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Supplementary appendix

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Title: Lack of effect of Bivalent COVID-19 mRNA Vaccine against Current Circulating Omicron Subvariants in Immunocompromised

Supplementary materials

METHODS

STUDY DESIGN AND ETHICS

The study was approved by the University Health Network research ethics board (protocol number 20-6069). All participants were organ transplant recipients on active immunosuppression. All patients provided written informed consent. Five groups of transplant recipients were recruited for the study.

1. Participants that received the fourth dose of an approved monovalent mRNA COVID-19 vaccine (both BNT162b2 mRNA [Pfizer-BioNTech] and mRNA-1273 [Moderna] were acceptable) from January to April 2022. Patients with previous documented COVID-19 were excluded.
2. Participants who received a bivalent vaccine between October and November of 2022 and had no history of previous COVID-19 infection. Pfizer-BioNTech Bivalent COVID-19 vaccine (Wild-type/Omicron BA.4/5) was administered to 24/25 patients (96%), and Moderna Spikevax® Bivalent COVID-19 vaccine (Ancestral Wuhan-Hu-1/Omicron BA.4/5) to 1/25 (4%) patients.
3. Participants who received a bivalent booster vaccine between October and November of 2022 and had a history of previous COVID-19 infection. Pfizer-BioNTech Bivalent COVID-19 vaccine (Wild-type/Omicron BA.4/5) was given to 15/22 (68.2%), and Moderna Spikevax® Bivalent COVID-19 vaccine (Ancestral Wuhan-Hu-1/Omicron BA.4/5) to 7/22 (31.8%) patients.

4. Participants diagnosed with COVID-19 during the Omicron BA.1 wave in Ontario, Canada (from December 2021 to February 2022).
5. Participants diagnosed with COVID-19 during the Omicron BA.5 wave in Ontario (from August to October, 2022).

Whole blood was collected for serum at 4-6 weeks after the administration of the last dose of the vaccine or 4-6 weeks after the diagnosis of COVID-19. Serum and peripheral blood mononuclear cells (PBMCs) were isolated from whole blood and cryopreserved for batch testing. SARS-CoV-2 infection was determined via SARS-CoV-2 nasopharyngeal polymerase chain reaction or rapid antigen test. Baseline characteristics were recorded and included demographics, comorbidities, transplant characteristics and immunosuppression as well as details of previous COVID-19 infection and vaccination.

NEUTRALIZING ANTIBODY TESTING:

The pseudovirus neutralization assay was performed as described previously.[1] Briefly viral packaging (psPAX2; Addgene, USA), the ZsGreen and luciferase reporter (pHAGE-CMV-Luc2-IRES-ZsGreen-W, provided by Jesse Bloom) and spike protein constructs (BA.4/5, BQ.1.1 and XBB.1.5) generated from consensus sequences from Outbreak.Info,[2] were co-transfected into HEK293TN cells. A viral titre assay for each pseudovirus was performed by infecting HEK293T-ACE2/TMPRSS2 cells. Patient serum samples were serially 3-fold diluted, then incubated with diluted virus at a 1:1 ratio prior to addition to cells. The infected cells were lysed after 48 hours using the BrightGlo Luciferase Assay System (Promega, USA), and luciferase activity was measured using a PerkinElmer Envision instrument (PerkinElmer, USA). Inhibitory

dilution with 50% virus neutralization (ID50) titers were calculated in Prism, ver. 9.2.0 (GraphPad Software, USA) using a nonlinear regression (log[inhibitor] versus normalized response – variable slope) algorithm and converted to a $\log_{10}$ scale. A positive neutralization assay was defined as any dilution that resulted in 50% viral neutralization as calculated based on the above generated curve (therefore the lower limit of detection is considered $\log_{10}$ ID50 > 0 where a value of 1.0 corresponds to absence of 50% neutralization as calculated for an undiluted specimen).

