APRIL levels are associated with disease activity in human chronic graft-*versus*-host disease

François Chasset,[1,2,3] Adèle de Masson,[1,2,3] Hélène Le Buanec,[1,2] Aliénor Xhaard,[2,4] Flore Sicre de Fontbrune,[2,4] Marie Robin,[2,4] Michel Rybojad,[2,3] Nathalie Parquet,[2,4,5] Anne C. Brignier,[2,4,5] Tereza Coman,[2,4] Djaouida Bengoufa,[2,6] Anne Bergeron,[2,7] Régis Peffault de Latour,[2,4] Martine Bagot,[1,2,3] Armand Bensussan,[1,2] Gérard Socié,[2,4,8*] and Jean-David Bouaziz[1,2,3*]*

*Have equally contributed to that work and share last author seniorship

[1]INSERM and UMRS 976, Laboratory of Onco dermatology, Immunology, and Cutaneous Stem cells, F-75739 Paris; [2]University Paris Diderot, Sorbonne Paris Cité; [3]Dermatology Department, Saint-Louis Hospital, Paris; [4]Hematology and Transplantation, Saint-Louis Hospital, Paris; [5]Therapeutic Apheresis Unit, Saint-Louis Hospital, Paris; [6]Immunobiology Department, Saint-Louis Hospital, Paris; and [7]Pneumology Department, Saint-Louis Hospital, Paris; [8]INSERM and UMRS1160, France

The online version of this letter has a Supplementary Appendix.

Correspondence: gerard.socie@aphp.fr

Supplemental table 1: Antibodies used for cell surface and intracellular flow cytometry staining

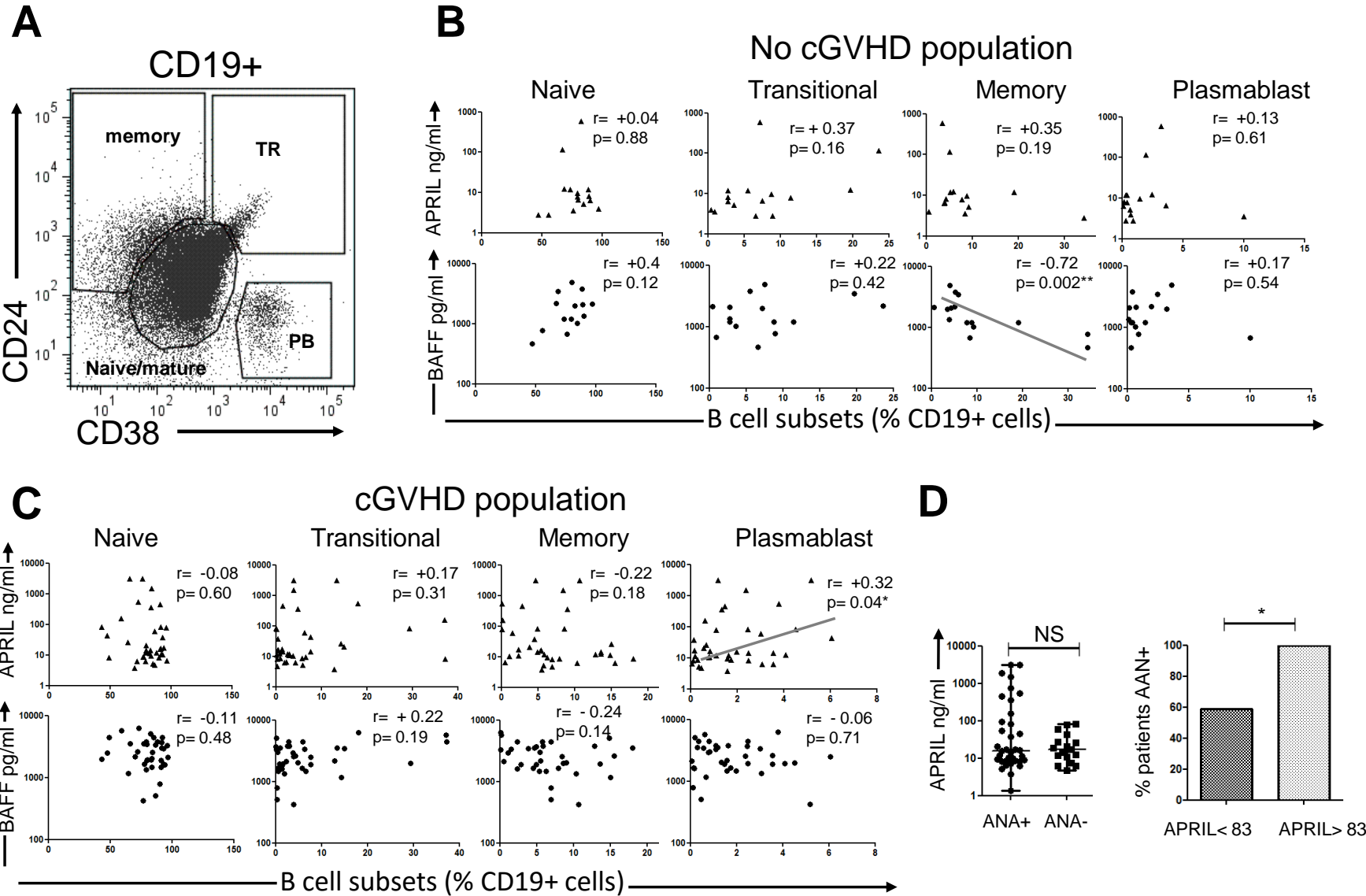| Specificity	Fluorophore	Clone	Manufacturer
Fixable Viability	eFluor® 506	NA[1]	eBioscience®
CD19	PerCP-Cy5.5	Hib19	eBioscience®
CD27	APC-eFluor 780	LG7F9	eBioscience®
CD24	FITC	ML5	BD Pharmigen®
CD38	APC	HIT2	BD Pharmigen®
CD267 TACI	PE	1A1	Miltenyi Biotec®
CD269 BCMA	APC	REA31	Miltenyi Biotec®
BAFF-R	FITC	7H22-E16	Miltenyi Biotec®

