Efficacy, safety and immunological profile of combining rituximab with belimumab for adults with persistent or chronic immune thrombocytopenia: results from a prospective phase IIb trial

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Supplemental Table 1. List of monoclonal antibodies used for flow cytometry.

Panel	Antigen	Fluorochrome	Clones	Company
T cell subsets	CXCR5	BB515	RF8B2	BD bioscience
	PD1	PE	EH12.1	BD bioscience
	CXCR3	PE-CY5	IC6/CXCR3	BD bioscience
	CCR6	PeCy7	11A9	BD bioscience
	CD45RA	APC	HI100	BD bioscience
	CD3	AF700	UCHT1	BD bioscience
	HLA-DR	APC-H7	G46-6	BD bioscience
	ICOS	BV421	DX29	BD bioscience
	CCR7	BV605	3D12	BD bioscience
	CD27	BV711	L128	BD bioscience
	CD4	BV786	SK3	BD bioscience
	CD38	PerCy5,5	HIT2	BD bioscience
B cell subsets	CD24	FITC	ML5	BD bioscience
	CD3	PE	UCHT1	BD bioscience
	CD14	PE	M5E2	BD bioscience
	CD16	PE	3G8	BD bioscience
	IgD	PE CF594	IA6-2	BD bioscience
	CD38	PerCy5,5	HIT2	BD bioscience
	CD27	PE CY7	M-T271	BD bioscience
	CD19	AF700	HIB19	BD bioscience
	CD10	APC	HI10A	BD bioscience

Supplemental Table 2. Serum level of total gammaglobulins and immunoglobulin (Ig) isotypes (IgG, IgA, IgM) during the study of rituximab and belimumab combined.

	W0	W12	W24	W36	W52	ΔValue W0/W24
Gammaglobulins	10,6	9	9	9,05	9	1,2
IgG	11	9,3	9,405	9,01	9,745	0,98
IgM	1,11	0,8	0,74	0,67	0,74	0,42
IgA	1,8	1,42	1,53	1,41	1,71	0,3

Median gammaglobulins, IgG, IgM and IgA titers at W0, 12, 24, 36 and 52. ΔValue W0/W24 represents the decrease of median gammaglobulin titers between W0 and W24.

Supplemental Table 3: Antibody titers to the 13 serotypes of pneumococcus serotypes contained in Prevenar 13® (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) for each patient at week 0 (W0) and W52.