T-CELL ASSESSMENT:

SARS-CoV-2 specific $CD4^+$ and $CD8^+$ T-cells were measured using an intracellular cytokine staining (ICS) flow cytometry assay, described elsewhere.[3] T-cell responses directed against Omicron BA.4/5 and BQ.1.1 were measured in n=35 transplant recipients and characterized in terms of monofunctional and polyfunctional (IL-2+ IFN-γ +) $CD4^+$ T-cells, and total IFN-γ expressing $CD8^+$ T-cells, as these reflect the principal T-cell subsets responding to SARS-CoV-2 infection and vaccination.[4] XBB.1.5 peptides became available late in the study and were tested in n=16 patients with remaining PBMC available. For this assay, 10^6 PBMC (per test) were incubated overnight with overlapping peptide pools corresponding to variant-specific SARS-CoV-2 spike proteins. Incubation also included co-stimulation with CD28/CD49d antibodies (BD Biosciences, USA) and treatment with a protein transport inhibitor (ThermoFisher Scientific, Canada) to prevent cytokine release. PMA/ionomycin (ThermoFisher Scientific) was used as a positive control, and FMO-controls were used to guide gating. Antigen-specific T-cells were determined after subtracting for background cytokine expression. A minimum of 100,000 live $CD3^+$ T-cell events were required for samples to be included in the flow analysis. The minimum threshold for detection of variant-

specific T-cell responses was 0.01%, as per prior studies (3). All peptides were purchased through JPT (Germany) and resuspended partially in DMSO and then PBS. Details on the GISAID sequences used and the mutational profiles associated with these products are available on the manufacturer's website. Antibodies were purchased from BD Biosciences, except for the anti-IL-2 antibody which was purchased from BioLegend.

STATISTICAL ANALYSIS

In this study, the primary outcome was the neutralizing antibody titer (expressed as ID50) against BA.4/5, BQ.1.1, and XBB.1.5 after receiving bivalent vaccine. Secondary outcomes included the (i) proportion of patients with detectable neutralization, and (ii) T-cell responses against BA.4/5, BQ.1.1, and XBB.1.5. Patients that received the bivalent vaccine were divided into two groups based on whether they had a previous history of documented COVID-19 (as determined by previous rapid antigen or PCR positivity). To assess the effectiveness of this vaccine, we compared the neutralizing antibody titer and the proportions with detectable neutralization in the non-infected bivalent vaccine group with the remaining four groups. To test differences in the median neutralizing antibody levels between multiple cohorts, we used Kruskal-Wallis test followed by Dunn's test and Holm-Šídák adjustment for multiple comparisons. We used the Bonett-Price 95% confidence interval for the difference of medians. We estimated the odds ratios of the probability of neutralization against BA.4/5, BQ.1.1, and XBB.1.5 for each cohort using the non-infected bivalent vaccine group as the reference. Statistical analyses were performed with Stata statistical software, version 15.1 (StataCorp, LLC., USA) and figures were created using Prism GraphPad version 9.4.1 (La Jolla, CA).

REFERENCES

1. Abe KT, Li Z, Samson R, Samavarchi-Tehrani P, Valcourt EJ, Wood H, et al. A simple protein-based surrogate neutralization assay for SARS-CoV-2. JCI insight. 2020 Oct;5(19).
2. Gangavarapu K, Latif AA, Mullen JL, Alkuzweny M, Hufbauer E, Tsueng G, et al. Outbreak.info genomic reports: scalable and dynamic surveillance of SARS-CoV-2 variants and mutations. Research square. United States; 2022.
3. Ferreira VH, Solera JT, Hu Q, Hall VG, Arbol BG, Rod Hardy W, et al. Homotypic and heterotypic immune responses to Omicron variant in immunocompromised patients in diverse clinical settings. Nat Commun. 2022 Aug;13(1):4489.
4. Moss P. The T cell immune response against SARS-CoV-2. Nat Immunol [Internet]. 2022;23(2):186–93. Available from: https://doi.org/10.1038/s41590-021-01122-w

SUPPLEMENTARY RESULTS

SEQUENCING OF VARIANTS OF THE PATIENTS FROM THE BIVALENT VACCINE AND PREVIOUS COVID-19 GROUP

Response: Of the 22 patients in the bivalent group who had previous COVID, variant determination was done in two (9%) and was: one Alpha and one Omicron BA.1. In other patients, four had a PCR performed in other centers and the variant was not tested, and 16 were diagnosed with a rapid antigen test. However, the likely variant could be determined based on the community prevalence (Ontario Genomic Surveillance: https://www.publichealthontario.ca/en/laboratory-services/test-information-index/covid-19-voc) during the timing of infection. Based on this, five cases of infection were likely Omicron BA.1, seven Omicron BA.2, and eight Omicron BA.4/5.

