Different humoral but similar cellular responses of patients with autoimmune inflammatory rheumatic diseases under disease-modifying anti-rheumatic drugs after COVID-19 vaccination – Supplementary material

Supplement materials and methods

Stimulation with overlapping peptide pools from SARS-CoV-2 S-protein

After thawing, PBMCs were rested overnight before being plated in a 96-UWell plate (Sarstedt) in RPMI media (Life Technologies) supplemented with 1% Penicillin-Streptomycin-Glutamine (Sigma Aldrich), and 10% FCS (PAN-Biotech). The cells were stimulated for 16 h at 37°C and after 2 h, Brefeldin A (1 µg/ml, Sigma Aldrich) was added. As a positive control, cells were stimulated with *Staphylococcus aureus* enterotoxin B SEB (1 µg/ml, Sigma Aldrich) and as a negative control, cells were left untreated.

Antibodies and staining procedure

PBMCs were stained with the following antibodies:

- Surface staining: Fixable Viability Dye eFluor-780 (ThermoFisher, USA), CCR7 [CD197]-PerCP-Cy5.5 (clone: G043H7; BioLegend, Germany), CD4-A700 (clone: OKT4; BioLegend), CXCR5-PE-Dazzle594 (clone: MOPC-21; BioLegend), CD8-V500 (clone: RPA-T8; BD) and CD45RA-BV605 (clone: HI100; BioLegend).
- Intracellular staining: Granzyme B-FITC (clone: GB11; BioLegend), IL-2-PE (clone: MQ1-17H12; BioLegend), CD137 [4-1BB]-PE-Cy7 (clone: 4B4-1; BioLegend), CD154 [CD40L]-A647 (clone: 24-31; BioLegend), TNFα-eFluor450 (clone: MAb11; eBioscience, Germany), IFN-γ-BV650 (clone: 4S.B3; BioLegend) and CD3-BV785 (clone: OKT3; BioLegend).

Harvested cells were washed with PBS and incubated with the surface staining antibodies at optimal concentrations at room temperature in the dark for 15 minutes. After washing with PBS, cell fixation and permeabilization were performed using the Fixation/Permeabilization Buffer Set Kit (Life Technologies, Germany) per manufacturer's instructions. Intracellular

staining was performed using staining antibodies at optimal concentrations at room temperature in the dark for 30 minutes, before a single wash with PBS.

Statistical Analysis

Descriptive statistic was used for summarizing demographics, clinical characteristics, humoral/cellular response. Flow cytometry data were analyzed using FlowJo version 10.7.1 (BD Biosciences, USA); gating strategy and representative dot plots are shown in Figure S1. Unspecific activation in unstimulated controls was subtracted from stimulated samples to account for specific activation in the presented frequencies. Negative values were set to zero.

Statistical analysis was performed using R, version 4.0.4. Continuous variables are described employing the median and interquartile range (IQR) and depicted in box plots; the maximum length of the whiskers corresponds to 1.5 times the interquartile range. Only two-sided tests were employed. Differences for a continuous variable between more than two sub-cohorts were analyzed using the Kruskal-Wallis rank sum-test, with the Dunn test without P value adjustment as post-hoc test. Differences between two sub-cohorts, e.g. difference between combined and monotherapy, were calculated employing the Mann-Whitney U test. The increase of a variable between two visits was evaluated employing the Wilcoxon rank sum test. Differences in compositional data were assessed employing multivariate analysis of variance (MANOVA) after applying the isometric log ratio transformation. Correlations between two continuous variables were evaluated employing Spearman's correlation coefficient. Multivariate linear regression analysis was implemented to assess the association of employed treatments with the humoral immune response after vaccination, while controlling for demographic characteristics and underlying rheumatic disease. P values for the independent variables of the multivariate model were calculated employing the t test; only significant effects pertaining to the immunosuppressive treatment are reported here. Significance threshold was set at 0.050; only significant P values are marked in the figures.

