

Supplementary Material

Immunophenotype and Transcriptome Profile of Patients with Multiple Sclerosis Treated with Fingolimod. Setting Up a Model for Prediction of Response in a 2-Year Translational Study.

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Supplementary Table 1. Lymphocyte subpopulations analyzed by flow cytometry.

PANEL	ANTIBODIES	SUBPOPULATIONS ANALYZED
1	anti-CD3 FITC, anti-CD56 PE, anti-CD20 Per-CP vio 770, anti-CD4 PE vio 770 and anti-CD8 APC	T Lymphocytes: CD3 ⁺ CD20 ⁻ CD56 ⁻ Helper T cells: CD3 ⁺ CD4 ⁺ CD20 ⁻ CD56 ⁻ Cytotoxic T cells: CD3 ⁺ CD8 ⁺ CD20 ⁻ CD56 ⁻ B lymphocytes: CD20 ⁺ CD3 ⁻ CD56 ⁻ Natural killer cells (NK): CD56 ⁺ CD20 ⁻ CD3 ⁻ , CD56 ^{dim} CD20 ⁻ CD3 ⁻ and CD56 ^{bright} CD20 ⁻ CD3 ⁻ Natural killer T cells (NKT): CD3 ⁺ CD56 ⁺ CD20 ⁻
2	anti-CD45RA Vio green, anti-CD39 FITC, anti-CCR7 PE, anti-CD8 PerCP, anti-CD127 PE Vio 770, anti-CD25 APC and anti-CD4 APC vio 770	Naive T cells (Tn): CD3 ⁺ CD4 ⁺ CCR7 ⁺ CD45RA ⁺ and CD3 ⁺ CD8 ⁺ CCR7 ⁺ CD45RA ⁺ Central memory T cells (TCM): CD3 ⁺ CD4 ⁺ CCR7 ⁺ CD45RA ⁻ and CD3 ⁺ CD8 ⁺ CCR7 ⁺ CD45RA ⁻ Effector memory T cells (TEM): CD3 ⁺ CD4 ⁺ CCR7 ⁻ CD45RA ⁻ and CD3 ⁺ CD8 ⁺ CCR7 ⁻ CD45RA ⁻ Effector memory RA T cells (TEMRA): CD3 ⁺ CD4 ⁺ CCR7 ⁻ CD45RA ⁺ and CD3 ⁺ CD8 ⁺ CCR7 ⁻ CD45RA ⁺ Regulatory T cells (Reg T): CD3 ⁺ CD4 ⁺ CD25 ^{high} CD127 ^{down}
3	anti-CD3 FITC, anti-CD27 PE, anti-CD20 Per-CP vio 770, anti-CD11b APC and anti-CD43 APC vio 770	CD20 Naive B cells (Naive B): CD20 ⁺ CD3 ⁻ CD27 ⁻ CD43 ⁻ CD20 Memory B cells (Mem B): CD20 ⁺ CD3 ⁻ CD27 ⁺ CD43 ⁻ B1 cells (B1): CD20 ⁺ CD3 ⁻ CD27 ⁺ CD43 ⁺ B1 CD11b+ cells: CD20 ⁺ CD3 ⁻ CD27 ⁺ CD43 ⁺ CD11b ⁺
4	anti-CD24 FITC, anti-CD27 PE, anti-CD38 PE-vio 770, anti-IgM APCand anti-CD19 APC vio770	CD19 Naive B cells (NaiveB): CD19 ⁺ CD27 ⁻ IgM ⁺ CD19 Memory B cells (MemB): CD19 ⁺ CD27 ⁺ Class switched memory B cells (CS MemB): CD19 ⁺ CD27 ⁺ IgM ⁻ Non class switched memory B cells (NoCS MemB): CD19 ⁺ CD27 ⁺ IgM ⁺ Immature B cells (Immat B): CD19 ⁺ CD27 ⁻ IgM ⁻ Transitional B cells (Transit B): CD19 ⁺ CD27 ⁻ CD24 ^{high} CD38 ^{high} Plasmablasts (PB): CD19 ⁺ CD24 ^{high} CD38 ^{high} IgM ⁻ Regulatory B cells (Reg B): CD19 ⁺ CD24 ^{high} CD27 ⁺ and CD19 ⁺ CD24 ^{high} CD27 ⁺ CD38 ^{int}
5	anti-CD3 Vio green, anti-CD27 PE, anti-CD138 PE-vio 770, anti-CD5 APC and anti-CD19 APC vio770	Plasmatic cells (PC): CD19 ⁺ CD3 ⁻ CD27 ⁺ CD138 ⁺ CD5+ B cells: CD19 ⁺ CD5 ⁺
6	Surface staining: anti-CD3 Vio green, anti-CD161 FITC, anti-CD8 PerCP, anti-CCR6 PE vio 770 and anti-CCR4 APC. Intracellular staining: anti-IL17A PE.	Th17 cells by surface markers vs intracellular staining: IL17 ⁺ , CD3 ⁺ CD8 ⁻ IL17 ⁺ and CD3 ⁺ CD8 ⁺ IL17 ⁺ CD3 ⁺ CD8 ⁺ CCR6 ⁺ CCR4 ⁺ vs CD3 ⁺ CD8 ⁺ CCR6 ⁺ CCR4 ⁺ IL17 ⁺ CD3 ⁺ CD8 ⁺ CD161 ⁺ CCR6 ⁺ CCR4 ⁺ vs CD3 ⁺ CD8 ⁺ CD161 ⁺ CCR6 ⁺ CCR4 ⁺ IL17 ⁺ CD3 ⁺ CD8 ⁺ CCR6 ⁺ CCR4 ⁺ vs CD3 ⁺ CD8 ⁺ CCR6 ⁺ CCR4 ⁺ IL17 ⁺ CD3 ⁺ CD8 ⁺ CD161 ⁺ CCR6 ⁺ CCR4 ⁺ vs CD3 ⁺ CD8 ⁺ CD161 ⁺ CCR6 ⁺ CCR4 ⁺ IL17 ⁺
7	Surface staining: anti-CD3 Vio green and anti-CD8 PerCP., Intracellular staining: anti-IL17A PE, anti-IFNγ FITC and anti-IL2 APC vio770	IFNγ ⁺ , CD3 ⁺ CD8 ⁻ IFNγ ⁺ and CD3 ⁺ CD8 ⁺ IFNγ ⁺ IL2 ⁺ , CD3 ⁺ CD8 ⁻ IL2 ⁺ and CD3 ⁺ CD8 ⁺ IL2 ⁺ IL17 ⁺ IFNγ ⁺ , CD3 ⁺ CD8 ⁻ IL17 ⁺ IFNγ ⁺ and CD3 ⁺ CD8 ⁺ IL17 ⁺ IFNγ ⁺

Supplementary Table 1. Lymphocyte subpopulations analyzed by flow cytometry.

Seven cytometry panels with a different combination of antibodies were designed to characterize 48 lymphocyte subpopulations according surface and intracellular markers. Prior to staining, all cells of each panel were pre-incubated with Fc-blocking reagent from Miltenyi Biotec. To exclude dead cells, 4',6-diamidino-2'-phenylindole dihydrochloride (DAPI) was added to the surface staining panels (1-5) and the fluorescent reactive dye of LIVE/DEAD™ Fixable Dead Cell Stain Kit (Thermo Fisher Scientific Inc.) in the intracellular staining panels (6-7) following the manufacturer's instructions.

Supplementary Table 2. Target and reference gene primers

Target Genes Primers	
Name	5'3'Sequence
FOXP3-F	CAAGGGCCAAGGAAGGG
FOXP3-R	CCAGGCTGATCCTTTTCTGT
AATF-F	TGGAAAAGCAACTCTTCCTGA
AATF-R	ACAAAGGTGGCCAGAAATT
GPI-F	GCTCGAAGTGTCAAAACCC
GPI-R	AAACATGTTTCGAGTCTGGGA
PSMD9-F	TGCGGATCACTGTCACATTC
PSMD9-R	AGTTCGGCTCTGTGAACACC
TNFSF8-F	GGCCCATGACTTCTTGAATG
TNFSF8-R	AACTCACCTGACAACGTCCC
FCRH1-F	GCTATCAAAAACAGCTCGGC
FCRH1-R	AGGTACCATCCCTGACCTGG
UBASH3A-F	TGGAGTTCAGGTCTGTGGCT
UBASH3A-R	TTCTGCCGGAAAACACAC
BCL3-F	GCACCACAGCAATATGGAGA
BCL3-R	CCTATACCCCATGATGTGCC
SSTR3-F	GAGGGTGGTCAGCAGTCAG
SSTR3-R	GAGAGGGGACAGCAGAATGA
MAPK8IP1-F	AGCTGATGCCACACTCATCA
MAPK8IP1-R	CTTCGCCTCCCAATTTCAG
Reference Genes Primers	
Name	5'3'Sequence
B2M-F	TCTCTGCTGGATGACGTGAG
B2M-R	TAGCTGTGCTCGCGCTACT
TFRC-F	CCACCAAACAAGTTAGAGAATGC
TFRC-R	TGTGGGGAAAGGGGCTGT
SDHA-F	TGGGAACAAGAGGGCATCTG
SDHA-R	CCACCACTGCATCAAATTCATG

Supplementary Table 2. Target and reference gene primers

Name and 5' and 3' sequences of the 10 target genes and 3 reference genes used.

