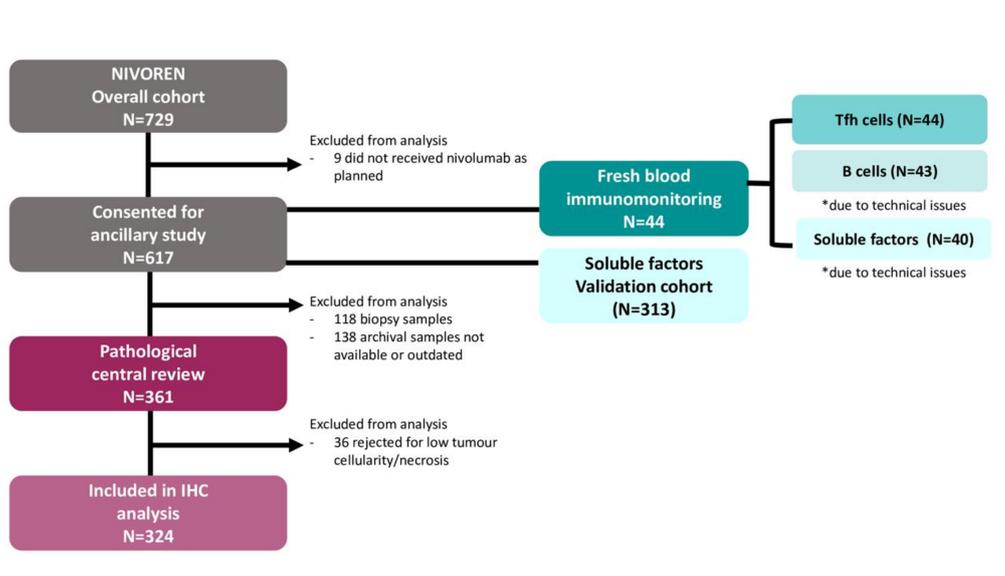


SUPPLEMENTARY MATERIALS:

Supplementary Methods:



Supplementary Figure S1. NIVOREN translational study flowchart.

Sample processing

Blood samples were collected in heparinized tubes for fresh whole blood immune phenotyping and plasma banking. For plasma banking, samples were centrifuged at 800 *g* for 10 minutes and stored at -80°C until analysis. After thawing, a second centrifugation was performed at 10 000 \times *g* for 10 minutes at $2-8^{\circ}\text{C}$ to remove platelets before conducting cytokine, soluble factors, and growth factors dosage.

Fresh whole blood immunophenotyping

Immune phenotyping was realized at the Laboratory of Immunomonitoring in Oncology (L.I.O.) of the Gustave Roussy Cancer Campus.

To avoid the loss or reduction or phenotype modifications due to the process of freezing/thawing and to reduce technical manipulations and the blood sampling needed to perform the tests, we performed fresh whole blood immune cell phenotyping. To ensure feasibility and reproducibility for use in longitudinal clinical studies, we designed and optimized the marker and dye selections of 6 10-colors flow cytometry panels to use in clinical research studies as previously described (39): Numeration, B cells, Polarization, Activation, Treg Phenotype and MDSC panels, described in the supplementary table S1.

For surface staining on blood, fresh whole blood (100 μ l) was incubated for 20 min at room temperature in the dark with liquid antibodies or in Duraclone® tubes. Erythrocytes lysis was performed adding 1 ml of Versalyse™ (Beckman) containing 25 μ l of Fixative Solution (Beckman) for liquid antibodies and 2 ml of Versalyse containing 50 μ l of Fixative Solution for Duraclone® tubes. After vortex, tubes were incubated for 20 min at room temperature in the dark. After adding 2 ml of phosphate-buffered saline (PBS) 1X, tubes were centrifuged 5 min at 500g, cells were resuspended in 3 ml of 1X PBS and centrifuged again. The pellet was resuspended in 250 μ l of 1X PBS and tubes were analyzed on a Gallios™ flow cytometer (Beckman, 10 colors, 3 lasers).

Supplementary Table S1. Flow cytometry panels.