Serotype																										
	1		3		4		5		6A		6B		7F		9V		14		18C		19A		19F		23F	
Concentration µg/ml																										
Patient	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52	W0	W52
1	7.3	4	5	1.4	11	6.7	5.3	3.9	2.7	1.7	7.4	4.9	19	6.6	40	18	103	84	6.4	2.7	2.6	2	38	29	18	10
2	9.37	2.4	1	0.66	1.8	0.36	3.9	0.71	2.8	0.67	7.8	1.3	9.8	2.4	1.9	0.27	6.6	1.44	17	4.4	25	6.6	8.8	2.6	2.7	0.38
3	23	13	0.2	0.22	6.6	4.1	12	6.9	99	73	82	39	16	5.8	3.2	1.7	48	52	17	10	26	20	11	6.7	177	70
4	12	10	1.7	1.8	9.8	8	15	13	69	58	8.5	6.7	3.1	2.7	12	8.8	20	15	4.5	3.5	14	15	18	15	11	8
5	1.6	0.54	0.89	0.43	5.4	1.4	1.8	1.1	45	16	13	4.2	20	7.9	8	3.8	51	21	4.9	2.6	11	7	21	8.4	21	7.4
6	67	93	0.51	0.29	1.5	0.42	30	29	1.5	1.1	2.4	0.97	1.6	0.79	0.93	0.47	21	23	1.4	0.79	2.5	1.4	3.3	2.4	1.6	1.2
7	23	12	1.8	1.5	33	19	11	6.9	16	13	26	17	41	26	22	12	170	78	33	20	12	8.5	115	54	95	40
8	0.65	0.24	0.33	0.24	1.4	0.62	0.52	0.22	1.6	0.91	3.2	1.7	0.98	0.45	0.64	0.24	133	68	4.7	1.9	14	4.5	27	14	25	5.5
9	12	3.2	1.8	3.6	16	4.7	36	2.7	3.8	1.7	13	2.5	15	5.3	14	4.3	5.3	1.2	19	16	8.6	3.4	7.6	2.7	17	26
10	5.8	0.39	0.27	<0.1	0.32	0.13	0.65	0.76	0.75	0.29	0.32	0.19	4.4	1.3	2.6	1.1	1.6	0.45	12	2	3.2	0.96	4.2	1.1	1	0.19
11	3	1.3	0.85	0.33	4.3	4.2	10	5.6	8.1	5.1	15	7.8	6.7	2.9	7.4	3.2	20	8.9	3.1	0.46	25	9.7	10	4.6	4.3	0.69
12	6.9	1.1	0.3	<0.1	2.9	0.52	1.6	0.35	2.7	1	5.2	1.6	6.9	0.55	2.5	0.25	25	6.7	7.1	1.9	25	7	17	4.5	86	12
13	4.7	0.53	0.84	0.13	2.3	0.43	1.9	0.13	3.5	0.38	11	0.76	28	5.9	4.1	0.55	26	5.9	3.4	0.22	19	1.1	21	1.5	107	8
14	8.1	25	0.45	1	1.7	2.3	0.98	8.6	4.1	5.3	1.7	2.9	3.5	8.5	1.7	4.5	4.7	7.8	11	14	1.3	2.4	1.9	2.8	0.45	2.5
15	2.8	1.1	0.39	0.23	2.6	0.5	1.7	0.52	2.2	1	2.7	0.94	12	4.7	1.4	0.22	3	0.61	1	0.35	30	12	4.7	1.4	1.9	0.38

Supplemental Table 4. Antiplatelet antibody testing. Results of direct monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay (ApDia, Turnhout, Belgium).

Patients	ITP	Results of MAIPA W0	Results of MAIPA W24	Clinical outcome at W24	Results of MAIPA W52	Clinical outcome at W52
1	Persistent	Not performed	Negative	CR	Negative	CR
2	Persistent	Anti-GpIIb/IIIa	Negative	CR	Negative	CR
3	Chronic	Anti-GpIIb/IIIa	Negative	CR	Negative	CR
4	Persistent	Negative	Not performed	CR	Not performed	CR
5	Persistent	Anti-GpIIb/IIIa	Anti-GpIIb/IIIa	CR	Negative	CR
6	Persistent	Anti-GpIIb/IIIa	Negative	NR	Negative	NR
7	Chronic	Anti-GpIIb.IIIa	Anti-GpIIb/IIIa	R	Anti-GpIIb/IIIa	R
8	Persistent	Anti-GpIIb/IIIa	Negative	CR	Negative	CR
9	Persistent	Anti-GpIIb/IIIa	Anti-GpIIb/IIIa	CR	Negative	CR
10	Chronic	Anti-GpIb/IX	Negative	R	Negative	R
11	Persistent	Negative	Negative	NR	Negative	NR
12	Persistent	Anti-GpIIb/IIIa	Anti-GpIIb/IIIa	CR	Negative	CR
13	Chronic	Undetermined	Negative	R	Negative	NR
14	Persistent	Anti-GpIIb/IIIa	Anti-GpIIb/IIIa	CR	Anti-GpIIb/IIIa	CR
15	Persistent	Negative	Negative	R	Negative	CR

