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ORIGINAL ARTICLE

An additional dose of viral vector COVID-19 vaccine and mRNA COVID-19 vaccine in kidney transplant recipients: A randomized controlled trial (CVIM 4 study)

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Immunogenicity following an additional dose of Coronavirus disease 2019 (COVID-19) vaccine was investigated in an extended primary series among kidney transplant (KT) recipients. Eighty-five KT participants were randomized to receive either an mRNA (M group; $n = 43$) or viral vector (V group; $n = 42$) vaccine. Among them, 62% were male, with a median (IQR) age of 50 (43–59) years and post-transplantation duration of 46 (26–82) months. At 2 weeks post-additional dose, there was no difference in the seroconversion rate between the M and V groups (70% vs. 65%, $p = .63$). A median (IQR) of anti-RBD antibody level was not statistically different between the M group compared with the V group (51.8 [5.1–591] vs. 28.5 [2.9–119.3] BAU/ml, $p = .18$). Furthermore, the percentage of participants with positive SARS-CoV-2 surrogate virus neutralization test results was not statistically different between groups (20% vs. 15%, $p = .40$). S1-specific T cell and RBD-specific B cell responses were also comparable between the M and V groups (230 [41–420] vs. 268 [118–510], $p = .65$ and 2 [0–10] vs. 2 [0–13] spot-forming units/10^6 peripheral blood mononuclear cells, $p = .60$). In conclusion, compared with an additional dose of viral vector COVID-19 vaccine, a dose of mRNA

Abbreviations: ACE2, angiotensin-converting enzyme 2; AE, adverse events; BAU, binding antibody unit; CI, confidence interval; CMI, SARS-CoV-2-specific cell-mediated immunity; CNI, calcineurin inhibitors; COVID-19, coronavirus disease 2019; ELISpot, enzyme-linked immunospot assay; HMI, SARS-CoV-2-specific humoral immunity; IFN-γ, interferon-γ; IgG, immunoglobulin G; IQR, interquartile range; KT, kidney transplant; LT, liver transplant; MMF, mycophenolate mofetil; MPA, mycophenolic acid; MPS, mycophenolate sodium; mRNA, messenger ribonucleic acid; N, SARS-CoV-2 nucleoprotein; OR, odds ratio; ORF, open reading frame; PBMC, peripheral blood mononuclear cell; RBD, receptor-binding domain; RT-PCR, reverse transcription-polymerase chain reaction; S, SARS-CoV-2 spike glycoprotein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot-forming units; SOT, solid organ transplantation; SVNT, SARS-CoV-2 surrogate virus neutralization test.

COVID-19 vaccine did not elicit significantly different responses in KT recipients, regarding either humoral or cell-mediated immunity. (TCTR20211102003).

KEYWORDS
BNT162b2, booster, ChAdOx1 nCoV-19, immunization, immunocompromised, mRNA-1273, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) poses an elevated threat for immunocompromised individuals.[1] Solid organ transplant (SOT) recipients are considered to be at greater risk for developing severe COVID-19, which carries a higher risk of mortality.[2,3] Although a standard two-dose COVID-19 vaccine regimen was implemented, suboptimal immunogenicity has been noticed across vaccine platforms. Inactivated COVID-19 vaccines produce relatively weak immune responses in kidney transplant (KT) recipients compared with adenoviral-vectored or messenger ribonucleic acid (mRNA)-based vaccines.[4–6] Therefore, the World Health Organization has recommended the use of an extended primary series, that is, the addition of an extra dose of COVID-19 vaccine, in these vulnerable populations.[7]

The overall anti-receptor-binding domain (RBD) seroconversion rate after an additional dose was found to increase by 10%–20%, depending on the previous COVID-19 vaccine regimen and the type of additional vaccine. Although most studies were predominately observational, and different cut-off values for seroconversion have been applied,[7] two randomized control trials of a third mRNA COVID-19 vaccine dose did reaffirm the increasing trend in immune responses observed after the first two mRNA COVID-19 vaccine doses in SOT recipients.[8,9] Most studies focused on a homologous vaccine regimen; heterologous regimens, especially with an inactivated vaccine, have been less investigated. Our study reported that those previously vaccinated with a heterologous regimen (inactivated followed by adenoviral vector vaccine) displayed a significantly greater response, although the response was worse in KT recipients than in immunocompetent individuals. Here, we aimed to compare the immunogenicity following an additional COVID-19 vaccine dose in KT recipients who had previously completed a primary COVID-19 vaccine series.

