SUPPLEMENT MATERIAL

Sensitivity analyses

Of the 132 patients treated with rituximab, 105 had serum albumin levels available at baseline and on follow up. Of these patients, 73 (69.5%) had serum levels < 2.5 mg/dl to start with. According to proteinuria criteria considered for primary outcome analyses, 41 (56.2%) progressed to the combined endpoint and 18 (24.7%) to complete remission alone. All patients of both groups also achieved serum albumin levels >3.2 mg/dl and eight additional patients achieved this target without progressing to any and point. In the first group median (IQR) time to normal serum albumin level was similar to time to the combined endpoint [7.13 (4.14-12.71) vs 7.14 (3.14-12.20), p=0.5023), Supplement Figure 7, Left Panel], whereas in the second group it remarkably preceded time to complete remission [5.91 (3.12-10.38) vs 17.98 (12.10-29.36) months, p<0.0001), Supplement Figure 7, Right Panel]. The probability of achieving normal serum albumin levels and the combined endpoint was significantly higher in subjects with undetectable than in those with detectable antibodies (Supplement Figure 8, Left Panel) whereas among subjects with detectable antibodies, it was significantly higher in the lowest and middle titer tertiles than in the highest one (Supplement Figure 8, Right Panel). Consistently, the antibody titer significantly predicted the probability of normal albuminuria and combined endpoint (p=0.002). Treatment effect was similar between patients with or without previous immunosuppression or receiving the four-dose or the B Cell-driven regimen (data not shown).

By using Western blot analyses, anti PLA_2R autoantibodies were detected in 6 out of 20 sera without detectable antibodies by ELISA screening. Additional analyses performed by considering these 6 patients in the subgroup with detectable antibodies by ELISA, confirmed that progression to the combined endpoint or to complete remission considered alone was similar across patients with detectable or undetectable antibodies and those without antibody evaluation. Again, the highest

probability of progression to both end points was observed in the lowest titer tertile and the lowest probability in the highest tertile

LEGENDS TO SUPPLEMENT FIGURES

Supplement Figure 1

Study flow-chart

Supplement Figure 2

Twenty-for urinary protein excretion at start of the six-month Run-In period (- 6 months) and at the time of rituximab treatment (time 0) in patient subgroups considered according to rituximab administration as first line therapy or as second line when previous treatments had failed in the past (Left Panel); or according to detectable, undetectable or not available autoantibodies (Middle Panel), or according to progression to the combined end point of complete and partial remission considered together or to complete remission considered alone or no remission (Right Panel).In no considered subgroup urinary protein excretion decreased during the Run-In period. Proteinuria did not change appreciably in subjects with undetectable or not available antibodies and in those eventually achieving complete remission, and increased significantly in all the other considered subgroups.

Supplement Figure 3

Fitted log-hazard ratio for the risk of complete or partial remission (Left Panel) or complete remission considered as a single endpoint (Right Panel) from modified fractional polynomials adjusted for gender and baseline log-transformed proteinuria and serum creatinine and anti PLA_2R autoantibody titer plotted against anti PLA_2R autoantibody titer at baseline. Among subjects with detectable antibodies the probability to achieve both endpoints progressively increases for decreasing levels of anti PLA_2R autoantibodies up to a peak in correspondence of the detection threshold of the method (14 RU/ml). In subjects with the lowest titer of detectable autoantibodies,

however, the probability of achieving both endpoints is higher than in subjects without detectable antibodies (identified by circles).

Supplement Figure 4

Fitted values of mean percent changes in proteinuria, serum albumin and anti PLA_2R antibody levels at different visits vs baseline estimated by fractional polynomial simple regression and relative equations. Y is the percent change in the considered variable vs baseline. X is [time (in months vs baseline) +1]/10.

Supplement Figure 5

Receiver Operating Characteristic curve for the prediction of the combined endpoint of complete or partial remission (Left Panel) or of complete remission considered as a single endpoint (Right Panel) after Rituximab treatment by anti PLA_2R antibody titer evaluation. At logistic regression analysis the association between 6-month percent reduction in anti PLA_2R autoantibody titer and progression to the combined endpoint (p=0.005) or to complete remission considered separately (p=0.044) were both statistically significant.

Supplement Figure 6

Kaplan-Meier curves for the proportion of participants with primary MN with the combined end point of complete or partial remission according to PLA2R1 (AG + GG vs AA, Left Panel) or *HLA-DQA1* (GG vs AA + AG, Right Panel) polymorphisms in the 72 participants with genetic evaluations and detectable anti PLA_2R autoantibodies at baseline. Adjustments are by gender, and baseline log-transformed proteinuria and serum creatinine and anti PLA_2R autoantibody levels. At multivariable analyses there is no significant association between PLA2R1 polymorphism and outcome and the GG genotype of the *HLA-DQA1* polymorphism is associated with a marginally

significant excess probability of complete or partial remission as compared to the AA and AG genotypes considered as whole.

Supplement Figure 7

Kaplan-Meier curves for the proportion of participants with primary MN and hypoalbuminemia at baseline (serum albumin <2.5 mg/dl) who progressed to the combined endpoint of complete or partial remission (Left Panel) or to Complete Remission alone (Right Panel) defined by proteinuria criteria only as for primary outcome analyses or by considering also progression to serum albumin levels >3.2 mg/dl. Achieving normal serum albumin levels preceded both endpoints defined by proteinuria criteria only, in particular in the subgroup with complete remission alone (Right Panel).