Supplementary Table 3. Clinical and MRI outcomes

	Time periods			Statistical significance ‡		
	Baseline	1 year	2 years	Baseline vs 1 year	1 year vs 2 years	Baseline vs 2 years
ARR »						
All	0.71+/-0.13	0.41+/-0.1	0.1+/-0.05	p=0.0079	p<0.001	p<0.001
NTZ group	0.07+/-0.06	0.45+/-0.16	0.09+/-0.07	p=0.0026	p=0.0026	p=0.5136
No NTZ group	1.02+/-0.22	0.42+/-0.14	0.07+/-0.06	p=0.0011	p=0.0010	p<0.00109
EDSS ¶						
All	2.63+/-1.59	2.68+/-1.73	2.41+/-1.59	p=0.8612	p=0.5353	p=0.412
NTZ group	3.17+/-1.68	3.39+/-1.72	3.3+/-1.54	p=0.8754	p=0.8545	p=0.9871
No NTZ group	2.18+/-1.4	2.09+/-1.53	1.71+/-1.27	p=0.5926	p=0.5396	p=0.2435
Number of GdE lesions »						
All	1.52+/-0.19	1.08+/-0.16	0.18+/-0.07	p=0.0102	p<0.001	p<0.001
NTZ group	0+/-0	0.69+/-0.2	0+/-0	p<0.001	p<0.001	p=0.9981
No NTZ group	1.59+/-0.27	1.27+/-0.24	0.31+/-0.12	p=0.0994	p<0.001	p<0.001
GdE lesion volume ¶						
All	0.27+/-0.89	0.22+/-0.56	0.03+/-0.16	p=0.6321	p=0.0141	p=0.0429
NTZ group	0+/-0	0.3+/-0.75	0+/-0	p<0.001	p<0.001	p=0.9900
No NTZ group	0.49+/-1.16	0.15+/-0.33	0.05+/-0.22	p=0.3823	p=0.2931	p=0.0467
Number of new T2-Weighted lesions »						
All	1.41+/-0.19	2+/-0.22	2.05+/-0.23	p=0.0881	p=0.4465	p=0.1532
NTZ group	0.78+/-0.21	1.91+/-0.33	0.09+/-0.07	p<0.001	p<0.001	p<0.001
No NTZ group	1.42+/-0.25	1.81+/-0.29	2.5+/-0.34	p=0.0683	p=0.0142	p=0.0002
T2-weighted lesion volume ¶						
All	9.52+/-7.17	9.64+/-6.85	9.84+/-7.51	p=0.8512	p=0.9498	p=0.9649
NTZ group	9.46+/-7.97	9.34+/-7.45	9.64+/-8.1	p=0.8831	p=0.9192	p=0.9869
No NTZ group	9.56+/-6.64	9.88+/-6.49	10+/-7.23	p=0.7126	p=0.8737	p=0.8815
T1-weighted lesion volume ¶						
All	1.06+/-1.36	1.07+/-1.32	1.1+/-1.38	p=0.4302	p=0.7467	p=0.7937
NTZ group	1.44+/-1.78	1.41+/-1.73	1.44+/-1.83	p=0.9731	p=0.8669	p=0.8842
No NTZ group	0.75+/-0.81	0.79+/-0.8	0.83+/-0.83	p=0.6429	p=0.9109	p=0.9839
Annual brain volume loss (BVL) ¶						
All	-	0.48+/-0.41	0.31+/-0.3	-	p=0.0492	-
NTZ group	-	0.45+/-0.4	0.3+/-0.31	-	p=0.0394	-
No NTZ group	-	0.5+/-0.43	0.33+/-0.3	-	p=0.0406	-
Percentage of relapse-free patients †£						
All	60	75	70	OR=2 (95%IC:0.76-5.2) p=0.2351	OR=5 (95%IC:1.27-19.6) p=0.03	OR=15 (95%IC:3.29-68.3) p=0.0005
NTZ group	94.44	66.6	61.12	OR= 0 (95% IC:0-0) p=0.06	OR=6 (95%IC:0.93-38.4) p=0.12	OR=1 p=1
No NTZ group	31.81	81.82	77.28	OR=12 (95%IC:2.43-59.2) p=0.003	OR=4 (95%IC:0.52-30.3) p=0.37	OR=~ p=0.0001
Percentage of CDP-free patients ‡£ ¥						
All	-	92.5	87.5	-	OR=1.5 (95%IC:0.25-8.8) p=1	-
NTZ group	-	95	92.5	-	OR=2 (95%IC:0.19-21.03) p=1	-
No NTZ group	-	97.5	95	-	OR=1 p=1	-
Percentage of patients with GdE lesions £						
All	17.5	22.5	2.94	OR= 1.5(95%IC:0.42-5.26) p=0.75	OR= 0 (95%IC:0-0) p=0.0078	OR= 0 (95%IC:0-0) p=0.03
NTZ group	0	22.2	0	OR=~ p=0.12	OR= 0 (95%IC:0-0) p=0.02	OR= ~ p=1
No NTZ group	31.81	22.72	5.26	OR= 0.5 (95%IC:0.09-2.63) p=0.68	OR=0 (95%IC:0-0) p=0.02	OR= 0(95%IC:0-0) p=0.03
Percentage of patients with T2w lesions £						
All	22.5	22.5	14.7	OR= 2(95%IC:0.19-21.03) p=1	OR=0.33 (95%IC:0.03-2.87) p=0.62	OR= 0(95%IC:0-~) p=1
NTZ group	11.1	16.6	6.66	OR=0.75 (95%IC:0.16-3.33) p=1	OR=0.5 (95%IC:0.04-2.63) p=0.68	OR=0.5 (95%IC:0.12-1.94) p=0.5
No NTZ group	31.81	27.27	21.05	OR=1 p=1	OR=0.42 (95%IC:0.11-1.59) p=0.34	OR=0.42 (95%IC:0.11-1.59) p=0.34
Percentage of patients with MRI activity £						
All	27.5	30	14.7	OR=1.6 (95%IC:0.4-6.86) p=0.72	OR=0.2 (95%IC:0.05-0.78) p=0.03	OR=0.33 (95%IC:0.09-1.15) p=0.14
NTZ group	11.11	27.7	6.66	OR= ~ p=0.25	OR= 0(95%IC:0-0) p=0.12	OR=0 (95%IC:0-~) p=1
No NTZ group	40.9	31.81	21.05	OR= 0.6 (95%IC:0.11-3.94) p=1	OR=0.33 (95%IC:0.07-1.52) p=0.28	OR=0.37 (95%IC:0.10-1.34) p=0.22

Supplementary Table 3. Clinical and MRI outcomes

Values of clinical and radiological variables that were measured at baseline, 1 and 2 years and statistical significance of the tests. ‡ For the numerical variables, p-values were calculated. For percentages, odds ratio, confidence interval and p-values were calculated. p<0.05 was considered statistically significant. » Count data for ARR, number of GdE lesions and number of new T2-weighted lesions were fitted to a zero-inflated Poisson model. Estimated ARR, number of GdE lesions and new T2-w lesions were calculated at different time periods (baseline, 1 year and 2 years). To assess the statistical significance between groups, we obtained the p-values by calculating the probability of observing a group rate given the other group rate as a null hypothesis. ¶ The values are the means+/-SD. P-values were calculated using the Wilcoxon signed-rank test to compare MRI and clinical measures before treatment and after 1 and 2 years. † Percentage estimates from the Kaplan-Meier cumulative survival curve for time to relapse. ‡ Percentage estimates from the Kaplan-Meier cumulative failure curve for time to confirmed disease progression. £ Odds ratio, confidence interval and p-values were calculated using the McNemar's test to compare paired proportions. ¥ Patients were CPD-free at baseline as part of study design.

Supplementary Table 4. Comparison of lymphocyte subpopulations between patients and controls

	Percentages *			p-values ¶	
	Patients (Baseline) n=40	MS controls n=10	HCS n=10	Patients vs. MS controls	Patients vs. HCS
T Lymphocytes	73.98+/-9.71	73.23+/-7.91	75.5+/-4.64	p=0.7342	p=0.8272
Helper T cells	48.36+/-9.86	44.17+/-9.05	45.46+/-5.3	p=0.1986	p=0.2072
Cytotoxic T cells	22.02+/-5.84	25.32+/-6.93	25.41+/-4.94	p=0.1524	p=0.0727
B Lymphocytes	14.43+/-4.79	12.92+/-8.14	10.32+/-3.11	p=0.1456	p=0.0079
NK cells	9.44+/-7.94	11.05+/-3.16	10.07+/-2.98	p=0.0896	p=0.1390
NKT cells	3.2+/-3.73	3.08+/-42.17	3.3+/-1.22	p=0.5605	p=0.0895
Nkbright (% of NK)	12.58+/-8.04	11.24+/-6.35	8.79+/-4.15	p=0.7342	p=0.1903
Nkdim (% of NK)	86.86+/-7.88	88.75+/-6.35	91.20+/-40.15	p=0.6276	p=0.1326
CD4 Naive T cells	23.01+/-9.33	19.18+/-6.37	21.99+/-5.65	p=0.1986	p=0.7342
CD4 Naive T cells	11.3+/-6.04	11.85+/-4.93	11.31+/-2.52	p=0.6276	p=0.8462
CD4 TCM cells	21.99+/-7.39	21.49+/-7.02	21.02+/-4.5	p=1.0000	p=0.6276
CD8 TCM cells	2.36+/-1.8	3.16+/-1.67	3.39+/-1.66	p=0.0727	p=0.0495
CD4 TEM cells	2.95+/-2.8	2.95+/-2.14	2.27+/-1.42	p=0.716	p=0.6801
CD4 TEMRA cells	0.41+/-0.78	0.54+/-0.71	0.17+/-0.15	p=0.2347	p=0.4669
CD8 TEM cells	2.71+/-2.15	2.65+/-1.17	3.18+/-2.18	p=0.5605	p=0.3826
CD8 TEMRA cells	5.66+/-4.23	7.67+/-1.9	7.52+/-2.92	p=0.0257	p=0.0618
Reg T cells	1.56+/-0.82	1.9+/-0.69	1.19+/-0.45	p=0.2161	p=0.2072
Naive B cells (% of CD20)	54.65+/-13	58+/-11.46	58.15+/-9.1	p=0.7896	p=0.6624
Mem B cells (% of CD20)	28.21+/-8.57	31.46+/-7.62	32.46+/-4.76	p=0.3320	p=0.1456
B1cells (% of CD20)	0.8+/-0.63	0.97+/-0.32	3.47+/-1.49	p=0.1743	p<0.001
B1cells CD11b (% of CD20)	0.72+/-0.52	0.87+/-0.28	3.21+/-1.55	p=0.1149	p<0.001
Naive B cells (% of CD19)	64.5+/-9.9	61.48+/-10.62	59.88+/-10.15	p=0.3442	p=0.2859
Mem B cells (% of CD19)	26.62+/-10.2	30.17+/-5.84	29.29+/-3.15	p=0.1094	p=0.2859
NoCS Mem B cells (% of CD19)	14.58+/-8.49	17.41+/-3.93	15.97+/-3.52	p=0.1524	p=0.3200
CS Mem B cells (% of CD19)	12.04+/-5.71	12.76+/-3.17	13.32+/-2.94	p=0.3892	p=0.2859
Immat B cells (% of CD19)	3.33+/-1.81	3.55+/-1.08	3.83+/-0.88	p=0.3960	p=0.1327
Transit B cells (% of CD19)	3.02+/-2.68	2.46+/-1.54	2.78+/-1.38	p=0.8462	p=0.8084
PB (% of CD19)	3.16+/-1.55	3.18+/-1.2	3.96+/-0.66	p=0.8084	p=0.0653
RegB1 (% of CD19)	14.09+/-10	18.41+/-7.64	18.58+/-9.34	p=0.0851	p=0.0896
RegB2 (% of CD19)	7.62+/-5.51	6+/-3.06	5.77+/-1.45	p=0.6276	p=0.3631
PC (% of CD19)	15.77+/-14.34	10+/-7.88	15.54+/-9.15	p=0.0653	p=0.6105
CD5+cells (% of CD19)	16.66+/-10.73	19.52+/-5.94	18.53+/-5.09	p=0.1206	p=0.1456
IL17+ cells	1.57+/-1.09	1.46+/-0.58	0.77+/-0.52	p=0.8366	p=0.0186
CD4+IL17+	0.99+/-0.6	0.95+/-0.39	0.58+/-0.42	p=0.9013	p=0.0309
CD8+IL17+	0.24+/-0.21	0.21+/-0.09	0.1+/-0.09	p=0.5443	p=0.0226
CD4+CCR6+CCR4+	2.7+/-2.45	2.93+/-1.37	2.15+/-1.93	p=0.2538	p=0.4568
CD4+CD161+CCR6+CCR4+	0.54+/-0.58	0.43+/-0.52	0.34+/-0.31	p=0.6374	p=0.4419
CD8+CCR6+CCR4+	10.72+/-4.43	13.85+/-5.08	12.42+/-6.98	p=0.0825	p=0.5683
CD8+CD161+CCR6+CCR4+	1.02+/-1.31	0.7+/-0.48	0.97+/-0.96	p=0.6552	p=0.9210
IFN+	20.53+/-10.72	21.27+/-4.6	20.05+/-6.61	p=0.3210	p=0.7659
IFN+CD4	7.18+/-3.72	6.89+/-1.92	6.46+/-2.7	p=0.9802	p=0.5683
IFN+CD8	9.74+/-6.56	11.15+/-4.31	10.91+/-5.98	p=0.2436	p=0.4273
IL2+	6.48+/-5.47	5.68+/-2.30	3.72+/-1.26	p=0.8041	p=0.1928
IL2+CD4	3.21+/-2.24	3.38+/-1.54	1.99+/-0.85	p=0.5852	p=0.1574
IL2+CD8	0.54+/-0.51	0.73+/-0.58	0.3+/-0.25	p=0.3091	p=0.2975
IL17+IFN+	0.3+/-0.33	0.28+/-0.1	0.16+/-0.13	p=0.8620	p=0.1237
IL17+IFN+CD4	0.12+/-0.09	0.13+/-0.06	0.09+/-0.09	p=0.3853	p=0.2975
IL17+IFN+CD8	0.17+/-0.15	0.1+/-0.06	0.05+/-0.04	p=0.2861	p=0.0431

Supplementary Table 4. Comparison of lymphocyte subpopulations between patients and controls

The percentages of 48 lymphocyte subpopulations were compared between the samples obtained from control subjects (naïve patients and healthy controls) and MS patients before fingolimod treatment. The percentages were calculated with respect to the total PBMCs or to other subpopulation, as specified in parentheses. The MS patients (naïve and non-naïve) exhibited a lower percentage of B1 cells than healthy controls. * The values are the mean +/- SD of each group. ¶ The p-values were calculated using the Mann-Whitney test to compare differences between subgroups. p<0.001 was considered statistically significant after Bonferroni's correction for multiple tests.