Supplementary figures

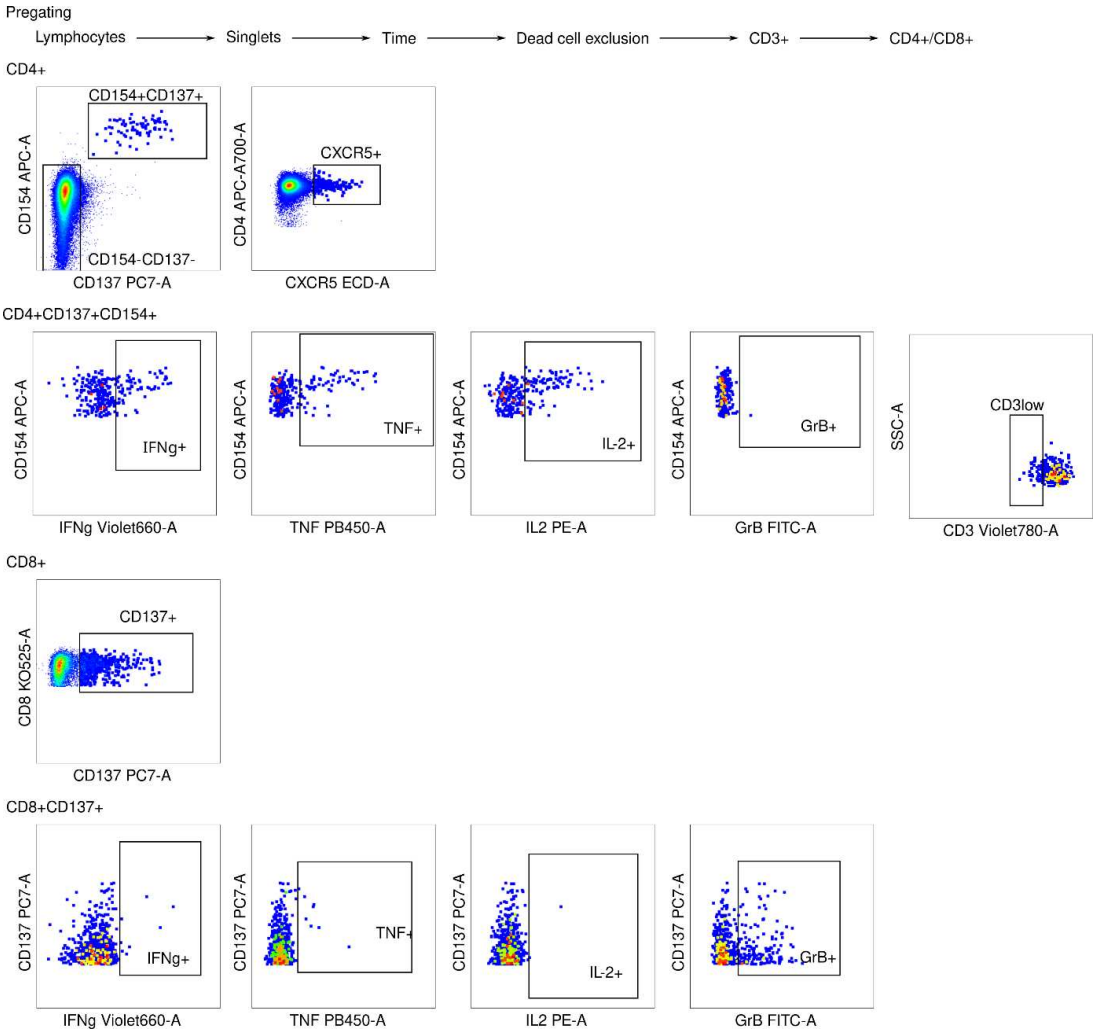

Figure S1. Gating strategy to identify S-protein reactive T-cells

PBMC were incubated for 16 hours with overlapping peptide pools (OPP) of the complete SARS-CoV-2 wild type (WT) S-protein. Brefeldin A was added after 2 hours. Stimulation with peptide diluent and *Staphylococcus aureus* enterotoxin B (SEB) as polyclonal stimulus served as negative and positive controls, respectively. Cells were acquired using a Cytoflex flow cytometer. Only an illustrative example of the SARS-CoV-2 stimulation is shown.

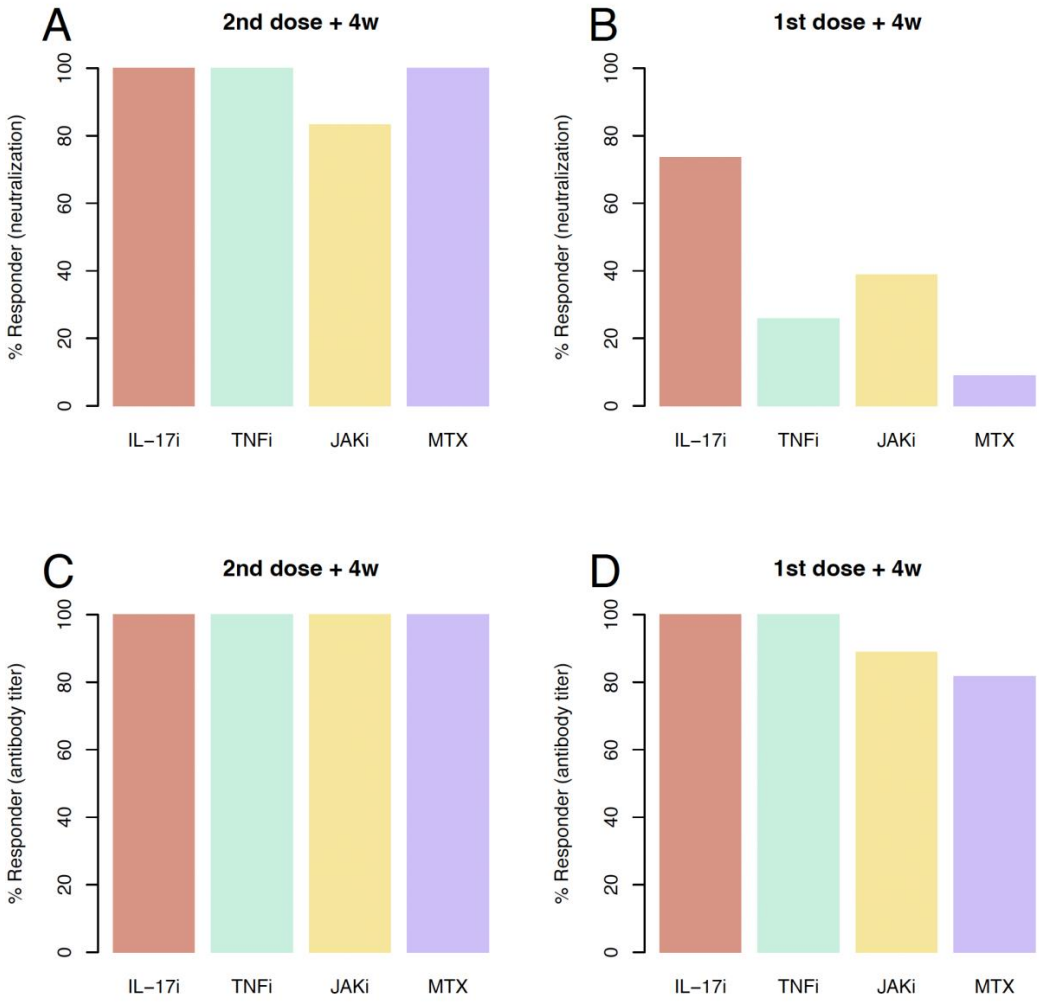

Figure S2.

Percent of patients who developed neutralizing antibodies (A and B) and serological immune response (C and D) against SARS-CoV-2 ancestral variant by different therapies after the second (2nd dose+4w) and first vaccinations (1st dose+4w). In the analysis were included only patients on monotherapy. IL-17i=interleukin 7 inhibitor. TNF=tumor necrosis factor. JAKi= Janus kinase inhibitor. MTX=methotrexate.