Supplement Figure 8

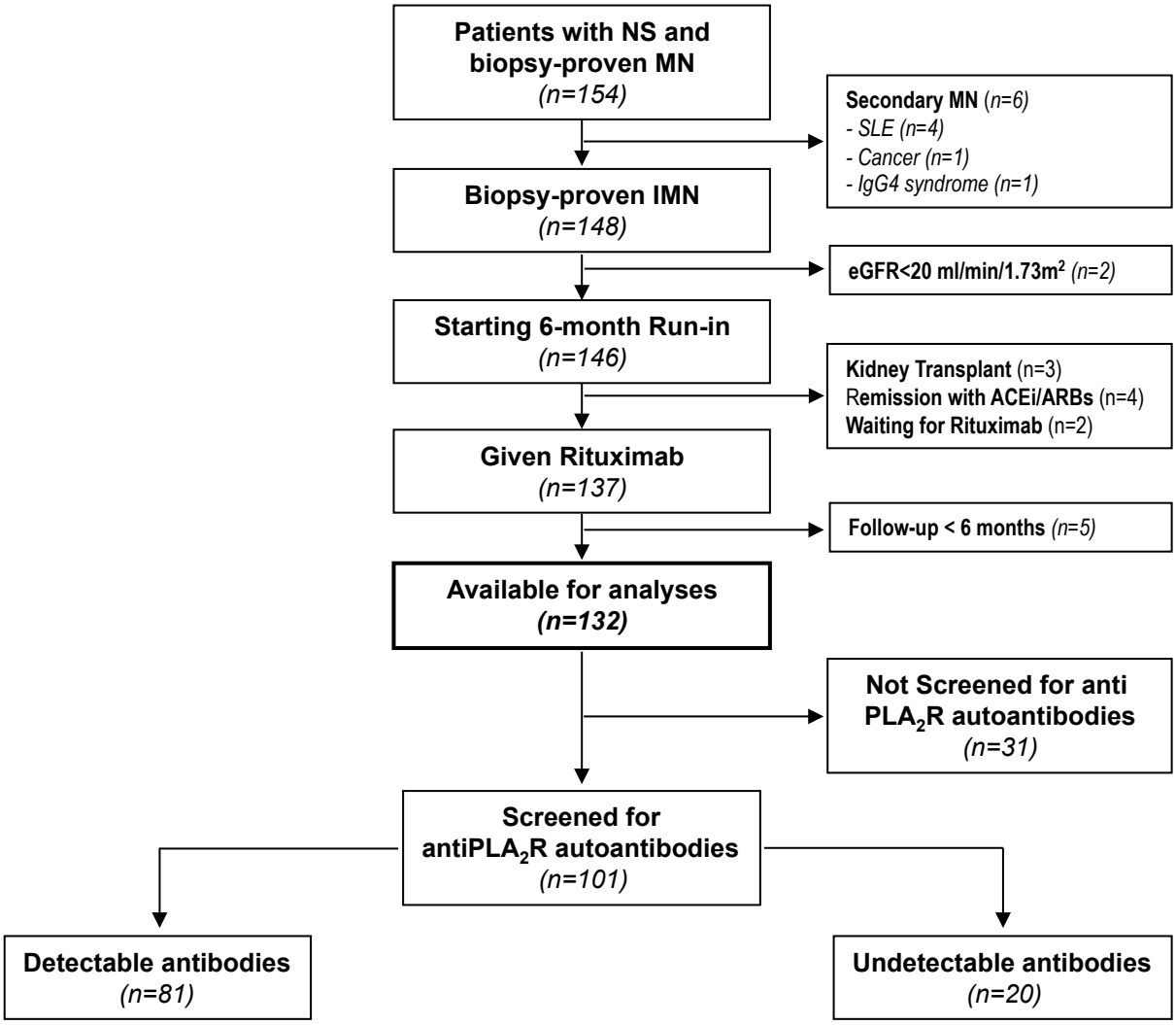
Kaplan-Meier curves for the proportion of participants with primary MN and hypoalbuminemia at baseline (serum albumin <2.5 mg/dl) who progressed to the combined endpoint of complete or partial remission and serum albumin levels >3.2 mg/dl in three subgroups with or without detectable anti PLA_2R autoantibodies or without anti PLA_2R antibody evaluations at baseline (Left Panel) and, among subjects with detectable antibodies at baseline, in three tertiles of baseline anti PLA_2R autoantibody titer (Right Panel). The probability of achieving normal serum albumin levels was lower in subjects with detectable than in those with undetectable antibodies (Left Panel, Upper Hazard Ratio) and, among subjects with detectable antibodies, it was higher in those in the Middle and Lowest Tertile than in those in the Highest titer tertile (Right Panel, Lower and Upper Hazard ratios, respectively). Undetectable (Left Panel) and Highest Tertile (Right Panel) were the reference groups.

