RMD Open
Rheumatic & Musculoskeletal Diseases

ORIGINAL RESEARCH

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

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To cite: Andreica I, Blazquez-Navarro A, Sokolar J, *et al*. Different humoral but similar cellular responses of patients with autoimmune inflammatory rheumatic diseases under disease-modifying antirheumatic drugs after COVID-19 vaccination. *RMD Open* 2022;**8**:e002293. doi:10.1136/rmdopen-2022-002293

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/rmdopen-2022-002293).

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Received 15 February 2022
Accepted 18 July 2022

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ABSTRACT

Objectives The effect of different modes of immunosuppressive therapy in autoimmune inflammatory rheumatic diseases (AIRDs) remains unclear. We investigated the impact of immunosuppressive therapies on humoral and cellular responses after two-dose vaccination.

Methods Patients with rheumatoid arthritis, axial spondyloarthritis or psoriatic arthritis treated with TNFi, IL-17i (biological disease-modifying antirheumatic drugs, b-DMARDs), Janus-kinase inhibitors (JAKi) (targeted synthetic, ts-DMARD) or methotrexate (MTX) (conventional synthetic DMARD, csDMARD) alone or in combination were included. Almost all patients received mRNA-based vaccine, four patients had a heterologous scheme. Neutralising capacity and levels of IgG against SARS-CoV-2 spike-protein were evaluated together with quantification of activation markers on T-cells and their production of key cytokines 4 weeks after first and second vaccination.

Results 92 patients were included, median age 50 years, 50% female, 33.7% receiving TNFi, 26.1% IL-17i, 26.1% JAKi (all alone or in combination with MTX), 14.1% received MTX only. Although after first vaccination only 37.8% patients presented neutralising antibodies, the majority (94.5%) developed these after the second vaccination. Patients on IL17i developed the highest titres compared with the other modes of action. Co-administration of MTX led to lower, even if not significant, titres compared with b/tsDMARD monotherapy. Neutralising antibodies correlated well with IgG titres against SARS-CoV-2 spike-protein. T-cell immunity revealed similar frequencies of activated T-cells and cytokine profiles across therapies.

Conclusions Even after insufficient seroconversion for neutralising antibodies and IgG against SARS-CoV-2 spike-protein in patients with AIRDs on different medications, a second vaccination covered almost all patients regardless of DMARDs therapy, with better outcomes in those on IL-17i. However, no difference of bDMARD/tsDMARD or csDMARD therapy was found on the cellular immune response.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Data on the effect of individual immunosuppressants on B-cell and T-cell immunity after mRNA vaccination in patients with autoimmune inflammatory rheumatic diseases (AIRDs) is still a matter of intense research.

WHAT THIS STUDY ADDS

⇒ This study comprehensively assesses the T-cell response after mRNA vaccination in patients with AIRDs determining activation patterns and cytokine profiles, along with direct measurement of humoral activity by establishing neutralising capacity of sera antibodies.

⇒ Although IL-17i compared with TNFi and Janus-kinase inhibitors seem to have the least impact on the development of humoral immunity, cellular immunity does not seem to be significantly affected by various immunosuppressive drugs.

⇒ While a second vaccination provided a significant boost of humoral immunity, it did not significantly influence the cellular immune response.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Future vaccination strategies should be adapted according to degree and type of immunosuppression in patients with AIRDs considering both the humoral and cellular immune response.

INTRODUCTION

Vaccination against the SARS-CoV-2 is strongly recommended for all patients with autoimmune inflammatory rheumatic diseases (AIRDs) on immunosuppressive therapy.[1] However, patients with AIRDs were greatly underrepresented in all vaccine approval studies.[2–5] Treatment of AIRDs is mainly based on glucocorticoids (GC), conventional

synthetic (cs—such as methotrexate (MTX)), biologic (b—such as tumour-necrosis factor inhibitors (TNFi) or interleukin-17 inhibitors (IL-17i)) and targeted synthetic (ts—such as Janus-kinase inhibitors (JAKi)) disease-modifying antirheumatic drugs (DMARDs).

Previous studies have shown that GC doses above 10 mg/day negatively affect the humoral vaccination response, while MTX reduces the humoral vaccination response against pneumococci and influenza.[6–9] On the other hand, TNFi is known to have a minor influence on the humoral vaccine-induced response against influenza.[7 10] In comparison, whereas the JAKi Baricitinib appears to have only a minor effect on the humoral vaccination response against pneumococcal conjugate vaccine,[11] treatment with the JAKi tofacitinib appears to have a negative effect.[12]

More recently, the humoral immune response after SARS-CoV-2 vaccination has been evaluated in patients with AIRDs. However, the impact of immunosuppressive therapies in patients with AIRDs on T-cell functionality elicited by COVID-19 vaccination is not completely elucidated. This is critical given the emergency of SARS-CoV-2 variants of concerns with potential to evade humoral immunity. Although the recommended immunosuppressive therapies appear to inhibit the development of a robust immune response postvaccination, their effects on COVID-19 mRNA vaccine-induced immunity are still contradictory.[13–15]

Recent evidence suggests an essential role for T-cell mediated immunity for vaccine-induced protection against COVID-19.[16 17] While some studies on CD20 inhibitors have shown a preserved T-cell response after SARS-CoV-2 vaccination despite reduced humoral response,[13 18 19] the effect of CD20 depletion remains inconclusive.[20 21]

In this study, we evaluated patterns of humoral and cellular immune responses after SARS-CoV-2 vaccination with mRNA vaccines in patients with AIRD treated with four different, frequently used modes of action (MoA), by assessing neutralisation and IgG development against SARS-CoV-2 spike-protein and analysing the polyfunctionality of SARS-CoV-2 specific T cells.

METHODS

Study participants

Patients diagnosed with rheumatoid arthritis (RA), axial spondyloarthritis (axSpA) and psoriatic arthritis (PsA) with a stable and low disease activity status measured by the DAS28 (Disease Activity Score 28) for RA and PsA patients, and by the BASDAI (Bath Ankylosing Spondylitis Disease Activity Index) for SpA patients, and a planned first dose of SARS-CoV-2 vaccination with BNT162b2 mRNA (Pfizer/BioNTech); mRNA-1273 (Moderna) or AZD1222 (AstraZeneca) receiving a bDMARDs (TNFi or IL-17i), tsDMARDs (JAKi) or csDMARD therapy (MTX) were consecutively recruited. Treatment of patients was by one MoA alone (monotherapy) or in combination with MTX (combination therapy), according to the treatment recommendations of each indication. Assessment of the specific effect of each drug on the magnitude of humoral response after vaccination was applied to patients on monotherapy with IL-17i, TNFi, JAKi or MTX only. Patients with active disease, therapy changes during and until 4 weeks after the second vaccination or known primary immunodeficiency were excluded. In addition, patients with a possible previous SARS-CoV-2 infection were excluded, as assessed by antibody titre against SARS-CoV-2 spike protein prior to study inclusion.