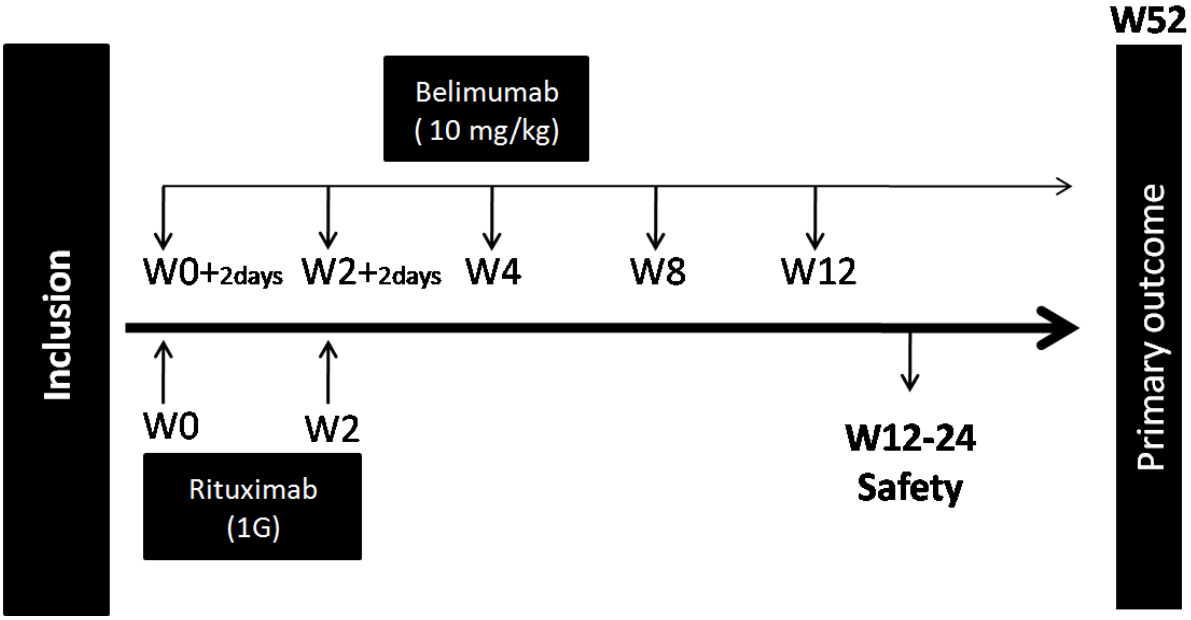
W, week; Gp, glycoprotein; CR, complete response, NR, no response; R, response

Supplemental Table 5. Baseline clinical characteristics of patients with immune thrombocytopenia (ITP) receiving rituximab alone.

Age/Sex	ITP duration (months)	Bleeding manifestations	Treatments received before inclusion	Nadir platelet count in the month before inclusion (x 10^9/L)	Anti-nuclear antibodies
77/F	9	Yes	CST, IVIG, romiplostim, eltrombopag	6	1/400
92/F	2	Yes	CST, IVIG, romiplostim, eltrombopag	26	1/80
67/F	9	Yes	CST, IVIG, romiplostim, eltrombopag	26	1/80
47/F	3	Yes	CST, IVIG	2	1/80
86/M	22	Yes	CST, IVIG, dapsone, eltrombopag	5	1/320
18/M	156	Yes	IVIG, Azathioprine	30	Neg
76/F	7	Yes	CST, eltrombopag, daspone	29	Neg
29/M	10	Yes	CST, dapsone	7	Neg
38/F	84	Yes	CST, IVIG, dapsone	22	1/500
75/M	3	Yes	CST, IVIG	6	Neg
40/M	3	Yes	CST	11	Neg
47/F	2	Yes	CST, IVIG, revolade	28	Neg