Supplementary Table 5. Comparison of baseline lymphocyte subpopulations between the NTZ group and No NTZ group

	Subgroups *		P-Values ¶
	NTZ group (n=18)	No NTZ group (n=22)	NTZ vs. No NTZ group
T Lymphocytes	73.4+/-10.97	74.46+/-8.79	p=0.8704
Helper T cells	47.55+/-11.41	49.03+/-8.61	p=0.8278
Cytotoxic T cells	22.08+/-5.92	21.98+/-5.91	p=0.7649
B Lymphocytes	14.17+/-5.19	14.65+/-4.54	p=0.5681
NK cells	10.38+/-6.73	8.66+/-8.89	p=0.0645
NKT cells	4.21+/-4.19	2.37+/-3.17	p=0.0625
Nkbright (% of NK)	12.86+/-8.27	12.91+/-8.05	p=0.9783
Nkdim (% of NK)	87.14+/-8.27	86.65+/-7.74	p=0.8490
CD4 Naive T cells	21.85+/-11.6	23.96+/-7.12	p=0.2212
CD4 Naive T cells	8.66+/-5.5	13.46+/-5.69	p=0.0060
CD4 TCM cells	22.6+/-7.46	21.49+/-7.48	p=0.4465
CD8 TCM cells	2.35+/-1.66	2.36+/-1.94	p=0.7649
CD4 TEM cells	2.9+/-1.99	3+/-3.37	p=0.3277
CD4 TEMRA cells	0.2+/-0.34	0.58+/-0.99	p=0.4465
CD8 TEM cells	3.65+/-2.62	1.94+/-1.27	p=0.0388
CD8 TEMRA cells	7.42+/-4.75	4.22+/-3.18	p=0.0134
Reg T cells	1.4+/-0.83	1.69+/-0.8	p=0.2212
Naive B cells (% of CD20)	51.34+/-12.9	57.36+/-12.73	p=0.1212
Mem B cells (% of CD20)	29.13+/-7.23	27.46+/-9.63	p=0.5680
B1cells (% of CD20)	0.73+/-0.37	0.86+/-0.79	p=0.6341
B1cells CD11b (% of CD20)	0.67+/-0.32	0.75+/-0.65	p=0.5498
Naive B cells (% of CD19)	60.69+/-9.32	67.61+/-9.45	p=0.0240
Mem B cells (% of CD19)	29.65+/-10.5	24.15+/-9.47	p=0.0428
NoCS Mem B cells (% of CD19)	17.72+/-9.07	12.01+/-7.22	p=0.0363
CS Mem B cells (% of CD19)	11.93+/-6	12.14+/-5.6	p=0.9350
Immat B cells (% of CD19)	2.88+/-1.43	3.69+/-2.03	p=0.2650
Transit B cells (% of CD19)	4.59+/-3.15	1.7+/-1.31	<p=0.001
PB (% of CD19)	3.32+/-1.57	3.02+/-1.56	p=0.3769
RegB1 (% of CD19)	16.7+/-12.03	11.96+/-7.61	p=0.1575
RegB2 (% of CD19)	7.68+/-6.55	7.57+/-4.65	p=0.8491
PC (% of CD19)	15.59+/-18.52	15.91+/-10.2	p=0.3277
CD5+cells (% of CD19)	16.97+/-15.23	16.4+/-5.1	p=0.4629
IL17+ cells	1.26+/-0.92	1.82+/-1.17	p=0.1574
CD4+IL17+	0.84+/-0.54	1.11+/-0.62	p=0.1740
CD8+IL17+	0.17+/-0.17	0.29+/-0.23	p=0.0868
CD4+CCR6+CCR4+	3.64+/-3.34	1.96+/-1.03	p=0.1486
CD4+CD161+CCR6+CCR4+	0.62+/-0.68	0.47+/-0.51	p=0.5520
CD8+CCR6+CCR4+	11.41+/-5.13	10.19+/-3.86	p=0.4445
CD8+CD161+CCR6+CCR4+	1.4+/-1.85	0.73+/-0.55	p=0.1408
IFN+	22.86+/-11.79	18.74+/-9.72	p=0.2882
IFN+CD4	7.35+/-3.68	7.06+/-3.82	p=0.6302
IFN+CD8	12.42+/-8.34	7.67+/-3.82	p=0.0474
IL2+	7.31+/-6.5	5.83+/-4.38	p=0.6420
IL2+CD4	3.4+/-2.03	3.03+/-2.47	p=0.4469
IL2+CD8	0.47+/-0.33	0.6+/-0.63	p=0.9775
IL17+IFN+	0.24+/-0.16	0.35+/-0.42	p=0.4273
IL17+IFN+CD4	0.09+/-0.09	0.14+/-0.09	p=0.0443
IL17+IFN+CD8	0.14+/-0.14	0.19+/-0.16	p=0.1567

Supplementary Table 5. Comparison of baseline lymphocyte subpopulations between the NTZ group and No NTZ group

The percentages of 48 lymphocyte subpopulations were compared between samples from NTZ and No NTZ patients. The percentages were calculated with respect to the total PBMCS or to other subpopulation, as specified in parentheses. The only significantly different subpopulation was the transitory B cells, for which a lower percentage was observed in the NTZ-group

* The values are the mean +/- SD of each group. ¶ The p-values were calculated using the Mann-Whitney test to compare differences between subgroups. p<0.001 was considered statistically significant after Bonferroni's correction for multiple tests.

Supplementary Table 6. Effects of fingolimod on lymphocyte subpopulations after 6 months of treatment

Lymphocyte subpopulations	Porcentajes			p-values (pre vs post-tratamiento)		
	Pre-tratamiento	Post-tratamiento	Fold-change	Todos los pacientes N=40	Grupo NTZ N=18	Grupo no-NTZ N=22
Linfocitos T (LT)	73,98+/-8,7	43,15+/-18,25	-1,71	<0.001	<0.001	<0.001
Células T colaboradoras (Th)	48,36+/-9,17	14,51+/-10,06	-3,33	<0.001	<0.001	<0.001
Células T citotóxicas (Tc)	22,02+/-6,01	22,13+/-11,31	1,00	0.9940	0.2343	0.2046
Linfocitos B (LB)	14,43+/-5,39	6,85+/-3,5	-2,10	<0.001	0.0013	<0.001
Células asesinas naturales (NK)	9,44+/-6,7	43,17+/-18,05	4,57	<0.001	<0.001	<0.001
Células T NK (NKT)	3,2+/-3,19	11,37+/-11,32	3,55	<0.001	<0.001	<0.001
NKbright (% de NKs)	12,89+/-7,34	5,03+/-2,89	-2,56	<0.001	0.0013	<0.001
NKdim (% de NKs)	86,87+/-7,25	94,67+/-2,85	1,08	<0.001	0.0016	0.0024
CD4 T naïve (CD4 Tn)	23,01+/-8,41	2,55+/-2,73	-9,02	<0.001	<0.001	<0.001
CD8 T naïve (CD8 Tn)	11,3+/-5,37	1,85+/-1,72	-6,10	<0.001	<0.001	<0.001
CD4 T de memoria central (CD4 TCM)	21,99+/-6,85	6,08+/-4,62	-3,61	<0.001	<0.001	<0.001
CD8 T de memoria central (CD8 TCM)	2,36+/-1,78	1,39+/-1,55	-1,69	<0.001	0.0017	<0.001
CD4 T de memoria efectora (CD4 TEM)	2,95+/-2,5	4,96+/-5,03	1,68	0.0074	0.0066	0.0017
CD4 T de memoria efectora RA (CD4 TEM)	0,41+/-0,71	0,91+/-1,37	2,21	<0.001	<0.001	0.0064
CD8 T de memoria efectora (CD8 TEM)	2,71+/-2	6,75+/-7,42	2,49	<0.001	0.0072	0.0030
CD8 T de memoria efectora RA (CD8 TEM)	5,66+/-3,81	12,14+/-7,08	2,14	<0.001	0.0023	<0.001
T reguladoras (T reg)	3,2+/-1,36	7,03+/-4,2	2,19	<0.001	0.0016	<0.001
B naïve (Bn1) (% de CD20)	54,65+/-12,13	72,78+/-13,51	1,33	<0.001	<0.001	<0.001
B de memoria (BMem1) (% de CD20)	28,21+/-8	15,71+/-7,19	-1,79	<0.001	<0.001	<0.001
Células LB1cells (% deCD20)	0,8+/-1,27	2,69+/-2,15	3,36	<0.001	<0.001	<0.001
Células LB1 CD11b+ (% de CD20)	0,72+/-1,19	0,84+/-0,74	1,16	0.6133	0.4380	0.3570
B naïve (Bn) (% de CD19)	64,5+/-10,06	81,2+/-7,61	1,25	<0.001	<0.001	<0.001
B de memoria (BMem2) (% de CD19)	26,62+/-8,81	11,53+/-6,69	-2,30	<0.001	<0.001	<0.001
B de memoria class switched (CS BMem) (% de CD19)	14,58+/-7,29	6,17+/-5,2	-2,36	<0.001	0.0019	0.0033
B de memoria non-class switched (NoCS BMem) (% de CD19)	12,04+/-4,96	5,47+/-2,75	-2,20	<0.001	0.0013	<0.001
Células B inmaduras (B inm) (% de CD19)	3,33+/-1,58	5,73+/-2,95	1,72	<0.001	0.0096	0.0033
Células B transicionales (BTransi) (% de CD19)	3,02+/-2,68	10,38+/-6,36	3,20	<0.001	0.0030	<0.001
Plasmablastos (PB) (% de CD19)	2,93+/-1,45	6,7+/-3,92	2,28	<0.001	0.0023	0.0019
Células B reguladoras (Breg1) (% de CD19)	2,47+/-2,02	1,15+/-1,11	-2,14	<0.001	0.0013	0.0037
Células B reguladoras (Breg1) (% de CD19)	1,33+/-0,94	0,61+/-0,63	-2,18	0.0012	0.0028	0.0037
Células plasmáticas (CP) (% de CD19)	15,77+/-12,76	14,7+/-13,46	-1,07	0.6271	0.6529	0.8484
Células B CD5+ (% de CD19)	16,66+/-9,32	27,8+/-9,11	1,66	<0.001	0.0017	<0.001
Células IL17+	1,57+/-0,98	0,54+/-0,41	-2,90	<0.001	0.0032	<0.001
Células CD4+IL17+	0,99+/-0,55	0,28+/-0,23	-3,53	<0.001	<0.001	<0.001
Células CD8+IL17+	0,24+/-0,19	0,1+/-0,11	-2,4	<0.001	0.0017	<0.001
Células CD4+CCR6+CCR4+	2,7+/-2,2	1,61+/-1,87	-1,67	0.0045	0.0067	0.0065
Células CD4+CD161+CCR6+CCR4+	0,54+/-0,54	0,43+/-0,44	-1,25	0.8137	0.2078	0.1808
Células CD8+CCR6+CCR4+	10,72+/-5,09	10,77+/-6,24	1,00	0.8752	0.5245	0.9032
Células CD8+CD161+CCR6+CCR4+	1,02+/-1,15	2,08+/-1,38	2,03	<0.001	0.0055	<0.001
Células IFN+	20,53+/-9,25	10,3+/-3,63	-1,99	<0.001	<0.001	0.0015
Células IFN+CD4	7,18+/-3,29	1,56+/-1,33	-4,60	<0.001	<0.001	<0.001
Células IFN+CD8	9,74+/-6,09	4,27+/-2,27	-2,28	<0.001	<0.001	<0.001
Células IL2+	6,48+/-4,66	2,62+/-2,82	-2,47	<0.001	0.0023	0.0014
Células IL2+CD4	3,21+/-2	0,86+/-0,91	-3,73	<0.001	<0.001	<0.001
Células IL2+CD8	0,54+/-0,5	0,54+/-0,83	1	0.2387	0.6417	0.1790
Células IL17+IFN+	0,3+/-0,28	0,41+/-0,3	1,36	0.0077	0.0554	0.0536
Células IL17+IFN+CD4	0,12+/-0,09	0,11+/-0,1	-1,09	0.5717	0.5245	0.9032
Células IL17+IFN+CD8	0,17+/-0,13	0,15+/-0,12	-1,13	0.8382	0.1354	0.6143

Supplementary Table 6. Effects of fingolimod on lymphocyte subpopulations after 6 months of treatment

The percentages of 48 lymphocyte subpopulations were compared between the samples obtained from MS patients before and after 6 months of fingolimod treatment. The percentages were calculated with respect to the total PBMCs or to another subpopulation, as specified in parentheses. Most lymphocyte subpopulations were modified by fingolimod in this study*. The values are the means +/- SD at each time interval. ¶ All values were calculated as the ratio between the final value and the initial value. A positive or negative sign indicates an increase or decrease, respectively. # The p-values were calculated using the Wilcoxon signed-rank test to compare differences in flow cytometry data before and after 6 months of therapy. p<0.001 was considered statistically significant after Bonferroni's correction for multiple tests.