Demographic and clinical data, including comorbidities and smoking status, were recorded. The vaccine doses were administrated in accordance with the current national recommendations.[22]

Sampling schedule

The sampling schedule is presented in figure 1. Baseline serum samples were collected at the same day of and directly before the first vaccination (‘first dose’). Further blood samples were obtained 4 weeks after the first vaccination (‘1st dose+4 weeks’). We determined at this time point the anti spike-protein antibodies against SARS-CoV-2 by Elecsys testing and the specific neutralising antibody as well as the cellular immune response. The time frame between the first and second vaccination was 3–6 weeks for mRNA vaccines or 12 weeks for vector-based vaccination based on the national recommendations. Four weeks after the second SARS-CoV-2 vaccination (‘2nd dose’), all patients were again analysed (‘2nd dose+4 weeks’), following the same procedure as in ‘1st dose+4 weeks’.

SARS-CoV-2 neutralisation assay and SARS-CoV-2 IgG antibody titres

Serum was generated from blood collected into S-Monovette Z-Gel (Sarstedt). The SARS-CoV-2 wild-type neutralisation assay was performed as previously described.[23] Briefly, pseudoviruses were incubated with twofold serial dilutions from 1:20 to 1:2560 of immune sera in 96-well plates prior to infection of Vero E6 cells (1×10^4 cells/well). At 18 hours postinfection, firefly luciferase activity was determined as a proxy for infection and the reciprocal antibody dilution causing 50% inhibition of the luciferase reporter was calculated (ND50). Detection range was defined to be between 1:20 and 1:2 560, meaning that patients with a ND50 less than 1:20 were classified as having no neutralising antibodies.

The Elecsys Anti-SARS-CoV-2 S (Roche Diagnostics, Switzerland) immunoassay was used for measurement of IgG to SARS-CoV-2 spike-protein. This assay measures the presence and amount of serum antibodies to the spike (S) antigen of SARS-CoV-2 and reports these in the units (U)/mL, which is equivalent to BAU/mL. The assay has a linear detection range up to 250 U/mL. By prediluting the samples 1:50, a concentration range up to 25 000 U/mL can be reached. This strategy was applied to samples obtained at ‘2nd dose+4 weeks’ to allow evaluation of

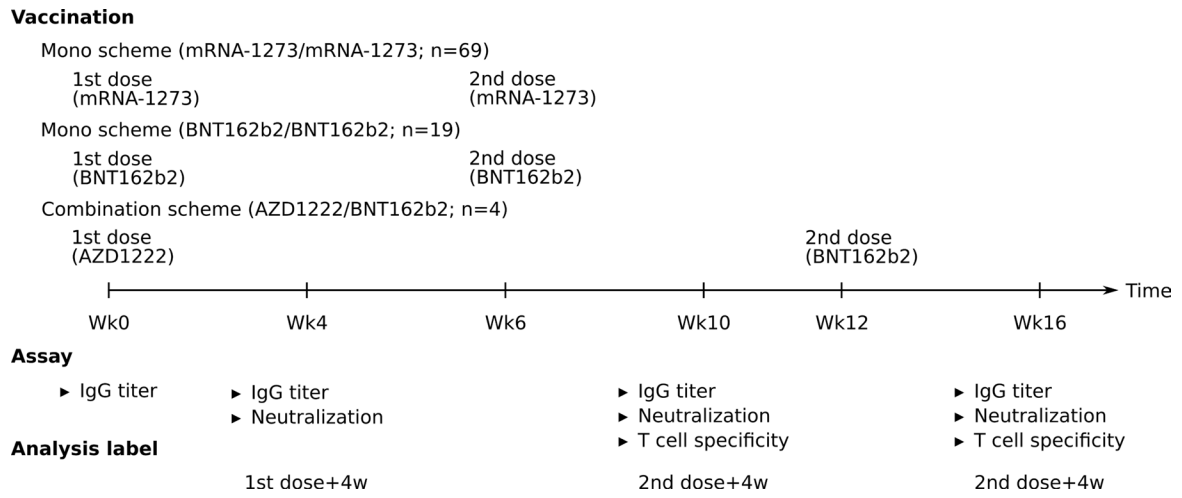

Figure 1 Sample schedule. Schedule of vaccinations and sample collection. The time frame between the first and second vaccination was 3 to 6 weeks for mRNA vaccines or 12 weeks for vector-based vaccination based on the national recommendations. Four weeks after the second SARS-CoV-2 vaccination, all patients were again analysed ('2nd dose+4 weeks'), following the same procedure as in '1st dose+4 weeks'. '1st dose', first vaccination, '2nd dose', second vaccination, '1st dose+4 weeks', 4 weeks after the first vaccination with an mRNA based or vector-based SARS-CoV-2 vaccine, '2nd dose+4 weeks', 4 weeks after the second vaccination with an mRNA vaccine.

a wider range after vaccination. Seroconversion was defined as a response equal to or greater than 0.8 U/mL.

Preparation of peripheral blood mononuclear cells and stimulation with overlapping peptide pools from SARS-CoV-2 S-protein

Peripheral blood mononuclear cells (PBMCs) were isolated from 30 mL blood collected in S-Monovette EDTA K blood collection tubes (Sarstedt) by gradient centrifugation as previously described.[24] Isolated PBMCs were stored at −80°C in 90% FCS (Fetal Calf Serum)+10% DMSO (Dimethyl sulfoxide) until stimulation with SARS-CoV-2 S-protein overlapping peptide pools as previously described.[24]

Flow cytometric analysis

Samples were stained as described in online supplemental file and acquired on a CytoFlex flow cytometer (Beckman Coulter, USA). Activated T-cells express CD137 on their surface and activated $CD4^+$ T-cells additionally express CD154.[25] Using these two markers, we identified antigen specific T-cells among $CD4^+$, $CD8^+$ or $CD4^+CXCR5^+$ T-cells. The expression level of the CD3-TCR-complex decreases in response to activation in a manner depending on the strength of the activation signal.[26–28] We therefore assessed the frequency of activated $CD154^+CD137^+$ $CD4^+$ T-cells with a low expression of CD3 ($CD3^{low}$) and compared these to the frequency of $CD3^{low}$ on unstimulated $CD154^-CD137^-$ $CD4^+$ T-cells. Circulating follicular T helper (cTfh) like cells are defined by expression of the surface chemokine receptor CXCR5 that endows $CD4^+$ T-cells with the ability to migrate to the germinal centre and provide B-cell help during the antibody optimisation process.[29] Polyfunctional T-cells, which express more than one effector molecule, have been described as a hallmark of protective immunity in viral infections.[30] Cytotoxic $CD8^+$ T-cells release effector molecules like perforin and granzyme B (GrB), which directly kill infected cells. In contrast to $CD4^+$ T-cells, activated $CD8^+$ T-cells do not express CD154 and the frequency of SARS-CoV-2 S-protein specific CD8+T cells can be assessed by their expression of CD137. Considering this, we analysed the IFN-γ, TNF, IL-2 and as effector molecule GrB expression, by intracellular staining (see online supplemental file), in parallel to differentiation stage phenotyping. Activation was considered when the stimulation index (the ratio stimulated to unstimulated) was 3 or higher.[24]