Target – Fluorochrome	Provider	Clone	Reference	Dilution	Panel
CD16 – FITC	Beckman Coulter	3G8	B49215	1/10	Numeration
CD56 – PE	Beckman Coulter	N901	A07788	1:10	Numeration Activation
CD19 – ECD	Beckman Coulter	J3-119	A07770	1/20	Numeration
CD244 – PC5,5	Beckman Coulter	C1.7	B21171	1/40	Numeration MDSC
CD14 – PC7	Beckman Coulter	RMO52	A22331	1/50	Numeration
CD8 APC	Beckman Coulter	B9.11	IM2469	1/50	Numeration
CD4 AA700	Beckman Coulter	13B8.2	B10824	1/50	Numeration
CD3 AA750	Beckman Coulter	UCHT1	A94680	1/100	Numeration
CD15 PB	Beckman Coulter	ASR	A74775	1/10	Numeration MDSC
CD45 KrO	Beckman Coulter	J33	B36294	1/20	Numeration
CD57 FITC	Beckman Coulter	NC1	IM0466U	1/10	Activation
CD160 – PE	Beckman Coulter	BY55	IM3657	1/10	Activation

CD69 ECD	Beckman Coulter	TP1.55.3	6607110	1/10	Activation
CD279 PC5,5	Beckman Coulter	PD1 .3	B36123	1/10	Activation
CD137 PC7	Biolegend	4B4-1	309818	1/20	Activation
CD134 (OX40) – PC5,5	Biolegend	ACT35	350010	1:20	Activation
CD3 – AA700	Beckman Coulter	UCHT1	B10823	1:20	Activation Polarization
CD5 – AA750	Beckman Coulter	BL1a	A78836	1/20	Activation
CD4 – PB	Beckman Coulter	13B8.2	A82789	1/50	Activation Polarization
CD8 – KrO	Beckman Coulter	B9.11	B00067	1/50	Activation
IgD – FITC	Beckman Coulter	IA6-2	B30652	1/50	B cells
CD10 – PE	Beckman Coulter	ALB1	A07760	1/10	B cells
CD5 – ECD	Beckman Coulter	BL1a	A33096	1/20	B cells
CD27 – PC5,5	Beckman Coulter	1A4CD27	B21444	1/20	B cells
CD38 – PC7	Beckman Coulter	LS198-4-3	A54189	1/50	B cells
IgM – APC	Beckman Coulter	SA-DA4	B30654	1/50	B cells
CD19 – AA700	Beckman Coulter	J3-119	A07770	1/20	B cells
CD24 – AA750	Beckman Coulter	ALB9	B10738	1/50	B cells

CD21 PB	Beckman Coulter	BL13	B09982	1/50	B cells
CD32 KrO	Beckman Coulter	2E1	B01177	1/20	B cells MDSC
CXCR3 – FITC	Biolegend	G025H7	353703	1:20	Polarization
CCR10 – PE	R&D Systems	6588-5	FAB3478P	1:10	Polarization
CCR7 – Dazzle	Biolegend	G043H7	353236	1/20	Polarization
CCR6 – PE-Cy5,5	Biolegend	G034E3	353405	1:10	Polarization
CCR4 – PC7	Biolegend	L291H4	359409	1:20	Polarization
CXCR5 – APC	Biolegend	J252D4	356907	1:20	Polarization
CD45RA – AA750	Beckman Coulter	2H4LDH11LDB9	A86050	1:20	Polarization
CD161 – BV510	Biolegend	HP-3G10	339922	1:20	Polarization
CD33 – FITC	Biolegend	HIM3-4	303304	1/20	MDSC
HLA-DR – ECD	Beckman Coulter	Immu-357	IM3636	1/20	MDSC
CD64 – PC7	Beckman Coulter	22	B06025	1/20	MDSC
CD11b APC	Beckman Coulter	Bear1	A87782	1/20	MDSC
CD14 AA700	Beckman Coulter	RMO52	A99020	1/20	MDSC
CD16 – AA750	Beckman Coulter	3G8	A66330	1/50	MDSC

