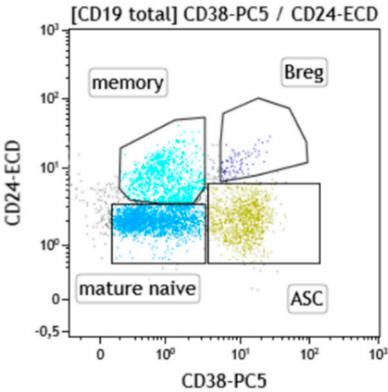
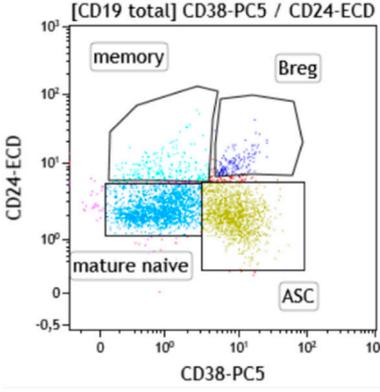


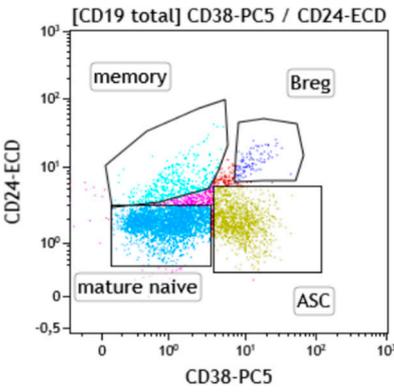
Supplementary Data



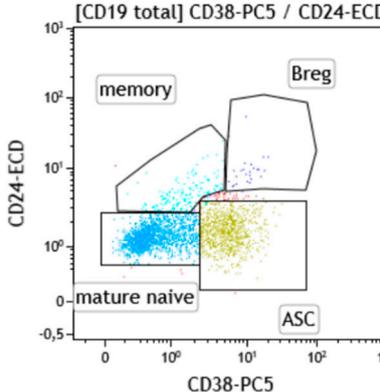
A.



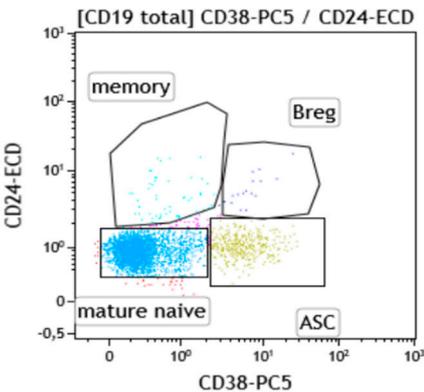
B. a)



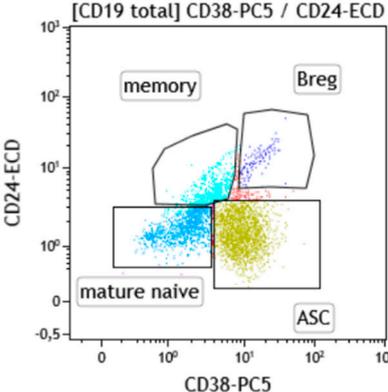
b)