Statistical analysis

Flow cytometry data were analysed using FlowJo V.10.7.1 (BD Biosciences, USA); gating strategy and representative dot plots are shown in online supplemental file. Statistical analysis was performed using R, V.4.0.4 and detailed in online supplemental file. Significance threshold was set at 0.050; only significant p values are reported in the figures. Since this study had an exploratory nature, we did not perform an adjustment for multiple testing.[31]

RESULTS

From a total of 105 initially recruited patients, 100 patients with RA (n=41), axSpA (n=34) and PsA (n=25) received the first SARS-CoV-2 vaccination beginning of May 2021. High baseline levels of antibodies against S-protein were found in two patients, indicating a possible recent infection, two patients did not attend the vaccination appointments and one patient could not meet the scheduled appointment of first vaccine dose due to an infection. Additionally, eight patients had to be excluded because of therapy changing between the first and second vaccination (figure 2). The total study

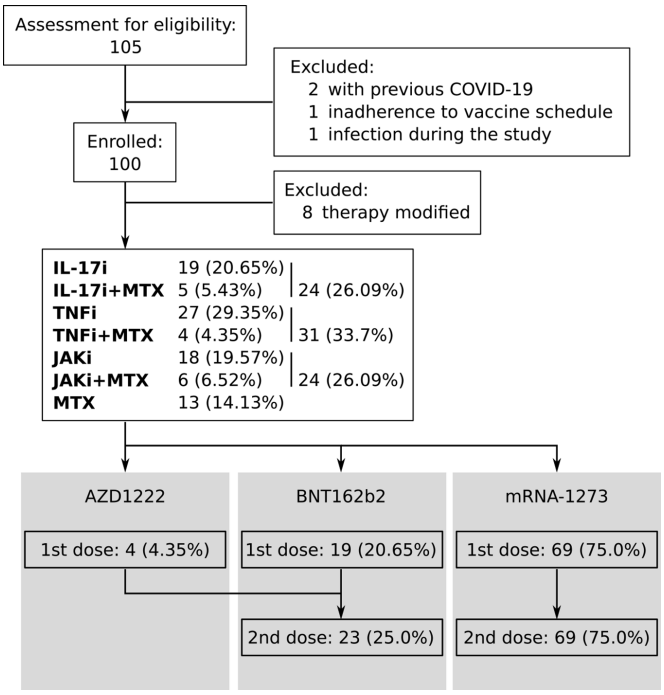

Figure 2 Cohort selection. IL, interleukin; i, inhibitor; JAK, Janus-kinase; MTX, methotrexate; TNF, tumour necrosis factor.

sample included the remaining 92 patients. No participants reported COVID-19 infection during the study.

The median age of participants was 50 (IRQ: 39–56), 46 (50.0%) were females, 29 (31.5%) were current smoker, 13 (14.1%) patients had an additional low dose GC therapy with a median dose of 5.0 mg prednisolone (IRQ: 2.5–5.0) per day (table 1). The distribution of patients by MoA and vaccine type is shown in figure 2.

In total, 69/92 (75.0%) patients received mRNA-1273 and 19/92 (20.7%) BNT162b2. 4/92 (4.3%) patients received AZD1222 vaccine as first vaccination and thereafter BNT162b2 which was due to changes in national vaccination recommendations. These four patients initially given AZD1222 were on MTX (n=2), monotherapy with IL-17i (n=1) or combination therapy of TNFi/MTX (n=1). For two patients with RA receiving BNT162b2 the second dose was given 3 weeks after the first vaccination, in accordance with the national recommendations at that time. Subsequently, no blood could be drawn at '1st dose+4 weeks' for these two patients.

The median time between the vaccination and blood sampling was 31 (IQR: 28–34) days after the first and 28 (IRQ: 28–28) days after the second vaccination.

At '2nd dose+4 weeks', 86/91 (94.5%) patients developed neutralising antibodies. Neutralising antibodies were found in all patients on IL-17i, TNFi and MTX monotherapy yet only in 15/18 (83.3%) patients on JAKi monotherapy (online supplemental figure S2A). In contrast, only 34/90 (37.8%) patients developed neutralising antibodies at '1st dose+4 weeks' (online supplemental figure S2B). Considering the seroconversion rate at '1st dose+4 weeks', there were 14/19 (73.7%) patients on IL-17i, 7/18 (38.9%) on JAKi, 7/27 (25.9%) on TNFi and 1/11 (9.1%) on MTX with detectable neutralising antibodies in sera (online supplemental figure S2B). Over time, an increase in neutralisation titres was observed for 83/89 (93.3%) patients .

Across the MoA, patients on IL-17i developed significantly higher neutralising antibodies at both, '2nd dose+4 weeks' (798 (IQR: 511–1.344)) (figure 3A) and '1st dose+4 weeks' (74 (IQR: 13–91)) (online supplemental figure S3A) compared with patients on TNFi or JAKi. For the patients on MTX monotherapy, a similar pattern to IL-17i was detected although without reaching statistical significance at '2nd dose+4 weeks' (figure 3A). When comparing neutralising antibodies titres of all other groups no statistically significant differences were found at both time points (figure 3A and online supplemental figure S3A). Addition of MTX to b/tsDMARDs monotherapy led to lower titres, irrespective of specific monotherapy; however, this difference did not reach statistical significance (figure 3B and online supplemental figure S3B). The median titre of neutralising antibodies in all patients rose significantly to 354 (IQR: 123–853) at '2nd dose+4 weeks' ($p<0.001$) compared with '1st dose+4 weeks'. Importantly, this effect was observed in all MoA groups (figure 3C).

Overall, 91/92 (98.9%) patients developed IgG antibodies against SARS-CoV-2 spike-protein at '2nd dose+4 weeks' vs 81/90 (90.0%) patients at '1st dose+4 weeks'. All patients on monotherapies seroconverted at '2nd dose+4 weeks' (online supplemental figure S2C) whereas at '1st dose+4 weeks', all patients on IL-17i and TNFi already developed anti-spike antibodies, while this was observed only in 15/18 (83.3%) JAKi and 7/11 (63.6%) MTX-treated patients (online supplemental figure S2D). Usage of different cut offs for the measurements at '1st dose+4 weeks' and '2nd dose+4 weeks' impeded on direct comparison over time. Of note, some patients developed unexpectedly high antibody titres after the first vaccine dose and exceeded the upper detection limit of 250 U/mL.

At '2nd dose+4 weeks' patients on IL-17i developed significantly higher anti-S antibody levels (8295 U/mL (IQR: 4586–11,237)) compared with the other three arms: JAKi (4405 U/mL (IQR: 1436–7265), TNFi (2,313 (IQR: 1156–3630) U/mL) and MTX (2010 U/mL (IQR: 693–9254)) (figure 3D). The same pattern was observed for '1st dose+4 weeks', although not significant (online supplemental figure S3C). At this time point MTX therapy resulted in a significant reduction in abundances of anti-spike IgG antibodies compared with the other MoA. Furthermore, MTX led to numerically but not significant lower antibody titres against spike-protein when administered in combination to a biologic agent (figure 3E and online supplemental figure S3D).