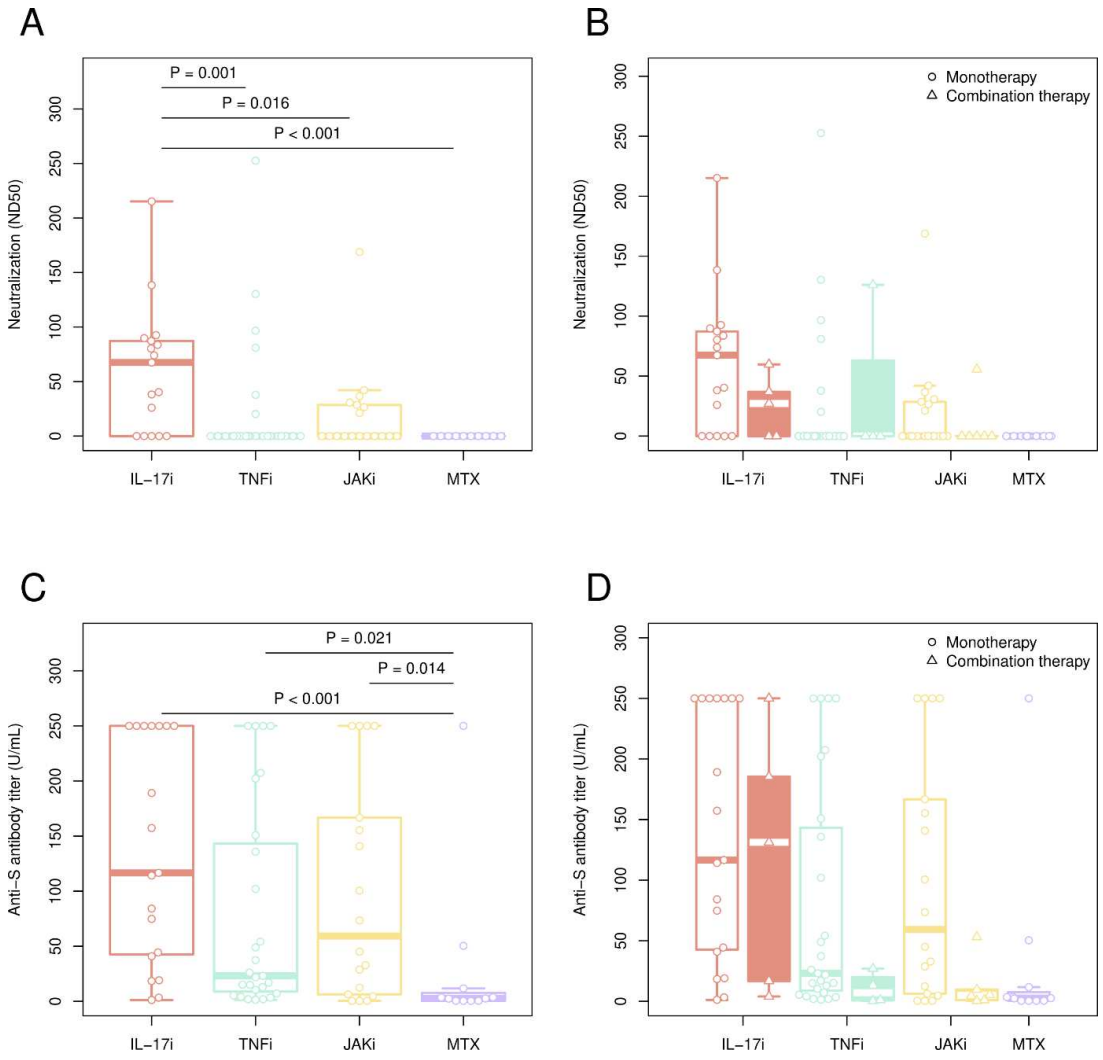

Figure S3. Neutralization titers and serological immune responses against SARS-CoV-2 ancestral strain by different therapies four weeks after the first vaccination (1st dose+4w).

Neutralization titers (ND50) against SARS-CoV-2 in plasma A) SARS-CoV-2 neutralization antibodies four weeks after the first vaccination (1st dose+4w) for all four groups of patients on monotherapy B) Comparison between SARS-CoV-2 neutralization antibodies four weeks after the first vaccination (1st dose+4w) for all four groups of patients on monotherapy (unfilled plots) and combination therapies (filled colored plots) **Serological immune responses against SARS-CoV-2** C) Spike-specific IgG titers four weeks after the first vaccination (1st dose+4w) for all four groups of patients on monotherapy D) Comparison between spike-specific IgG titers four weeks after the first vaccination (1st dose+4w) for all four groups of patients on monotherapy (unfilled plots) and combination therapies (filled

colored plots). The box plots indicate the 75th, 50th, and 25th quantile, and the whiskers have a maximum length of 1.5 times the interquartile range. Each point represents individual values; small triangles represent the additional patients on combination therapy. The following numbers of patients are presented: IL17i (n=19- 1st dose+4w), TNFi (n=27- 1st dose+4w), JAKi (n=18 -1st dose+4w) and MTX (n=11- 1st dose+4w), IL17i/MTX (n=5 -1st dose+4w), TNFi/MTX (n=4- 1st dose+4w), JAKi/MTX (n=6- 1st dose+4w). ND50=50% inhibitory dilution. U/ml=units/milliliter. IL17i=interleukin-17 inhibitor. TNFi=tumor necrosis factor inhibitor. JAKi= Janus kinase inhibitor. MTX=methotrexate.