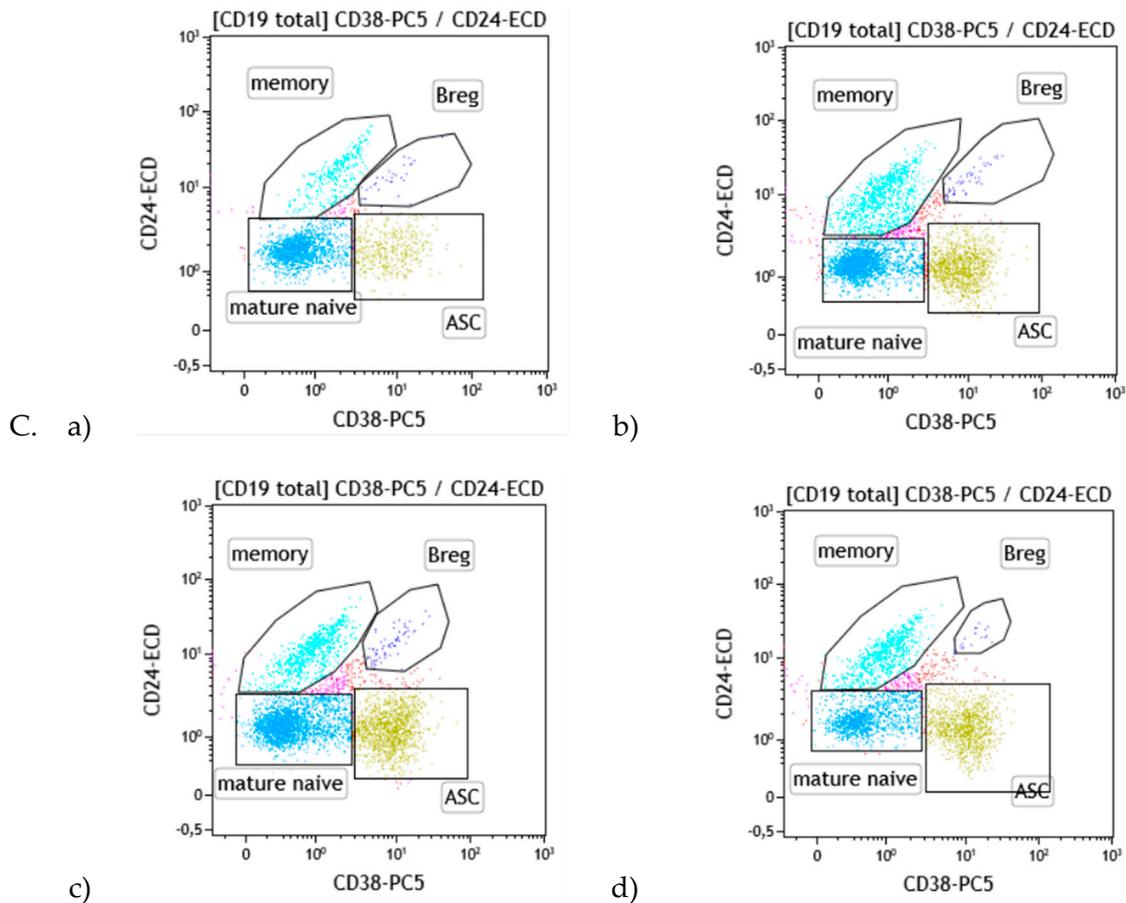
c)



d)



e)



Supplemental Figure S1: Representative plots (raw data) of flow cytometry from one healthy donor, one responder and one non-responder. Every plot represents the circulating B cell subpopulations analysed (mature naïve, memory, B regs and ASC) out of the total CD19+ B cells considering that the X-axis depicts the number ($\times 10^3$) of CD38+ B cells in ascending order and Y-axis depicts the number ($\times 10^3$) of CD24+ B cells in ascending order. A. plot of flow cytometry of a Healthy Donor. B. plot of flow cytometry of a Responder, figure (a) corresponding at timepoint 0, figure (b) at timepoint 1, figure (c) at timepoint 2, figure (d) at timepoint 3, figure (e) at timepoint 4. C. plots of flow cytometry of a Non-responder, figure (a) corresponding to the timepoint 0, figure (b) corresponding to the timepoint 1, figure (c) to the timepoint 2, figure (d) to the timepoint 3.

Target antigen	Clone	Isotype	Fluorochrome	Brand
CD19	J3-119	IgG1 Mouse	PE	Beckman Coulter
CD24	ALB9	IgG1 Mouse	ECD	Beckman Coulter
CD38	LS198-4-3	IgG1 Mouse	PC5	Beckman Coulter
CD21	BL13	IgG1 Mouse	FITC	Beckman Coulter
CD11c	BU15	IgG1 Mouse	PE	Beckman Coulter
T bet	4B10	IgG1 Mouse	FITC	BioLegend