BREAKTHROUGH COVID-19 IN PATIENTS AFTER BIVALENT AND MONOVALENT VACCINE

No patients from the bivalent vaccine without previous COVID-19 had a breakthrough infection in the three months after being vaccinated. A total of 2/22 (9.1%) of the bivalent and previous COVID-19 group, and 10/86 (11.6%) of the monovalent vaccine group patients had breakthrough within 3 months from the vaccine. All the cases were mild and none required hospitalization. The median time from vaccine to the breakthrough was 59.5 days (IQR, 33 to 86) in the bivalent and infection group, and 67.5 days (IQR, 29 to 84) in the monovalent vaccine group. One of the two patients of the bivalent and infection group (50%) and 2/10 (22.2%) of the monovalent group received early antiviral therapy with three doses of remdesivir.

SUPPLEMENTARY TABLES

Table S1. Demographic and Clinical Characteristics of the Patients.					
Characteristics	4th dose monovalent vaccine (N=86)	Bivalent booster (N=25)	Bivalent and previous COVID-19 (N=22)	Post-BA.1 infection (N=75)	Post-BA.5 infection (N=50)
Age – years, median (IQR)	65·3 (55·2 to 71·1)	63·6 (49 to 69·5)	61·5 (49 to 67·1)	54·9 (47·1 to 64·1)	57·8 (45·8 to 66·3)
Female sex – no. (%)	34 (39·5%)	11 (44%)	7 (31·8%)	25 (33·3%)	23 (46%)
Type of transplant – no. (%)					
Kidney	24 (27·9%)	6 (24%)	7 (31·8%)	29 (38·7%)	17 (34%)
Lung	17 (19·8%)	4 (16%)	5 (22·7%)	18 (24%)	9 (18%)
Liver	17 (19·8%)	7 (28%)	3 (13·6%)	11 (14·7%)	14 (28%)
Heart	18 (20·9%)	4 (16%)	5 (22·7%)	6 (8%)	6 (12%)
Combined organs†	10 (11·6%)	4 (16%)	2 (9·1%)	11 (14·7%)	4 (8%)
Retransplant – no. (%)	6 (7·1%)	2 (8%)	0 (0%)	13 (17·3%)	2 (4%)
Years since transplant, median (IQR)	6·3 (2·7 to 12·6)	7 (3·7 to 13·5)	4·9 (3·3 to 11·2)	5·9 (2·3 to 9·8)	5·2 (1·8 to 15·6)
Comorbid conditions – no. (%)					
Hypertension	54 (64·3%)	15 (60%)	17 (77·3%)	55 (73·3%)	35 (70%)
Diabetes mellitus	14 (16·7%)	6 (24%)	4 (18·2%)	28 (37·3%)	10 (20%)
BMI ≥30 kg/m^2	14 (16·7%)	3 (12%)	3 (13·6%)	17 (22·7%)	11 (22%)
Coronary artery disease	15 (17·9%)	4 (16%)	5 (22·7%)	11 (14·7%)	9 (18%)
Chronic cardiac failure	1 (1·2%)	0 (0%)	1 (4·5%)	5 (6·7%)	0 (0%)
Chronic lung disease	9 (10·7%)	2 (8%)	4 (18·2%)	11 (14·7%)	6 (12%)
Chronic kidney disease					
eGFR<60 mL/min /1.73m^2	47 (56%)	14 (56%)	15 (68·2%)	29 (38·7%)	11 (22%)
eGFR<30 mL/min /1.73m^2	11 (13·1%)	3 (12%)	1 (4·5%)	0 (0%)	7 (14%)
Malignancy	0 (0%)	0 (0%)	0 (0%)	1 (1·3%)	4 (8%)
Other Immunosuppression	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (4%)
No. of comorbidities, median (IQR)	2 (1 to 3)	2 (1 to 3)	2 (1 to 3)	2 (1 to 3)	2 (1 to 3)
Immunosuppressant – no. (%)					
Prednisone	65 (75·6%)	16 (64%)	16 (72·7%)	64 (85·3%)	34 (68%)
Tacrolimus	63 (73·3%)	19 (76%)	17 (77·3%)	64 (85·3%)	36 (72%)
Cyclosporine	20 (23·3%)	4 (16%)	4 (18·2%)	10 (13·3%)	7 (14%)
Mycophenolate	56 (65·1%)	17 (68%)	12 (54·5%)	62 (82·7%)	29 (58%)