Table 1 supplementary. Univariable and multivariable Cox analysis of the association between baseline predictors and the combined endpoint of Complete or Partial remission

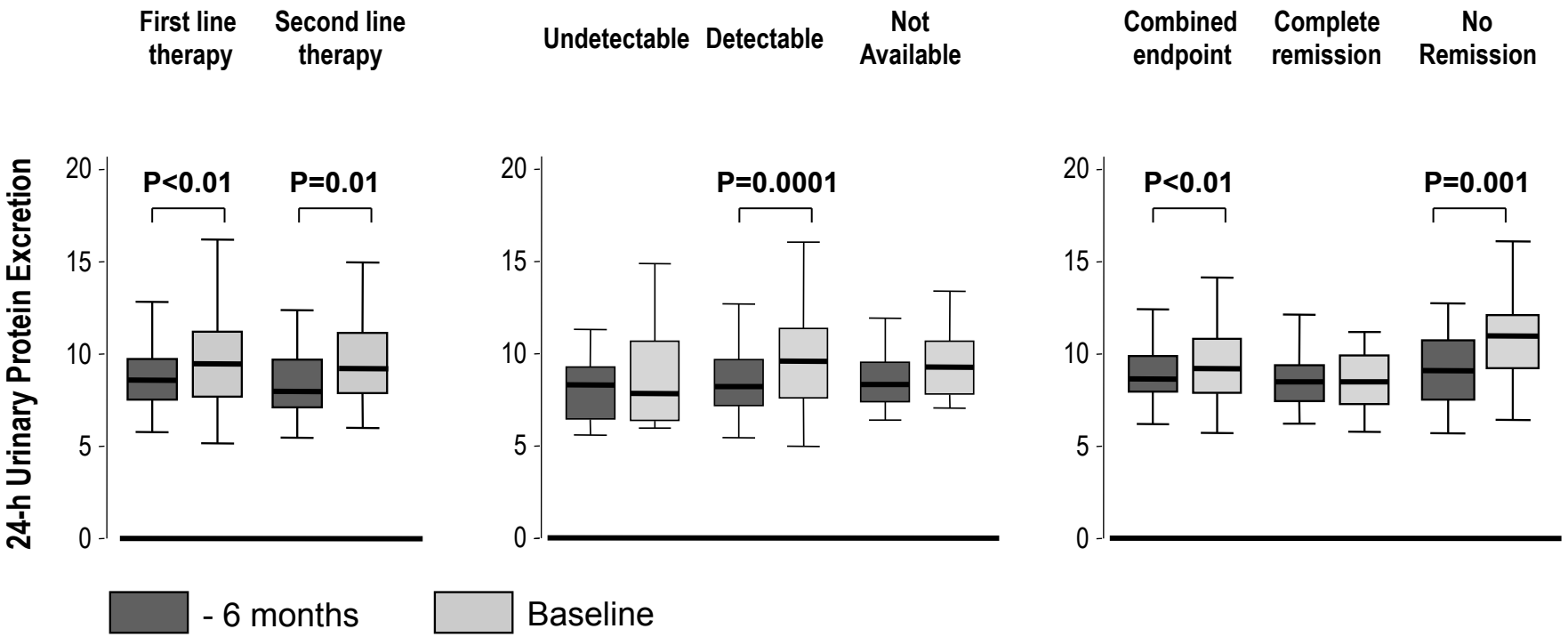
Characteristics	Complete or Partial remission (n=84/132)									
	Univariable		**Multivariable 1 #**		**Multivariable 2**		**Multivariable 3**		**Multivariable 4 modified FP**	
	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value	Hazard ratio (95% CI)	p-value
Age (*y*)	0.992 (0.978-1.007)	0.294								
Female gender	3.079 (1.957-4.844)	<0.0001	1.187 (0.552-2.552)	0.661	1.531 (0.753-3.114)	0.24	1.384 (0.708-2.705)	0.341	1.338 (0.662-2.704)	0.418
Body weight (Kg)	0.982 (0.965-0.999)	0.041								
Previous immunosuppression	0.814 (0.514-1.289)	0.381								
Map (*mmHg*)	0.985 (0.966-1.005)	0.148								
Serum creatinine (*mg/dl*) *	0.249 (0.132-0.473)	<0.0001	0.278 (0.100-0.777)	0.015	0.212 (0.088-0.511)	0.001	0.222 (0.094-0.523)	0.001	0.202 (0.084-0.485)	<0.0001
Serum albumin (*g/dl*)	2.087 (1.415-3.079)	<0.0001								
Total cholesterol (*mg/dl*)	0.996 (0.992-0.999)	0.007								
Triglycerides (*mg/dl*) *	0.264 (0.164-0.425)	<0.0001								
Proteinuria (*g/24h*) *	0.302 (0.211-0.431)	<0.0001	0.334 (0.192-0.580)	<0.0001	0.342 (0.201-0.582)	<0.0001	0.379 (0.227-0.633)	<0.0001	0.362 (0.218-0.600)	<0.0001
AntiPLA$_2$R (detectable, n=81)*	0.539 (0.395-0.736)	<0.0001	0.597 (0.443-0.807)	0.001					0.612 (0.456-0.812)	0.001
AntiPLA$_2$R (tertile vs undetectable) §										
lowest	1.303 (0.644-2.637)	0.461			0.858 (0.392-1.879)	0.702				
middle	0.721 (0.340-1.527)	0.393			0.832 (0.357-1.940)	0.671				
highest	0.326 (0.137-0.778)	0.012			0.234 (0.090-0.607)	0.003				
AntiPLA$_2$R (detectable vs undetectable)	0.712 (0.377-1.345)	0.295					0.594 (0.289-1.223)	0.158	0.160 (0.034-0.746)	0.02

* log transformation of the covariate. § The categorical variable was based on four subgroups, undetectable and the tertile of the detectable AntiPLA$_2$R (undetectable <14 RU/ml, lowest tertile: 14 to 86 RU/ml; middle tertile: 87 to 204 RU/ml; highest tertile: > 204 RU/ml).