2 | MATERIALS AND METHODS

2.1 | Study design

A non-blinded, randomized, control trial (CVIM 4 study) was conducted at a single transplant center at the Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand, from November 2021 to January 2022. Eligible adult KT recipients were randomized using a stratified (by previous vaccine regimen) block randomization approach to receive either an mRNA vaccine (BNT162b2 [Pfizer-BioNTech] or mRNA-1273 [Moderna]; M group) or the ChAdOx1 nCoV-19 vaccine (AstraZeneca; V group).

At 2 weeks post-vaccination, participant humoral immunity (HMI) was evaluated by anti-RBD immunoglobulin G (IgG) level and by percentage of neutralizing antibody inhibition measured with a surrogate viral neutralization test (%SVNT), and participant cell-mediated immunity (CMI) was evaluated by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific, interferon-γ (IFN-γ)-producing T and B cell responses measured with an enzyme-linked immunospot (ELISpot) assay. Anti-RBD seroconversion predictors were assessed, and post-vaccination adverse events (AEs) were monitored.

The primary endpoint was the seroconversion rate of the anti-SARS-CoV-2 RBD IgG antibody levels at 2 weeks after an additional dose of each COVID-19 vaccine. The secondary endpoints were the rate of SVNT positivity, S1-specific T cell and receptor-binding domain-specific B cell responses, and safety profiles following the vaccination.

Those who had participated in the CVIM 1, CVIM 2, and CVIM 3 studies (Figure S1), in which vaccination regimens consisting of primary series two doses of CoronaVac (CVIM 1; 3–5 weeks apart)[4] or followed by one dose of ChAdOx1 nCoV-19 vaccine (CVIM 2; 11–13 weeks after)[10] or primary series two doses of ChAdOx1 nCoV-19 vaccine (CVIM 3; 11–13 weeks apart), were invited to participate. Adults aged ≥18 years old with stable kidney allograft function on an immunosuppressive regimen, at least 30 days posttransplant were considered eligible (see Supplementary protocol for full inclusion/exclusion criteria). Potential participants were excluded if they were suspected of having a respiratory tract infection or any concurrent infection, were receiving intense immunosuppressants for kidney allograft rejection or had a previous COVID-19 history. All included patients were verbally screened for active respiratory tract infection and COVID-19 exposure, but did not provide samples for SARS-CoV-2 reverse transcription-polymerase chain reaction (RT-PCR) to exclude COVID-19. The patients' current immunosuppressive regimens were continued during the study. Patient demographic and transplant characteristics were collected. A low therapeutic dose of mycophenolic acid (MPA) was defined as ≤1 g/day of mycophenolate mofetil (MMF) or ≤720 mg/day of mycophenolate sodium (MPS).[11] A low C_0 level of calcineurin inhibitor (CNI) was defined as ≤5 ng/ml tacrolimus or ≤150 ng/ml cyclosporine.[12]

2.2 | SARS-CoV-2 humoral immune responses

Anti-SARS-CoV-2 RBD IgG antibody levels were measured using the Abbott SARS-CoV-2 IgG II Quantification assay (Abbott SARS-CoV-2 IgG II Quant assay; Abbott). Plasma samples were run on the Abbott Alinity instrument following the manufacturer's instructions.

