Supplementary data for ***"Additional heterologous versus homologous booster vaccination in immunosuppressed patients without SARS-CoV-2 antibody seroconversion after primary mRNA vaccination: a randomized controlled trial"***

Bonelli M, *et al. Ann Rheum Dis* 2022;0:1–8. doi: 10.1136/annrheumdis-2021-221558

Table of contents

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Supplementary Methods

ASSESSMENTS

Quantification of CD19⁺ peripheral B-cells.

Immunological phenotyping was performed by flow cytometry (FACSCanto II, Becton Dickinson, San Jose, California, USA) using the whole blood first stain and then lyse and wash method (Becton Dickinson). Lymphocyte subsets were characterized with a combination of the following monoclonal antibodies (all provided by Becton Dickinson): fluorescein isothiocyanate (FITC)-labelled anti-CD3, phycoerythrin (PE)-labelled anti-CD16⁺56⁺, peridinin-chlorophyll-protein (PerCP)-cy5.5-labelled anti-CD4, PE-Cy7-labelled anti-CD19, allophycocyanin (APC)-Cy7-labelled anti-CD8, V450-labelled anti-human leukocyte antigen (HLA)-DR, V500-labelled anti-CD45 and APC-labelled anti-CD14. Results were expressed as percentage of $CD19^+$ B-cells among total lymphocytes.

Anti-SARS-CoV-2 antibody testing.

The Elecsys Anti-SARS-CoV-2 S immunoassay was used for the quantitative determination of antibodies to the receptor-binding domain (RBD) of the viral spike (S) protein (1,2). The quantitation range is between 0·4 and 2500·0 BAU/mL (binding antibody units per milliliter). A value greater than 0·8 BAU/mL was considered as positive. Tests were performed on a Cobas e801 analyser (Roche Diagnostics, Rotkreuz, Switzerland) at the Department of Laboratory Medicine, Medical University of Vienna (certified acc. to ISO 9001:2015 and accredited acc. to ISO 15189:2012).

T-cell responses.

For T-cell stimulation (see below), PepMix SARS-CoV-2 peptide pools were purchased from JPT (Berlin, Germany). The pools cover the entire sequences of the SARS-CoV-2 S protein and comprise 15-mer peptides overlapping by 11 amino acids (aa). The S peptides are split into two subpools S1 (aa 1–643) and S2 (aa 633–1273). Peptides were dissolved in dimethyl sulfoxide and diluted in AIM-V medium for use in enzyme-linked immunosorbent spot (ELISpot) assays.

For ex vivo *T-cell IFN-γ ELISpot assay*, PBMCs from patients before and after the third vaccination were thawed and processed on the same day. A total of $1–2\times10^5$ cells per well were incubated with SARS-CoV-2 peptides (2 µg/mL; duplicates), AIM-V medium (negative control; 3–4 wells) or phytohemagglutinin (PHA) (L4144, Sigma; 0·5 µg/mL; positive control) in 96-well plates coated with 1·5 µg anti-IFN-γ (1-D1K, Mabtech) for 24 hours. After washing, spots were developed with 0·1 µg biotin-conjugated anti-IFN-γ (7-B6-1, Mabtech), streptavidin-coupled alkaline phosphatase (Mabtech, 1:1000) and 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (Sigma). Spots were counted using a Bio-Sys Bioreader 5000 Pro-S/BR177 and Bioreader software generation 10. Data were calculated as spot-forming cells (SFCs) per 10^6 PBMCs after subtracting of the spots from the negative control (mean spot numbers from three to four unstimulated wells). T-cell responses were considered positive if spot counts were greater than the mean SFCs plus three times the standard deviation from pre-pandemic controls, as defined previously (3).

SARS-CoV-2 neutralization test (NT).

The NT was performed as described previously (4). Two-fold serial dilutions of heat-inactivated serum samples were incubated with 50–100 TCID50 SARS-CoV-2 for 1h at 37°C before the mixture was added to Vero E6 (ATCC ® CRL-1586) cell monolayers. Incubation was continued for three days. NT titers were expressed as the reciprocal of the serum dilution required for protection against virus-induced cytopathic effects. NT titers ≥10 were considered positive.

Supplementary Table 1: Patient characteristics stratified by seroconversion

Seroconversion	no	yes
n	40	15
Age (mean (SD))	60·6 (16·7)	58·0 (17·0)
Gender: female n (%)	30 (75·0)	11 (73·3)
Diagnosis n (%)		
Arthritis	18 (45·0)	3 (20·0)
Connective tissue diseases	12 (30·0)	4 (26·7)
IgG4-related disease	2 (5·0)	2 (3·3)
Multiple sclerosis	3 (7·5)	3 (20·0)
Vasculitis	5 (12·5)	3 (20·0)
Months between RTX and screening (mean (SD))	6·04 (5·78)	7·69 (1·41)
Weeks between 2nd vaccination and screening (mean (SD))	7·80 (3·47)	6·43 (1·62)
Patients with detectable B-cells: n (%)	6 (15·0)	12 (80·0)
Peripheral B-cells, percentage (median [IQR])	0·0 [0·0, 0·0]	0·8 [0·4, 1·5]
Concomitant medication, n (%)		
Any csDMARD	23 (57·5)	3 (20·0)
Methotrexate	9 (22·5)	1 (6·7)
Mycophenolate mofetil	5 (12·5)	1 (6·7)
Azathioprine	4 (10·0)	1 (6·7)
Leflunomide	4 (10·0)	0 (0·0)
Hydroxychloroquine	4 (10·0)	0 (0·0)
Immunoglobulin therapy	1 (2·5)	1 (6·7)
Prednisone	12 (30·0)	3 (20·0)
Booster vaccination n (%)		
ChAdOx1 nCoV-19	21 (52·5)	6 (40·0)
BNT162b2	15 (37·5)	6 (40·0)
mRNA-1273	4 (10·0)	3 (20·0)
SARS-Cov-2 Spike antibodies BAU/mL (median [IQR])	0·0 [0·0, 0·0]	15·7 [4·7, 25·8]