Supplementary table 7. Differentially expressed genes (DEGs) before treatment in responder vs non-responder patients.

Clinical response at 1 year

	Next generation RNA sequencing (RNA-seq)					q-PCR			
	Mean of expression levels		log2 FC ‡	p-value*	p-value adj ~	Relative expression ratio †		FC ¶	p-value ‡
	NEDA patients	EDA patients				NEDA patients	EDA patients		
FOXP3	3.26+/-0.42	1.67+/-0.49	1.14	1.48E-05	0.007	0.0099+/-0.0082	0.0013+/-0.0015	7.46	0.001
AATF	25.8+/-2.06	18.71+/-0.45	0.46	1.68E-05	0.007	0.0220+/-0.0094	0.0195+/-0.0314	1.12	0.047
CXCL9	7.93+/-2.32	0.44+/-0.89	4.15	1.47E-05	0.007				
GPI	25.6+/-2.78	18.4+/-0.84	0.47	0.00016	0.03	2.5499+/-3.5412	1.940+/-2.6596	1.31	0.023
PSMD9	18.29+/-1.08	12.88+/-1.44	0.68	0.00044	0.048	6.3500+/-0.1652	4.8713+/-5.8970	1.3	0.063
TNFSF8	84.69+/-18.07	43.51+/-17.92	1.01	0.00033	0.044	2.0279+/-2.1090	1.1060+/-1.1371	1.83	0.001
IL21	55.97+/-40.57	16.28+/-6.88	1.78	0.00041	0.048				
IFNG	8628.55+/-3645.31	3857.11+/-689.08	1.21	0.00026	0.038				
IL17A	32.06+/-10.47	8.35+/-3.88	0.46	2.43E-05	0.008				
FCRL1	0.74+/-0.31	2.79+/-1.33	-1.88	7.78E-06	0.005	0.0610+/-0.0656	0.1521+/-0.1718	-2.49	0.048
UBASH3A	1.01+/-0.18	2.72+/-1.33	-1.45	1.20E-05	0.006	0.2476+/-0.2377	0.1900+/-0.2422	1.3	0.051
BCL3	3.13+/-0.83	7.5+/-2.58	-1.33	2.20E-05	0.008	0.0144+/-0.0239	0.0406+/-0.0374	-2.82	0.032
SSTR3	0.39+/-0.11	0.97+/-0.37	-1.16	1.37E-05	0.019	0.0002+/-0.0001	0.0008+/-0.0010	-3.60	0.032
MAPK8IP1	0.55+/-0.09	1.03+/-0.26	-1.17	4.93E-05	0.014	0.0010+/-0.0037	0.0020+/-0.0018	-1.93	0.002
FCRL2	0.35+/-0.32	1.55+/-0.76	-2.16	9.19E-05	0.023				
LEP	0.16+/-0.07	0.74+/-0.69	-3.1	0.00018	0.034				
NLRP1	9.83+/-2.19	17.38+/-3.99	-0.82	0.00036	0.046				

Clinical response at 2 years

	Next generation RNA sequencing (RNA-seq)				
	Mean of expression levels		log2 FC ‡	p-value*	p-value adj ~
	NEDA patients	EDA patients			
FUT1	1.11+/-0.24	0.59+/-0.14	2.42	3.83E-07	0.001
IFNG	9322.2+/-3809.21	4189.92+/-1021.97	1.31	1.61E-05	0.015
SPP1	70.1+/-21.08	22.2+/-13.83	1.74	2.75E-05	0.021
SLC4A4	0.42+/-0.1	0.15+/-0.06	1.62	3.60E-05	0.025
B3GAT1	2.13+/-0.95	0.74+/-0.37	1.48	5.94E-05	0.033
RASIP1	0.5+/-0.13	0.18+/-0.09	1.60	5.92E-05	0.033
TNFRSF9	60.15+/-21.29	23.87+/-9.71	1.43	6.10E-05	0.033
MUM1	1.09+/-0.18	2.02+/-0.58	-0.96	0.00010	0.045
EGR4	6.56+/-1.08	16.65+/-6.6	-1.51	4.92E-08	0.000
NFKBIZ	83.85+/-7.28	137.42+/-16.45	-0.69	1.07E-06	0.003
CYP4V2	3.57+/-0.16	6.25+/-1.5	-1.77	1.38E-06	0.003
TCL1A	0.47+/-0.31	2.65+/-1.75	-2.61	2.62E-06	0.005
AFF3	2.19+/-0.59	5.19+/-1.7	-1.35	8.33E-06	0.011
DHRS1	0.94+/-0.12	1.82+/-0.4	-1.14	1.30E-05	0.014
CCDC88B	6.99+/-2.03	11.28+/-1.53	-0.83	3.19E-05	0.024
MMP2	0.06+/-0.04	0.41+/-0.29	-2.67	7.13E-05	0.037
ADORA2A	14.2+/-3.75	19.7+/-4.92	-0.82	8.96E-05	0.042
JAK3	7.43+/-1.14	13.11+/-2.81	-1.00	8.86E-05	0.042

Supplementary table 7. Differentially expressed genes (DEGs) before treatment in responder vs non-responder patients.

In samples collected before treatment, the gene expression levels of responder vs non-responder patients (at 1 and 2 years) were compared. In this table, the most representative DEGs in responder patients at 1 and 2 years are shown, as well as the q-PCR validation. ‡ The log2 (fold-change) or log2FC is the log-ratio of a gene expression values in two different biological conditions, in this case NEDA status vs EDA status. * The estimated significance level (p-value) of differential gene expression was calculated with DESeq2, which uses a negative binomial distribution. ~ p-values were corrected to account for multiple hypotheses testing using Benjamini and Hochberg false discovery rate (FDR) adjustment. Genes with an FDR less than or equal to 0.05 were selected as differentially expressed. † Relative expression of the target genes versus three reference genes using the $2^{-\Delta\Delta Ct}$ method. ¶ Fold change is the ratio of the gene expression values. ‡ p-value was calculated using the t-test to compare means of expression levels.

Supplementary Table 8. Significantly enriched gene sets (GSEA)

	ES	NES	nominal p-value	FDR
BIOCARTA_BCELLSURVIVAL_PATHWAY	0.27	1.30	0.0020	0.0237
BIOCARTA_CCR3_PATHWAY	0.29	1.48	0.0655	0.2037
BIOCARTA_CCR5_PATHWAY	0.25	1.24	0.0412	0.1861
BIOCARTA_CXCR4_PATHWAY	0.25	1.41	0.0000	0.0037
BIOCARTA_CYTOKINE_PATHWAY	-0.30	-1.65	0.0082	0.0874
BIOCARTA_ERK_PATHWAY	0.27	1.60	0.0493	0.1391
BIOCARTA_GATA3_PATHWAY	-0.27	-1.29	0.0663	0.1369
BIOCARTA_IL10_PATHWAY	0.32	1.60	0.0332	0.1401
BIOCARTA_IL12_PATHWAY	0.11	0.62	0.0663	0.1369
BIOCARTA_IL17_PATHWAY	-0.21	-1.00	0.0409	0.1564
BIOCARTA_INFLAM_PATHWAY	-0.18	-1.18	0.2087	0.1619
BIOCARTA_MAPK_PATHWAY	0.23	2.52	0.0000	0.0021
BIOCARTA_NKCELLS_PATHWAY	0.61	3.26	0.0000	0.0000
BIOCARTA_NKT_PATHWAY	-0.17	-1.06	0.0480	0.1252
BIOCARTA_PYK2_PATHWAY	0.20	1.24	0.0020	0.0237
BIOCARTA_RAC1_PATHWAY	0.28	1.58	0.0491	0.1437
BIOCARTA_RAS_PATHWAY	0.35	2.01	0.0000	0.0295
BIOCARTA_RELA_PATHWAY	0.28	1.36	0.0123	0.2372
BIOCARTA_RHO_PATHWAY	0.41	2.70	0.0000	0.0013
BIOCARTA_TGFB_PATHWAY	0.34	1.81	0.0155	0.0717
BIOCARTA_TH1TH2_PATHWAY	-0.18	-0.92	0.0560	0.2463
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	0.20	2.07	0.0059	0.0108
KEGG_APOPTOSIS	0.15	1.66	0.0207	0.0654
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.20	2.02	0.0084	0.0136
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	0.25	2.25	0.0000	0.0045
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	0.12	2.28	0.0000	0.0042
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	0.30	3.35	0.0000	0.0000
KEGG_MAPK_SIGNALING_PATHWAY	0.12	2.08	0.0042	0.0109
KEGG_MELANOMA	0.21	1.97	0.0085	0.0179
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.24	3.15	0.0000	0.0000
KEGG_OXIDATIVE_PHOSPHORYLATION	-0.13	-1.68	0.0402	0.0920
KEGG_SPHINGOLIPID_METABOLISM	0.25	1.80	0.0204	0.0404
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.11	1.35	0.1269	0.1913
KEGG_TGF_BETA_SIGNALING_PATHWAY	0.14	1.52	0.0671	0.1045
REACTOME_ACTIVATION_OF_NF_KAPPAB_IN_B_CELLS	-0.18	-1.66	0.0333	0.0574
REACTOME_ANTIGEN_PROCESSING_CROSS_PRESENTATION	0.15	1.54	0.0565	0.1139
REACTOME_APOPTOSIS	0.07	0.92	0.0513	0.2377
REACTOME_CD28_CO_STIMULATION	0.19	1.31	0.1630	0.2426
REACTOME_CLASS_I_MHC_MEDIATED_ANTIGEN_PROCESSING_PRESENTATION	0.10	1.74	0.0195	0.0556
REACTOME_COMPLEMENT_CASCADE	0.32	1.83	0.0139	0.0394
REACTOME_DOWNSTREAM_SIGNALING_EVENTS_OF_B_CELL_RECEPTOR_BCR	-0.08	-0.91	0.0502	0.2355
REACTOME_ERK_MAPK_TARGETS	0.31	1.72	0.0208	0.0612
REACTOME_IL_2_SIGNALING	0.23	1.73	0.0295	0.0590
REACTOME_INNATE_IMMUNE_SYSTEM	0.20	3.46	0.0000	0.0000
REACTOME_MHC_CLASS_II_ANTIGEN_PRESENTATION	0.20	2.22	0.0041	0.0060
REACTOME_NCAM1_INTERACTIONS	-0.15	-1.02	0.0082	0.0874
REACTOME_PI3K_AKT_ACTIVATION	0.20	1.43	0.0988	0.1686
REACTOME_SIGNALING_BY_RHO_GTPASES	0.28	3.37	0.0000	0.0000
REACTOME_SIGNALING_BY_TGF_BETA_RECEPTOR_COMPLEX	0.09	0.83	0.0663	0.1369
REACTOME_SIGNALING_BY_THE_B_CELL_RECEPTOR_BCR	0.05	0.63	0.0041	0.0060
REACTOME_SIGNALING_TO_ERKS	0.32	2.25	0.0000	0.0053
REACTOME_SIGNALING_TO_RAS	0.32	1.97	0.0062	0.0231
REACTOME_SPHINGOLIPID_METABOLISM	0.28	2.52	0.0000	0.0010

Supplementary Table 7. Significantly enriched gene sets (GSEA)

Enrichment score (ES), normalized enrichment score (NES), nominal p-value and false discovery rate (FDR) of pathways related to multiple sclerosis, autoimmunity and fingolimod mechanisms of action that achieved significant enrichment in GSEA (FDR q-values < 0.25).