The assay is a chemiluminescent microparticle immunoassay for quantitative detection of SARS-CoV-2 spike protein RBD-specific IgG in human serum. Quantitative anti-RBD IgG results are reported in binding antibody units (BAU)/ml; seroconversion was defined as levels of ≥7.1 BAU/ml. A sensitivity of 91.6% and a specificity of 99.4% were reported with this particular threshold.[13]

The functionality of the anti-SARS-CoV-2 spike protein S1 RBD antibody was determined by using a SARS-CoV-2 NeutraLISA surrogate neutralization test assay (Euroimmun). The neutralizing antibodies in plasma compete with biotinylated angiotensin-converting enzyme 2 (ACE2) receptor for binding to the SARS-CoV-2 spike protein S1/RBD domain. Later, further incubation with peroxidase-labeled streptavidin catalyzes a color reaction of the bound ACE2. The intensity of the formed color is inversely proportional to the neutralizing antibody concentration in the sample. Neutralizing antibody inhibition is reported in percentages; positive results were defined as having ≥35% inhibition. With this threshold, a diagnostic sensitivity of 95.9% and a specificity of 99.7% were reported.[14]

2.3 | SARS-CoV-2-specific cell-mediated immune responses

Heparinized whole blood samples were collected from participants, and peripheral blood mononuclear cells (PBMCs) were isolated using the EasySep™ Direct Human PBMC Isolation Kit (Stemcell Technologies) in accordance with the manufacturer's protocol. Isolated cells were counted, and each cell suspension was normalized to a final concentration of 2.5×10^6 cells/ml in AIM V media (Gibco).

IFN-γ production by activated PBMCs was measured using ELISpot assays conducted with a Human IFN-γ ELISpot PRO kit (ALP) (Mabtech). ELISpot plates were washed four times with 200 µl/well of Dulbecco's phosphate-buffered saline (PBS; Gibco) and then blocked with AIM V media (Thermo Fisher Scientific, Waltham, MA) for ≥30 min. PBMCs (2.5×10^5/100 µl of AIM V) were stimulated under four conditions: (1) AIM V negative control; (2) SARS-CoV-2 S1 domain of the spike protein scanning peptide pool (Mabtech); (3) SNMO (SARS-CoV-2 spike protein, nucleoprotein, membrane protein, open reading frame [ORF]-3a, and ORF-7a proteins) peptide pool (Mabtech); and (4) anti-CD3 antibodies as a positive control. The final concentration of each peptide was 2 µg/ml. After incubation for 40 h at 37°C and 5% CO_2, the cells were removed, and IFN-γ production was determined using an enzyme-conjugated detection mAb (7-B6-1-ALP) for 2 h at room temperature, followed by treatment with 100 µl of ready-to-use BCIP®/NBTLiquid substrate (Pierce). After each step, the plates were washed five times with 200 µl/well of Dulbecco's PBS. Results are reported as IFN-γ-producing spot-forming units (SFUs) per 10^6 PBMCs for each peptide pool.[15]

Anti-SARS-CoV-2 RBD IgG antibody-secreting cells (memory B cells) were measured using ELISpot assays with a Human IgG (SARS-CoV-2, RBD) ALP (Mabtech). For memory B cell stimulation, PBMCs were incubated for 3 days with the toll-like receptor (TLR) agonist R848 and recombinant human IL-2 (human memory B cell stimpack; Mabtech AB) in RPMI-1640 medium with 10% FCS. The ELISpot plates were then washed four times with 200 µl/well of Dulbecco's PBS (Gibco) and blocked with RPMI-1640 medium with 10% FCS for ≥30 min. To detect B cells secreting anti-SARS-CoV-2 RBD IgG or secreting any IgG (total IgG), 5×10^5 or 5×10^4 stimulated B cells per well, respectively, were used. The cells were removed after an 18-h incubation at 37°C and 5% CO_2, and RBD-specific IgG was determined via incubations with RBD-WASP for 2 h, followed by anti-WASP-ALP for 1 h, whereas total IgG was determined via incubations with an MT78/145-biotin for 2 h, followed by Streptavidin-ALP for 1 h. All wells were finally treated with 100 µl of ready-to-use BCIP®/NBTLiquid substrate (Pierce). After each step, plates were washed five times with 200 µl/well of Dulbecco's PBS. Results are reported as B cells secreting anti-SARS-CoV-2 RBD IgG and those secreting any IgG (total IgG).[15]

Emerged spots were counted using an ImmunoSpot analyzer (Cellular Technology Limited), and spot quality was checked using ImmunoSpot Software v5.0.9.15.