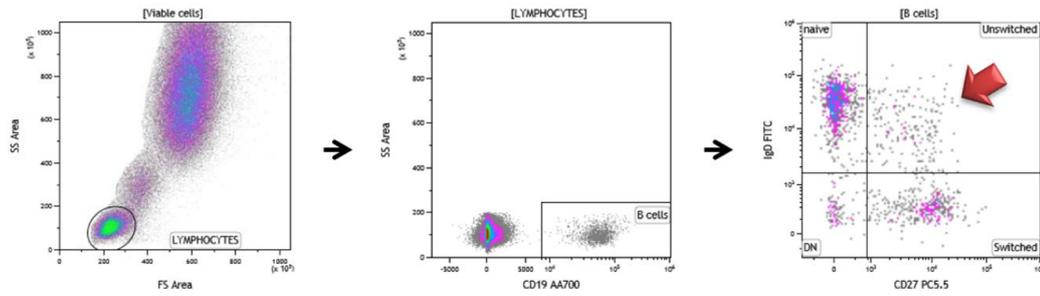
Plasma cytokine and soluble proteins concentrations

Cytokine and soluble proteins concentrations were measured in duplicate in plasma samples using the Meso-Scale Discovery (MSD) immunoassay (Rockville, MD, United States) according to the manufacturer's protocol. Eight different MSD kits were used for each plasma sample: "V-PLEX Human Proinflammatory Panel 1" to measure TNF-alpha, IFN-gamma, IL-6, IL-8 and IL-10, "V-PLEX Human Vascular Injury Panel 2" to measure VCAM-1, "V-PLEX Human Cytokine Panel 1" to measure IL-7 and VEGF, "V-PLEX Human Chemokine Panel 1" to measure MDC, "R-PLEX Human BCA-1/BLC/CXCL13 Assay" to measure BCA-1/CXCL13, "R-PLEX Human APRIL/TNFSF13 Assay" to measure APRIL, "R-PLEX Human 4-1BB/TNFRSF9 Assay" to measure 4-1BB and "U-PLEX Immuno-Oncology Assay" to measure BAFF and SDF-1alpha. Samples were measured using the MESO QuickPlex SQ120 and data, expressed in pg/ml of protein, were analyzed using the DISCOVERY WORKBENCH software (version 4.0). The lower limits of detection (LLOD) are described in Table S2.