Supplementary table 9. Posterior predictive response in model validation

Sample	Posterior predictive mean	Posterior predictive lower 95%	Posterior predictive upper 95%	Real response
1166	0,014973171	1,24E-04	0,08193146	0
1175	0,864864671	5,75E-01	0,99025255	1
1198	0,011318419	1,31E-08	0,09943895	0
1172	0,145597607	9,71E-03	0,46624478	0
1174	0,004772552	4,51E-06	0,03063313	0
1254	0,238005954	7,14E-03	0,65438967	0
1301	0,033617637	6,29E-07	0,27805955	0
1315	0,844556423	4,85E-01	0,99252926	0
1330	0,893776563	5,57E-01	0,99724563	1
1354	0,015081467	1,76E-09	0,16324828	0
1564	0,03029961	1,18E-07	0,27908216	0
1571	0,183560574	1,74E-02	0,55312179	0
1595	0,073613074	4,90E-04	0,31758959	0
1598	0,008079493	8,71E-08	0,07094498	0
1627	0,003240338	1,86E-11	0,0278112	0
1630	0,578044005	2,27E-01	0,86406943	0
1631	0,018487652	8,83E-05	0,09886409	0
1655	0,559918281	7,94E-02	0,97243389	1
1666	0,203399182	4,04E-02	0,45546709	0
1699	0,603018825	2,52E-01	0,89530839	0
1706	0,921410004	7,13E-01	0,99623156	1
1725	0,108755784	9,80E-04	0,51386938	0
1729	0,972366882	7,99E-01	0,99996937	1
1731	0,960468038	7,52E-01	0,99985463	1
1739	0,844811946	3,23E-01	0,99823225	1
1744	0,001923548	8,49E-13	0,01758241	0
1745	0,006102073	3,57E-06	0,04538053	0
1874	0,05124762	1,24E-03	0,22009736	0
1934	0,041527435	1,10E-03	0,17619654	0

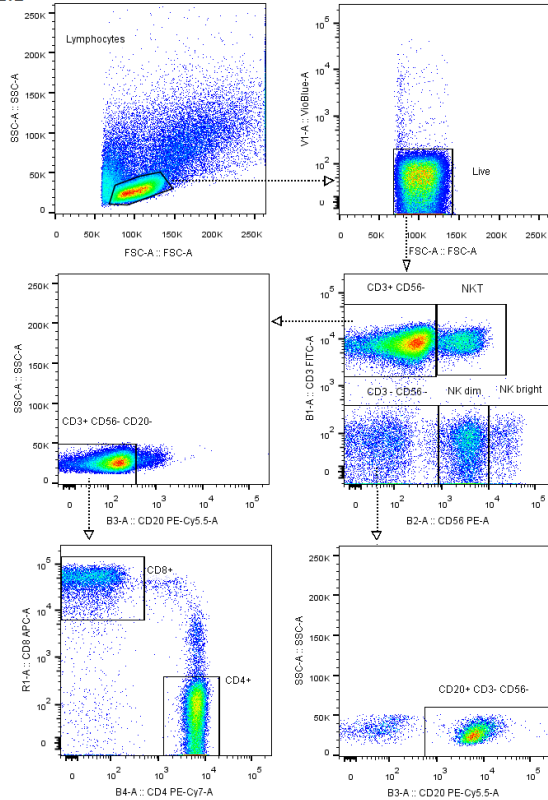
Supplementary table 9. Posterior predictive response in model validation.

The table shows the mean, lower and upper posterior predictive for each sample in the validation. Real response is 0 for non-responder and 1 for responder patients (NEDA-4 at 2 years).

Supplementary Figure S1. Gating strategy.

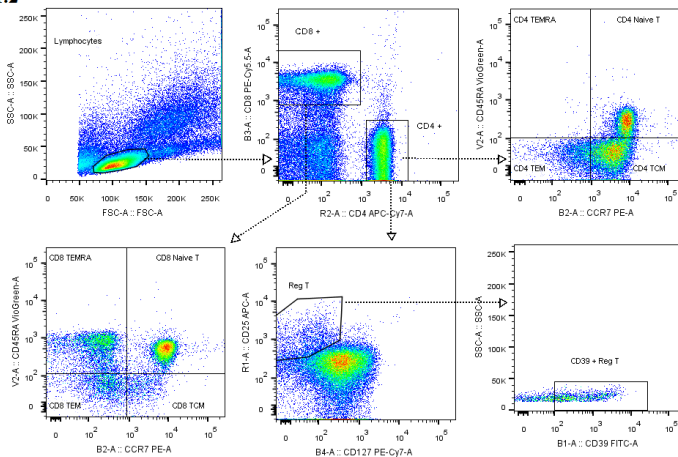
(a, b in S1.1-S1.7) Sample data from a control are shown. The lymphocyte population was gated on a forward scatter (FSC)/side scatter (SSC) plot. Live lymphocytes were further gated by 4',6-diamidine-2'-phenylindole dihydrochloride (DAPI) in S1.1-S1.5 and by the fluorescent reactive dye from the LIVE/DEAD™ Fixable Dead Cell Stain Kit (Thermo Fisher Scientific Inc.) in S1.6-S1.7.

S1.1



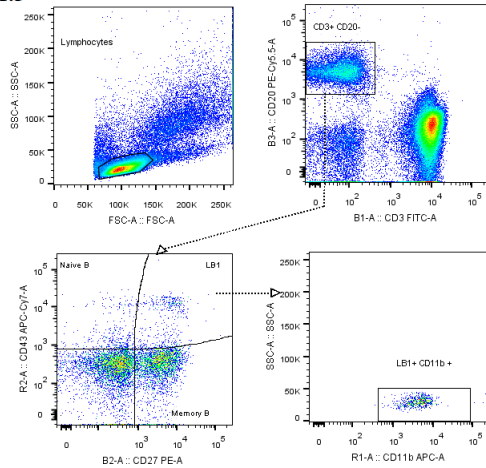
S1.1 Gating strategy in panel 1: (c) Live lymphocytes were then further gated to determine CD3⁺CD56⁻, CD3⁺CD56dim (NKdim), CD3⁺CD56bright (NKbright), CD3⁺CD56⁻ and CD3⁺CD56⁺ (NKT). (d) CD20⁻ from CD3⁺CD56⁻ (T lymphocytes). (e) CD4⁺ (Helper T cells) and CD8⁺ (cytotoxic T cells) from T lymphocytes and (f) CD20⁺ cells from CD3⁺CD56⁻ (B lymphocytes).

S1.2



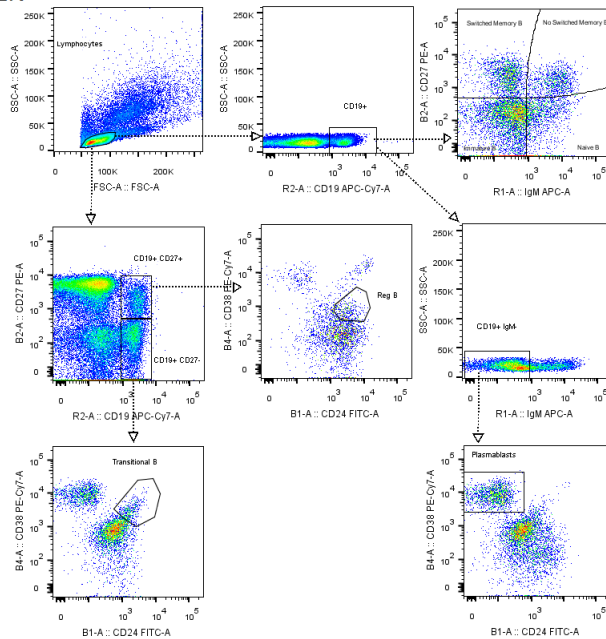
S1.2 Gating strategy in panel 2: (c) Live lymphocytes were then further gated to determine CD4⁺ (Helper T cells) and CD8⁺ (cytotoxic T cells), (d) CCR7⁺CD45RA⁻ (CD4TEM cells), CCR7⁺CD45RA⁺ (CD4TEMRA cells), and CCR7⁺CD45RA⁺ (CD4 naïve T cells) from CD4⁺ cells. (e) CCR7⁺CD45RA⁻ (CD8TEM cells), CCR7⁺CD45RA⁻ (CD8TCM cells), CCR7⁺CD45RA⁺ (CD8TEMRA cells), and CCR7⁺CD45RA⁺ (CD8 naïve T cells) from CD8⁺ cells. (f) CD127^{down}CD25^{high} from CD4 cells (regulatory T cells). (g) CD39⁺ cells from regulatory T cells.

S1.3



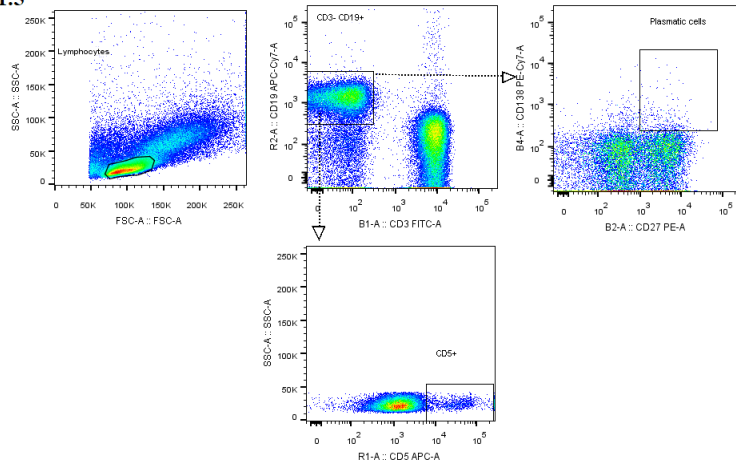
S1.3 Gating strategy in panel 3: (c) Live lymphocytes were then further gated to evaluate CD3⁺CD20⁺ cells, (d) CD27⁺CD43⁻ (memory1 B cells), CD27⁻CD43⁺ (naïve 1 B cells), and CD27⁺CD43⁺ (LB1 cells) from CD3⁺CD20⁺ cells (e) CD11b⁺ cells from LB1 cells.

S1.4



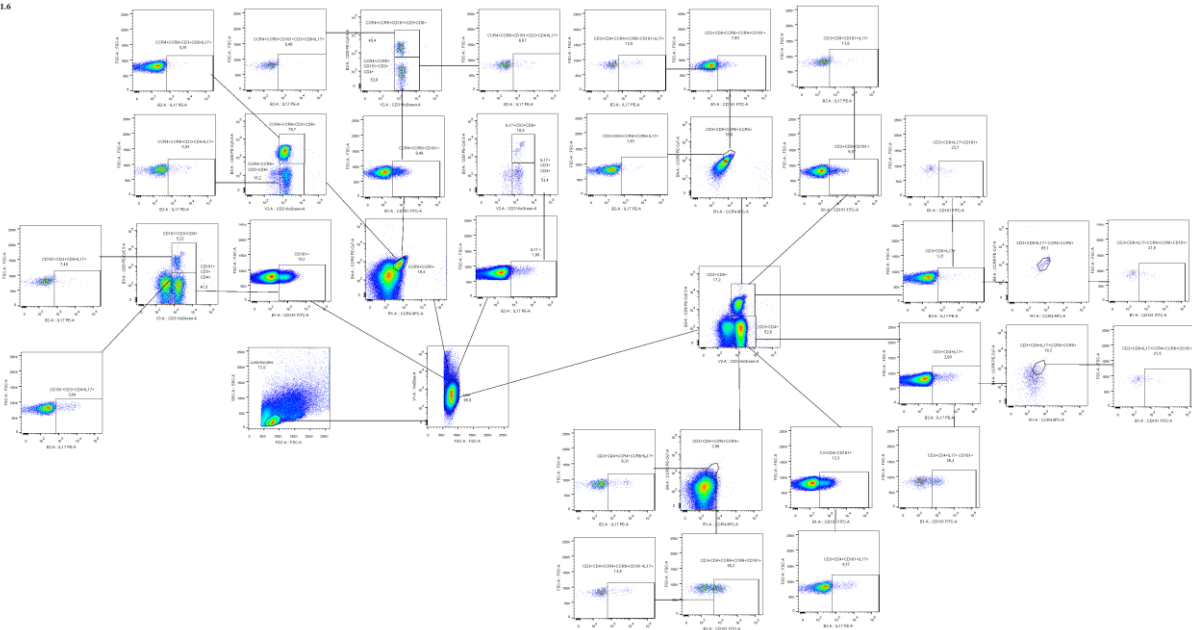
S1.4 Gating strategy in panel 4: (c) Live lymphocytes were then further gated to determine CD19⁺ cells, (d) IgM⁻CD27⁻ (transitory B cells), IgM⁺ CD27⁻ (naïve 2 B cells), IgM⁻ CD27⁺ (switched memory B cells), and IgM⁺ CD27⁺ (non-switched memory B cells) from CD19⁺ cells. (e) IgM⁻ cells from CD19⁺. (f) CD24⁻CD38^{high} cells from CD19⁺IgM⁻ (plasmablasts). (g) CD24⁻CD38^{high} cells from CD19⁺IgM⁻ (plasmablasts). (h) CD19⁺CD27⁻ and CD19⁺CD27⁺ from live lymphocytes. (i) CD24^{high} CD38^{int} from CD19⁺CD27⁺ (regulatory B cells) and (j) CD24^{high}CD38^{high} from CD19⁺CD27⁻ (transitory B cells).