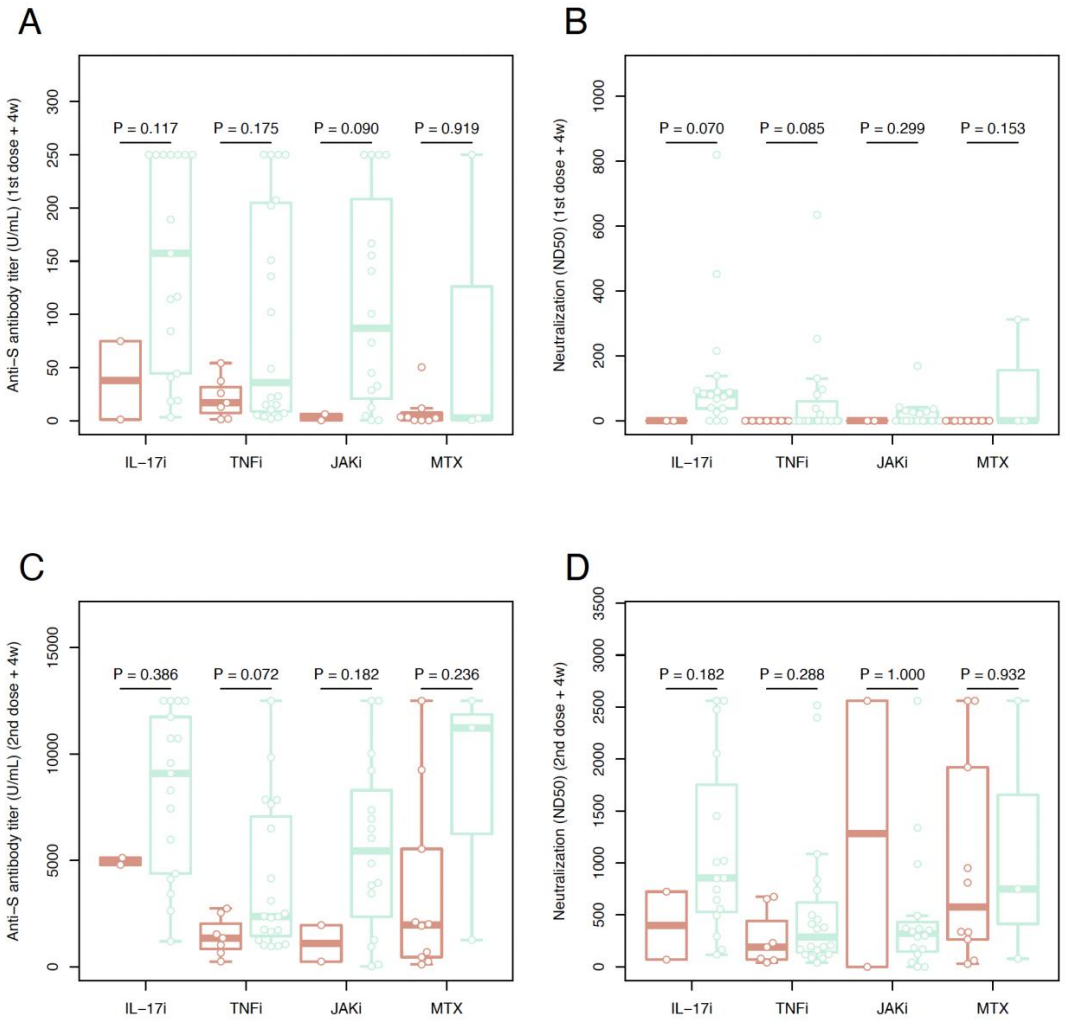

Figure S4. Comparison of serological immune response (A and C) against SARS-CoV-2 ancestral variant and neutralization titers (B and D) for each therapy group after the first dose (A and B) and the second dose (C and D) of mRNA-1273 (green) and BNT162b2 (red) vaccines.

Serological immune responses against SARS-CoV-2 A) Spike-specific IgG titers four weeks after the 1st vaccination (1st dose+4w) for all therapy groups explored C) Spike-specific IgG titers four weeks after the 2nd vaccination (2nd dose+4w) for all therapy groups explored. **Neutralization titers (ND50) against SARS-CoV-2 in plasma** B) SARS-CoV-2 neutralization four weeks after the 1st vaccination (1st dose+4w) for all therapy groups explored D) SARS-CoV-2 neutralization four weeks after the 2nd vaccination (2nd dose+4w) for all therapy groups explored. The box plots indicate the 75th, 50th, and 25th quantile, and the whiskers

have a maximum length of 1.5 times the interquartile range. Each point represents individual values. ND50=50% inhibitory dilution. U/ml=units/milliliter. IL17=interleukin17-inhibitor. TNFi=tumor necrosis factor inhibitor. JAKi= Janus kinase inhibitor. MTX=methotrexate.

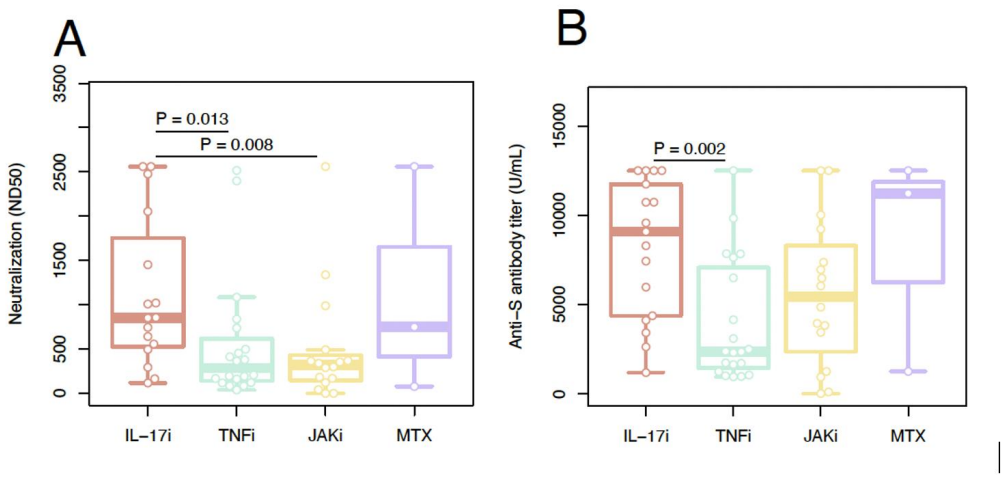

Figure S5. Neutralization titers (A) and serological immune responses (B) against SARS-CoV-2 ancestral variant by different therapies four weeks after the second vaccination with mRNA-1273 (2nd dose+4w)

ND50=50% inhibitory dilution. U/ml=units/milliliter. IL-17i=interleukin 17 inhibitor. TNF=tumor necrosis factor. JAKi= Janus kinase inhibitor. MTX=methotrexate.