Neutralisation titres correlated positively with the anti-spike IgG titres after both first and second vaccination (figure 3F–G). The multivariate analysis unveiled that age negatively influenced the magnitude of anti-spike IgG

Table 1 Demographics, comorbidities and therapy for all patients

	All	IL-17i	IL-17i+MTX	TNFi	TNFi+MTX	JAKi	JAKi+MTX	MTX
Patients (n)	92	19	5	27	4	18	6	13
Age (years)	50 (39–56)	42 (36–53)	37 (32–38)	51 (42–56)	58 (54–61)	50 (43–56)	55 (49–59)	54 (37–64)
Female sex	46 (50.0%)	6 (31.6%)	3 (60.0%)	10 (37.0%)	3 (75.0%)	13 (72.2%)	3 (50.0%)	8 (61.5%)
BMI	28 (24–31)	28 (25–30)	27 (24–28)	29 (24–32)	27 (26–37)	26 (22–32)	31 (28–35)	27 (23–30)
Smoker	29 (31.5%)	13 (68.4%)	1 (20.0%)	17 (63.0%)	0 (0.0%)	5 (27.8%)	1 (16.7%)	3 (23.1%)
RA	36 (39.1%)	0 (0.0%)	0 (0.0%)	3 (11.1%)	2 (50.0%)	14 (77.8%)	5 (83.3%)	12 (92.3%)
axSpA	34 (37.0%)	11 (57.9%)	0 (0.0%)	21 (77.8%)	0 (0.0%)	2 (11.1%)	0 (0.0%)	0 (0.0%)
PsA	22 (23.9%)	8 (42.1%)	5 (100.0%)	3 (11.1%)	2 (50.0%)	2 (11.1%)	1 (16.7%)	1 (7.7%)
DAS28	2.28 (1.54–3.00)	1.93 (0.70–2.59)	1.55 (0.49–2.83)	2.85 (2.47–2.89)	2.62 (2.09–3.34)	2.45 (1.71–3.57)	1.42 (1.18–1.91)	2.38 (1.66–2.86)
BASDAI	3.5 (2.0–6.0)	3.4 (2.2–6.1)	NA	5.2 (2.6–6.0)	NA	4.9 (2.5–5.4)	NA	1.4 (1.4–1.4)
CRP (mg/dL)	0.2 (0.1–0.3)	0.2 (0.1–0.4)	0.1 (0.1–0.1)	0.2 (0.1–0.3)	0.7 (0.2–2.8)	0.2 (0.1–0.3)	0.2 (0.1–0.2)	0.2 (0.1–0.4)
Patients with concomitant GC (n)	13 (14.1%)	1 (5.3%)	1 (20.0%)	0 (0.0%)	0 (0.0%)	5 (27.8%)	3 (50.0%)	3 (23.1%)
Prednisolone dosage (mg)	5.0 (2.5–5.0)	5.0 (5.0–5.0)	3.0 (3.0–3.0)			5.0 (2.5–5.0)	2.0 (2.0–4.0)	5.0 (4.0–5.0)
Time since diagosis (years)	5.0 (2.0–10.0)	5.5 (4.25–10)	11.0 (10.0–12.0)	6 (2–11)	13.0 (4–25.0)	6.5 (4–10.25)	6.0 (4.5–9.0)	1.5 (1.0–2.0)
Prime								
AZ1222 (AstraZeneca)	4 (4.3%)	1 (5.3%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	2 (15.4%)
mRNA-1273 (Moderna)	69 (75.0%)	17 (89.5%)	5 (100.0%)	20 (74.1%)	2 (50.0%)	16 (88.9%)	6 (100.0%)	3 (23.1%)
BNT162b2 mRNA (Pfizer/BioNTech)	19 (20.7%)	1 (5.3%)	0 (0.0%)	7 (25.9%)	1 (25.0%)	2 (11.1%)	0 (0.0%)	8 (61.5%)
Boost								
mRNA-1273 (Moderna)	69 (75.0%)	17 (89.5%)	5 (100.0%)	20 (74.1%)	2 (50.0%)	16 (88.9%)	6 (100.0%)	3 (23.1%)
BNT162b2 mRNA (Pfizer/BioNTech)	23 (25.0%)	2 (10.5%)	0 (0.0%)	7 (25.9%)	2 (50.0%)	2 (11.1%)	0 (0.0%)	10 (76.9%)
Comorbidities								
None	54 (58.7%)	14 (73.7%)	5 (100.0%)	13 (48.1%)	2 (50.0%)	9 (50.0%)	5 (83.3%)	6 (46.2%)
Hypertension	23 (25.0%)	4 (21.1%)	0 (0.0%)	6 (22.2%)	1 (25.0%)	7 (38.9%)	0 (0.0%)	5 (38.5%)
Heart failure	4 (4.3%)	1 (5.3%)	0 (0.0%)	2 (7.4%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)
Lung disease	12 (13.0%)	0 (0.0%)	0 (0.0%)	6 (22.2%)	1 (25.0%)	2 (11.1%)	0 (0.0%)	3 (23.1%)
Diabetes mellitus	8 (8.7%)	2 (10.5%)	0 (0.0%)	2 (7.4%)	1 (25.0%)	1 (5.6%)	1 (16.7%)	1 (7.7%)
Osteoporosis	3 (3.3%)	1 (5.3%)	0 (0.0%)	1 (3.7%)	0 (0.0%)	1 (5.6%)	0 (0.0%)	0 (0.0%)

For quantitative variables, data are provided as median (IQR). For categorical variables the count (% frequency) is provided.
axSpA, axial spondyloathritis; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BMI, body mass index; CRP, C reactive protein; DAS28, Disease Activity Score 28; GC, glucocorticoid; i, inhibitor; IL, interleukin; JAK, Janus kinase; MTX, methotrexate; NA, not available; PsA, psoriatic arthritis; RA, rheumatoid arthritis.