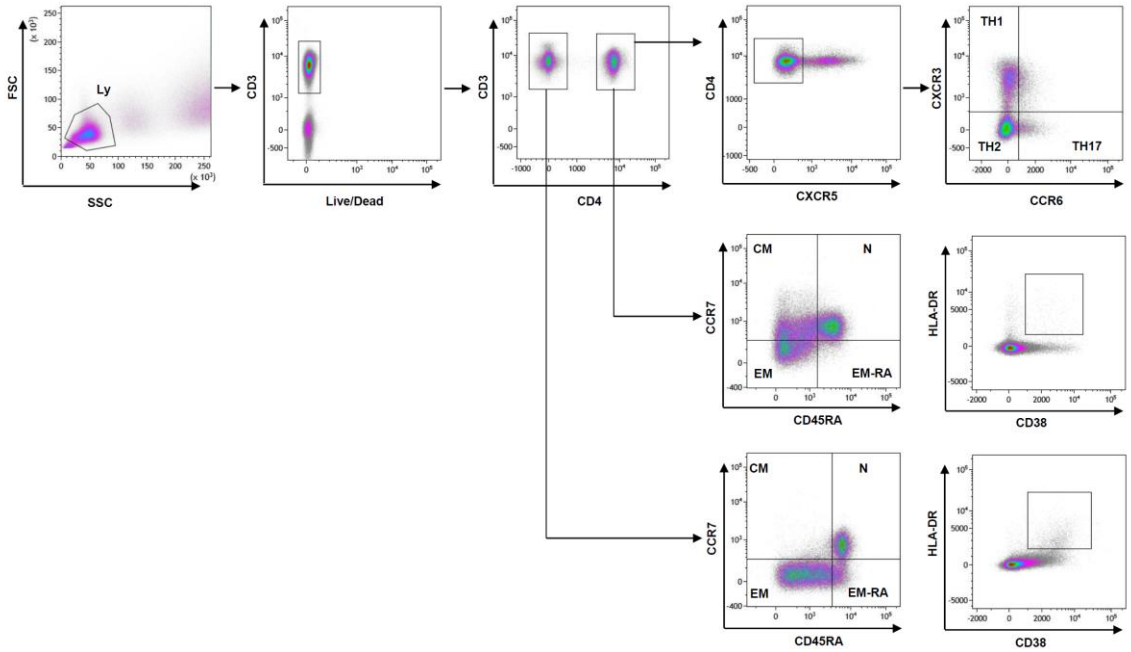
F, female; M, male; IVIG, intravenous immunoglobulins; CST, corticosteroids; Neg, negative