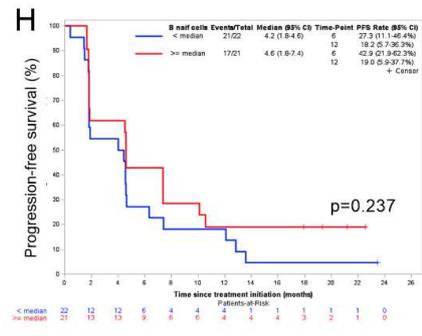
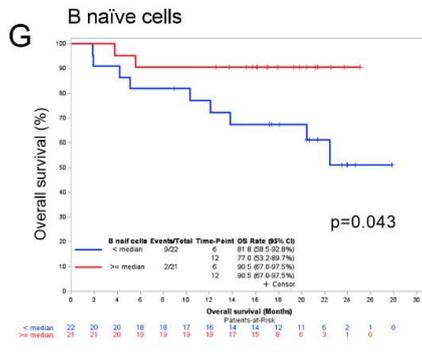
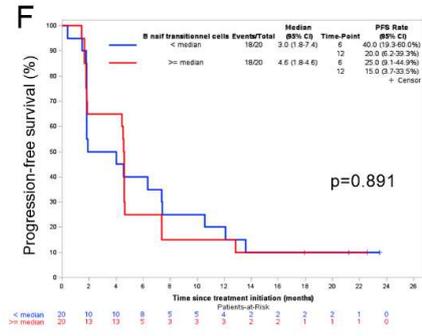
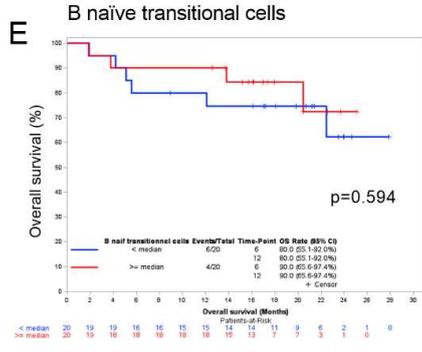
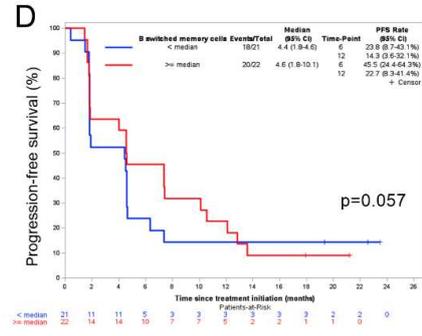
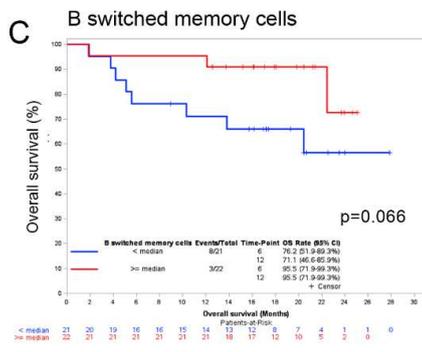
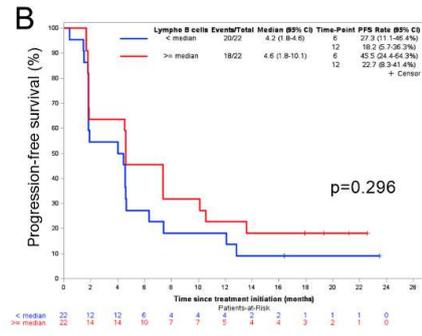
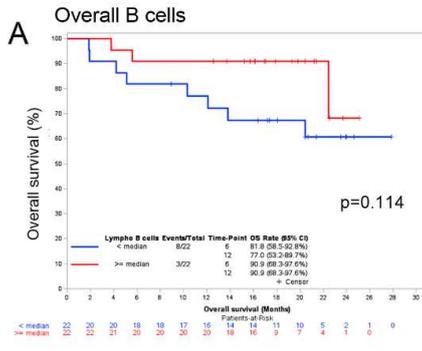
Supplementary Table S2. Plasma cytokines and soluble factor's concentration.

Target Cytokine / Soluble protein	Kit	Catalog number	Sample dilution	LLOD	Panel
TNF-alpha	V-PLEX Human Proinflammatory Panel 1	N05049A-1	1:2	0,04 pg/ml	Proinflammatory
IL-6	V-PLEX Human Proinflammatory Panel 1	N05049A-1	1:2	0,06 pg/ml	Proinflammatory
IL-8	V-PLEX Human Proinflammatory Panel 1	N05049A-1	1:2	0,07 pg/ml	Proinflammatory
IL-10	V-PLEX Human Proinflammatory Panel 1	N05049A-1	1:2	0,04 pg/ml	Proinflammatory
IFN-gamma	V-PLEX Human Proinflammatory Panel 1	N05049A-1	1:2	0,37 pg/ml	Proinflammatory
VEGF-A	V-PLEX Human Cytokine Panel 1	N05050A-1	1:2	1,12 pg/ml	Cytokine
IL-7	V-PLEX Human Cytokine Panel 1	N05050A-1	1:2	0,12 pg/ml	Cytokine
VCAM-1	V-PLEX Human Vascular Injury Panel 2	N45198B-1	1:1000	6,00 pg/ml	Vascular Injury