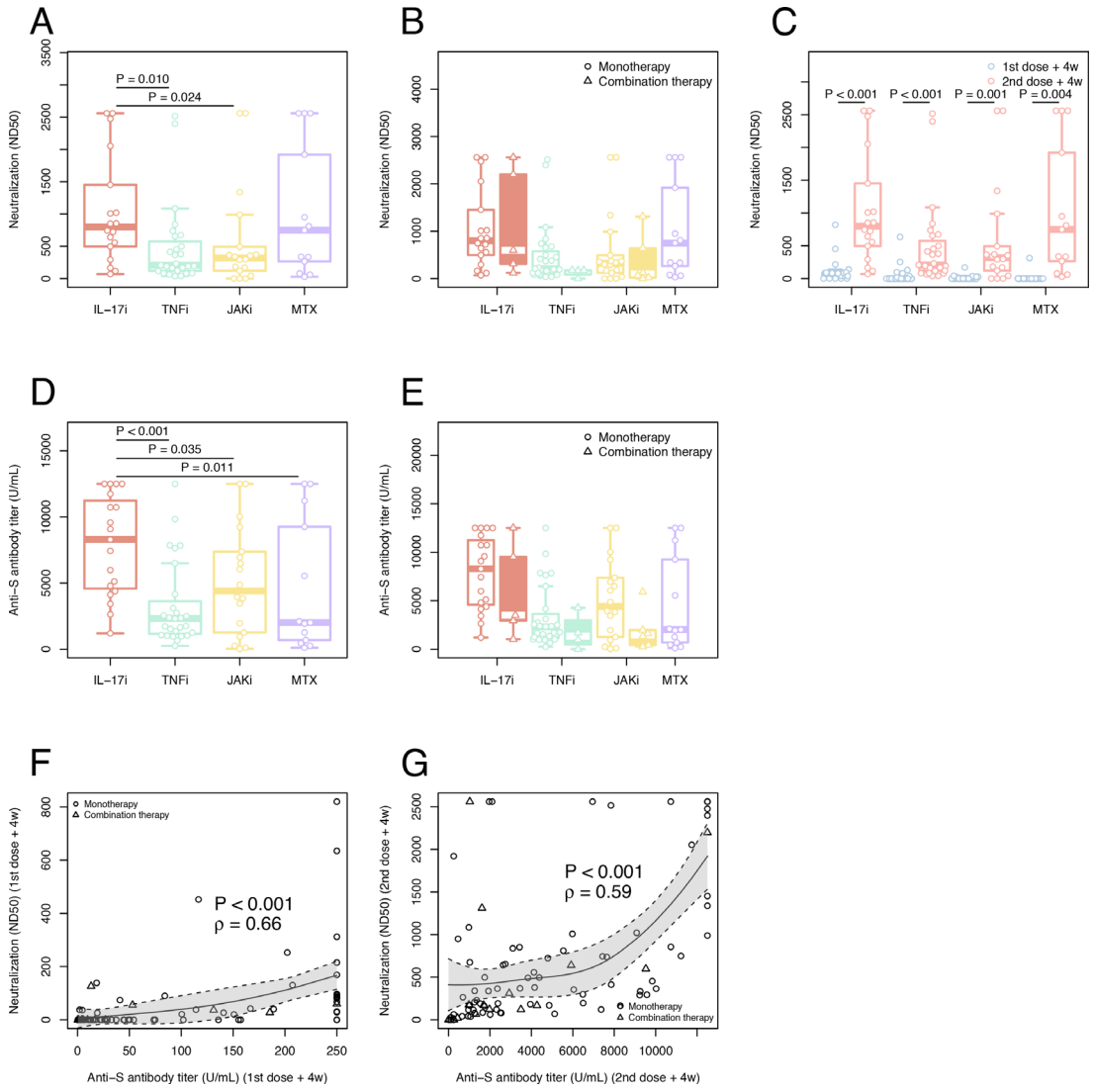

Figure 3 Serological immune responses and neutralisation titres against SARS-CoV-2 wild variant in time and by different therapies. Neutralisation titres (ND50) against SARS-CoV-2 in plasma (**A**) SARS-CoV-2 neutralisation antibodies at '2nd dose+4 weeks' for all four groups of patients on monotherapy (**B**) Comparison between SARS-CoV-2 neutralisation antibodies at '2nd dose+4 weeks' for all four groups of patients on monotherapy (unfilled plots) and combination therapies (filled coloured plots) (**C**) Kinetic of SARS-CoV-2 neutralisation antibodies for all four groups of patients on monotherapy with IL17i, TNFi, JAKi and MTX. Serological immune responses against SARS-CoV-2 (**D**) Spike-specific IgG titres at '2nd dose+4 weeks' for all four groups of patients on monotherapy (**E**) Comparison between spike-specific IgG titres at '2nd dose+4 weeks' for all four groups of patients on monotherapy (unfilled plots) and combination therapies (filled coloured plots) (**F**) Correlation of spike-specific IgG titres and SARS-CoV-2 neutralisation antibodies at '1st dose+4 weeks' (**G**) Correlation of spike-specific IgG titres and SARS-CoV-2 neutralisation antibodies at '2nd dose+4 weeks'. The box plots indicate the 75th, 50th and 25th quantile, and the whiskers have a maximum length of 1.5 times the IQR. Each point represents individual values, small triangles represent the additional patients on combination therapy. The following number of patients are presented: IL17i (n=19, '1st dose+4 weeks', n=18, '2nd dose+4 weeks'), TNFi (n=27, '1st dose+4 weeks', n=27, '2nd dose+4 weeks'), JAKi (n=18 '1st dose+4 weeks', n=18 '2nd dose+4 weeks') and MTX (n=11 '1st dose+4 weeks', n=13 '2nd dose+4 weeks'), IL17i/MTX (n=5 '2nd dose+4 weeks'), TNFi/MTX (n=4 'second dose+4 weeks'), JAKi/MTX (n=6 '2nd dose+4 weeks'). ND50, 50% inhibitory dilution; IL, interleukin; TNF, tumour necrosis factor; JAK, Janus-kinase inhibitor; MTX, methotrexate; ρ, Spearman's correlation coefficient.

antibodies (p=0.049) as shown in online supplemental tables S1 and S2. Other variables, including smoking habits, comorbidities, underlying disease or comedication with GC, did not have any impact on these humoral responses. Importantly, the use of mRNA-1273 was significantly associated with increased response with respect to IgG antibodies (p=0.022) but not for neutralisation.

Almost 90% of study patients treated with IL-17i and JAKi were vaccinated with mRNA-1273 vaccine, but only 75% of TNFi-treated patients received this vaccine. We analysed the vaccination response split on mRNA-1273 and BNT162b2 vaccines for each therapy group. Higher antibodies titres and neutralisation were developed under mRNA-1273 than BNT162b2 vaccine in all patients with

exception of those on JAKi therapy (online supplemental figure S4). However, analysis of different therapy groups including only the patients vaccinated with mRNA-1273 vaccine indicated comparable changes in antibody abundances (online supplemental figures S5 and S6).

Of note, the combination therapies reduced antibody titres (p=0.040), however, leaving antibody neutralisation capacity unchanged. A significant negative effect was found for TNFi in both neutralisation capacity (p=0.042) and antibody titres against SARS-CoV-2 spike-protein (p=0.016) if we have considered the IL-17i as reference (see online supplemental tables S1 and S2). The observed differences were not caused by differences in the T cell compartment (online supplemental figure S7).