Azathioprine	11 (12·8%)	1 (4%)	3 (13·6%)	2 (2·7%)	5 (10%)
Sirolimus	11 (12·8%)	5 (20%)	3 (13·6%)	2 (2·7%)	6 (12%)
ATG last 3 months	0 (0%)	0 (0%)	0 (0%)	1 (1.3%)	3 (6%)
Rituximab last year	0 (0%)	0 (0%)	1 (4.5%)	1 (1.3%)	3 (6%)
COVID-19 vaccines – no. (%)					
<3	0 (0%)	0 (0%)	0 (0%)	18 (24%)	4 (8%)
≥3	86 (100%)	25 (100%)	19 (100%)	57 (76%)	46 (92%)
Number of COVID-19 vaccines doses, median (IQR)	4 (4 to 4)	5 (5 to 6)	5 (5 to 6)	3 (3 to 3)	4 (3 to 4)
Time since last COVID-19 vaccine – days, median (IQR)	35 (28 to 42)	34 (29 to 39)	37 (33 to 46)	102 (54 to 134)	189 (73 to 299)
Previous COVID-19 – no. (%)	0 (0%)	0 (0%)	22 (100%)	–	–

†Combined organs include 27 kidney-pancreas, 2 kidney-liver, 1 kidney-heart, and 1 kidney-pancreas-liver.

BMI: body mass index (kg/m^2), IQR: Interquartile range, SD: Standard deviation. ATG: Anti-thymocyte globulin. Chronic kidney disease denotes GFR<60ml/min/m2 or GFR<30ml/min/m2. Malignancies included 1 cervical carcinoma, 1 post-transplant lymphoproliferative disorder, 1 diffuse large B Cell lymphoma, 1 disseminated melanoma, and 1 rectal cancer.

Table S2. Neutralization against BA.4/5, BQ.1.1, and XBB.1.5 in different clinical settings

Outcomes	4th dose monovalent vaccine (N=86)	Bivalent booster (N=25)	Bivalent and previous COVID-19 (N=22)	Post-BA.1 infection (N=75)	Post-BA.5 infection (N=50)
BA.4/5 humoral response					
Neutralization titer – median (IQR)	0·85 (0 to 2·58)	2·55 (0·81 to 3·30)	3·19 (2·68 to 3·64)	2·44 (0 to 3·25)	3·22 (2·84 to 3·68)
Adjusted p value *	0·02	Reference	0·07	0·5	0·02
Detected neutralization – no. (%)	43 (50·0%)	18 (72·0%)	21 (95·5%)	52 (69·3%)	46 (92·0%)
Odds ratio of neutralization detected (95% CI) †	0·39 (0·15 – 1·03)	Reference	8·17 (1·15 – 72·8)	0·88 (0·33 – 2·35)	4·47 (1·23 – 16·1)
BQ.1.1 humoral response					
Neutralization titer – median (IQR)	0 (0 to 1·67)	0 (0 to 2·53)	2·31 (0 to 2·89)	1·66 (0 to 2·23)	2·62 (2·03 to 2·97)
Adjusted p value *	0·1	Reference	0·08	0·3	<0·001
Detected neutralization – no. (%)	22 (25·6%)	9 (36·0%)	15 (68·2%)	39 (52·0%)	42 (84%)
Odds ratio of neutralization detected (95% CI) †	0·61 (0·24 – 1·55)	Reference	3·81 (1·15 – 12·6)	1·93 (0·77 – 4·81)	9·33 (3·12 – 28·0)
XBB.1.5 humoral response					
Neutralization titer – median (IQR)	0 (0 to 0)	0 (0 to 2·16)	2·13 (0 to 2·66)	1·71 (0 to 2·42)	2·53 (0 to 3·25)
Adjusted p value *	0·07	Reference	0·14	0·25	0·003
Detected neutralization – no. (%)	15 (17·4%)	10 (40·0%)	16 (72·7%)	39 (52·0%)	37 (74·0%)
Odds ratio of neutralization detected (95% CI) †	0·32 (0·12 – 0·82)	Reference	4 (1·19 – 13·4)	1·63 (0·66 – 4·01)	4·27 (1·56 – 11·8)

CI: Confidence interval

* Displays the adjusted p values of the comparison between the median neutralization titer against each Omicron subvariant of the bivalent vaccine without previous COVID-19 group (reference) and the median of each of the other groups. P values were estimated with Dunn's test with Holm-Sidák adjustment for multiple comparisons.

Table S3: T cell activity against the different Omicron subvariants in transplant recipients that received a bivalent mRNA vaccine (Original strain / Omicron BA.4/5) by the presence of previous COVID-19.