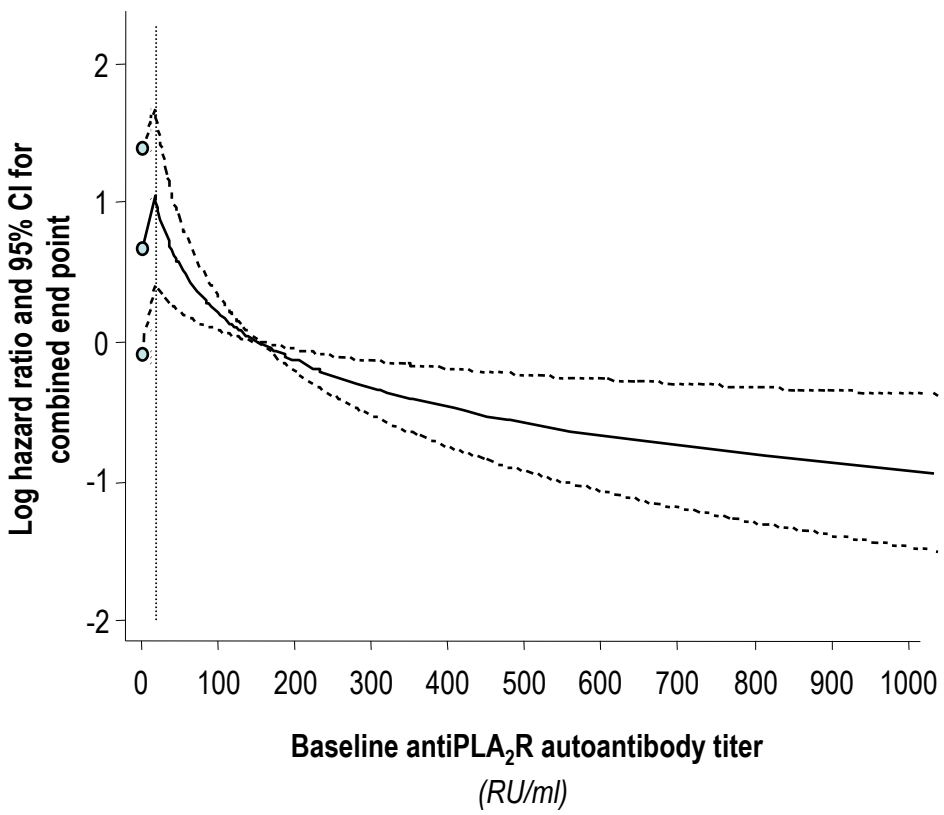
Multivariable survival Cox model adjusted for: 1. gender, serum creatinine, proteinuria and antiPLA$_2$R autoantibodies as continuous variables, among patients with detectable autoantibodies; 2. gender, serum creatinine, proteinuria and antiPLA$_2$R autoantibodies as tertile vs undetectable variables; 3. gender, serum creatinine, proteinuria and antiPLA$_2$R autoantibodies as binary variable (detectable vs undetectable); 4. gender, serum creatinine and proteinuria. The statistical analysis was performed using a modified Fractional polynomial for antiPLA$_2$R autoantibodies with spike at zero.