2.4 | Safety

All participants were monitored for 30 min post-vaccination to observe any local or systemic AEs. Solicited AEs were then assessed via phone call on days 3 and 7 post-vaccination, and unsolicited AEs were self-recorded by participants. All AEs were graded for severity by the investigators. Participants with potentially serious AEs or respiratory tract infections were investigated for diagnosis and treatment at the facility per standard of care.

2.5 | Statistical analyses

Categorical variables are summarized as frequency with percentage and continuous variables are expressed as the median with interquartile range (IQR). The variables are summarized by randomized group. The chi-squared test, Fisher's exact test, or Mann–Whitney U test were used to assess differences between randomized groups in the distribution of categorical and continuous variables as appropriate. The distribution of anti-RBD IgG level, percentage of neutralization inhibition, and numbers of S1-specific T cells and RBD-specific B cells in SFUs/10^6 PBMCs are presented as dot plots with bars representing the median with IQR, generated by GraphPad Prism 6.0 (GraphPad Software, Inc.). An immunogenicity and safety issues were compared between the M and V groups in the intention-to-treat and per-protocol analyses. The associated factors and anti-RBD seroconversion were analyzed using univariable logistic regression models and are presented as a Forest plot. *p*-values of <.05 were considered to indicate a significant difference. Statistical analyses were performed with Stata statistical software, version 15 (StataCorp, LLC). We hypothesized that at least 80% of participants who were randomized to receive an additional mRNA vaccine would be seroconverted by 2 weeks post-vaccination compared with the

expected 50% of those who received a viral vector vaccine. For two independent study groups, to achieve a power of 80% and an alpha level of .05 at 1:1 randomization, a sample size of 78 patients is required (39 patients each group). We enrolled 85 patients in accounting for a loss to follow-up of 10%.[16,17]

2.6 | Ethics approval

This study was registered under the Thai Clinical Trials Registry (identified number: TCTR20211102003). The study was reviewed and approved by the Institutional Review Board of the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (approval number: MURA2021/940). All participants provided signed consent prior to study participation.

3 | RESULTS

3.1 | Clinical characteristics

This study assessed 116 eligible patients; after 31 of them were excluded (Figure 1), 85 patients underwent randomization by *computer*-generated random numbers (M group, $n = 43$; V group, $n = 42$). Among them, 63% were male, with a median (IQR) age of 50 (43–59) years and median (IQR) post-transplantation duration of 46 (26–82) months. Twenty-six (34%) participants received two doses of CoronaVac followed by one dose of ChAdOx1 nCoV-19 vaccine, while 51 (66%) patients received two doses of ChAdOx1 nCoV-19 vaccine. Fifty-seven (77%) patients had received a deceased allograft; they have been maintained on tacrolimus (68%), cyclosporine (30%), corticosteroids (100%), mycophenolate mofetil (53%), mycophenolate sodium (29%), sirolimus (1%), and everolimus (6%). The clinical characteristics of the KT recipients are shown in Table 1. There was no difference in clinical or transplant characteristics or in existing anti-RBD antibody levels between groups. However, a non-statistically significant higher proportion of patients who were vaccinated within their first year post-transplantation were noted in the M group ($p = .06$). Seven patients received the COVID-19 vaccine in the first year after transplantation. In addition, six patients received interleukin-2 receptor antagonists for induction therapy. Of those, five and one patient were in the M and V groups, respectively.

One patient from the V group later withdrew their consent, and seven patients were lost during follow-up (four and three patients in the V and M groups, respectively). Therefore, there were 37 patients in the V group and 40 in the M group in our intention-to-treat (ITT) analysis. Six patients assigned to the V group requested to receive the mRNA vaccine for personal reasons. Two and four of them received BNT162b2 and mRNA-1273, respectively. All but two patients in the M group, who received mRNA-1273, were vaccinated with BNT162b2. Therefore, there were 31 and 40 patients in the V and M groups, respectively, in our per-protocol (PP) analysis.