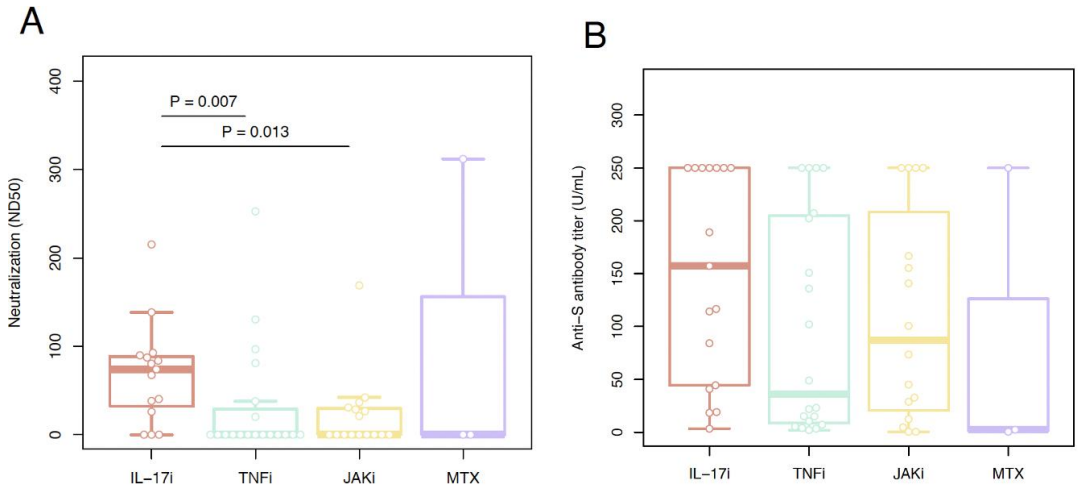

Figure S6. Neutralization titers (A) and serological immune responses (B) and against SARS-CoV-2 ancestral variant by different therapies four weeks after the first vaccination with mRNA-1273 (1st dose+4w)

ND50=50% inhibitory dilution. U/ml=units/milliliter. IL-17i=interleukin 17 inhibitor. TNF=tumor necrosis factor. JAKi= Janus kinase inhibitor. MTX=methotrexate.

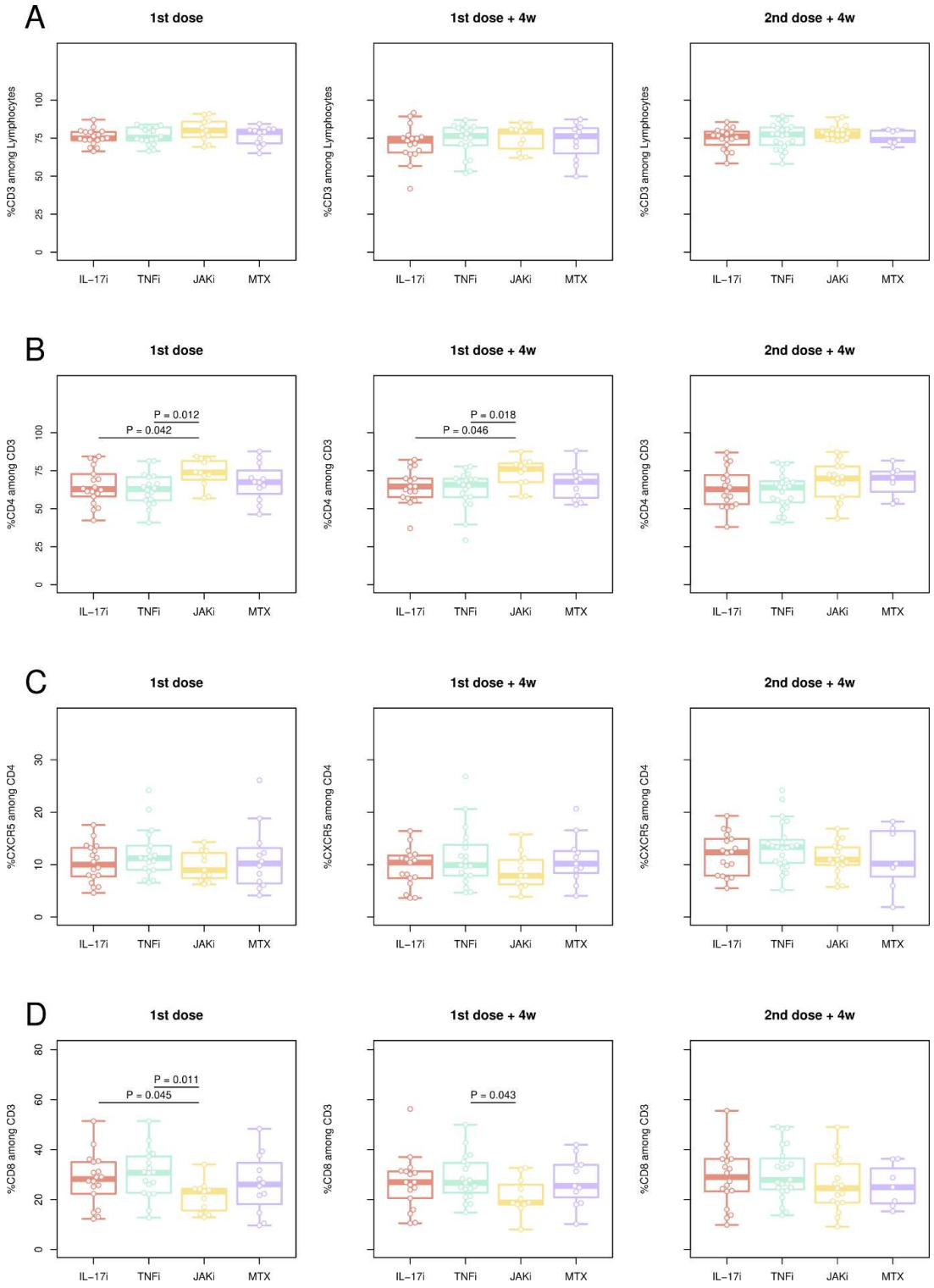
A
1st dose
1st dose + 4w
2nd dose + 4w
%CD3 among Lymphocytes
0
25
50
75
100
IL−17i
TNFi
JAKi
MTX
B
%CD4 among CD3
P = 0.012
P = 0.042
P = 0.018
P = 0.046
C
%CXCR5 among CD4
0
10
20
30
D
%CD8 among CD3
0
20
40
60
80
P = 0.011
P = 0.045
P = 0.043

Figure S7. T-cell compartment is similar across therapies.