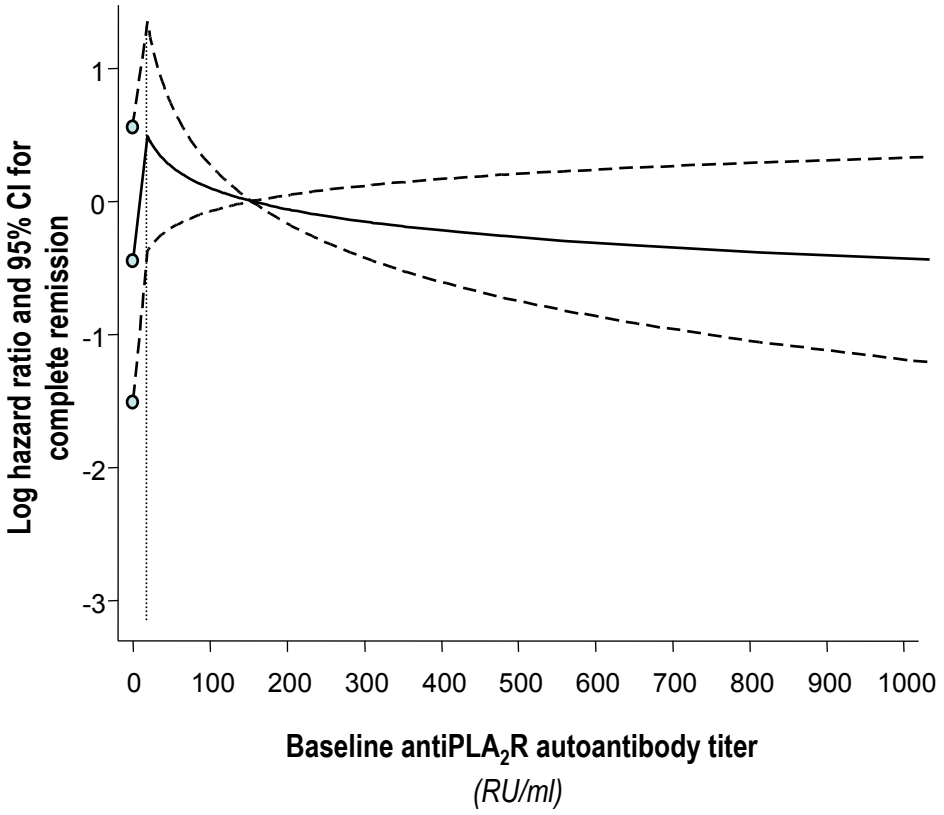


Supplement Figure 1



Supplement Figure 2





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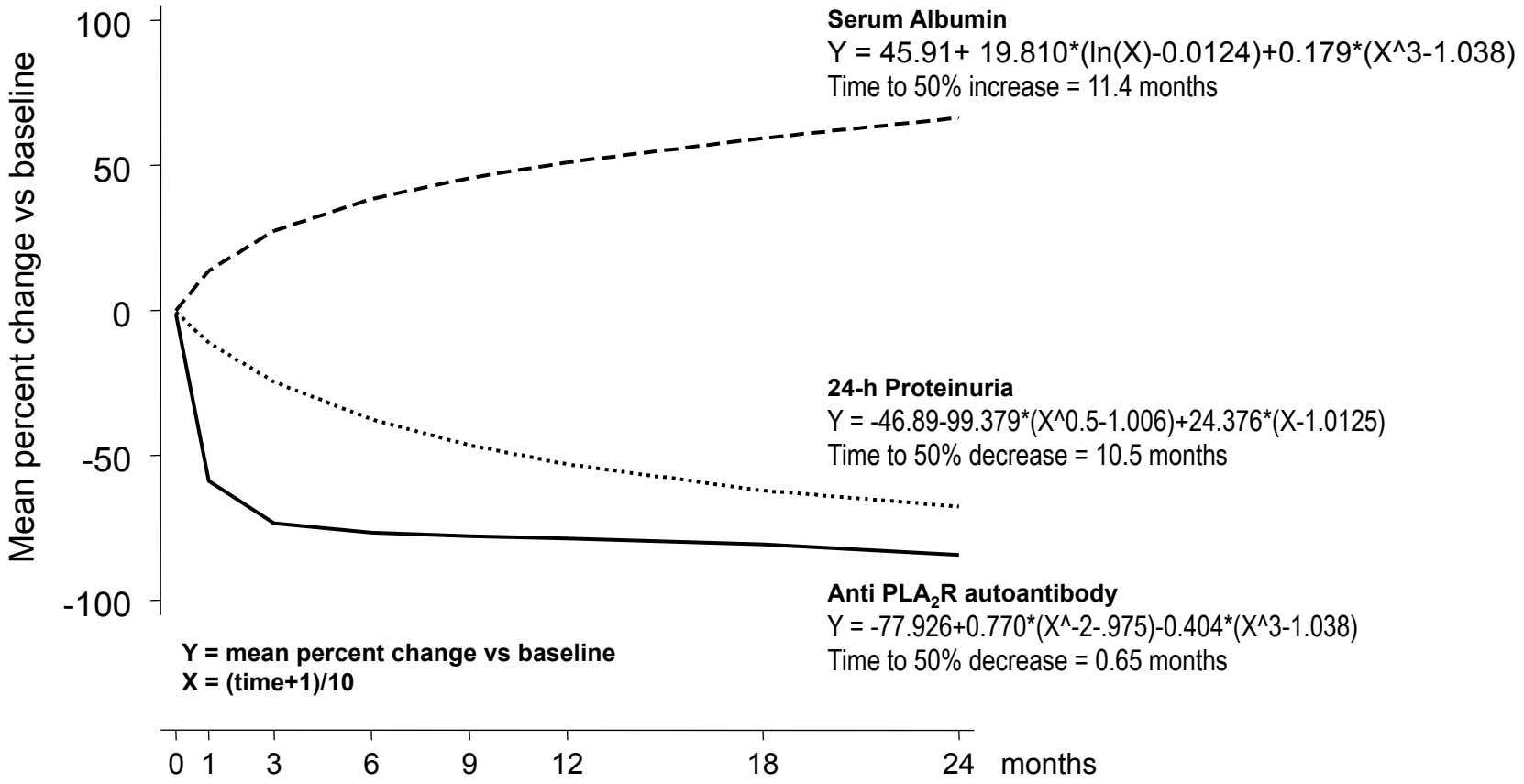