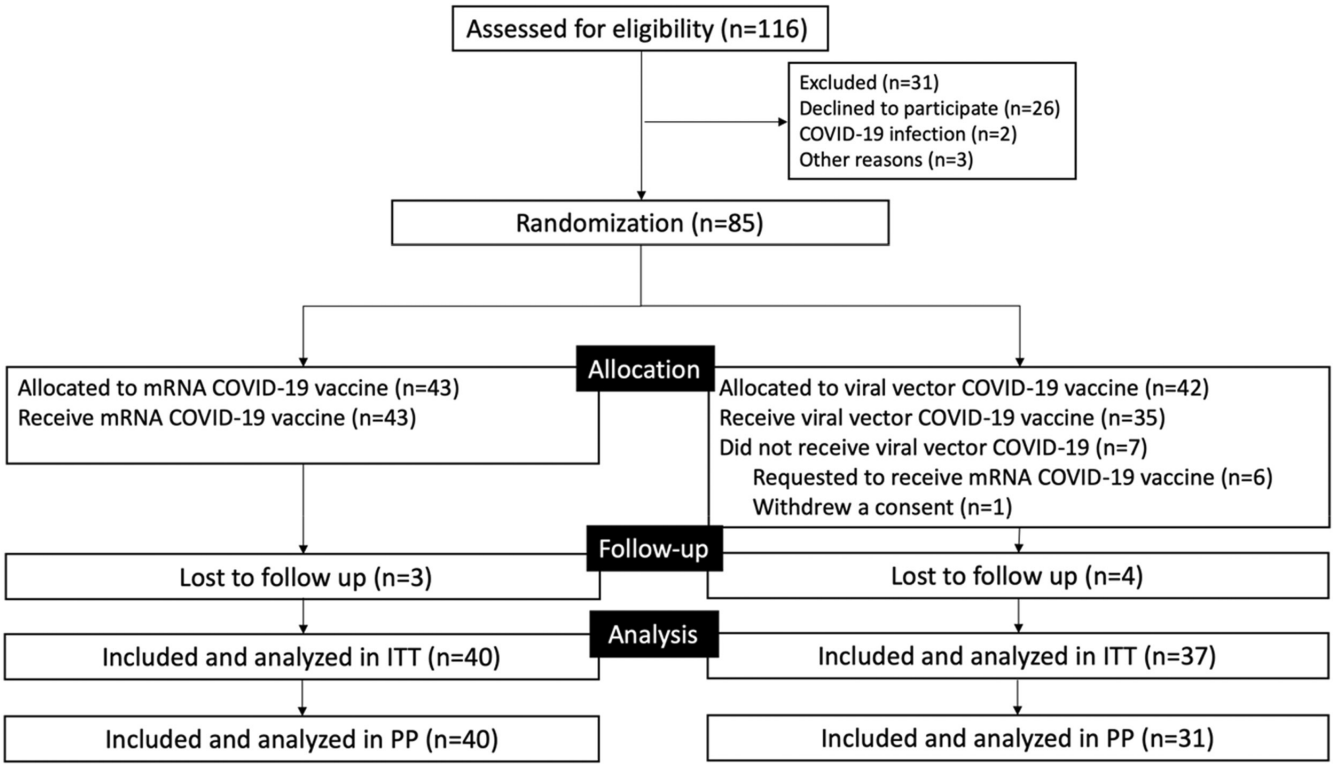

FIGURE 1 Study flow chart.