A) The frequency of CD3+ T-cells among lymphocytes before the first dose (1st dose), four weeks after the 1st dose (1st dose+4w), and four weeks after the second dose (2nd dose+4w). B) The frequency of CD4+ T-cells among CD3+ at the three different time points. C) The frequency of CD8+ T-cells among CD3+ at the three different time points. C) The frequency of circulating T-follicular helper cells like cells (CXCR5+) among CD4+ at the three different time points. The box plots indicate the 75th, 50th, and 25th quantile, and the whiskers have a maximum length of 1.5 times the interquartile range. Each point represents individual values. Only significant comparisons are marked.

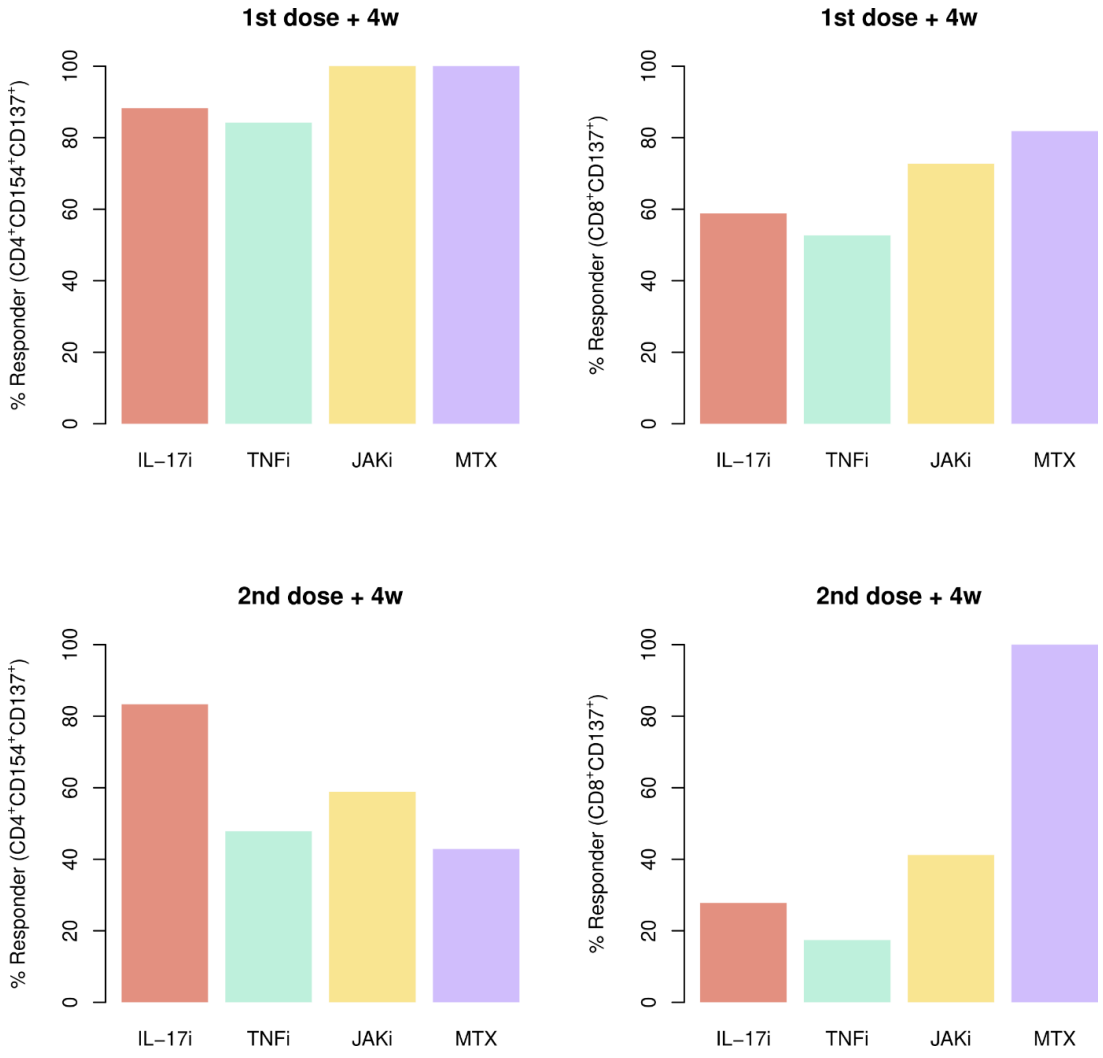

Figure S8. Number of cellular responders is similar across therapies.

Cellular response defined as a population of CD4+CD154+CD137+ or CD8+CD137+ with a stimulation index (the ratio stimulated to unstimulated) of at least 3.