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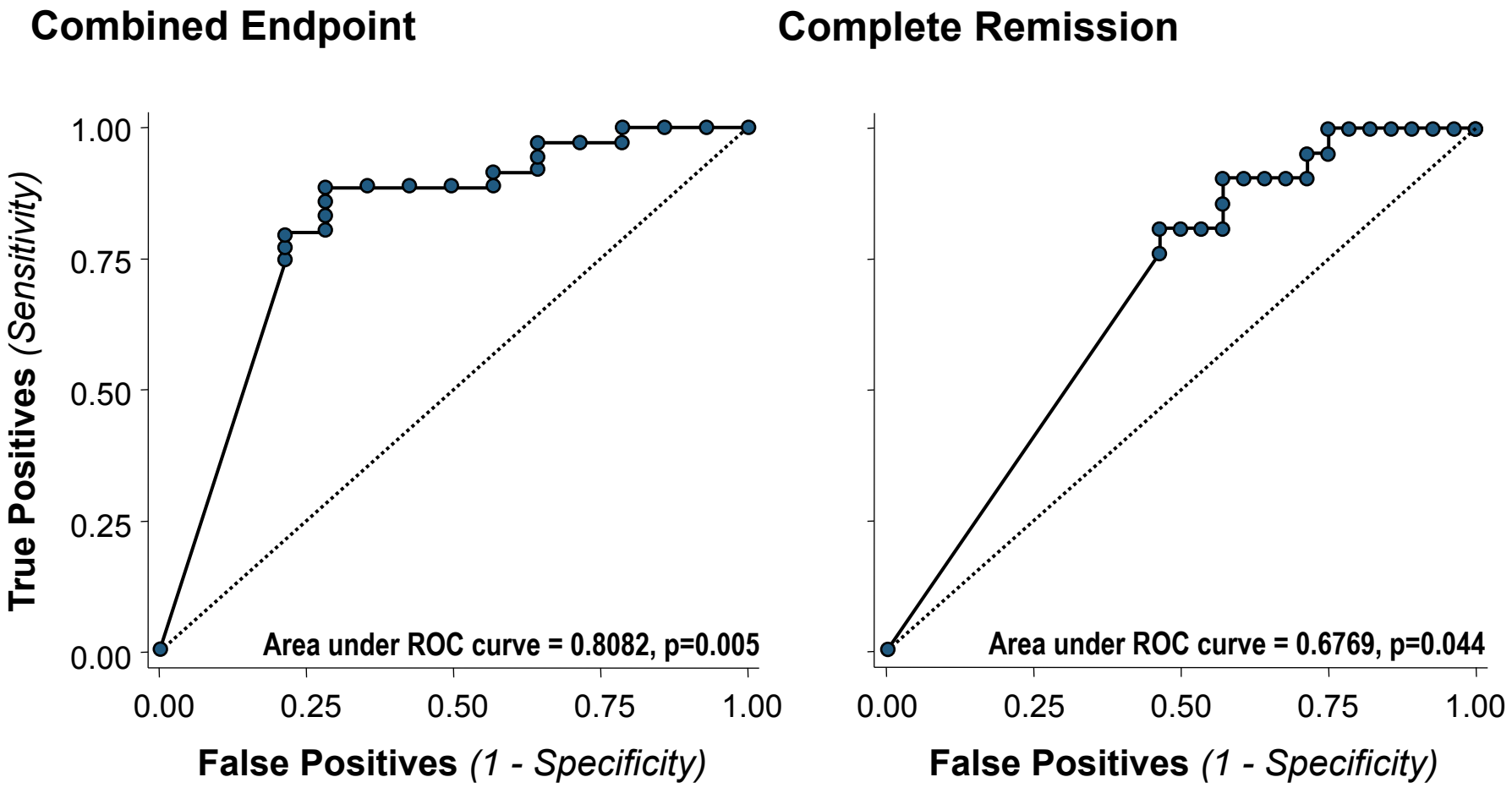

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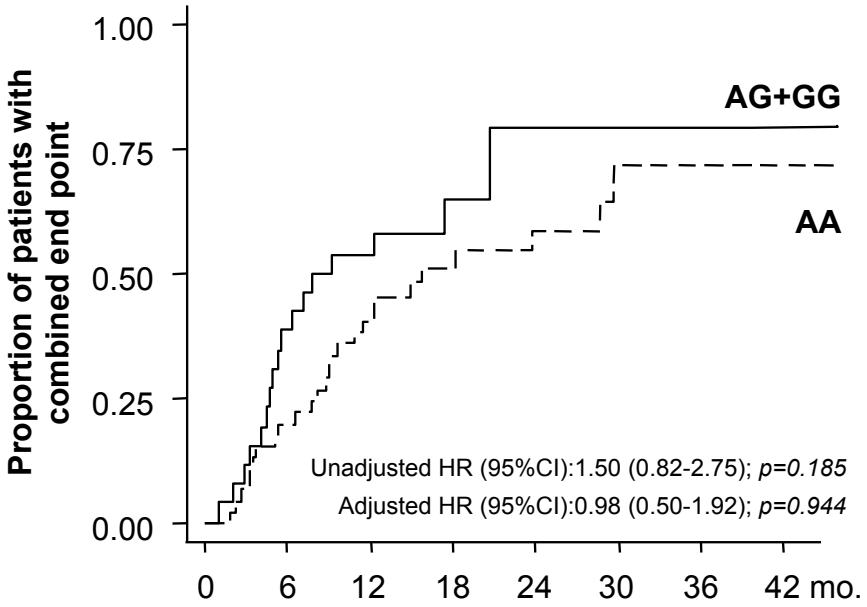
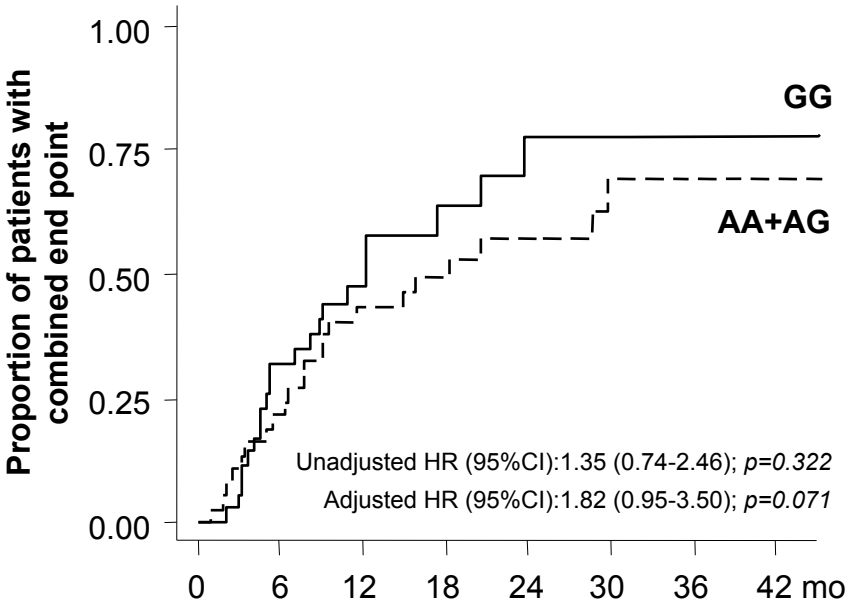