Variable	No previous COVID-19 (N=22)	Previous COVID-19 (N=13)	Difference of medians (95% CI)
BA.4/5 T cell activity			
IFN-γ CD4	182 (25·6 to 248·6)	184 (117·4 to 452)	-2 (-304·8 to 300·7)
IL-2 CD4	897·5 (241·2 to 2706·9)	1134·6 (539·1 to 2202·2)	-237·2 (-1720·8 to 1246·5)
Poly CD4	355·8 (87·6 to 1225·7)	322·4 (129·1 to 875·1)	33·4 (-593·7 to 660·5)
Total IFN-γ CD8	67·2 (1 to 453·4)	127·3 (1 to 351·9)	-60·2 (-254·3 to 134)
BQ.1.1 T cell activity			
IFN-γ CD4	116·1 (31·1 to 289·9)	227·9 (169·2 to 536·4)	-111·9 (-419·6 to 195·9)
IL-2 CD4	1077 (266·1 to 2472·9)	927·9 (521·5 to 2334·4)	149·1 (-1308·2 to 1606·4)
Poly CD4	378·7 (106·4 to 1032·7)	284·8 (199 to 639·2)	93·85 (-570·6 to 758·3)
Total IFN-γ CD8	86·9 (1 to 535·6)	84 (15·5 to 311·8)	2·85 (-305·1 to 310·8)
XBB.1.5 T cell activity			
IFN-γ CD4	189·3 (101·5 to 258·6)	155·2 (107·3 to 278·9)	34·1 (-642·1 to 710·3)
IL-2 CD4	972·6 (948·3 to 2946·8)	775·8 (296 to 2844·1)	196·8 (-6542·5 to 6936·1)
Poly CD4	421·3 (352·8 to 990·3)	229·3 (64·6 to 1214·2)	192 (-4056·7 to 4440·7)
Total IFN-γ CD8	634·8 (100·2 to 1352·7)	165·9 (1 to 3964·1)	468·9 (-21283·4 to 22221·2)

Abbreviations: CI: Confidence Interval; IFN-γ: Interferon gamma; IL: Interleukin

SUPPLEMENTARY FIGURES:

Figure S1. Variant-specific t-cell responses in patients that received bivalent vaccine divided into those with and without a history of covid-19.

IFN-γ monofunctional (A), IL-2 monofunctional (B) and polyfunctional (IFN-γ^+ and IL-2^+) $CD4^+$ T-cells (C), and total IFN-γ expressing $CD8^+$ T cells (D) were measured in transplant recipients with and without prior COVID-19. Antigen-specific responses were measured against BA.4/5, BQ.1.1 and XBB.1.5. Abbreviations: COV=COVID-19, IFN-γ – interferon gamma, IL-2 – interleukin 2, poly – polyfunctional