Supplemental Figure 1: Study design**.**



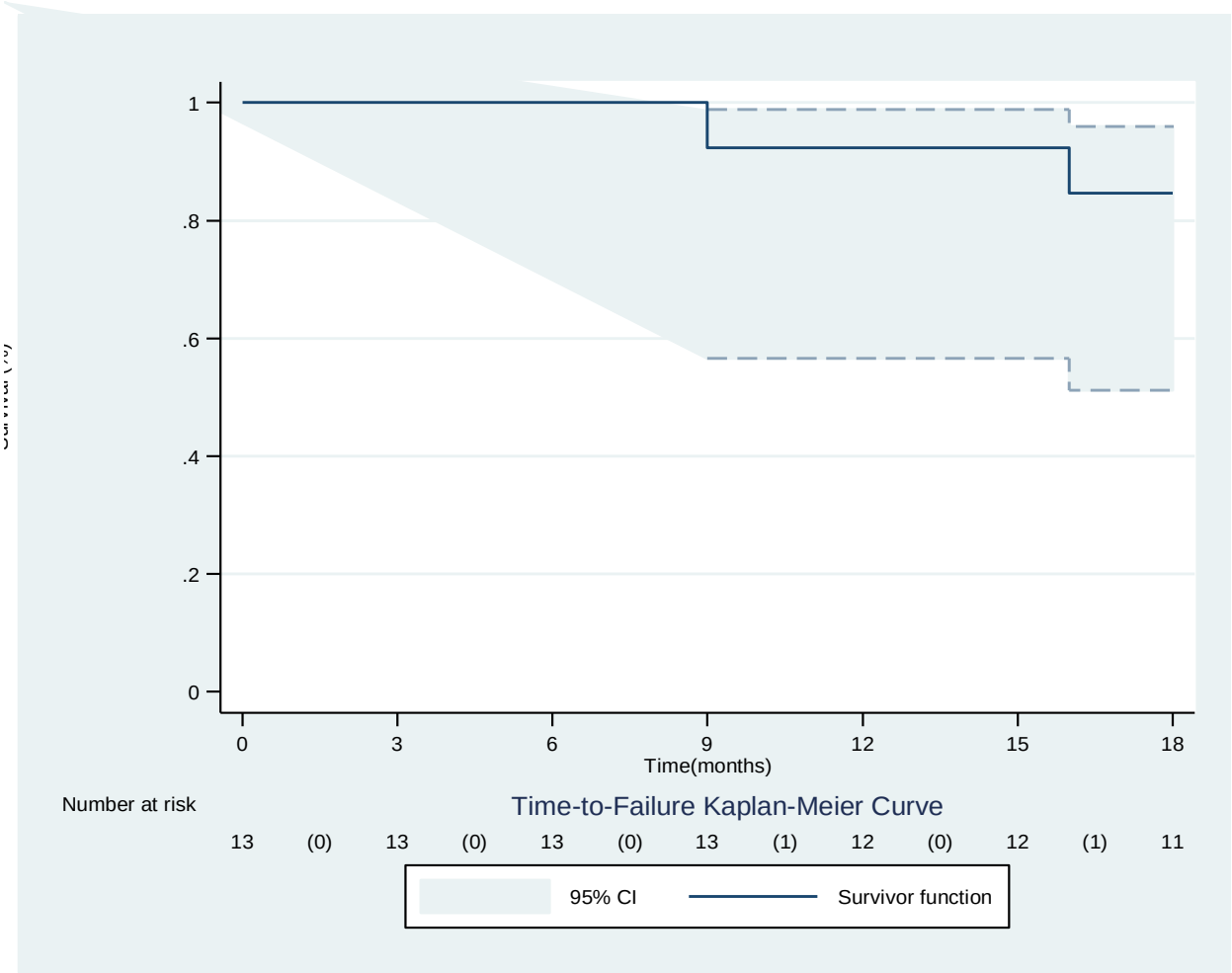
Eligible patients that gave consent and were verified for inclusion criteria received two intravenous infusions of 1 g of Rituximab (Mabthera®) at W0 and W2. Before RTX, premedication with 100 mg intravenous methylprednisolone and 5 mg dexchlopherinamine was administered. Five belimumab (Benlysta ®) infusions were administered (W0 + 2 days, W2 + 2 days, W4, W8, W12) at 10mg/kg doses intravenously. The first two injections were administered 2 days after Rituximab infusions. The adopted experimental scheme of belimumab was once used in systemic lupus erythematosus in accordance with European regulation. Follow-up was scheduled at W24, W36, and W52. The study was performed at the French national reference center for adult immune cytopenias at Henri Mondor university hospital, Créteil, France.

