A third vaccine dose substantially improves humoral and cellular SARS-CoV-2 immunity in renal transplant recipients with primary humoral non-response

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Concise Methods

Population and vaccination

With regard to the eminent risk of a severe course of COVID-19, we decided to offer a booster vaccine dose to all our renal transplant recipients with primary humoral non-response after two vaccine doses at the Transplant Center of Ruhr-University Bochum. All patients gave written informed consent for the off-label use of a third dose. Immunomonitoring of vaccine responses has been approved by the ethical committee of the Ruhr University of Bochum (Reg.-Nr. 20-7126). Patients who met inclusion criteria:

a) the vaccination (2 doses of mRNA vaccine applied within > 3 weeks) should be completed and patients received their 2nd vaccine dose four weeks prior the assessment of serological response;

b) no antibodies binding to SARS-CoV-2 S protein should be detected by ELISA measured using Elecsys Anti-SARS-CoV-2 S and detection level of <0.80 U/ml

were included in the study. To exclude a natural SARS-COV-2 infection occurring between the 2^{nd} and 3^{rd} vaccination, the antibody titers against the SARS-COV-2 nucleocapsid-protein in were determined in all patients. None of the patients showed detection of nucleocapsid-protein-specific antibodies. Blood samples of 10 renal transplant recipients were collected after written informed consent was obtained. For reasons of availability, the first two SARS-CoV-2 vaccinations were BNT162b2 (Pfizer, New York City, USA and BioNTech, Mainz, Germany) with 28 days apart. The booster vaccination was mRNA-1273 (Moderna, Massachusetts, USA) and injected 4 to 12 weeks following the second vaccination. Blood samples were obtained right before the first two administrations, and four weeks after the second regular administration (defined as “initial timepoint” in Suppl. Figure). No adjustment

of immunosuppression was performed within vaccination procedure. Finally, the last blood samples were obtained 14 days following the third dose of mRNA vaccine.

Measurement of SARS-CoV-2 spike and nucleocapsid antibodies

We determined SARS-CoV-2 spike and nucleocapsid antibody titer using Elecsys Anti-SARS-CoV-2-S (Roche, Mannheim, Germany) with Cobas e immunoassay analyzers (Roche Diagnostics, Basel, Switzerland). Testing and dilutions was conducted in accordance with the manufacturers` recommendations.

For the virus neutralization assay, a propagation-incompetent VSV*ΔG(FLuc) pseudovirus system bearing the SARS-CoV-2 spike protein in the envelope was incubated with serial dilutions of immune sera prior to infections of Vero E6 cells as previously described (Zettl et al.). At 18 hours post infection, firefly luciferase (FLuc) reporter activity was determined and the reciprocal antibody dilution causing 50% inhibition of the luciferase reporter calculated (PVN50). For technical reason the neutralization assay showed inconclusive result in one sample so that only nine out of ten patients can be shown in Fig 1B.

Analysis of SARS-CoV-2-reactive T cells by flow cytometry

Analysis of SARS-CoV-2 Spike protein reactive T cells was performed by flow cytometry as previously described (Thieme et al., 2020). Briefly, peripheral blood mononuclear cells (PBMC) were isolated from peripheral blood applying Ficoll-Hypaque density gradient centrifugation and isolated PBMC were stimulated with SARS-CoV-2-PepTivator peptide-pools solved in water (Miltenyi Biotec). Untreated PBMC were used as negative control to assess unspecific

background activation. After 2h of stimulation, Brefeldin-A (Sigma-Aldrich) was added and the stimulation stopped after 16h. Surface- and intracellular-staining for flow cytometry was performed using fixation and permeabilization (ThermoFisher) and antibodies for markers listed below. Samples were measured on a CytoFlex flow cytometer (Beckman-Coulter).