S1.5



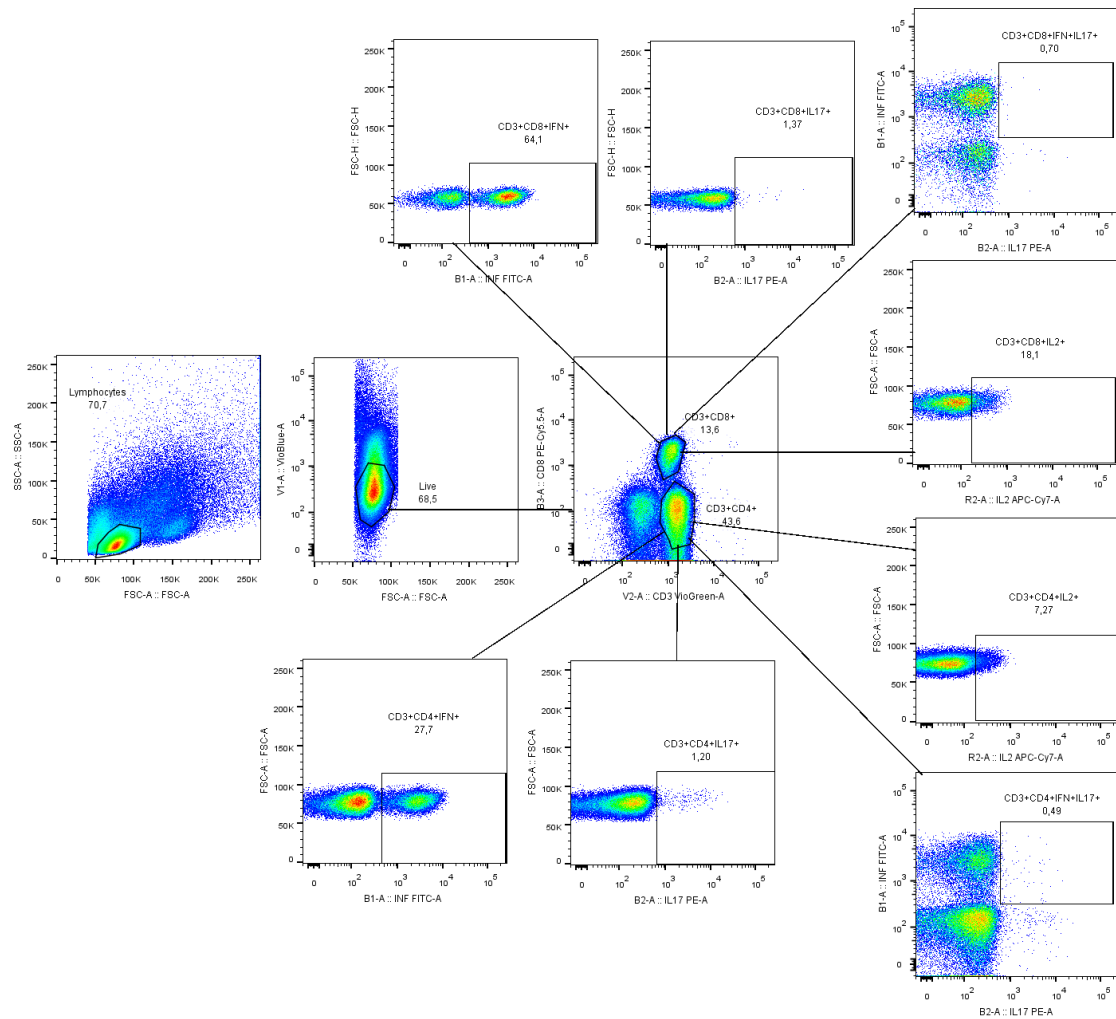
S1.5 Gating strategy in panel 5: (c) Live lymphocytes were then further gated to evaluate CD19+CD3⁻ cells and (d) CD138⁺CD27⁺ cells from CD19+CD3⁻ cells (plasmatic cells). (e) CD5⁺ cells from CD19+CD3⁻ cells.

S1.6



S1.6 Gating strategy in panel 6. All possible combinations between surface (CD3, CD8, CD161, CCR6 and CCR4) and intracellular (IL-17) markers for Th17 cells allowing us define the percentage of IL-17-producing cells among Th17 cells measured by surface markers.

S1.7

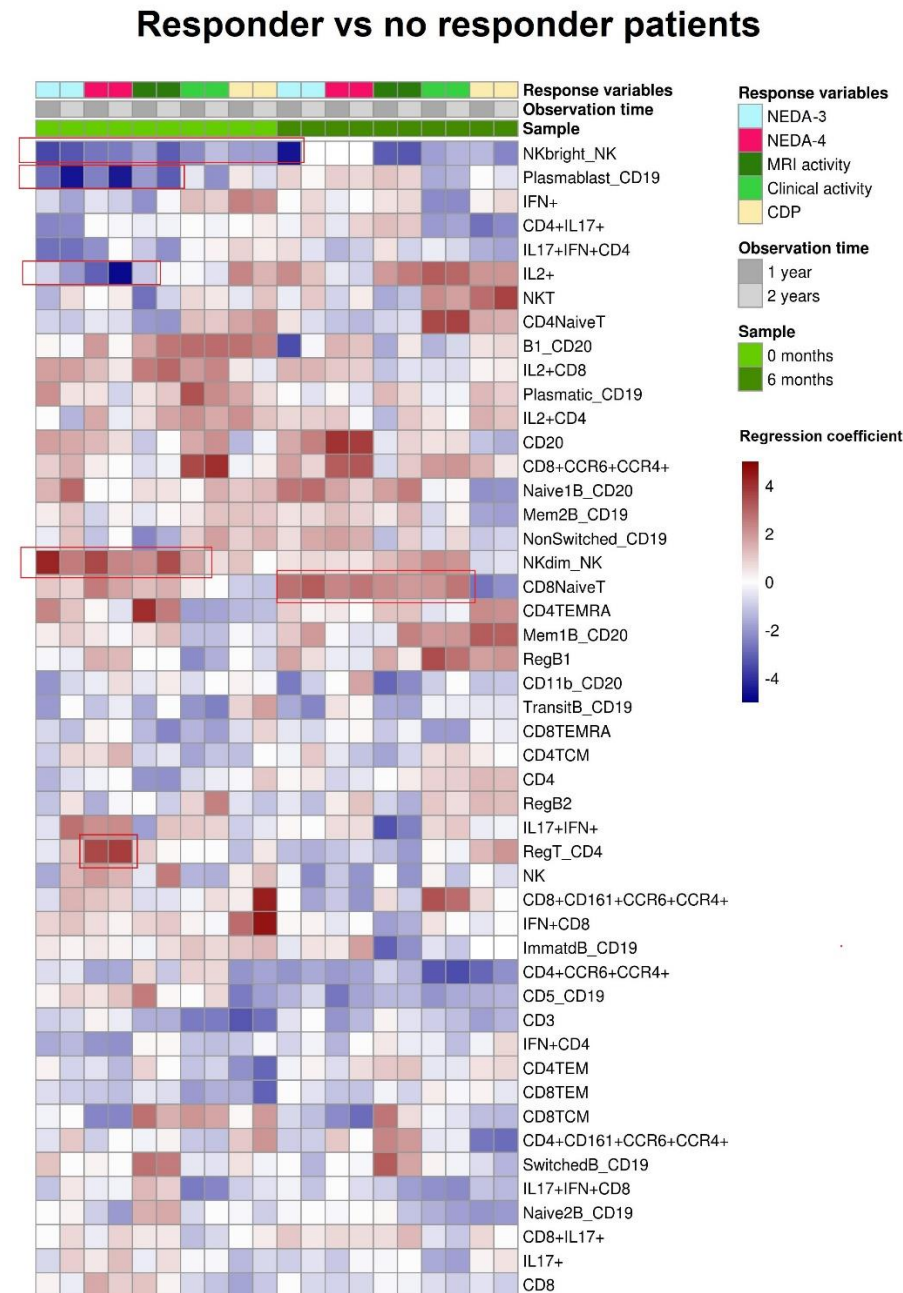


S1.7 Gating strategy in panel 7: All possible combinations between surface (CD3 and CD8) and intracellular (IL-17, IL-2 and IFN) markers allowing us to define the percentages of IL-17, IL-2 and IFN-producing cells among helper T and cytotoxic T cells.

Lymphocyte subpopulation correlations



Supplementary Figure S3. Heatmap of logistic regression coefficients between lymphocytes subpopulations and several response measures.



Supplementary Figure S3. Heatmap of logistic regression coefficients between lymphocytes subpopulations and several response measures.

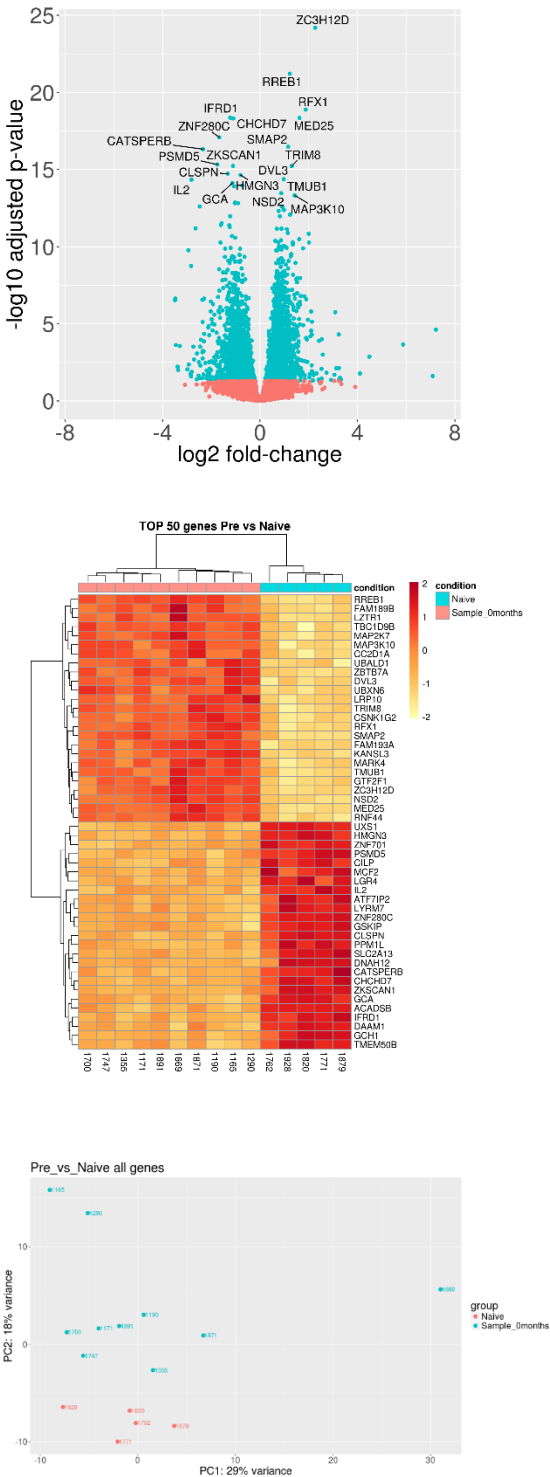
Heatmap of the logistic regression coefficients for each of the lymphocyte subpopulations as predictors for the response variables of interest (NEDA-3, NEDA4, MRI activity, clinical activity and CDP) organized by columns and marked with the color key “Response variables” in first line. These response variables were determined at 1 and 2 years and have been discriminated by columns and marked with the color key “Observation time” in the second line. The lymphocytes subpopulations obtained before and at 6 months of treatment are shown in columns 0-10 (light green) and 11-20 (dark green) respectively as indicated by color key “Sample” in third line. Coefficients are represented using the mean of the posterior distributions, as indicated by the color scale, with red representing a positive correlation with the predictors shown as columns and blue indicating a negative correlation. The subpopulations with the best coefficients for most of the target variables are locked in the red rectangle

[illegible]