Supplemental Figure 2. Gating strategy of T cell panel**.**

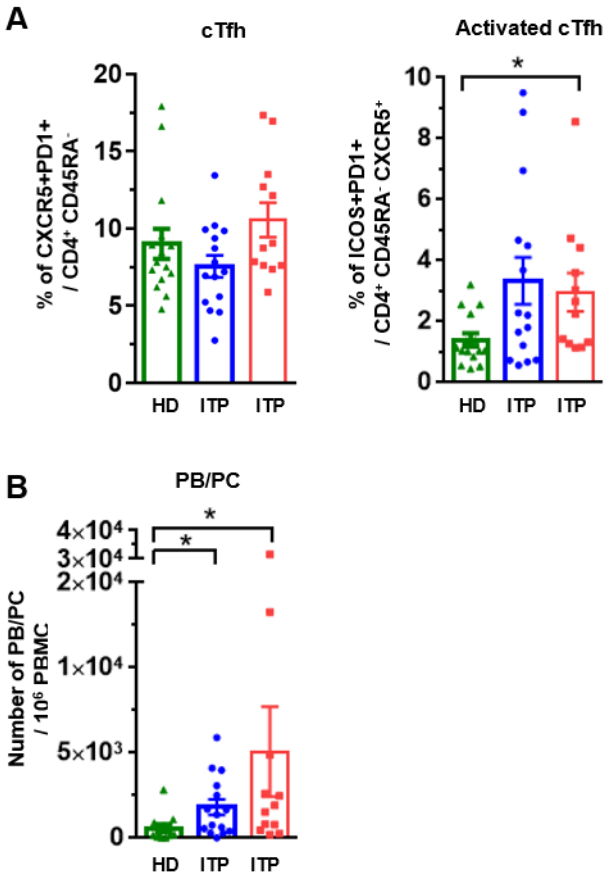


After gating on CD3+ we defined $CCR7^{+}CD45RA^{+}$ naive (T_N), $CCR7^{-}CD45RA^{-}$ memory (T_{EM}), and $CCR7^{+}CD45RA^{-}$ central memory (T_{CM}) in CD4+ and CD8+ cells. CD38 and HLA-DR activation markers were gated on CD4+ or CD8+ T cells.

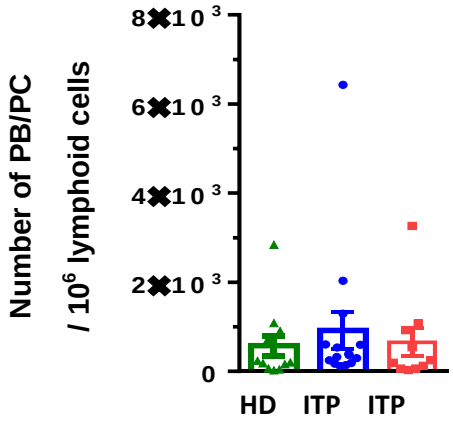
Supplemental Figure 3. Kaplan-Meier analysis of time-to-failure among initial responders to rituximab + belimumab treatment (N=13).



Supplemental Figure 4. Circulating T follicular helper (cTfh) cells and plasmablasts/plasma cells (PBs/PCs) in patients with immune thrombocytopenia (ITP) who were receiving the combination therapy or rituximab alone at baseline versus healthy donors (HD). (A) Percentage of cTfh and activated cTfh cells at week 0 in ITP patients before combination therapy (blue), rituximab treatment (red) and in healthy donors (green). (B) Number of PBs/PCs at week 0 in ITP patients before combination therapy (blue), rituximab treatment (Red) and in healthy donors (green). Data are mean±SEM. *P<0.05



Supplemental Figure 5. Number of **plasmablasts/plasma cells (**PB/PC) in healthy donors (green) and at week W52 in ITP patients after combination therapy (blue) or rituximab treatment (Red). Data are mean±SEM.



Supplemental Figure 6: Frequencies of T cell subsets, activation and polarization in ITP patients treated with the combination therapy or rituximab alone. (A) Percentage of $CD4^+$ and $CD8^+$ among CD3+ cells from whole blood obtained at week 0, 4, 12, 24, 36 and 52 in patients receiving rituximab and belimumab (in blue) or rituximab alone (in red) (B) Naïve $CD4^+$ and $CD8^+$ ($CCR7^+CD45RA^+$). (C) Effector memory $CD4^+$ and $CD8^+$ ($CCR7^-CD45RA^-$). (D) Central memory $CD4^+$ and $CD8^+$ ($CCR7^+CD45RA^-$). (E) Activated CD4+ and CD8+ T cells are defined as $HLA\text{-}DR^+CD38^+$. (F) Polarization of memory $CD4^+$ T cells using chemokine receptors, TH1 ($CXCR3^+CCR6^-$), TH2 ($CXCR3^-CCR6^+$) and TH17 ($CXCR3^-CCR6^+$) $CD4^+$ T cells. Data are mean±SEM.