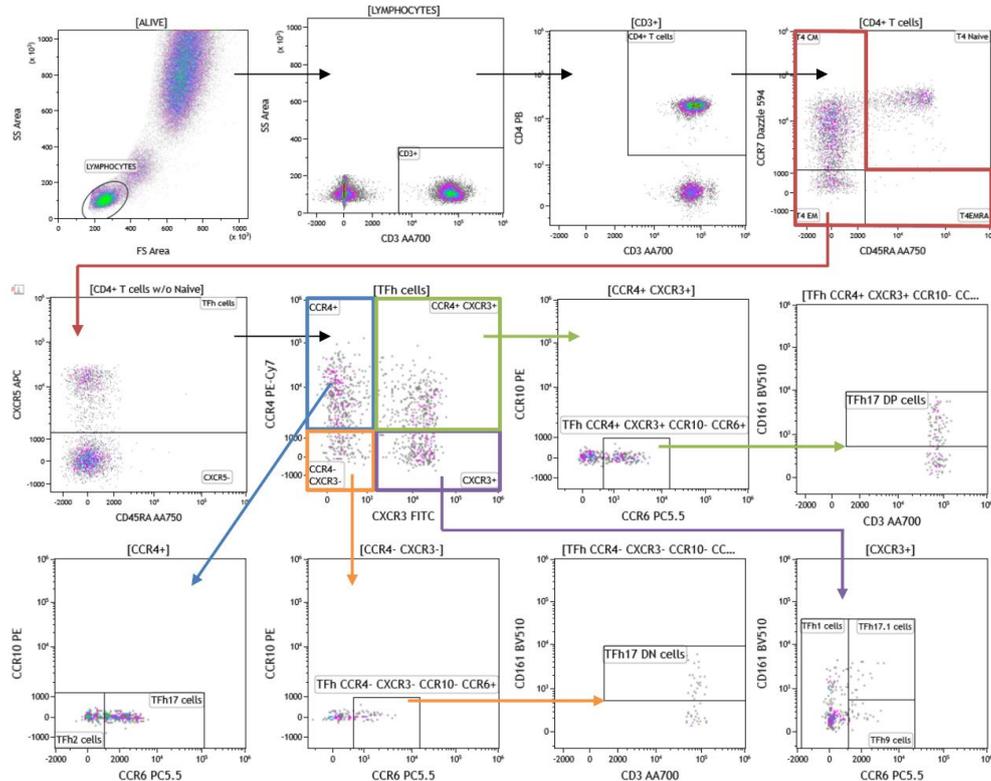
Target Cytokine / Soluble protein	Kit	Catalog number	Sample dilution	LLOD	Panel
MDC	V-PLEX Human Chemokine Panel 1	N05047A-1	1:4	1,22 pg/ml	Chemokine
4-1BB	R-PLEX Human 4-1BB/TNFRSF9	L45SA-1	1:4	1,2pg/ml	4-1BB
APRIL	R-PLEX Human APRIL/TNFSF13	L45SA-1	1:32	87 pg/ml	APRIL
BCA-1/CXCL13	R-PLEX Human BCA-1/BLC	L45SA-1	1:2	1,0 pg/ml	BCA-1/CXCL13
BAFF	U-PLEX Immuno-Oncology	N05227A-1	1:4	0,05 pg/ml	Immuno-Oncology
SDF	U-PLEX Immuno-Oncology	N05227A-1	1:4	278 pg/ml	Immuno-Oncology

Supplementary Results:

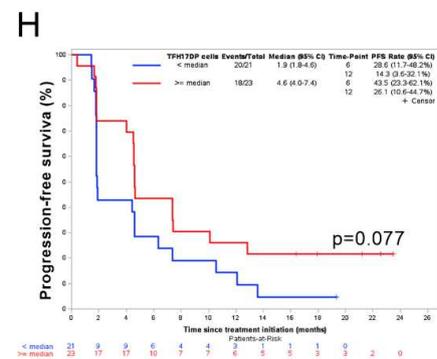
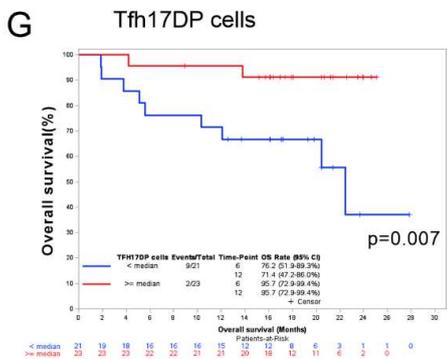
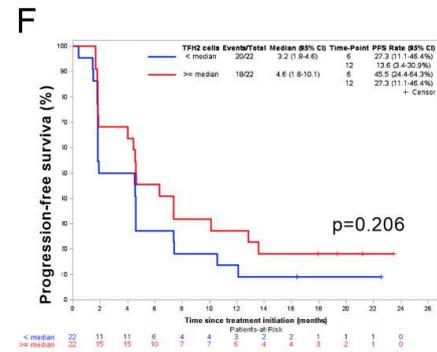
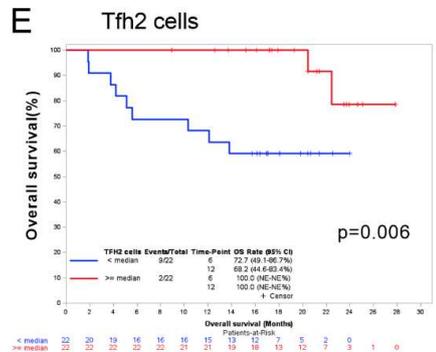
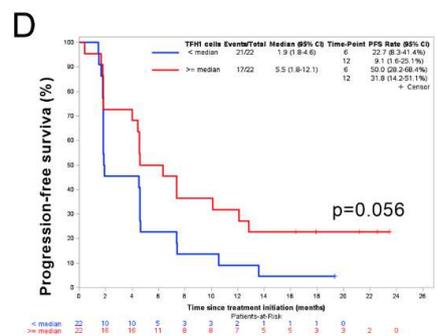
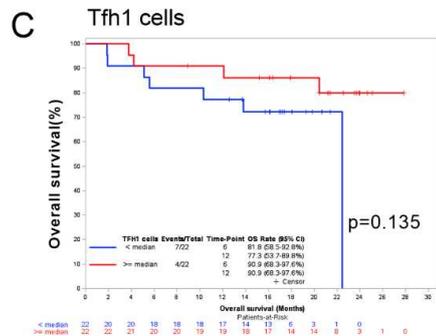
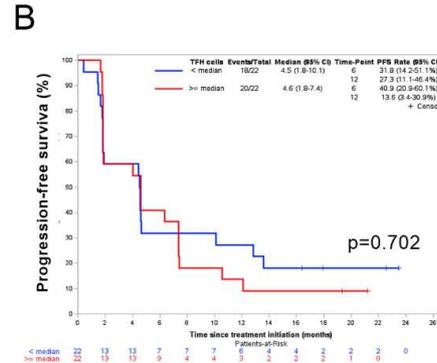
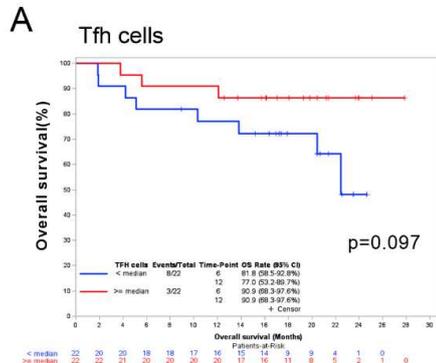
Supplementary Figure S2. Gating strategy for NSWm B cells (CD19+ CD27+ IgD+ IgM+) according to Pitoiset et al(39).



Supplementary Figure S3. (A-B), Kaplan-Meier for OS and PFS according to B cells concentration levels (high: \geq median; low: $<$ median) in m-ccRCC patients treated with nivolumab. (C-D), Kaplan-Meier for OS and PFS according to SwM B cells concentration levels (high: \geq median; low: $<$ median) in m-ccRCC patients treated with nivolumab. (E-F), Kaplan-Meier for OS and PFS according to naïve transitional B cells concentration levels (high: \geq median; low: $<$ median) in mRCC patients treated with nivolumab. (G-H), Kaplan-Meier for OS and PFS according to naïve B cells concentration levels (high: \geq median; low: $<$ median) in m-ccRCC patients treated with nivolumab.