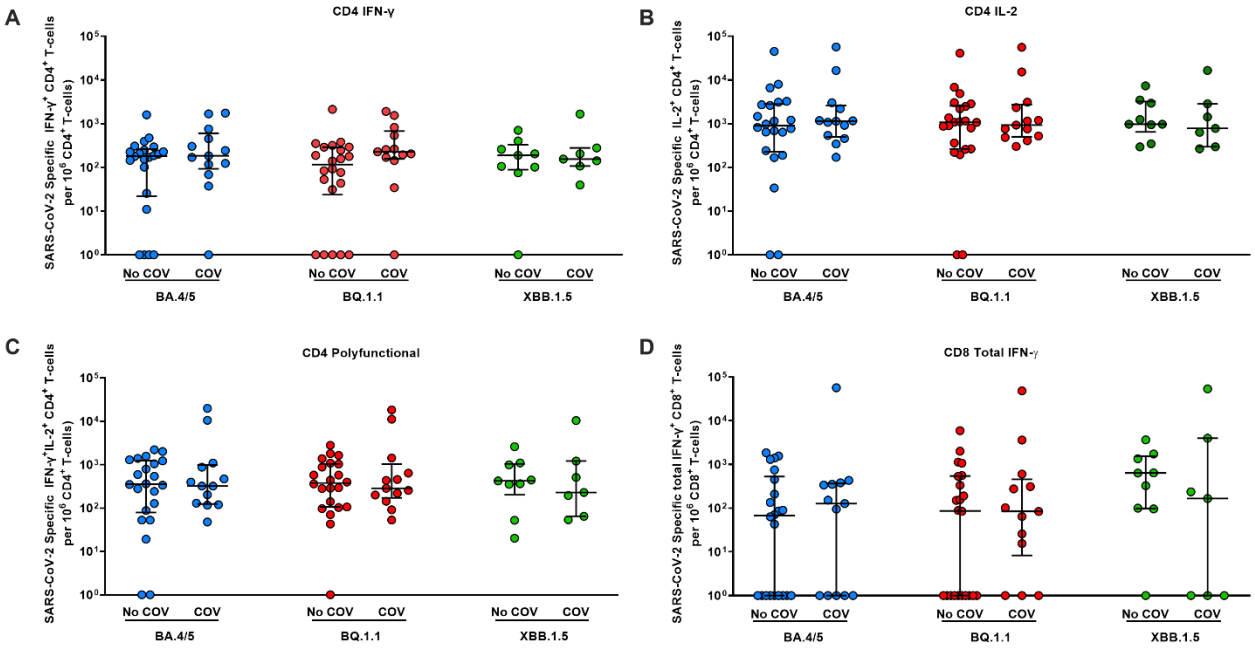

Figure S2. Comparison of bivalent T-cell responses to previous monovalent immunization.

Polyfunctional (IFN-γ^+ and IL-2^+), IFN-γ monofunctional and IL-2 monofunctional $CD4^+$ T-cells, and total IFN-γ expressing $CD8^+$ T cells were measured in n=9 individuals to their previous monovalent immunization, and their subsequent bivalent immunization. T-cells responses are shown against BA.4/5 (A) and BQ.1.1 (B). A two-sided Wilcoxon matched-pairs signed-rank test with Holm–Šídák correction for multiple comparisons was used. Adjusted p-values are shown above each respective comparison. Abbreviations: SARS-CoV-2 - severe acute respiratory syndrome Coronavirus-2, IFN-γ – interferon gamma, IL-2 – interleukin 2, poly – polyfunctional.