We observed a trend for reduction in frequency of SARS-CoV-2 specific CD4$^+$ T-cells in circulation between '1st dose+4 weeks' and '2nd dose+4 weeks' irrespective of the therapy; however, this tendency was significant only for patients treated with MTX (figure 4A). Despite the strong differences observed in antibody titres, we found similar frequencies of activated CD4$^+$ T-cells under all MoA at '2nd dose+4 weeks' (figure 4B). At '1st dose+4 weeks', SARS-CoV-2 specific CD4$^+$ or CD8$^+$ T-cells were identified in 68/73 (93.2%; IL-17i: 88.24%; TNFi: 84.21%; JAKi: 100.0%; MTX: 100.0%) and 49/73 patients (67.1%; IL-17i: 58.82%; TNFi: 52.63%; JAKi: 72.73%; MTX: 81.82%), respectively. At '2nd dose+4 weeks', these numbers were 49/78 (62.8%; IL-17i: 83.33%; TNFi:

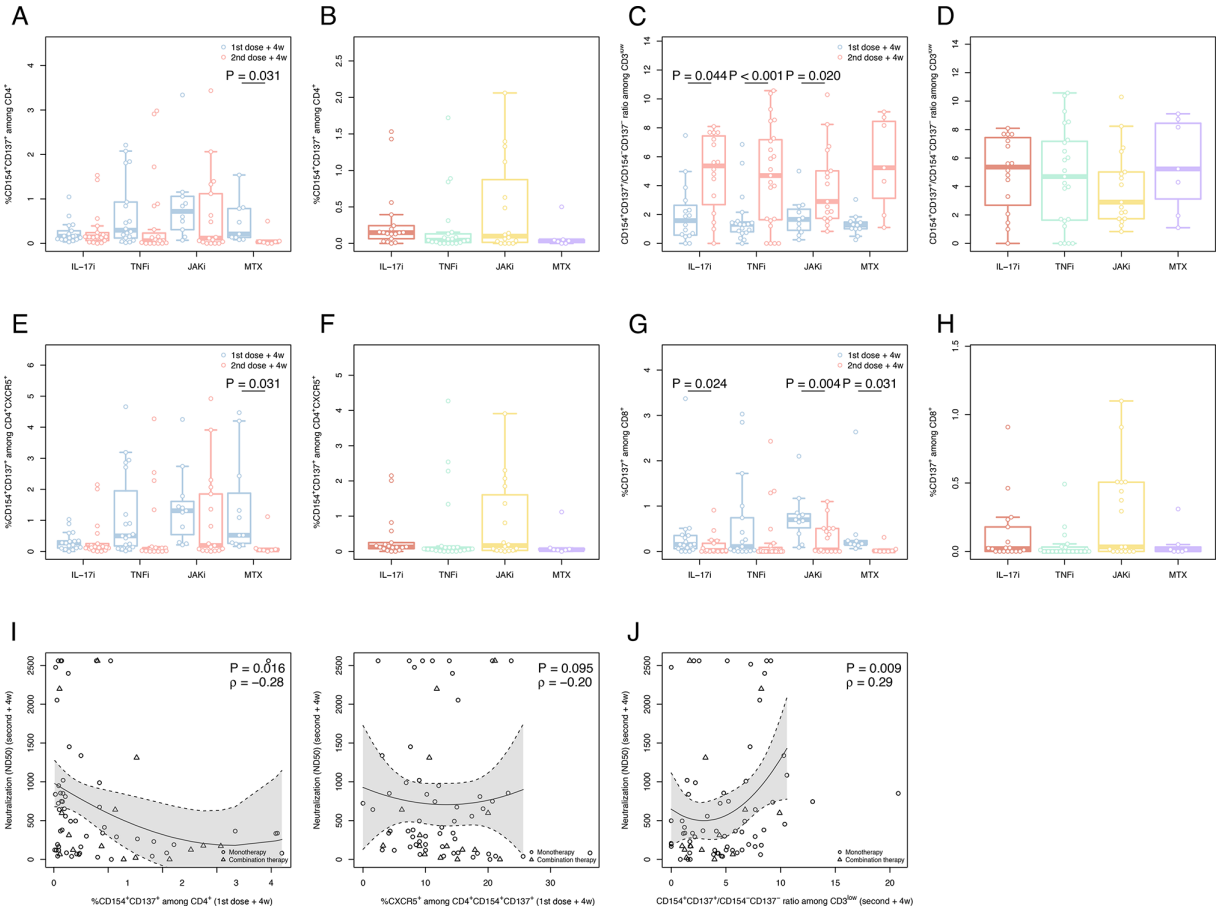

Figure 4 Frequencies of activated T cells is similar for all therapy groups. PBMCs were stimulated with peptides spanning the SARS-CoV-2 spike protein and cell activation was evaluated by multiparametric flow cytometry. Activation of CD4$^+$ T cells by SARS-CoV-2 S-protein (**A**) Kinetic of SARS-CoV-2 spike-reactive CD4$^+$ T cells, defined by coexpression of CDC154$^+$CD137$^+$ for all four groups of patients on monotherapy. (**B**) SARS-CoV-2 spike-reactive CD4$^+$ T cells at '2nd dose+4 weeks'. (C) Kinetic of ratio of CD3^{low} expression on activated to non-activated CD4$^+$ T cells. (**D**) Ratio of CD3^{low} expression on activated to non-activated CD4$^+$ T cells at '2nd dose+4 weeks'. (**E**) Kinetic of SARS-CoV-2 spike-reactive CD4$^+$ cTfh-like, defined by coexpression of CDC154$^+$CD137$^+$ on CD4$^+$CXCR5$^+$ T cells. (**F**) SARS-CoV-2 spike-reactive CD4$^+$ cTfh-like cells at 'second dose+4 weeks'. Activation of CD8$^+$ T cells by SARS-CoV-2 S-protein (G) Kinetic of SARS-CoV-2 spike-reactive CD8$^+$ T cells, defined by coexpression of CD137$^+$. (**H**) SARS-CoV-2 spike-reactive CD8$^+$ T cells at '2nd dose+4 weeks'. Association to final neutralisation capacity (I) Correlation of SARS-CoV-2 spike-reactive CD4$^+$ T cells at '1st dose+4 weeks'and neutralisation capacity at '2nd dose+4 weeks'. (**J**) Correlation of CD3^{low} expressing CD4$^+$ T cells at '2nd dose+4 weeks' and neutralisation capacity at '2nd dose+4 weeks'. The box plots indicate the 75th, 50th and 25th quantile, and the whiskers have a maximum length of 1.5 times the IQR. Each point represents a patient. Gr, granzyme; IL, interleukin; JAK, Janus kinase inhibitor; MTX, methotrexate; PBMCs, peripheral blood mononuclear cells; TNF, tumour necrosis factor; ρ, Spearman's correlation coefficient.

47.83%; JAKi: 58.82%; MTX: 42.86%) and 22/78 (28.2%; IL-17i: 27.78%; TNFi: 17.39%; JAKi: 41.18%; MTX: 100.0%), respectively (online supplemental figure S8).

Furthermore, we found an increase in the ratio of $CD3^{low}$ on activated to unstimulated $CD4^+$ T-cells between '1st dose+4 weeks' and '2nd dose+4 weeks', indicating an expansion of cells with a high avidity towards the SARS-CoV-2 S-protein (figure 4C). At '2nd dose+4 weeks' this ratio was above 1 in 70/78 (89.7%) patients and similar across the studied therapies (figure 4D). Only 8/60 (13.3%) patients displayed a decrease or only slight increase of <10% between the two time points, indicating a different progression in some patients.