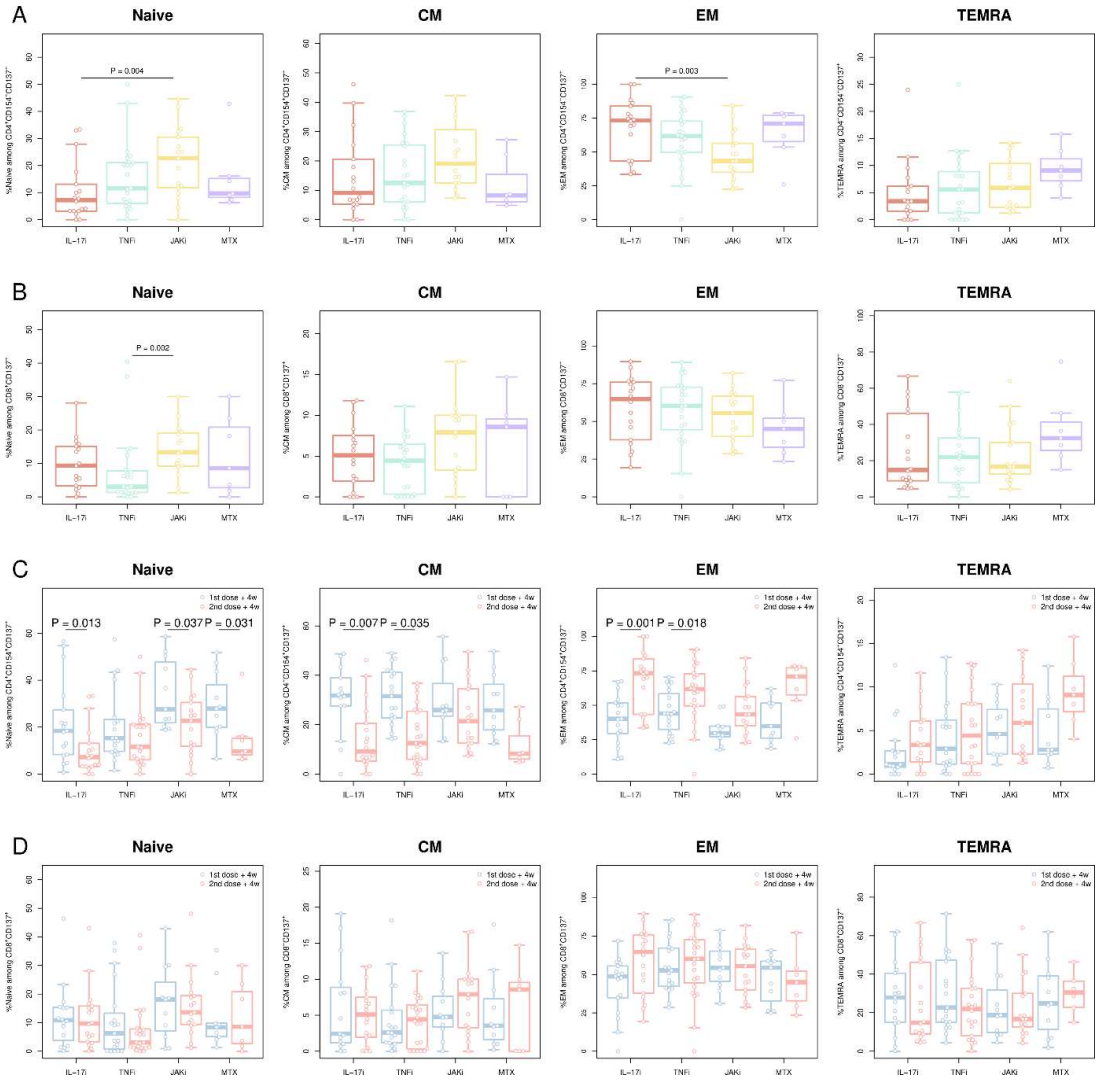

Figure S9. T-cell memory phenotype is similar across therapies after the second vaccination (2nd dose+4w).

T-cells memory is divided into four populations by their expression of CCR7 and CD45RA: Naïve (CD45RA+CCR7+), central memory (CM; CD45RA–CCR7+), effector memory (EM; CD45RA–CCR7–), and EM expressing CD45RA (TEMRA; CD45RA+CCR7–). A) The frequency of naïve, central memory (CM), effector memory (EM), and EM expressing CD45RA (TEMRA) among CD4+ T-cells. B) The frequency of naïve, central memory (CM), effector memory (EM), and EM expressing CD45RA (TEMRA) among CD8+ T-cells. C) Dynamics of the frequency of naïve, central memory (CM), effector memory (EM), and EM expressing CD45RA (TEMRA) among CD4+ T-cells. D) Dynamics of the frequency of naïve, central

memory (CM), effector memory (EM), and EM expressing CD45RA (TEMRA) among CD8+ T-cells.

The box plots indicate the 75th, 50th, and 25th quantile, and the whiskers have a maximum length of 1.5 times the interquartile range. Each point represents individual values. Only significant comparisons are marked.