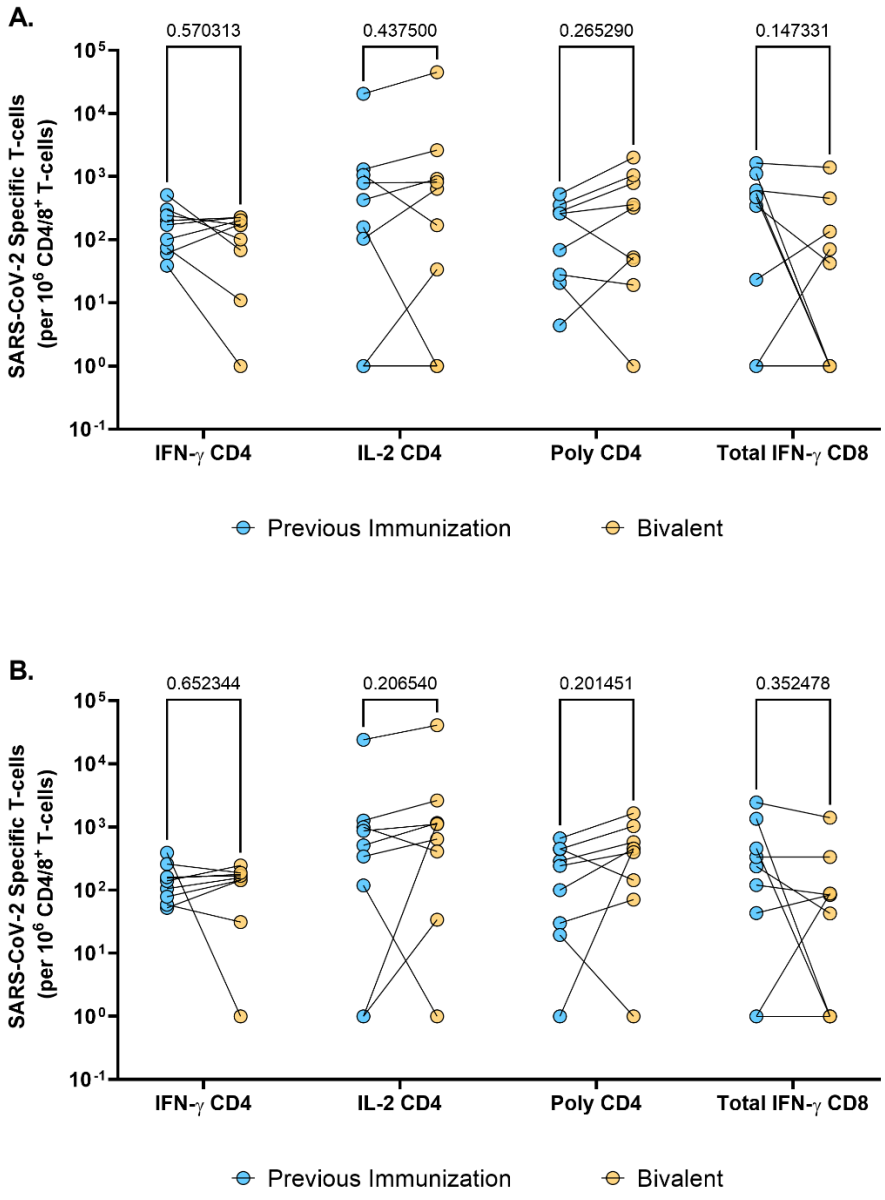

Figure S3. Immune response in subgroup of n=36 solid organ transplant recipients who had neutralization tested after receiving the fourth dose of a monovalent mRNA vaccine and after the bivalent vaccine.

Scatter dot plots with connectors of the neutralizing antibody titer against Omicron BA.4/5, BQ.1.1, and XBB.1.5 four to six weeks after receiving the 4th dose of a monovalent mRNA COVID-19 vaccine and same patients four to six weeks after receiving a bivalent booster vaccine. Each dot represents one patient, and the connectors join each patient antibody titer after receiving each vaccine. The yellow dots represent the patients that were diagnosed of COVID-19 in between the doses. The top horizontal line represents the 75th percentile, the next, the median, and the bottom one the 25th percentile. The vertical line delimits the interquartile range.

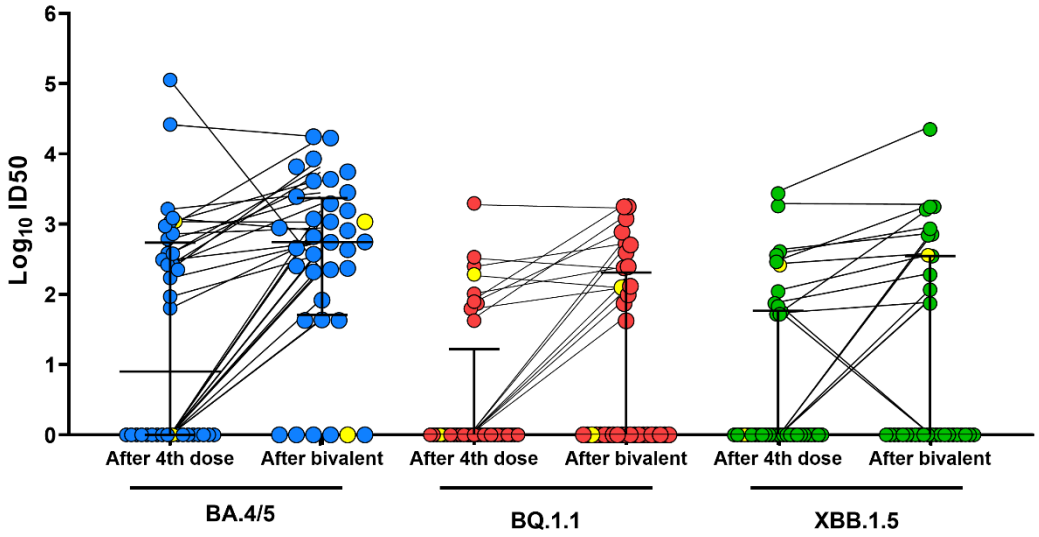

Figure S4. Comparison of BA.4/5-directed T-cell responses in a cohort of n=20 SOT solid organ transplant recipients post fourth dose without prior history of COVID-19 and in n=22 patients after receiving bivalent vaccination (also without prior history of COVID-19).

Scatter dot plots of the BA.4/5-directed T-cell responses four to six weeks after receiving the 4th dose of a monovalent mRNA COVID-19 vaccine (n=20) and after four to six weeks after receiving a bivalent booster vaccine (n=22). Each dot represents one independent patient. The top horizontal line represents the 75th percentile, the next, the median, and the bottom one the 25th percentile. The vertical line delimits the interquartile range.