Supplemental Table S1: Flow cytometry antibodies used in the experiments

Patient s	Gender	Age	Malignancy	Histological subtype	PD-L1 (%)	Outcom e
1 †	M	62	Uca	High-grade urothelial carcinoma		PD
2 †	M	65	NSCLC	Lung adenocarcinoma	<1%	PD
3 †	F	47	NSCLC	Lung adenocarcinoma		PD
4 †	M	72	NSCLC	Lung adenocarcinoma low differentiation	<1%	PD
5	M	70	NSCLC	Lung adenocarcinoma low differentiation		R
6 †	M	54	NSCLC	Squamous cell lung carcinoma, low differentiation	10%	PD
7	M	65	Uca	Infiltrating urothelial carcinoma, high-grade		R
8 †	M	75	Uca	High-grade urothelial carcinoma		PD
9	F	65	NSCLC	Lung adenocarcinoma		R
10	F	53	RCC	clear cell renal cell carcinomas		R
11	M	62	NSCLC	Squamous cell lung carcinoma, intermediate differentiation		R
12	M	80	NSCLC	Squamous cell lung carcinoma, intermediate-low differentiation	98%	R
13	F	63	NSCLC	Lung adenocarcinoma	5%	R
14 †	F	64	NSCLC	Lung adenocarcinoma		R
15	F	56	NSCLC	Squamous cell lung carcinoma, low differentiation		PD
16 †	M	65	NSCLC	Lung adenocarcinoma	<1%	PD
17 †	M	55	Uca	High-grade urothelial carcinoma		Non-evaluabl e
18	M	77	NSCLC	Squamous cell lung carcinoma, intermediate differentiation	>50%	R
19	M	60	SCCHN	Squamous cell laryngeal cancer		R
20	M	64	NSCLC	Lung adenocarcinoma low differentiation		PD

Supplemental Table S2: Supplementary data for all patients on histology and molecular features for each cancer type (PD: progressive disease, R: response; complete response; partial response; stable disease, †: indicates deceased patients, M: male, F: female, Uca: urothelial carcinoma, NSCLC: non-small cells lung cancer, RCC: renal cell carcinoma, SCCHN: squamous cell carcinoma of head and neck)

CD19+	Mean ± SEM of HDs	Mean ± SEM of R	Mean ± SEM of NR	P value HD VS R	P value HD VS NR	P value R VS NR
Timepoint 0	31.53 ± 1.682 N=8	27.62 ± 2.449 N=10	36.03 ± 5.127 N=10	0.23	0.4614	0.1563
Timepoint 1	-	25.03 ± 1.951 N10	29.03 ± 2.065 N=10	-	-	0.13
Timepoint 2	-	24.88 ± 2.118 N=10	25.64 ± 3.22 N=9	-	-	0.84

Timepoint 3	-	22.51 ± 2.396 N=9	22.39 ± 1.024 N=4	-	-	0.97
Timepoint 4	-	25.98 ± 1.458 N=9	19.87 ± 1.085 N=2	-	-	0.09

Supplemental Table S3. Statistical analysis of CD19+ B cells among healthy donors (HD), responders (R) and non-responders (NR). No significant difference was noticed.

Mature naive	Mean ± SEM of HDs	Mean ± SEM of R	Mean ± SEM of NR	P value HD VS R	P value HD VS NR
Timepoint 0	33.73 ± 4.612 N=8	46.52 ± 2.701 N=10	40.83 ± 5.175 N=10	0.0232	0.333
Timepoint 1	-	43.58 ± 3.815 N=10	43.18 ± 6.266 N=10	0.1161	0.2635
Timepoint 2	-	55.04 ± 5.815 N=10	52.51 ± 7.075 N=9	<i>0.0139</i>	0.0472
Timepoint 3	-	58.96 ± 5.211 N=9	49.95 ± 11.99 N=4	0.00027	0.1517
Timepoint 4	-	42.95 ± 4.209 N=9	31.76 ± 11.16 N=2	0.1597	0.8575

Supplemental Table S4. Statistical analysis of mature naïve B cells among healthy donors (HD), responders (R) and non-responders (NR). Significant differences observed at timepoint 0, 2 and 3 for responders and at timepoint 2 for non-responders compared to healthy donors. Remarkable differences between responders and non-responders were not noticed at any timepoint. Significant p-values are depicted bolted and in italics.