Supplementary Figure S4. Gating strategy for Tfh cells.



Supplementary Figure S5. (A-B) Kaplan-Meier for OS and PFS according to TFh cells levels (high: \geq median; low: $<$ median) in mRCC patients treated with nivolumab. (C-D) Kaplan-Meier for OS and PFS according to Tfh1 cells levels (high: \geq median; low: $<$ median) in mRCC patients treated with nivolumab. (E-F) Kaplan-Meier for OS and PFS according to Tfh2 cells levels (high: \geq median; low: $<$ median) in mRCC patients treated with nivolumab. (G-H) Kaplan-Meier for OS and PFS according to Tfh17DP cells levels (high: \geq median; low: $<$ median) in mRCC patients treated with nivolumab.

Supplementary Table S3. Discovery cohort. Association between soluble factors concentration levels and OS/PFS.

Cytokines	Selected threshold	OS HR (IC95%)	PFS HR (IC95%)
		p value	p value
IFN	Median	P= 0.9767	P=0.9033
< 6.5	< median	1	1
≥6.5	≥median	0.98 (0.28-3.42)	1.04 (0.53-2.03)
IL10	Median	P=0.0723	P=0.5148
< 0.4	< median	1	1
≥ 0.4	≥ median	3.24 (0.83-12.61)	0.80 (0.41-1.59)
IL6	3 rd quartile	P=0.0112	P=0.6459
< 3.8	< Q3	1	1
≥3.8	≥Q3	4.41 (1.26-15.43)	1.19 (0.55-2.55)
IL7	Median	P=0.4302	P=0.1525*
< 5.9	< median		
≥ 5.9	≥ median	1.66 (0.47-5.87)	1.60 (0.81-3.16)
IL8	3 rd quartile	P=0.5240	P=0.5493
< 15.9	< Q3	1	1
≥ 15.9	≥ Q3	1.55 (0.40-6.05)	1.26 (0.57-2.80)
MDC	1 st quartile	P=0.1283	P=0.3263
< 561	< Q1	1	1
≥ 561	≥ Q3	0.39 (0.11-1.38)	1.50 (0.64-3.50)
MDC	Median	P=0.7810	P=0.1337
< 636.3	< median	1	1
≥ 636.3	≥ median	0.84 (0.24-2.94)	1.64 (0.83-3.21)
TNF	Median	P=0.4646	P=0.1067
< 3.4	< median	1	
≥ 3.4	≥ median	1.61 (0.45-5.81)	0.59 (0.30-1.16)
VCAM 1	3 rd quartile	P=0.0714	P=0.8266

< 638470.3	< Q3	1	1
≥ 638470.3	≥ Q3	3.16 (0.84-11.84)	0.92 (0.42-2.03)
VCAM 1	Median	P=0.1973	P=0.2766
< 494344.2	< median	1	1
≥ 494344.2	≥ median	2.39 (0.61-9.39)	0.70 (0.36-1.37)
VEGF	3 rd quartile	P=0.6616	P=0.0967
< 63.7	< Q3	1	1
≥ 63.7	≥ Q3	1.36 (0.34-5.36)	1.83 (0.86-3.88)
BCA-1/CXCL13	3 rd quartile	P=0.0076	P=0.6285
< 56.3	< Q3	1	1
≥ 56.3	≥ Q3	4.74 (1.35-16.64)	1.21 (0.54-2.68)
4 1BB 2	Median	P=0.4302	P=0.8037
< 58.4	< median	1	1
≥ 58.4	≥ median	1.66 (0.47-5.87)	0.92 (0.47-1.79)
APRIL	Median	P=0.5677	P=0.8280
< 4777.9	< median	1	1
≥ 4777.9	≥ median	0.69 (0.20-2.46)	1.07 (0.55-2.10)
BAFF	3 rd quartile	P=0.0114	P=0.9974
< 1316.6	< Q3	1	1
≥ 1316.6	≥ Q3	4.39 (1.26-15.32)	1.00 (0.45-2.21)

Supplementary Table S4. Discovery cohort. Association between soluble factors concentration levels and ORR.