csDMARD, conventional synthetic disease modifying antirheumatic drug, defined here as concomitant treatment with at least one of the following: methotrexate, mycophenolate mofetil, azathioprine, leflunomide, hydroxychloroquine.

Supplementary Table 2: Patients characteristics with assessed T-cell response

	Vector	mRNA
n	20	16
Age (mean (SD))	56·6 (13·8)	56·3 (20·0)
Gender: female n (%)	15 (75·0)	14 (87·5)
Diagnosis n (%)		
Arthritis	7 (35·0)	6 (37·5)
Connective tissue diseases	6 (30·0)	5 (31·2)
IgG4-related disease	1 (5·0)	2 (12·5)
Multiple sclerosis	3 (15·0)	1 (6·2)
Vasculitis	3 (15·0)	2 (12·5)
Months between RTX and screening (mean (SD))	6·6 (5·3)	5·4 (4·0)
Weeks between 2nd vaccination and screening (mean (SD))	7·2 (2·8)	6·2 (1·9)
Detectable B-cells: n (%)	6 (30·0)	5 (31·2)
Concomitant medication, n (%)		
Any csDMARD	6 (30·0)	10 (62·5)
Mycophenolate mofetil	1 (5·0)	2 (12·5)
Leflunomide	3 (15·0)	1 (6·2)
Hydroxychloroquine	0 (0·0)	3 (18·8)
Methotrexate	2 (10·0)	5 (31·2)
Azathioprine	0 (0·0)	1 (6·2)
Immunoglobulin therapy	1 (5·0)	1 (6·2)
Prednisone	6 (30·0)	6 (37·5)
Primary vaccination with mRNA-1273 n (%)	3 (15·0)	1 (6·2)
Seroconversion: n (%)	5 (25·0)	4 (25·0)
T-cell response at screening (median [IQR])	248 [54, 705]	113 [33, 394]
T-cell response at week one (median [IQR])	459 [133, 722]	305 [171, 416]

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Supplementary Table 3: Integrative analysis of humoral and/or T-cell responses stratified by vector or mRNA vaccine.

pre Group	post Group	n (Vector)	n (mRNA)
AB (-), T (-)	AB (+), T (+)	2	2
AB (-), T (+)	AB (+), T (+)	3	1
AB (-), T (-)	AB (+), T (-)	0	1
AB (-), T (+)	AB (-), T (+)	12	9
AB (-), T (-)	AB (-), T (+)	3	1
AB (-), T (-)	AB (-), T (-)	0	2

Pre-Group: Before third dose, Post-Group: after third dose, AB: Antibody, T: T-cell response, +: positive,-: negative, n (Vector): Number of patients in the vector group, n (mRNA): number of patients in the mRNA group.

Supplementary Table 4: Patient characteristics stratified by T-cell response

T-cell response	no	yes
n	3	33
Age (mean (SD))	59·7 (19·1)	56·2 (16·7)
Gender: female n (%)	3 (100)	26 (79)
Diagnosis n (%)		
Arthritis	2 (66·7)	11 (33·3)
Connective tissue diseases	1 (33·3)	10 (30·3)
Vasculitis	0 (0·0)	5 (15·2)
Multiple sclerosis	0 (0·0)	4 (12·1)
IgG4-related disease	0 (0·0)	3 (9·1)
Months between RTX and screening (mean (SD))	8·5 (2·1)	5·8 (4·9)
Weeks between 2nd vaccination and screening (mean (SD))	7·24 (2·5)	6·7 (2·5)
Patients with detectable B-cells: n (%)	1 (33·3)	10 (30·3)
Concomitant medication, n (%)		
Any csDMARD	3 (100·0)	13 (39·4)
Methotrexate	2 (66·7)	5 (15·2)
Mycophenolate mofetil	0 (0·0)	3 (9·1)
Leflunomide	0 (0·0)	4 (12·1)
Hydroxychloroquine	0 (0·0)	3 (9·1)
Azathioprine	1 (33·3)	0 (0·0)
Immunoglobulin therapy	0 (0·0)	2 (6·1)
Prednisone	2 (66·7)	10 (30·3)
Booster vaccination n (%)		
ChAdOx1 nCoV-19	0 (0·0)	20 (60·6)
BNT162b2	2 (66·7)	13 (39·4)
mRNA-1273	1 (33·3)	0 (0·0)
Seroconversion: n (%)	1 (33·3)	8 (24·2)
T-cell response at week one (median [IQR])	20 [14, 31]	389 [190, 675]

csDMARD, conventional synthetic disease modifying antirheumatic drug, defined here as concomitant treatment with at least one of the following: methotrexate, mycophenolate mofetil, azathioprine, leflunomide, hydroxychloroquine.

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