Memory	Mean ± SEM of HDs	Mean ± SEM of R	Mean ± SEM of NR	P value HD VS R	P value HD VS NR	P value R VS NR
Timepoint 0	32.44 ± 5.864 N=8	13.07 ± 1.854 N=10	23.47 ± 5.958 N=10	0.0033	0.3058	0.113
Timepoint 1	-	9.128 ± 1.368 N=10	19.94 ± 4.4108 N=10	-	-	0.0224
Timepoint 2	-	7.740 ± 1.938 N=10	11.29 ± 3.367 N=9	-	-	0.3621

Timepoint 3	-	9.798 ± 1.979 N=9	9.145 ± 4.032 N=4	-	-	0.872
Timepoint 4	-	9.668 ± 1.404 N=9	17.09 ± 12.08 N=2	-	-	0.2

Supplemental Table S5. Statistical analysis of memory B cells among healthy donors (HD), responders (R) and non-responders (NR). Between responders and non-responders statistical significance was revealed only at timepoint 1, whereas also statistical significance was noticed at baseline between healthy donors and responders. Significant p-values are depicted bolted and in italics.

Bregs	Mean ± SEM of HDs	Mean ± SEM of R	Mean ± SEM of NR	P value HD VS R	P value HD VS NR	P value R VS NR
Timepoint 0	24.811 ± 1.032 N=8	5.645 ± 2.416 N=10	2.937 ± 1.205 N=10	0.77	0.26	0.32
Timepoint 1	-	4.723 ± 1.453 N=10	2.559 ± 0.9959 N=10	-	-	0.23
Timepoint 2	-	3.305 ± 0.7281 N=10	1.776 ± 0.752 N=9	-	-	0.16
Timepoint 3	-	3.587 ± 1.391 N=9	1.762 ± 1.339 N=4	-	-	0.4087
Timepoint 4	-	4.906 ± 0.946 N=9	7.3 ± 6.25 N=2	-	-	0.4614

Supplemental Table S6. It was observed no statistically significant result from the analysis of Bregs among healthy donors (HD), responders (R) and non-responders (NR).

ASCs	Mean ± SEM of HDs	Mean ± SEM of R	Mean ± SEM of NR	P value HD VS R	P value HD VS NR	P value R VS NR
Timepoint 0	20.62 ± 4.008 N=8	24.51 ± 4.327 N=10	24.86 ± 4.914 N=10	0.5283	0.5278	0.9572
Timepoint 1	-	33.86 ± 4.779 N=10	26.01 ± 5.863 N=10	-	-	0.3130
Timepoint 2	-	25.48 ± 4.528 N=10	21.43 ± 5.721 N=9	-	-	0.5828

Timepoint 3	-	21.08 ± 3.793 N=9	29.1 ± 11.19 N=4	-	-	0.3986
Timepoint 4	-	29.49 ± 4.587 N=9	31.8 ± 3.575 N=2	-	-	0.8267

Supplemental Table S7. The results from the statistical analysis among healthy donors (HD), responders (R) and non-responders (NR) revealed no significant difference at any timepoint of the study.

ABCs	Mean ± SEM of HDs	Mean ± SEM of R	Mean ± SEM of NR	P value HD VS R	P value HD VS NR	P value R VS NR
Timepoint 0	0.0088 ± 0.0021 N=8	0.0088 ± 0.0025 N=10	0.0551 ± 0.0213 N=10	-	<i>0.06</i>	<i>0.04</i>
Timepoint 1	-	-	0.0297 ± 0.007 N=10	-	<i>0.01</i>	-
Timepoint 2	-	-	0.0283 ± 0.0072 N=9	-	<i>0.02</i>	-
Timepoint 3	-	-	0.058 ± 0.0359 N=4	-	<i>0.03</i>	-
Timepoint 4	-	0.003 ± 0.0008 N=8	-	<i>0.02</i>	-	-

Supplemental Table S8. ABCs' statistical analysis revealed significant differences at baseline between healthy donors (HD) and non-responders (NR) and responders (R) and non-responders. Important differences were also noticed at timepoints 1, 2 and 3 between HD and NR and at timepoint 4 between HD and R. Significant p-values are depicted bolted and in italics.

MFI Tbet	Mean ± SEM of R	Mean ± SEM of NR	P value R VS NR
Timepoint 0	0.3856 ± 0.0428 N=10	0.7989 ± 0.0745 N=10	<i>0.0002</i>

Supplemental Table S9. According to MFI Tbet statistical analysis, a significant difference was observed only at the baseline between responders (R) and non-responders (NR).