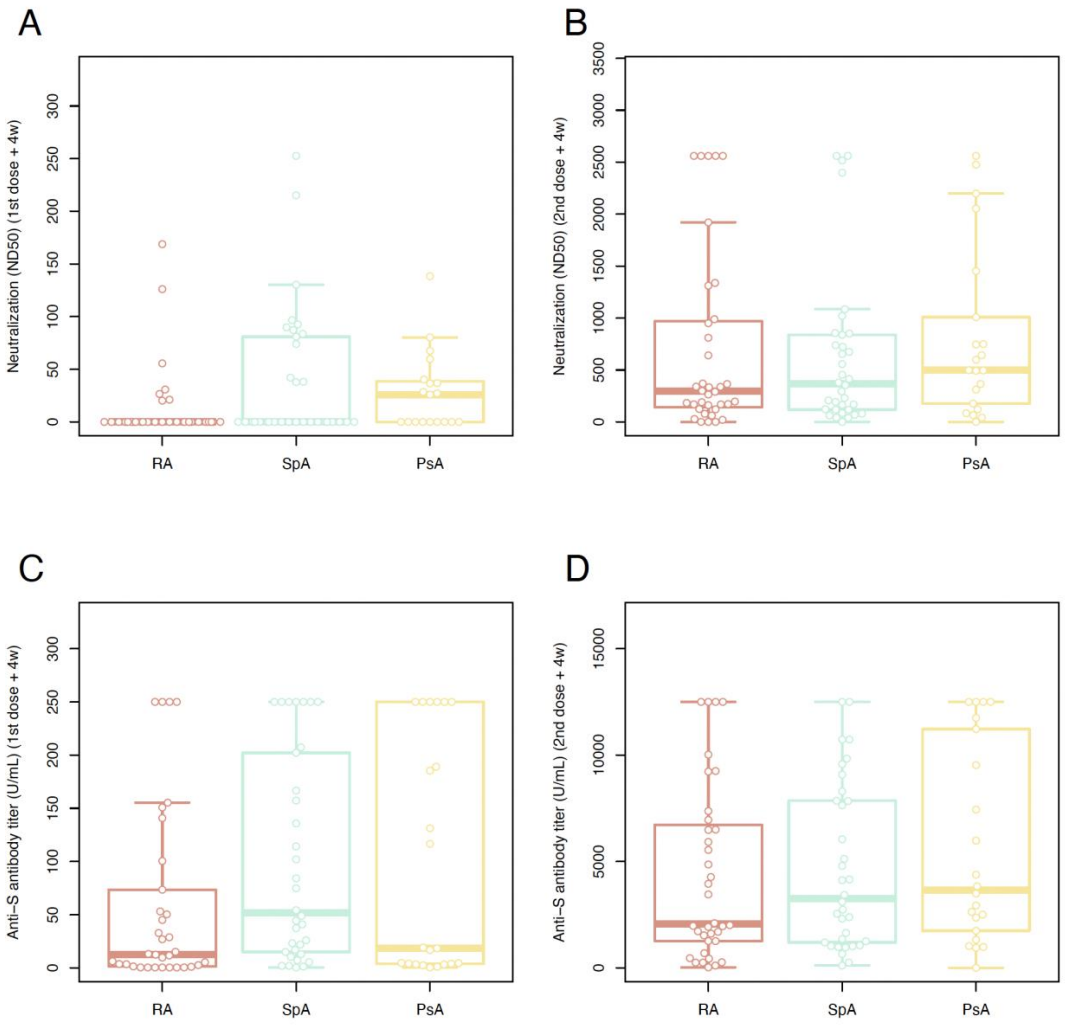

Figure S10. Neutralization titers and serological response against SARS-CoV-2 S-protein after the first dose (1st dose+4w) and second dose (2nd dose+4w) vaccine in all three AIRDs explored.

Neutralization titers (ND50) against SARS-CoV-2 in plasma A) SARS-CoV-2 neutralization antibodies four weeks after the 1st vaccination (1st dose+4w) B) SARS-CoV-2 neutralization antibodies four weeks after the 2nd vaccination (2nd dose+4w). **Serological immune responses against SARS-CoV-2** C) Spike-specific IgG titers four weeks after the 1st vaccination (1st dose+4w) D) Spike-specific IgG titers four weeks after the 2nd vaccination (2nd dose+4w). The box plots indicate the 75th, 50th, and 25th quantile, and the whiskers have a maximum length of 1.5 times the interquartile range. Each point represents individual

values. ND50=50% inhibitory dilution. U/ml=units/milliliter. RA=Rheumatoid Arthritis. SpA=Spondyloarthritis. PsA=Psoriasis Arthritis.

Table S1. Univariate and multivariate analysis of serological immune responses against SARS-CoV-2 spike protein when referenced to IL-17i

	Univariate			Multivariate		
	Estimate	Std. Deviat	P value	Estimate	Std. Deviation	P value
(Intercept)	-	-	-	8992.9	3283.6	0.008
Male sex	890.9	863.3	0.305	601.0	864.6	0.489
Age	-123.5	31.9	0.000	-76.7	38.2	0.049
BMI	-96.1	88.8	0.283	9.2	78.3	0.906
Diagnosis: SpA	357.3	993.7	0.720	-1460.7	1451.4	0.318
Diagnosis: PsA	1324.4	1124.4	0.242	-606.8	1543.5	0.695
Therapy: TNFi	-4336.5	1044.2	0.000	-3023.4	1226.2	0.016
Therapy: JAKi	-3144.0	1108.7	0.006	-2835.2	1614.6	0.083
Therapy: MTX	-2845.0	1322.6	0.034	-1277.3	2018.5	0.529
Combination Therapy	-1872.0	1158.7	0.110	-2766.7	1322.7	0.040
Corticosteroids	737.7	1244.0	0.555	404.2	1251.7	0.748
Vaccine: Moderna	2985.9	952.0	0.002	2766.1	1177.5	0.022
Comorbidity: Hypertension	-1478.7	990.5	0.139	-991.8	1034.6	0.341
Comorbidity: Heart	-486.1	2128.5	0.820	882.0	2186.1	0.688
Comorbidity: Lung	-2129.4	1269.5	0.097	-86.4	1257.7	0.945
Comorbidity: Diabetes	-822.2	1538.5	0.594	195.2	1598.1	0.903
Comorbidity: Osteoporosis	2138.3	2434.2	0.382	1408.3	2411.5	0.561
Comorbidity: Cancer	-3859.2	4167.6	0.357	-1922.2	4253.3	0.653
Smoker	-186.2	941.9	0.844	68.1	936.6	0.942

Table S2. Univariate and multivariate analyses of neutralizing capacity when referenced to IL-17i

	Univariate			Multivariate		
	Estimate	Std. Deviat	P value	Estimate	Std. Deviation	P value
(Intercept)	-	-	-	2182.3	702.3	0.003
Male sex	82.7	171.8	0.632	131.1	185.5	0.482
Age	-19.6	6.6	0.004	-16.3	8.1	0.050
BMI	-27.4	18.2	0.137	-12.0	16.6	0.474
Diagnosis: SpA	-59.4	196.6	0.763	-227.7	309.5	0.464
Diagnosis: PsA	120.5	225.7	0.595	-27.2	328.1	0.934
Therapy: TNFi	-626.6	214.2	0.004	-538.5	259.7	0.042
Therapy: JAKi	-535.9	227.1	0.021	-557.5	342.0	0.108
Therapy: MTX	-46.0	270.0	0.865	-213.7	429.0	0.620
Combination Therapy	-163.4	231.1	0.481	-191.2	282.5	0.501
Corticosteroids	212.9	244.7	0.387	222.0	265.0	0.405
Vaccine: Moderna	48.5	197.8	0.807	6.2	249.4	0.980
Comorbidity: Hypertension	-107.9	197.6	0.586	43.7	219.0	0.843
Comorbidity: Heart	-396.3	417.4	0.345	-340.3	462.8	0.465
Comorbidity: Lung	242.4	252.9	0.340	472.4	266.3	0.080
Comorbidity: Diabetes	-219.6	302.8	0.470	-60.6	339.2	0.859
Comorbidity: Osteoporosis	536.0	478.3	0.265	624.4	510.7	0.226
Comorbidity: Cancer	-666.0	821.9	0.420	-215.1	901.0	0.812
Smoker	-177.4	184.5	0.339	-95.0	199.3	0.635