TABLE 1 Patient characteristics

Clinical characteristics	mRNA vaccine group ($n = 40$)	Viral vector vaccine group ($n = 37$)	p value
Age (years), median (IQR)	50 (41–57)	51 (43–59)	.57
Male sex, *n* (%)	25 (63)	22 (59)	.78
Posttransplant duration (months), median (IQR)	38 (20–66)	53 (30–90)	.22
Within 1-year posttransplant, *n* (%)	6 (15)	1 (3)	.06
Deceased allograft, *n* (%)	31 (78)	26 (70)	.47
Immunosuppressants, *n* (%)			
Tacrolimus, *n* (%)	27 (68)	23 (62)	.62
C_0 level [ng/ml], median (IQR)	5.5 (4.7–6.3)	5.5 (4.3–6.1)	.47
Cyclosporine, *n* (%)	11 (28)	12 (32)	.64
C_0 level [ng/ml], median (IQR)	100 (100–150)	100 (94–150)	.58
Low C_0 level of calcineurin inhibitors[a]	18 (47)	23 (66)	.11
Mycophenolate mofetil, *n* (%)	20 (50)	21 (57)	.55
Dose [mg/day], median (IQR)	1375 (1000–1500)	1500 (1000–1550)	.52
Mycophenolate sodium, *n* (%)	12 (30)	10 (27)	.77
Dose [mg/day], median (IQR)	900 (720–1080)	720 (675–1080)	.49
Low therapeutic dose of mycophenolic acid[b]	15/32 (47)	13 (42)	.28
Sirolimus, *n* (%)	1 (3)	0 (0)	1.00[c]
Everolimus, *n* (%)	4 (10)	1 (3)	.19
Prednisolone, *n* (%)	40 (100)	37 (100)	1.00[c]
Dose (mg/day), median (IQR)	5 (5–5)	5 (5–5)	.62
Absolute lymphocyte count (cells/μl), median (IQR)	2043 (1515–2625)	2025 (1485–2612)	.92
Previous vaccine regimen, *n* (%)			
Two-dose CoronaVac then ChAdoOx1 nCoV-19	14 (35)	12 (32)	.81
Two-dose ChAdoOx1 nCoV-19	26 (65)	25 (68)	
Existing anti-RBD antibody level [BAU/ml], median (IQR)	1.5 (0.4–25.9)	2.4 (0.6–12.9)	.67

Abbreviations: IQR, interquartile range; RBD, receptor-binding domain.

[a]Denominator is the number of participants who received a calcineurin inhibitor ($n = 73$).

[b]Denominator is the number of participants who received mycophenolic acid ($n = 63$).

[c]Fisher's exact test.

3.2 | Anti-RBD antibody seroconversion

Immunogenicity comparisons between the two study arms are presented in Table 2 and Figure 2. In an ITT analysis (Table 2), there was no difference in the seroconversion rate between the M and V groups (70% vs. 65%, $p = .63$). The SARS-CoV-2-specific HMI response, which was assessed by a median (IQR) of anti-RBD antibody levels at 2 weeks post-additional vaccine dose was not statistically different between the M group compared with the V group (51.8 [5.1–591] vs. 28.5 [2.9–119.3] BAU/ml, $p = .18$).

Overall, 52 (68%) participants achieved seroconversion. Of the 49 KT recipients who were previously seronegative, 24 (49%; 14 and 10 patients in the V and M groups, respectively) converted to seropositive after receiving an additional dose of vaccine. Assessed potential predictors of anti-RBD seroconversion in KT recipients following an additional dose of COVID-19 vaccine are presented in Figure 3 and Table S1. Those who had undergone KT more than year prior and had a higher absolute lymphocyte count had a significantly greater chance of seroconversion after receiving an additional vaccine dose (odds ratio [OR], 16.11; 95% confidence interval [CI], 1.82–142.69; $p = .01$, and OR, 1.64; 95% CI, 1.13–2.37; $p = .01$ per 500 cells/μl, respectively). Additionally, KT recipients who were maintained on a non-mycophenolic acid-based regimen were marginally more likely to achieve seroconversion after an additional vaccine dose (OR, 8.00; 95% CI, 0.98–65.10; $p = .05$). However, age, sex, previous or additional vaccine platform, mycophenolic acid dose, and trough CNI concentration were not statistically associated with seroconversion.

The same trends were observed in a PP analysis. This again did not result in a significantly greater seroconversion rate (70% vs. 61%, $p = .44$). The median (IQR) of anti-RBD antibody levels was also not significantly different in the M group compared with the V group (51.8 [5.1–591] vs. 28.3 [2.4–87.1] BAU/ml, $p = .09$).