	Objective Response : complete response		Patients	Test
	or partial response			
	No N=32	YES N=7	N=39	
IFN >= P50				Fisher
< 6.5	15 (46.9%)	4 (57.1%)	19 (48.7%)	Exact
≥ 6.5	17 (53.1%)	3 (42.9%)	20 (51.3%)	P = 0.695
IL10 >= P50				Fisher
< 0.4	18 (56.3%)	2 (28.6%)	20 (51.3%)	Exact
≥ 0.4	14 (43.8%)	5 (71.4%)	19 (48.7%)	P = 0.235
IL6 >= P75				Fisher
< 3.8	25 (78.1%)	4 (57.1%)	29 (74.4%)	Exact
≥ 3.8	7 (21.9%)	3 (42.9%)	10 (25.6%)	P = 0.344
IL7 >= P50				Fisher
< 5.9	15 (46.9%)	5 (71.4%)	20 (51.3%)	Exact
≥ 5.9	17 (53.1%)	2 (28.6%)	19 (48.7%)	P = 0.407
IL8 >= P75				Fisher
< 15.9	25 (78.1%)	5 (71.4%)	30 (76.9%)	Exact
≥ 15.9	7 (21.9%)	2 (28.6%)	9 (23.1%)	P = 0.653
MDC >= P25				Fisher
< 561	9 (28.1%)	1 (14.3%)	10 (25.6%)	Exact
≥ 561	23 (71.9%)	6 (85.7%)	29 (74.4%)	P = 0.653
MDC >= P50				Fisher
< 636.3	14 (43.8%)	5 (71.4%)	19 (48.7%)	Exact
≥ 636.3	18 (56.3%)	2 (28.6%)	20 (51.3%)	P = 0.235
TNF >= P50				
< 3.4	18 (56.3%)	2 (28.6%)	20 (51.3%)	

	Objective Response : complete response or partial response		Patients N=39	Test
	No N=32	YES N=7		
	≥ 3.4	14 (43.8%)	5 (71.4%)	19 (48.7%)
VCAM 1 >= P75				Fisher
< 638470.3	26 (81.3%)	3 (42.9%)	29 (74.4%)	Exact P = 0.057
≥ 638470.3	6 (18.8%)	4 (57.1%)	10 (25.6%)	
VCAM 1 >= P50				Fisher
< 494344.2	17 (53.1%)	2 (28.6%)	19 (48.7%)	Exact P = 0.407
≥ 494344.2	15 (46.9%)	5 (71.4%)	20 (51.3%)	
VEGF >= P75				Fisher
< 63.7	23 (71.9%)	6 (85.7%)	29 (74.4%)	Exact P = 0.653
≥ 63.7	9 (28.1%)	1 (14.3%)	10 (25.6%)	
BCA-1/CXCL13 >= P75				Fisher
< 56.3	24 (75.0%)	5 (71.4%)	29 (74.4%)	Exact P = 1.000
≥ 56.3	8 (25.0%)	2 (28.6%)	10 (25.6%)	
4 1BB 2 >= P50				Fisher
< 58.4	17 (53.1%)	3 (42.9%)	20 (51.3%)	Exact P = 0.695
≥ 58.4	15 (46.9%)	4 (57.1%)	19 (48.7%)	
APRIL >= P50				Fisher
< 4777.9	16 (50.0%)	3 (42.9%)	19 (48.7%)	Exact P = 1.000
≥ 4777.9	16 (50.0%)	4 (57.1%)	20 (51.3%)	
BAFF >= P75				Fisher
< 1316.6	23 (71.9%)	6 (85.7%)	29 (74.4%)	Exact P = 0.653
≥ 1316.6	9 (28.1%)	1 (14.3%)	10 (25.6%)	
SDF >302				

	Objective Response : complete response or partial response		Patients N=39	Test
	No N=32	YES N=7		
	< Detection threshold	17 (53.1%)	5 (71.4%)	22 (56.4%)
> Detection threshold	15 (46.9%)	2 (28.6%)	17 (43.6%)	Exact P = 0.438