CD19+	Mean \pm SEM of HDs	Mean \pm SEM of irAEs groups	Mean \pm SEM of Non-irAEs groups	P value HD VS irAEs groups	P value HD VS non-irAEs group
Timepoint 0	31.53 \pm 1.682 N=8	-	-	-	-
Timepoint 3	-	-	24.69 \pm 1.591 N=9	-	<i>0.009</i>
Timepoint 4	-	23.52 \pm 1.734 N=4	25.63 \pm 2.006 N=7	<i>0.01</i>	<i>0.04</i>

Supplemental Table S10. Statistical analysis of CD19+ subpopulation among healthy donors (HD), patients who developed immune-related adverse events (irAES group) and patients who did not develop irAEs (non-irAEs group). Significant differences were observed between HDs and irAEs group at timepoint 4 and between HDs and non-irAes group at timepoints 3 and 4. Significant p-values are depicted bolted and in italics.

Mature naive B cells	Mean \pm SEM of HDs	Mean \pm SEM of irAEs groups	Mean \pm SEM of Non-irAEs groups	P value HD VS irAEs groups	P value HD VS non-irAEs group
Timepoint 0	33.73 \pm 4.612 N=8	-	-	-	-
Timepoint 2	-	58.52 \pm 7.188 N=6	52.70 \pm 5.308 N=14	<i>0.0103</i>	<i>0.0258</i>
Timepoint 3	-	61.69 \pm 6.993 N=5	52.56 \pm 6.104 N=9	<i>0.005</i>	<i>0.0292</i>

Supplemental Table S11. Mature naïve B cells statistical analysis among healthy donors (HD), patients who developed immune-related adverse events (irAES group) and patients who did not develop irAEs (non-irAEs group). Both irAEs and non-irAEs groups revealed remarkable differences at timepoints 2 and 3. Significant p-values are depicted bolted and in italics.

Memory B cells	Mean ± SEM of HDs	Mean ± SEM of irAEs groups	Mean ± SEM of Non-irAEs groups	P value HD VS irAEs groups	P value HD VS non-irAEs group
Timepoint 0	32.44 ± 5.864 N=8	-	18.17 ± 3.554 N=15	-	<i>0.03</i>
Timepoint 1	-	15.03 ± 3.922 N=6	14.71 ± 2.960 N=15	<i>0.04</i>	<i>0.006</i>
Timepoint 2	-	10.29 ± 2.919 N=6	9.647 ± 2.372 N=14	<i>0.01</i>	<i>0.0004</i>
Timepoint 3	-	12.32 ± 4.270 N=5	9.983 ± 2.259 N=9	<i>0.03</i>	<i>0.002</i>
Timepoint 4	-	10.12 ± 1.132 N=4	11.53 ± 3.462 N=7	<i>0.02</i>	<i>0.01</i>

Supplemental Table S12. Significant differences were noticed at almost all timepoints between healthy donors (HDs) and patients who developed irAEs (irAEs group) and between HD and patients who did not develop irAEs (non-irAEs group). No important difference was observed when the irAEs and non-irAEs groups were compared. Significant p-values are depicted bolted and in italics.

Bregs	Mean ± SEM of HDs	Mean ± SEM of Non-irAEs groups	P value HD VS non-irAEs group
Timepoint 0	4.811 ± 1.032 N=8	-	-
Timepoint 2	-	2.146 ± 0.5906 N=14	<i>0.025</i>

Supplemental Table S13. Patients who did not develop irAEs (non-irAEs group) confirmed significantly lower counts of Bregs compared to healthy donors (HDs) at timepoint 2. The significant p-value is depicted bolted and in italics.

ABCs	Mean \pm SEM of HDs	Mean \pm SEM of Non-irAEs groups	P value HD VS non-irAEs group
Timepoint 0	0.0088 \pm 0.0021 N=8	-	-
Timepoint 1	-	0.0289 \pm 0.0065 N=13	<i>0.03</i>
Timepoint 2	-	0.03217 \pm 0.008766 N=12	<i>0.04</i>

Supplemental Table S14. From the statistical analysis of the ABC subset, significant differences were observed only at timepoints 1 and 2 between healthy donors (HDs) and patients who did not develop immune-related adverse events (non-irAEs group). Significant p-values are depicted bolted and in italics.