We,therefore asked if activation of cTfh-like could explain the differences observed for the antibody titres. Activation of the cTfh-like followed the pattern of the overall activation. We observed a tendency for reduction of $CXCR5^+$ $CD4^+$ T-cells in the frequency between '1st dose+4 weeks' and '2nd dose+4 w', which was significant only for patients under MTX (figure 4E). No effect of the different therapies was observed for the activation of cTfh-like (figure 4F).

For all MoA with exception of TNFi, we observed a strong decrease in the frequency of SARS-CoV-2 specific $CD8^+$ T-cells from '1st dose+4 weeks' to '2nd dose+4 weeks' (figure 4G). Similar to the frequency of all activated $CD4^+$ T-cells, we observed no difference in activation, irrespective of the immunosuppressive therapy (figure 4H).

We also investigated if there was some association between the cellular immunity and the final humoral outcome. We found a weak but significant correlation between the frequencies of activated $CD4^+$ T-cells and the antibody ND50 at '2nd dose+4 weeks' (figure 4I left). Further association was found between activated cTfh-like at '1st dose+4 weeks' and the antibody ND50 at '2nd dose+4 weeks' (figure 4I right). We also found a positive correlation between the frequency of $CD3^{low}$ at '2nd dose+4 weeks' and ND50 at '2nd dose+4 weeks' (figure 4J). Collectively, this points to a possible negative effect of preformed memory on the neutralising capacity after vaccination.

We next evaluated the memory phenotype of the activated cells after the second vaccination dose (online supplemental figure S9). Depending on the expression of the CD45 isoform CD45RA and the chemokine receptor CCR7, T-cells can be separated into four distinct populations: naïve ($CD45RA^+CCR7^+$), central memory (CM; $CD45RA^-CCR7^+$), effector memory (EM; $CD45RA^-CCR7^-$) and EM expressing CD45RA (TEMRA; $CD45RA^+CCR7^-$). We found that the frequency of activated $CD4^+$ T-cells with a naïve and CM phenotype followed a similar pattern among the different therapy groups. This pattern was inversed for EM, where patients under JAKi had the lowest frequency of activated $CD4^+$ T cells with an EM phenotype (online supplemental figure S9A–B). When evaluating the changes over time, we found a similar pattern across all patient groups: a shift from activated $CD4^+$ T-cells with a naïve and CM phenotype, towards an EM phenotype (online supplemental figure S9C). Interestingly, no dynamics were observed for activated $CD8^+$ T-cells (online supplemental figure S9D). This shows that $CD4^+$ memory developed as expected irrespective of the immune suppressive therapy.

We observed modest levels of cytokine production by $CD4^+$ (figure 5A) and CD8+T cells (figure 5B) irrespective of therapy group and with no striking differences. A similar pattern was observed when we evaluated the coexpression of the measured cytokines, and no significant differences were found (figure 5C–D)

DISCUSSION

In this prospective study, we evaluated the interplay of the humoral and the cellular immune response after 2 doses of SARS-CoV-2 vaccines in a well-characterised AIRD cohort treated with four different, frequently used MoA. Our study provides a comprehensive analysis of immune reactivity of patients with AIRDs to mRNA vaccines in context of immunosuppression and thereby provides (1) essential insights into responses of patients with AIRDs to these new vaccines and (2) contributes novel views on impact of immune-targeted biologics on vaccine immunity.

One strength of this study is the homogeneous and clearly defined patient subcohorts with stable disease over the entire duration of the study. Accordingly, the influence of underlying diseases could be minimised even if in general the specific influence of the immunopathology of individual AIRDs cannot be excluded. However, no significant difference was shown in a crude subanalysis of the humoral immune response (IgG antibodies against spike-protein and neutralising capacity) split on the three explored AIRDs in this study (online supplemental figure S10). Furthermore, we profiled levels of anti-spike antibodies and in addition determined the neutralising antibodies against SARS-CoV-2 at all measuring time points. Additionally, we studied the T-cell responses comprehensively by determining multiple cytokine products and monitored different cell populations, including subsets with direct cytotoxic potential and populations bridging T-cell and B-cell activation, notably cTfh-like. To our knowledge, our study offers unprecedented details about cellular immune responses following COVID-19 vaccination in patients with AIRDs.

Several reports have recently shown similar antibody titres for patients with AIRDs after the second vaccination as compared with healthy controls,[32 33] whereas other studies have reported contradictory results, notably lower titres.[34 35] These inconsistencies can be explained by the different MoA investigated in these studies. Our results demonstrate a more rapid and robust induction of humoral immunity in patients treated with IL-17i as compared with those on TNFi and JAKi. These findings suggest a more favourable outcome of IL-17i over other MoA during vaccination against SARS-CoV-2. Although usage of MTX during influenza vaccination interferes