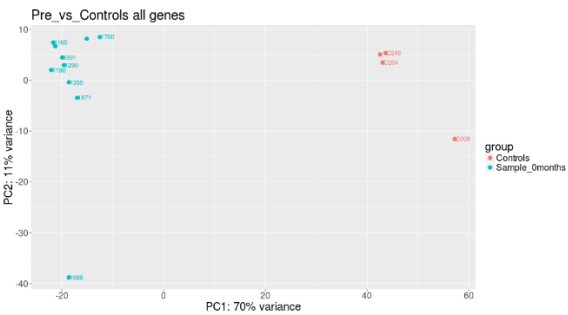
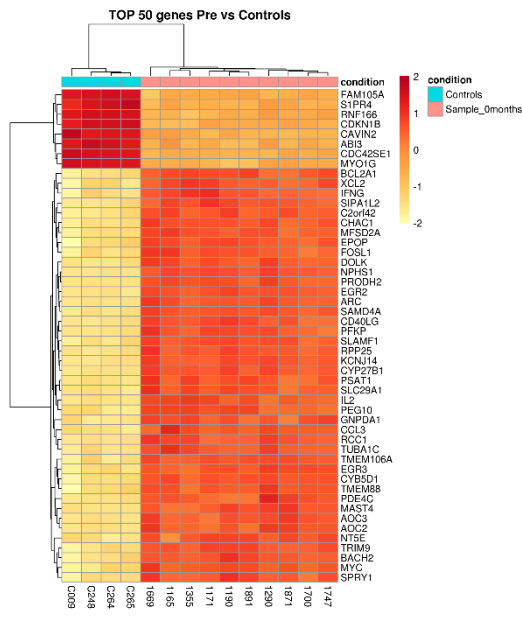
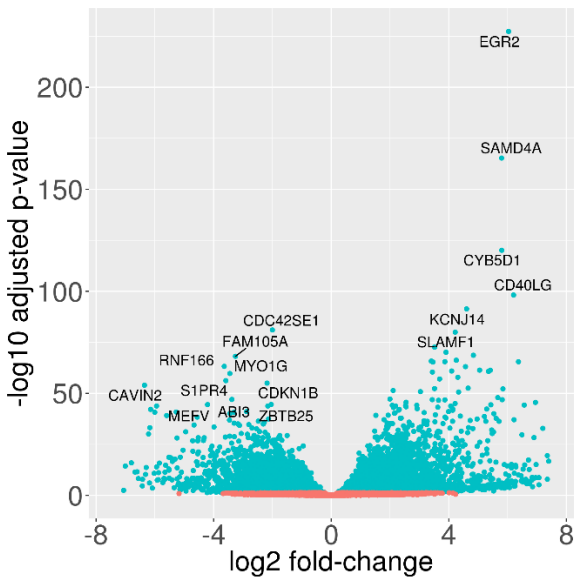
The gene expression fold-change was mapped in color from red for up-regulated genes to blue for down-regulated genes in response to fingolimod after 6 months of treatment. For each gene, the fold change was calculated for 5 subgroups (all patients, NEDA patients at 1 year, NEDA patients at 2 years, EDA patients at 1 year and EDA patients at 2 years) to assess the differential regulation between R and NR patients. * Genes with significant differences between responder and non-responder patients. Pathways were obtained from wikipaths and drawn using the PathVisio program **A.** S1P pathway (ontology terms: PW0000960). The only gene in this pathway that exhibited differential regulation after 6 months of treatment was MAPK3, which was up-regulated in R patients and down-regulated in NR patients (at 1 and 2 years). **B.** Th17 pathway (ontology terms: PW0000960). IL17-A was down-regulated after 6 months only in R patients. **C- D.** Oxidative stress response pathway (*Homo sapiens*) adapted from wikipaths (ontology terms: PW0000378). The only gene in this pathway that exhibited differential regulation after 6 months of treatment was FOS, which was significantly down-regulated only in NR patients at 2 years.

Supplementary figure S5. Differentially Expressed Genes (DEGs): volcano plots, top 50 genes and PCA.

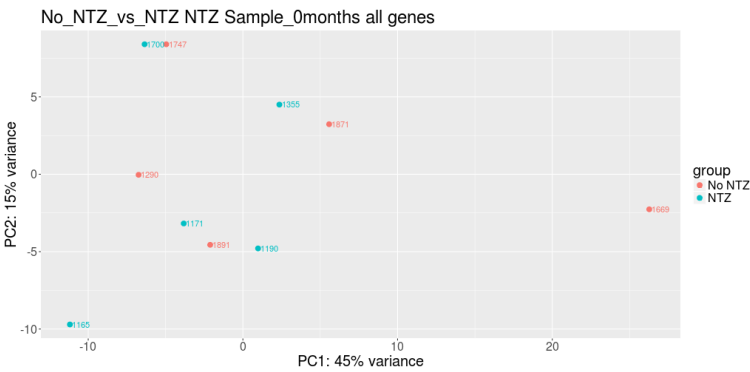
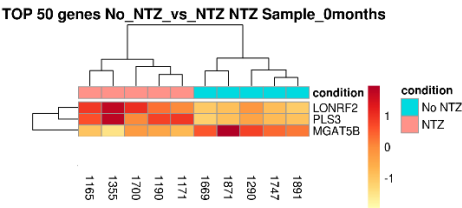
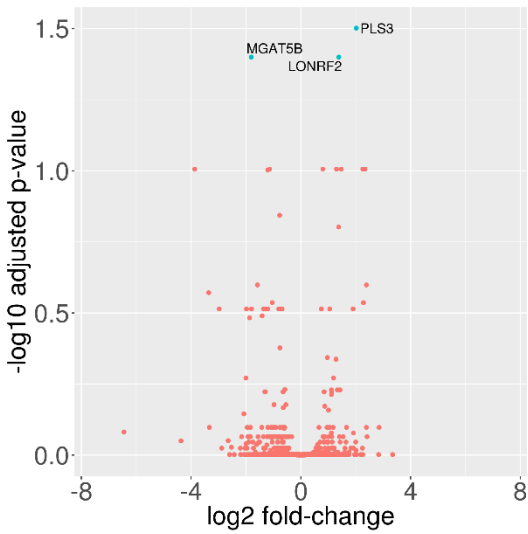
S4.1. DEGs of samples obtained from naive patients vs MS patients (previously treated with natalizumab or interferon beta) before fingolimod treatment.

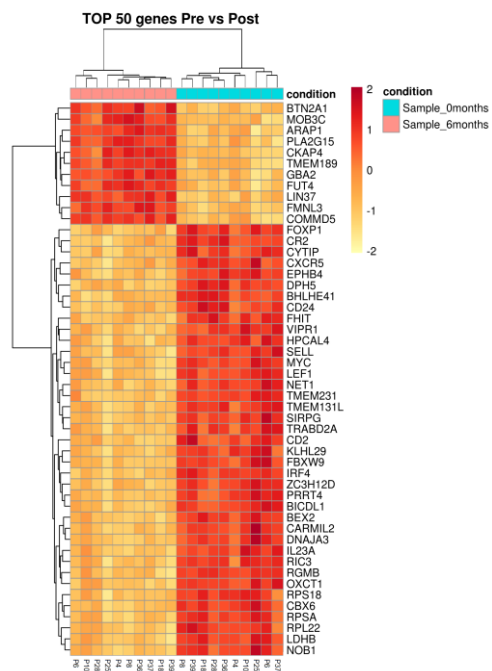


S4.2. DEGs of samples obtained from healthy controls vs MS patients (previously treated with natalizumab or interferon beta) before fingolimod treatment.

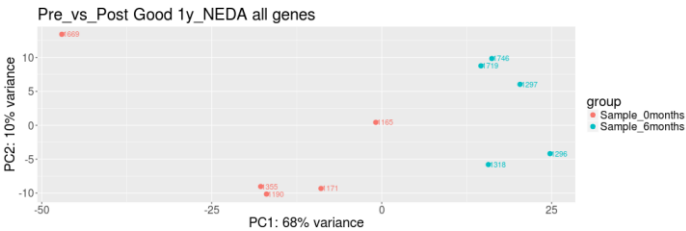
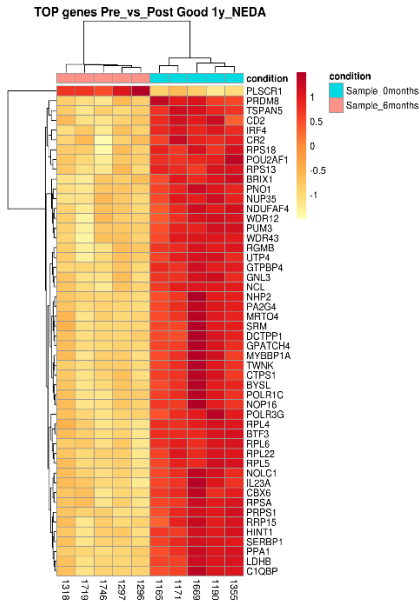
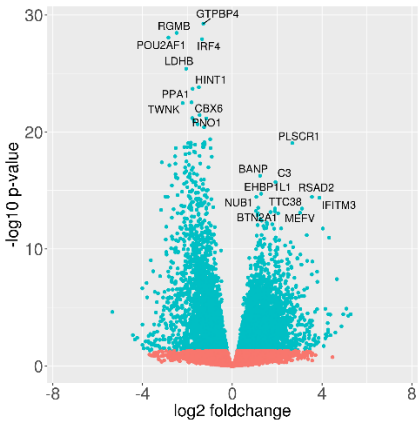


S4.3. DEGs of samples obtained from NTZ-group vs No-NTZ group before fingolimod treatment.

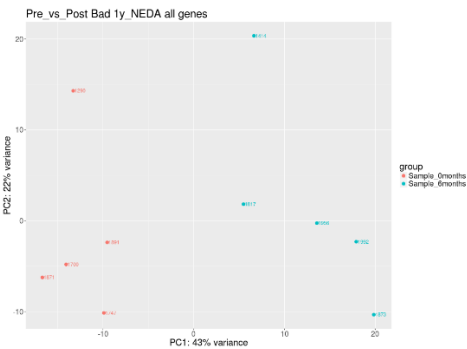
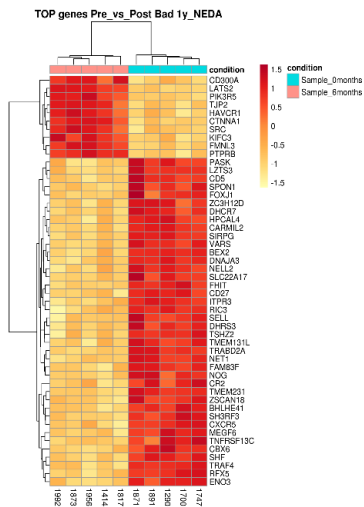
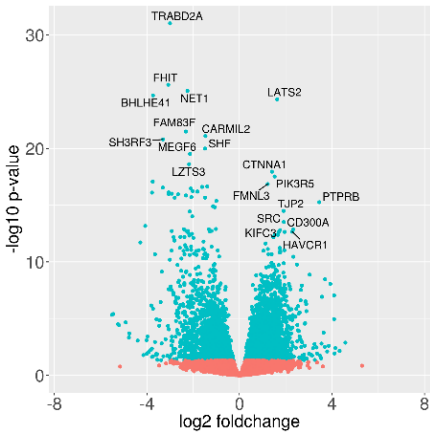




S4.5. DEGs of samples obtained before and after (6 months) fingolimod treatment exclusively in MS patients who achieved NEDA-4 status at 1 year.