Patiens at risk according to PLA2R1 polymorphism

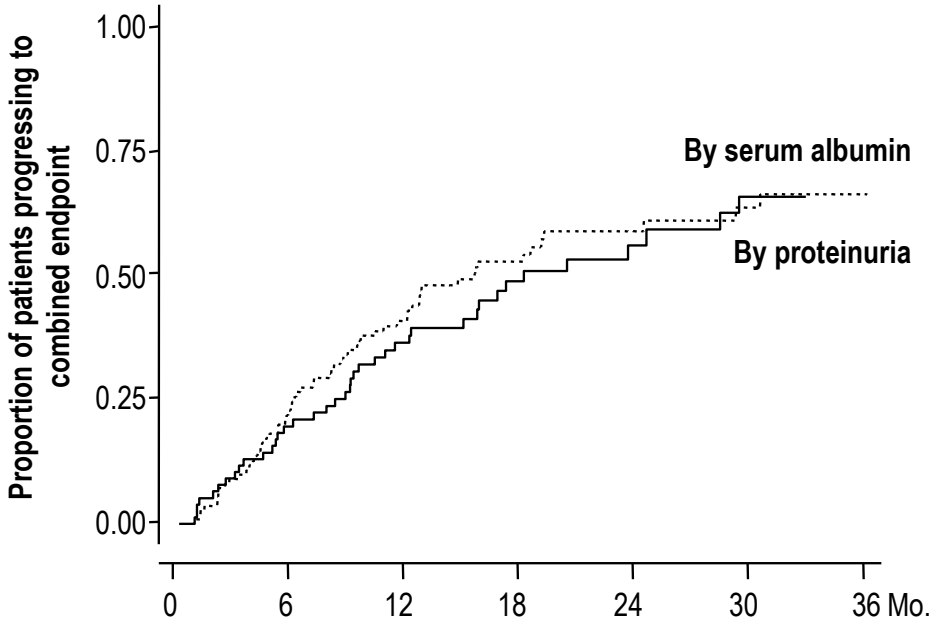
AG+GG	26	16	11	5	3	3	2	1
AA	46	35	26	16	10	4	4	3



Patients at risk according to HLA-DQA1 polymorphism

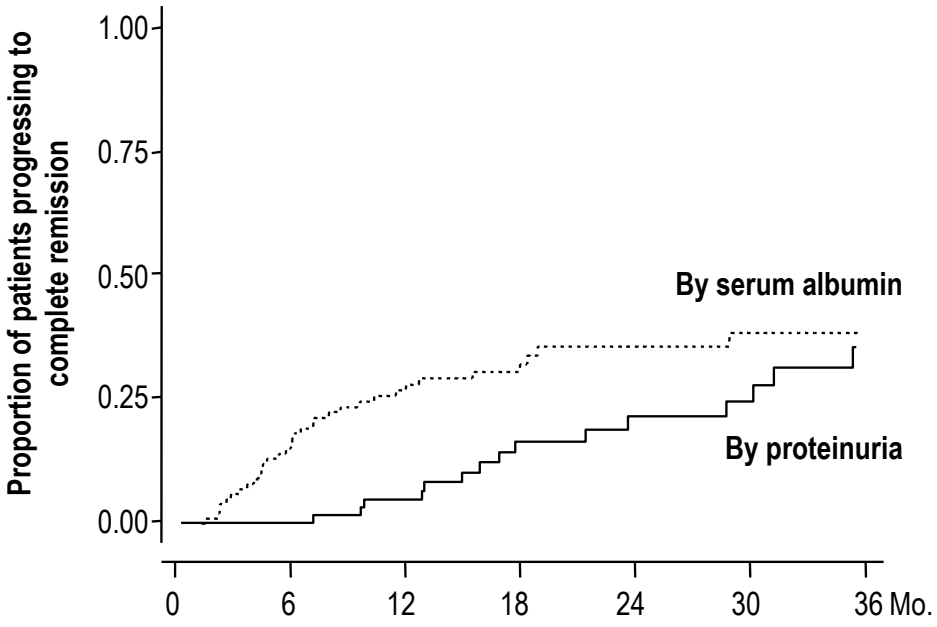
GG	35	22	16	6	3	2	1	1
AA+AG	37	29	21	15	10	5	5	3