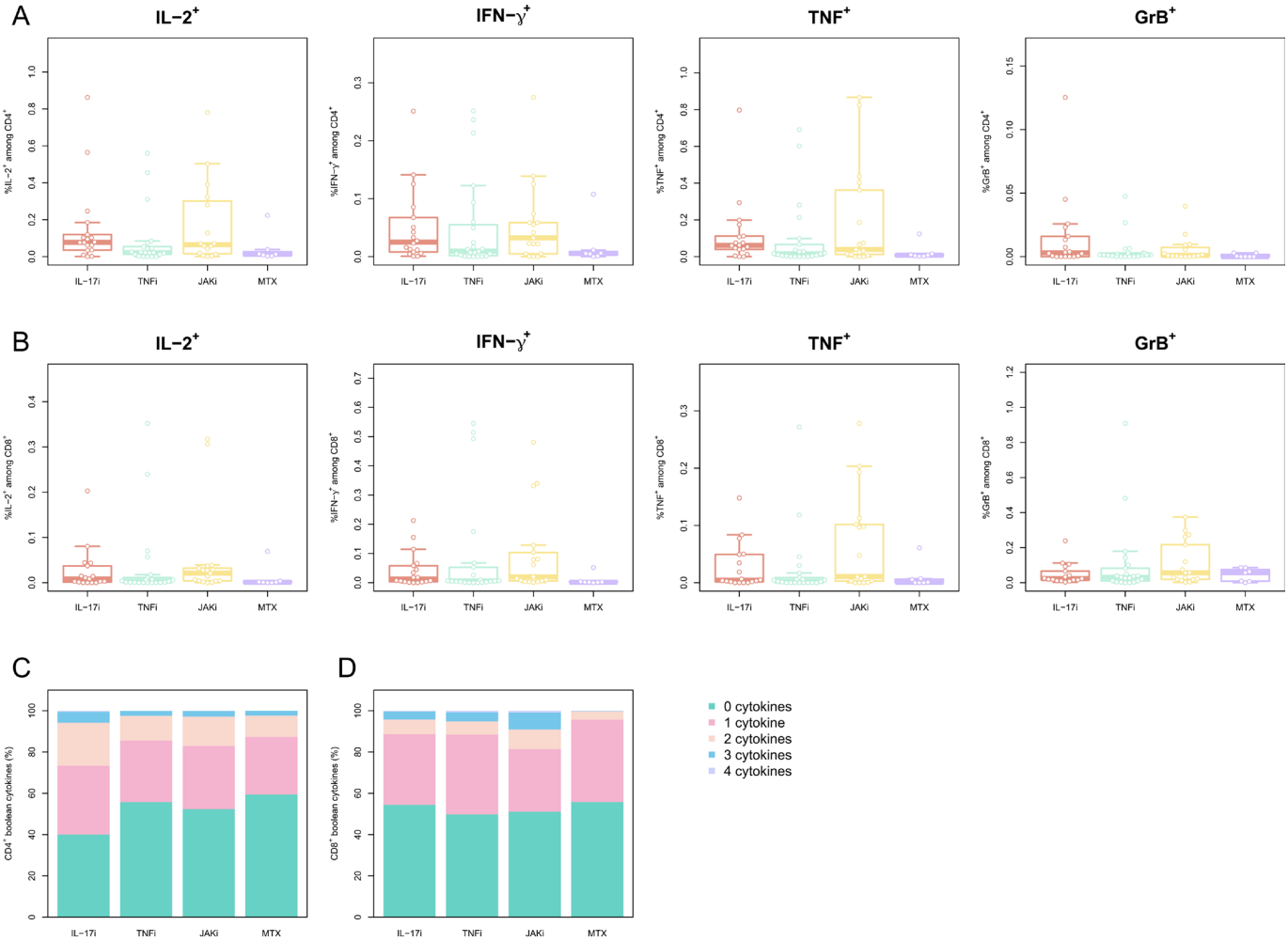

Figure 5 Frequency of the cytokines IL-2, IFN-γ, TNF and GrB for SARS-CoV-2 spike-reactive CD4+ (**A**) CD8+ (**B**) T cells at '2nd dose+4 weeks'. Boolean combinations of assayed cytokines for CD4+ and CD8+ T cells (**C,D**). The box plots indicate the 75th, 50th and 25th quantile, and the whiskers have a maximum length of 1.5 times the IQR. Each point represents a patient. Gr, granzyme; IL, interleukin; JAK, Janus kinase inhibitor; MTX, methotrexate; PBMCs, peripheral blood mononuclear cells; TNF, tumour necrosis factor.

with vaccine-induced immunity we did not observe negative effects of MTX monotherapy following full vaccination for COVID-19.[8] This is in line with recent observations for similar vaccines.[32 33] Although antibodies are important in limiting an infection, only neutralisation prevents viral entry. A clear correlation was found between total IgG levels and neutralising activity at each sampling time point indicating a superior protection in patients on IL-17i.

Combination of MTX with bDMARDs having distinct MoA resulted in reduced levels of antibodies against SARS-CoV-2 spike protein and importantly also lower neutralisation activity even if the difference did not reach statistical significance. Accordingly, such drug combinations should be avoided whenever possible to promote superior humoral responses in patients with AIRDs. This is particularly relevant nowadays due to emergence and spread of new SARS-CoV-2 variants of concerns.

Cellular immunity is an integral part of the antiviral response and may be sufficient to clear a SARS-CoV-2 infection in the absence of adequate humoral immunity.[36 37] To assess a lasting cellular immunity, we evaluated the levels of T-cell activation defined by the expression of the activation markers CD154+ and CD137+ on CD4+ T-cells and CD137+ on CD8+ T-cells, and in addition established the polyfunctional profile of T-cells. This approach yields considerably more information than cytokine capture assays alone and enables a thorough immunological characterisation of cellular immunity after vaccination. Most studies evaluated selected cytokine profiles or single T-cell populations.[13 33 38 39] Although the humoral responses varied, the cellular immunity was similar for all MoA evaluated here. Similar findings have been reported by Mahil *et al*[33] with no better response after the second vaccination in patients with psoriasis under therapy with IL-17i, TNFi, MTX and IL-23i as compared with healthy controls. Similarly, the OCTAVE study has reported unchanged T-cell responses after the second vaccine dose in patients with inflammatory arthritis.[40] Others have found diminished T-cell responses compared with healthy controls after two doses of BNT162b2 in patients with RA treated with IL-6 inhibitors, TNFi or abatacept.[38]

A limitation of our study is the lack of a control group. However, our purpose was primarily to compare the different immunosuppressants in terms of their impact on development of vaccine-induced humoral and cellular immune responses in thoroughly diagnosed patients on stable treatment and low disease activity status. Furthermore, we did not adjust for the therapeutic dose of DMARD therapy, which could affect the vaccine efficacy as measured on IgG titres and neutralising capacity. When we compared the dose of the individual therapeutics of the patients not experiencing seroconversion, we found no hint that the therapeutic dose could greatly skew the study outcome (data not shown). All but 4 patients received an mRNA-based vaccine for the first immunisation, so confounding effects are rather unlikely. Antibodies are emerging as a correlate of protection for SARS-CoV-2 vaccines and non-neutralising antibody may be protective.[41] We assessed solely neutralising capacity and this only for ancestral SARS-CoV-2. Follow-up studies may evaluate effects of MoA employed herein using systems serology. An additional limitation is the formal exclusion of patients with a preformed memory to SARS-CoV-2, either through asymptomatic infection or previous exposures to human coronaviruses. However, by excluding patients with pre-existing antibodies against the S-protein, we believe to have limited this confounder. Asymptomatic infections during the study could not be excluded due to the lack of anti-nucleocapsid antibody assay. However, due to the short time period between baselines and antibodies measure, the existing hygiene rules and considering the time period in which this study was conducted (with predominance of the alpha and delta variants), we assume that this does not significantly impact the results of this study.

Literature data have shown higher antibody response among recipients of the mRNA-1273 vaccine than among recipients of the BNT162b2 vaccine.[42 43] Looking for confounders we did not find any significant difference between the two vaccines.

In summary, our data demonstrate that even after insufficient neutralising capacity and antibody response against SARS-CoV-2 spike-protein between patients of different immunosuppressive therapies, particularly for MTX and JAKi after first vaccination, a second vaccination can cover almost all patients regardless of DMARDs therapy. While IL-17i at the very least alters antibody development and a combination of b/tsDMARDs with MTX, even when not significant, reduces the amount of antibody development, the T-cell responses remain unchanged across the examined immunosuppressive therapies. These observations are critical for rheumatic patient care and vaccine deployment in various rheumatic disease groups.

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Acknowledgements We thank all the patients who participated in this study. We thank the study nurses Gordana Brnos and Silke Kunkel for their support in the implementation of the study. We thank Sarah Skrzypczyk, Jan Zapka, Julia Kurek, Eva Kohut for their technical assistance and Dr. Toralf Roch for commenting the manuscript. We acknowledge support by the DFG Open Access Publication Funds of the Ruhr-Universität Bochum.

Contributors Conception and design of the study: IA, UK, TW, NB, US and XB. Data acquisition: IA, JS, AB-N and US. Analysis and interpretation: IA, AB-N, MA, JS, SP, EVB, US, UK and XB. Drafting and revising the manuscript critically: IA, AB-N, US, UK, SP and XB. Final approval of the manuscript: all Authors. US and XB are last authors. IA accepts full responsibility for the finished work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by Ethics committee Ruhr-Universität Bochum, Germany Approval number 20-7143. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

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