NA[1]: Not applicable

Supplemental table 2. Clinical and biological parameters associated with High APRIL levels

Variables	Low APRIL levels	High APRIL levels	p value (univariate analyze)	p value (multivariate analyze)
Severe cGVHD; n (%)	18 (40.9)	10 (90.9)	0.005*	0.03*
Lichenoid cGVHD; n (%)	21 (48.8)	6 (66.6)	0.46	-
Sclerodermiform cGVHD; n (%)	20 (46.5)	2 (22.2)	0.27	-
Ocular cGVHD; n (%)	12 (29.3)	3 (33.3)	1	-
Oral mucosa cGVHD; n (%)	20 (50)	2 (22.2)	0.26	-
Digestive tract cGVHD; n (%)	5 (11.3)	2 (18.1)	0.61	-
Hepatic cGVHD; n (%)	9 (20.4)	3 (27.27)	0.68	-
Pulmonary cGVHD; n (%)	8 (18)	6 (54.5)	0.02*	0.2
ANA +; n (%)	25 (60.9)	11 (100)	0.01*	0.9
Corticosteroids; n (%)	32 (78)	6 (54.5)	0.14	0.07

* Statistically significant; ANA: Antinuclear autoantibody

Supplemental Figure 1. Correlation between circulating BAFF and APRIL levels and B cell subsets and association with antinuclear antibodies.

Supplemental Figure 1. Correlation between BAFF and APRIL circulating levels and B cell subsets and association with antinuclear antibodies.

(A). Representative flow cytometry dot plot gating on $CD19^+$ B cell populations according to CD24 and CD38 immunostaining. (B). Correlation between circulating BAFF levels and B-cell subsets among no cGVHD patients. (C). Correlation between circulating APRIL levels and B-cell subsets among cGVHD patients. (D). Median APRIL circulating levels between patients with and without antinuclear antibodies (ANA) and frequency of ANA positivity between patients with circulating APRIL levels > or < 83 ng/ml. Correlations were measured using the Spearman test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS: Non-significant