Supplement Figure 6



Patients at risk

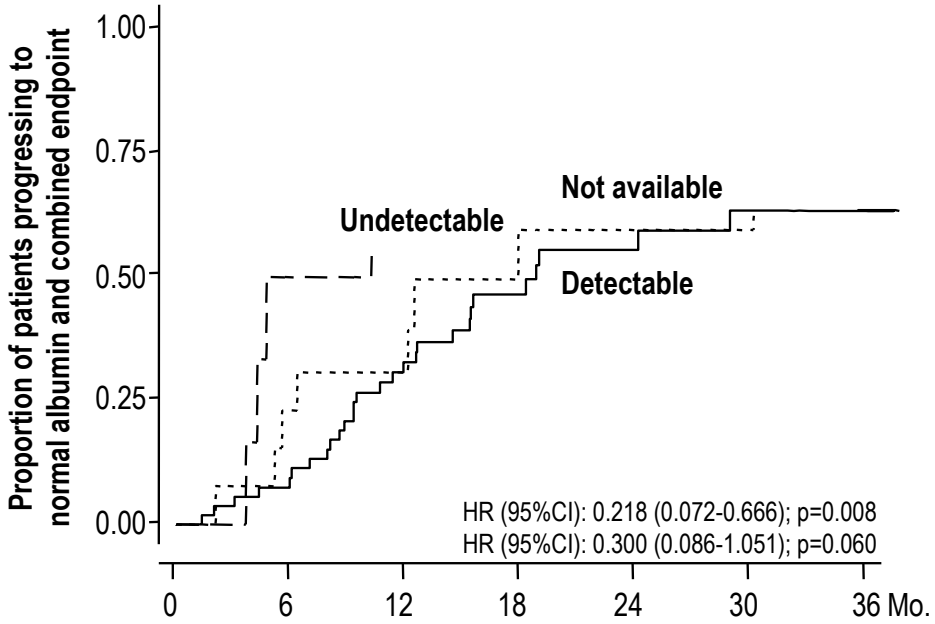
Proteinuria	73	55	40	24	14	9	6
Albumin	*73*	*60*	*43*	*25*	*14*	*10*	6



Patients at risk

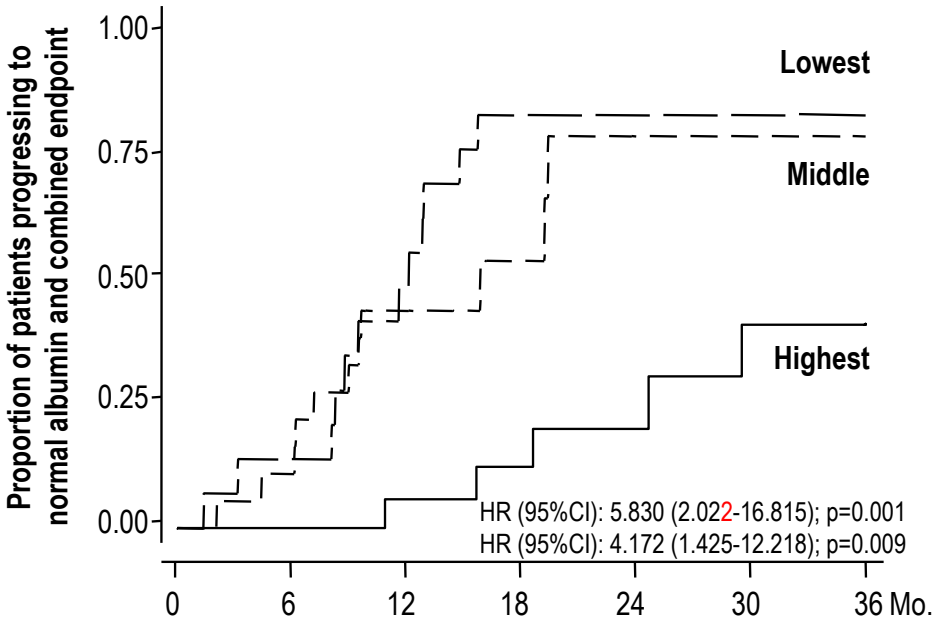
Proteinuria	73	69	59	42	33	25	18
Albumin	*73*	*64*	*51*	*37*	*26*	*19*	14

Supplement Figure 7



Patients at risk

Undetectable	6	2	0	0	0	0	0
Detectable	54	48	35	20	12	9	6
Not available	13	10	8	5	2	1	0



Patients at risk

Lowest	15	13	8	3	2	02	2
Middle	19	17	10	4	1	1	1
Highest	20	18	17	13	9	6	3

Supplement Figure 8