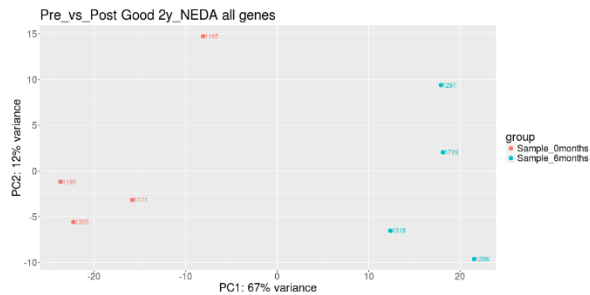
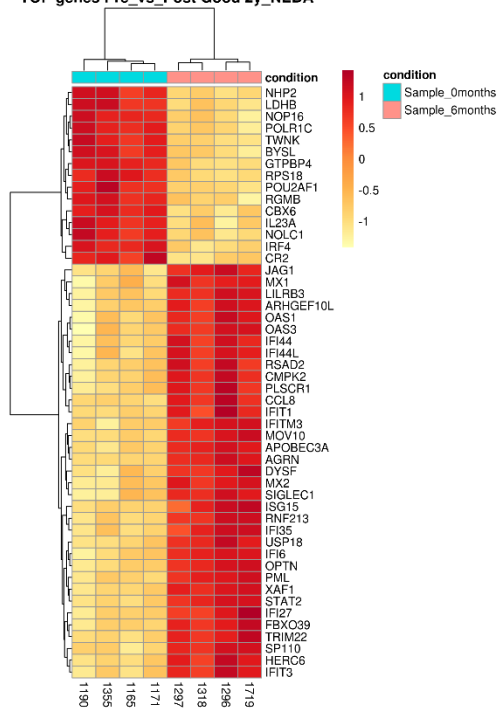
S4.6. DEGs of samples obtained before and after (6 months) fingolimod treatment exclusively in MS patients who did not achieve NEDA-4 status at 1 year.



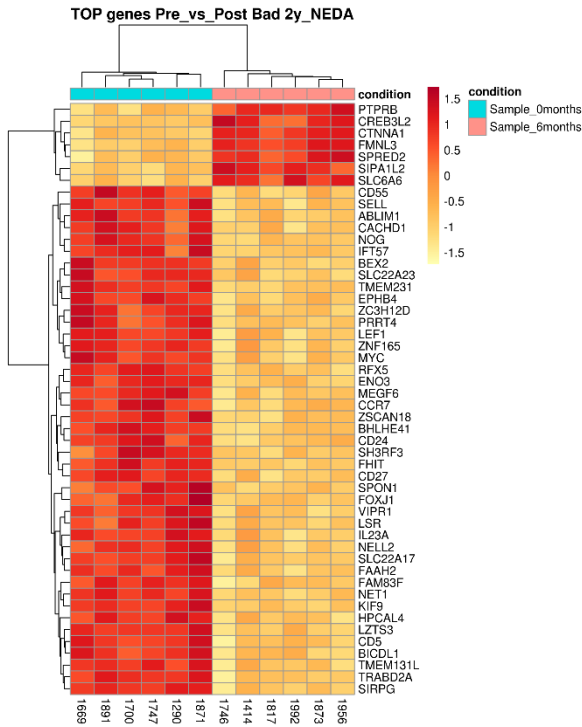
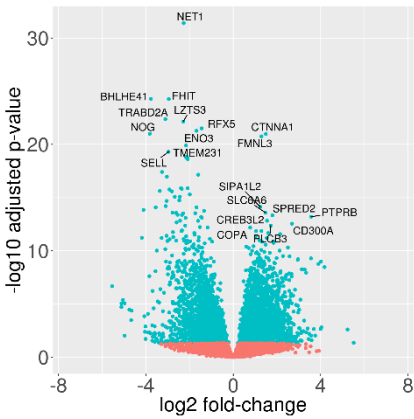
S4.7. DEGs of samples obtained before vs after (6 months) fingolimod treatment in MS patients who achieved NEDA-4 status at 2 years.



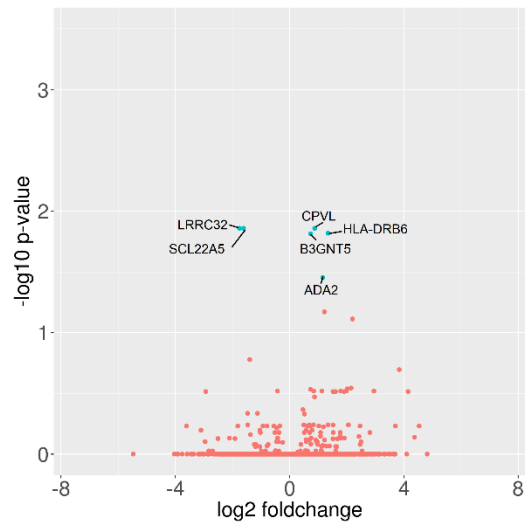
TOP genes Pre_vs_Post Good 2y_NEDA



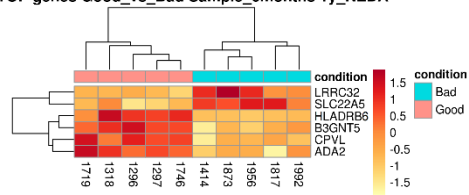
S4.8. DEGs of samples obtained before and after (6 months) fingolimod treatment in MS patients who did not achieve NEDA-4 status at 2 years.



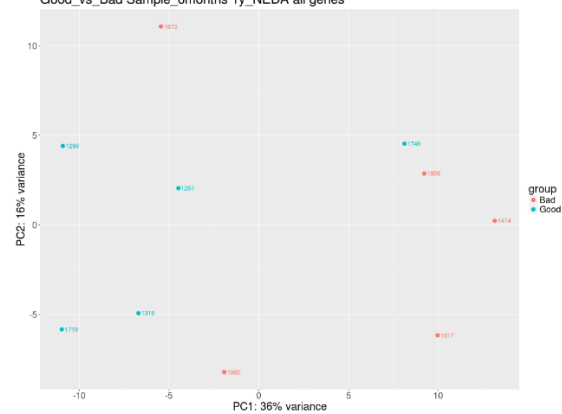
S4.9. DEGs after 6 months of fingolimod in samples obtained from NEDA-4 vs EDA-4 patients when it was calculated at 1 year.



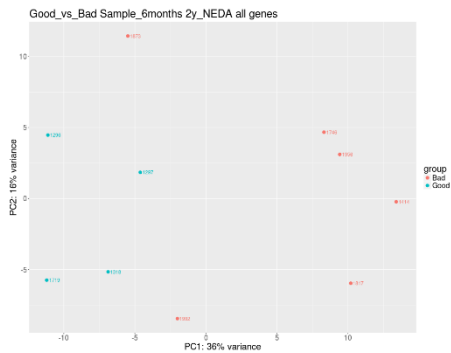
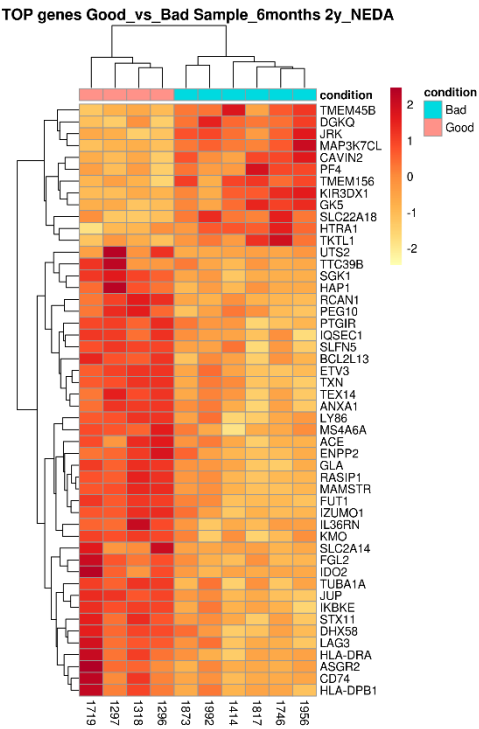
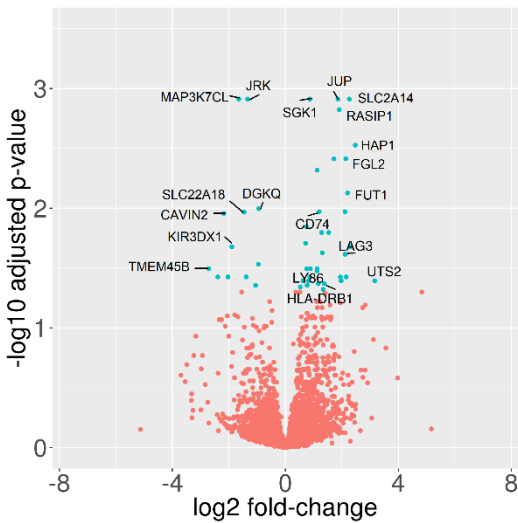
TOP genes Good_vs_Bad Sample_6months 1y_NEDA



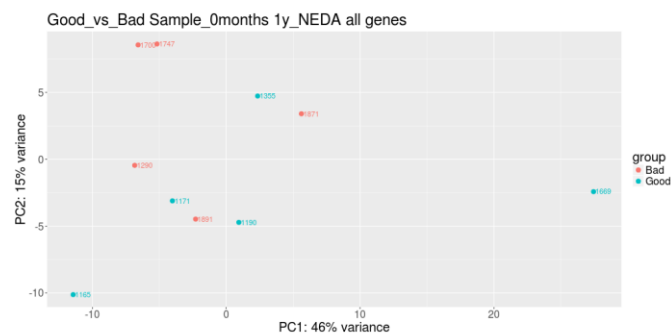
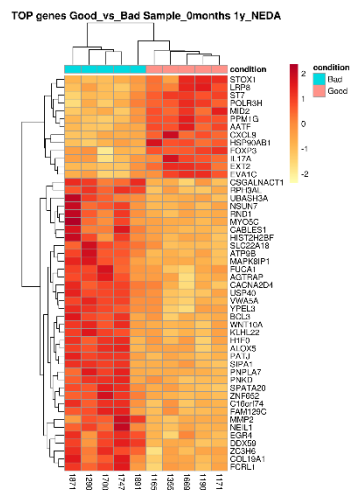
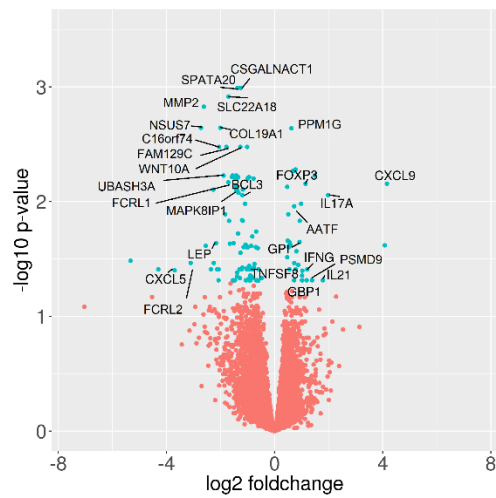
Good_vs_Bad Sample_6months 1y_NEDA all genes



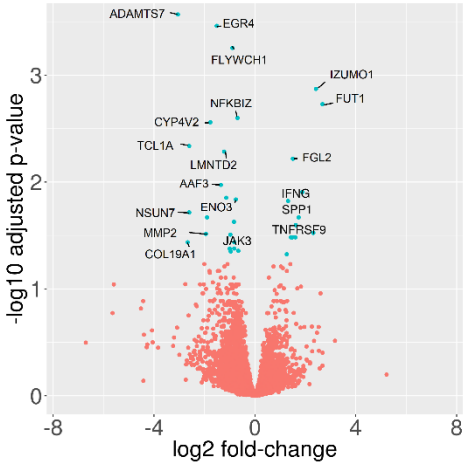
S4.10. DEGs after 6 months of fingolimod treatment in samples obtained from NEDA-4 vs EDA-4 patients when it was calculated at 2 years.



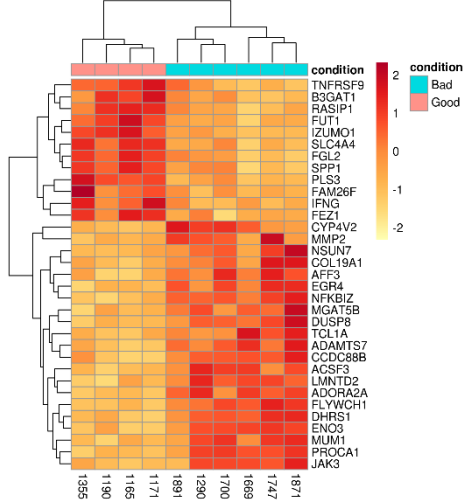
S4.11. DEGs before fingolimod treatment in samples obtained from NEDA-4 vs EDA-4 patients when it was calculated at 1 year.



S4.12. DEGs before fingolimod treatment in samples obtained from NEDA-4 vs EDA-4 patients when it was calculated at 2 years.



TOP genes Good_vs_Bad Sample_0months 2y_NEDA



Good_vs_Bad Sample_0months 2y_NEDA all genes