Flow cytometry data were analyzed using FlowJo version 10.6.2 (BD Biosciences). Single stains and fluorescence-minus-one controls were used for gating. $CD4^+$ T cells expressing CD154 and CD137 and $CD8^+$ T cells expressing CD137 in combination with production of at least one of IL2/IL4/IFNγ/TNFα/GrzB were defined as reactive T cells. Unspecific activation in unstimulated controls was subtracted from stimulated samples to account for SARS-CoV-2-specific activation in the presented frequencies. Responses were considered if they are identifiable as a visible cell cluster exceeding 10 T cells and 0.01% of CD4 or CD8 T-cells, respectively.

Statistical Analysis

Flow cytometry data was analyzed using FlowJo version 10.7.1 (BD Biosciences, USA. Statistical analysis was performed using R, version 4.0.4. Continuous variables are depicted as box plots; the maximum length of the whiskers corresponds to 1.5 times the interquartile range. Differences for a continuous variable between the sub-cohorts were analysed using the paired, two-sided t-test. Significance threshold was set at 0.05.

References:

S1 Zettl F. et al. *Rapid Quantification of SARS-CoV-2-Neutralizing Antibodies Using Propagation-Defective Vesicular Stomatitis Virus Pseudotypes.* Vaccines, 2020 Jul 15;8 (3):p. 386

S2 Thieme, C.J., et al., *Robust T Cell Response Toward Spike, Membrane, and Nucleocapsid SARS-CoV-2 Proteins Is Not Associated with Recovery in Critical COVID-19 Patients.* Cell Rep Med, 2020. **1**(6): p. 100092.

Supplementary Table 1: Study population

	Age	Sex	Immunosuppressive regimen					Months since TX	Cause of ESRD	GFR (CKD-EPI; ml/min/1.73²)	Weeks between second and third vaccination	SARS-COV-2-IgG (U/ml) [a]	SARS-COV-2-IgG (U/ml) [a]	SARS-COV-2-IgG (U/ml) [a]	SARS-CoV2-Spike-reactive CD4+ T cells (%) [b]	SARS-CoV2-Spike-reactive CD4+ T cells (%) [b]
			CNI	mTOR-I	Belatacept	MPA	GC					4 weeks after first dose	4 weeks after second dose	2 weeks after third dose	4 weeks after second dose	2 weeks after third dose
1	51	M		X		X	X	35	IgAN	42.0	12	<0.80	<0.80	561.1	0.07	0.15
2	52	M	X			X	X	11	NS	46.1	10	<0.80	<0.80	<0.80	0.01	0.11
3	66	F	X			X	X	9	FSGS	31.3	10	<0.80	<0.80	76.7	<0.01	0.81
4	69	M			X	X	X	190	NS	18.1	10	<0.80	<0.80	<0.80	<0.01	0.01
5	41	M	X			X	X	102	IgAN	55.2	10	<0.80	<0.80	<0.80	<0.01	<0.01
6	66	M	X			X	X	51	unknown	50.1	4	<0.80	<0.80	<0.80	0.04	0.63
7	54	M	X				X	20	unknown	56.9	10	<0.80	<0.80	521.8	0.21	0.83
8	74	F	X			X	X	27	MPGN	24.2	10	<0.80	<0.80	6280	<0.01	0.02
9	76	M	X			X	X	48	IgG4	55.6	10	<0.80	<0.80	463.4	<0.01	0.02
10	46	M	X			X	X	132	IgAN	54.6	6	<0.80	<0.80	1044	0.05	0.13

[a] reference <0.80 U/ml (Roche *Elecsys Anti-SARS-CoV-2-S*)

[b] % of $CD154^{+}CD137^{+}$ among CD4+ T cells, values < 0.01 (cut off) considered negative

TX – transplantation. ESRD – end-stage renal disease; F – female; M – male; CNI – calcineurin inhibitor; mTOR-I – mTOR inhibitor; GC – glucocorticoids; GFR – glomerular filtration rate; CKD-EPI – Chronic Kidney Disease Epidemiology Collaboration; IgAN – IgA-Nephropathy; NS – nephrosclerosis; FSGS – focal segmental glomerulosclerosis; MPGN – idiopathic membranoproliferative glomerulonephritis; IgG4 – IgG4-related tubulointerstitial nephritis