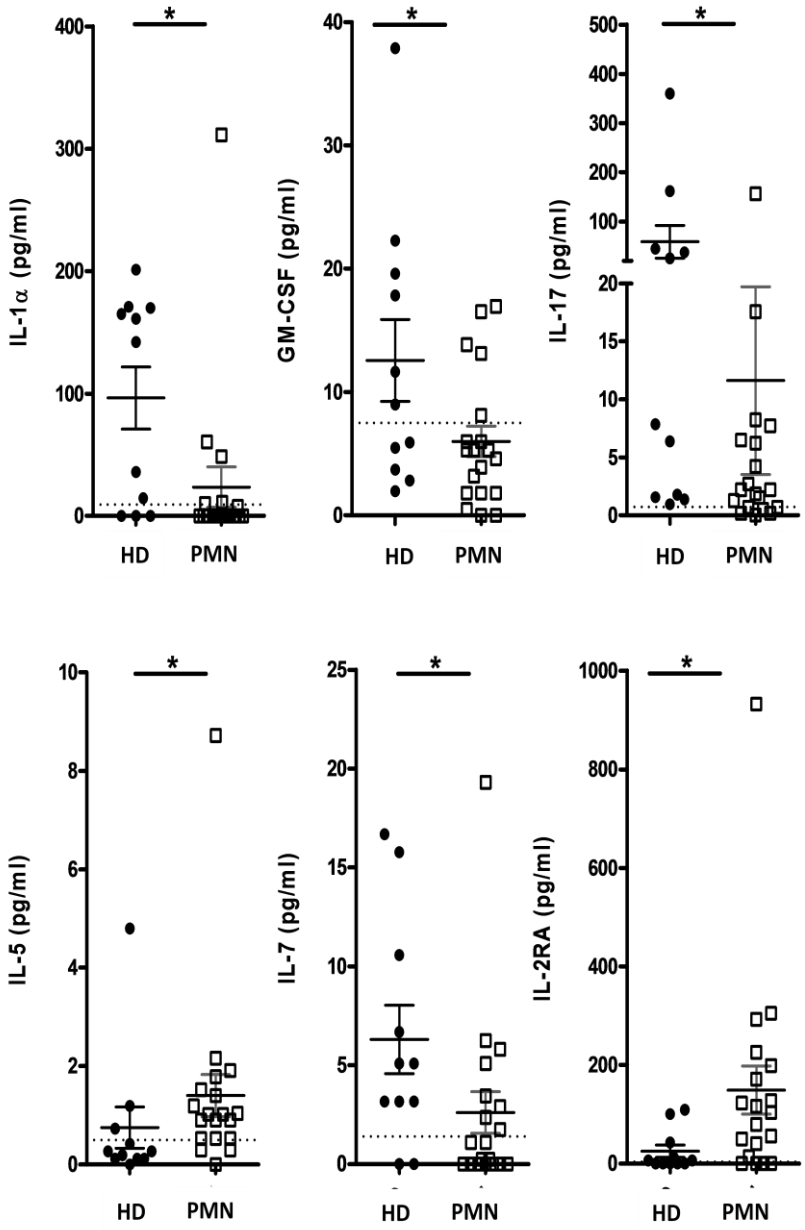
Supplementary figures and legends

Supplementary Figure 1



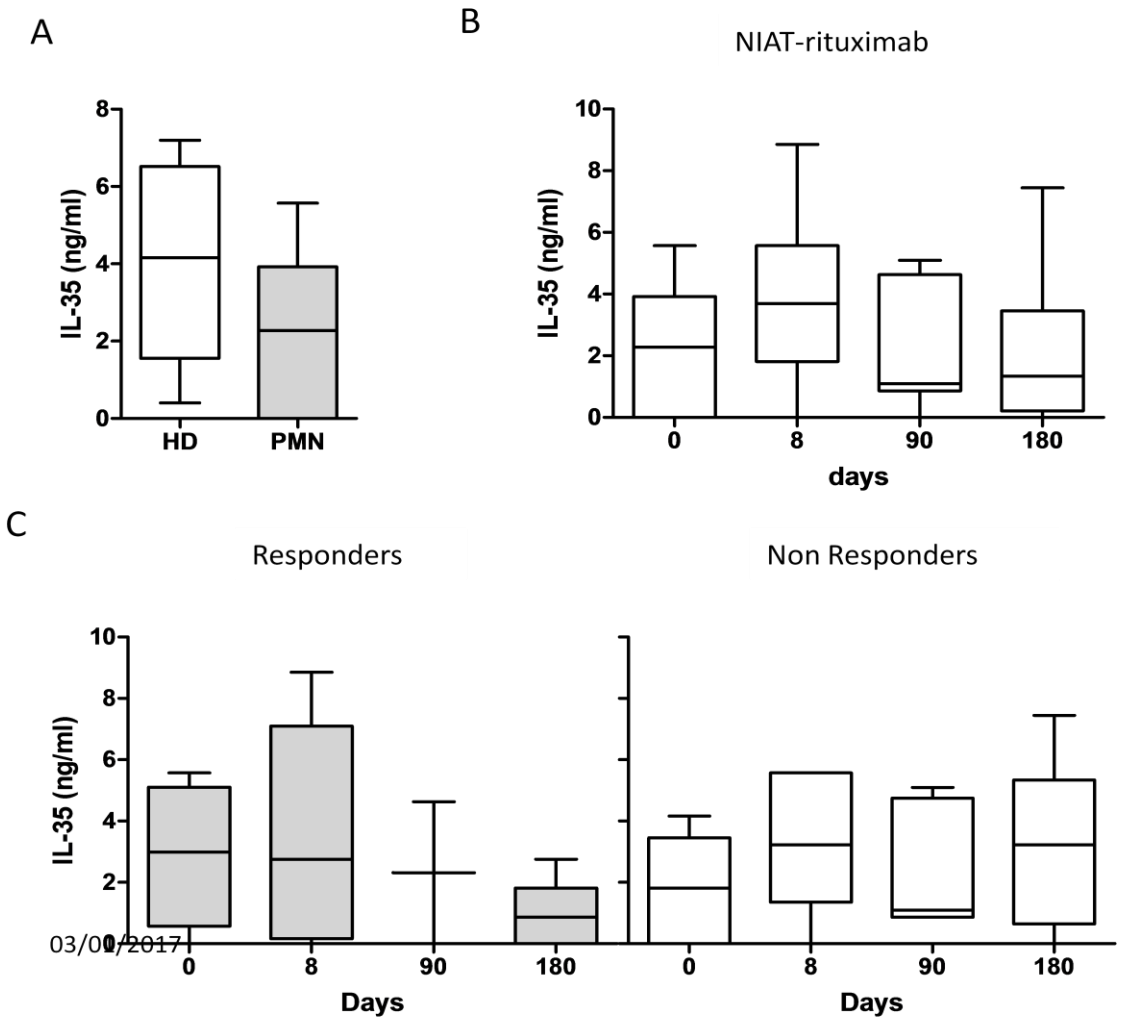
Supplementary Figure 1: Comparison of Treg phenotype in PMN patients and healthy donors (HD). Studies were performed by multicolor flow cytometry analysis. Tregs were gated in CD4+ T cells and were identified as CD25hiCD127lo/-cells. HLA-DR, CD45RA, LAP, GITR, CTLA-4 and Helios expression is represented as the percentage of positive cells in Treg. Each box plot represents the median and the 25th and 75th centiles. Error bars represent the smallest and the largest values. No difference was seen between PMN patients and healthy donors.

Supplementary Figure 2:



Supplementary Figure 2: Comparison of plasma level of cytokines in PMN patients and healthy donors. IL-1α, GM-CSF, IL-5, IL-7, IL-17, and IL-2RA were measured in the plasma of patients (n=11) and of healthy donors (n=9). Differences with healthy donors were compared using Student's t-test when distribution was normal or with non-parametric unpaired Mann-Whitney U test. *p<0.05; **p<0.01; ***p<0.001

Supplementary Figure 3:



Supplementary Figure 3: Plasma level of IL-35 in PMN patients treated with NIAT-rituximab. A) Quantitative measurement of IL-35 in the plasma of patients (n=9) and healthy donors (n=11). B) IL-35 levels in patients over time from NIAT-rituximab administration (day 0; n=9), to day 8 (n=11), day 90 (n=8) and day 180 (n=12). C) Kinetic of IL-35 levels in responders (day 0, n=6; day 8, n=6; day 90, n=2; day 180, n=7) and non-responders (day 0, n=5; day 8, n=6; day 90, n=6; day 180, n=6). The time is from rituximab administration. Each box plot represents the median and the 25th and 75th centiles. Error bars represent the smallest and the largest values. No difference was seen with healthy donors and during follow-up using non-parametric Mann-Whitney U test.