TABLE 2 Immunogenicity in KT recipients after receiving an additional dose of mRNA or viral vector COVID-19 vaccine

	mRNA vaccine group	Viral vector vaccine group	*p* value
Intention-to-treat analysis	*N* = 40	*N* = 37	
Rate of seroconversion, *n* (%)	28 (70)	24 (65)	.63
Anti-RBD IgG (BAU/ml), median (IQR)	51.8 (5.1–591)	28.5 (2.9–119.3)	.18
%SVNT, median (IQR)	47 (0–98)	19 (8–81)	.24
Rate of SVNT positivity, *n* (%)	20 (50)	15 (41)	.40
S1-specific T cells (SFUs/10^6 PMBCs), median (IQR)	230 (41–420)	268 (118–510)	.65
RBD-specific B cells (SFUs/10^6 PMBCs), median (IQR)	2 (0–10)	2 (0–13)	.60
Per protocol analysis	N = 40	N = 31	
Rate of seroconversion, *n* (%)	28 (70)	19 (61)	.44
Anti-RBD IgG [BAU/ml], median (IQR)	51.8 (5.1–591)	28.3 (2.4–87.1)	.09
%SVNT, median (IQR)	47 (0–98)	14 (7–73)	.18
Rate of SVNT positivity, *n* (%)	20 (50)	12 (39)	.34
S1-specific T cells [SFUs/10^6 PMBCs], median (IQR)	230 (41–420)	232 (116–400)	.91
RBD-specific B cells [SFUs/10^6 PMBCs], median (IQR)	2 (0–10)	2 (0–9)	.11

Abbreviations: BAU, binding antibody unit; IgG, immunoglobulin G; IQR, interquartile range; KT, kidney transplant; PBMC, peripheral blood mononuclear cell, RBD, receptor-binding domain; S1, S1 domain of spike protein; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFUs, spot-forming units; %SVNT, percentage of neutralizing antibody inhibition measured with a surrogate SARS-CoV-2 neutralization test.

3.3 | Other outcomes

In an ITT analysis, the %SVNT and rate of positivity were not statistically different between the M and V groups ($p > .05$). Regarding the SARS-CoV-2-specific CMI response, the S1-specific T cell and RBD-specific B cell responses were comparable among the two groups (230 [41–420] vs. 268 [118–510] SFUs/10^6 PMBCs, $p = .65$ and 2 [0–1] vs. 2 [0–13] SFUs/10^6 PMBCs, $p = .60$, respectively). In a PP analysis, there was no difference in the %SVNT, S1-specific T cell responses, or RBD-specific B cell responses between the two groups ($p > .05$).

3.4 | Safety

There were no severe local or systemic AEs within the first 30 min after vaccination in any participants. Solicited AEs for each group are presented in Table 3 and Figure S2. In the V and M groups, 6% and 7%, respectively, reported having solicited AEs within the first 3 days post-vaccination ($p = .23$), the majority of which were classified as grade 1 in severity. Pain at the injection site was the most common symptom reported in both groups, with a significantly greater number of patients in the M group experiencing this compared with the V group ($p = .02$). A lower percentage of participants reported solicited AEs on day 7, with approximately 75% being free of complaints in each group ($p = .94$). Among those who experienced some side effects, all the AEs they reported were mild and had similar patterns between groups. Two patients, one in each group, reported an unsolicited AE, which was hemiparesis (grade 2) on the day after receiving the additional vaccine dose. Both instances were spontaneously resolved without medical intervention within 24 h, and further investigation found no permanent neurological deficit.

4 | DISCUSSION

We here report a randomized control study that directly compared the immunogenicity and safety of an additional COVID-19 vaccine dose in KT recipients who received different primary COVID-19 vaccination series. Those fully vaccinated with a standard regimen were randomly offered an extra dose of either the viral vector or an mRNA COVID-19 vaccine. The mRNA vaccine did not perform significantly better than the viral vector vaccine regarding the induction of seroconversion, neutralization inhibition, or SARS-CoV-2-specific T cell or B cell responses. However, our study revealed that approximately half of the patients seroconverted against RBD after an additional dose of COVID-19 vaccine. Higher lymphocyte numbers and a longer posttransplant duration prior to COVID-19 vaccination may be favorable predictors of improved COVID-19 vaccine immunogenicity in KT recipients. Furthermore, no worrisome short-term safety issues were indicated.

Owing to suboptimal immune responses in SOT recipients after two doses of COVID-19 vaccine, an additional dose was recommended to extend the primary vaccine series for these

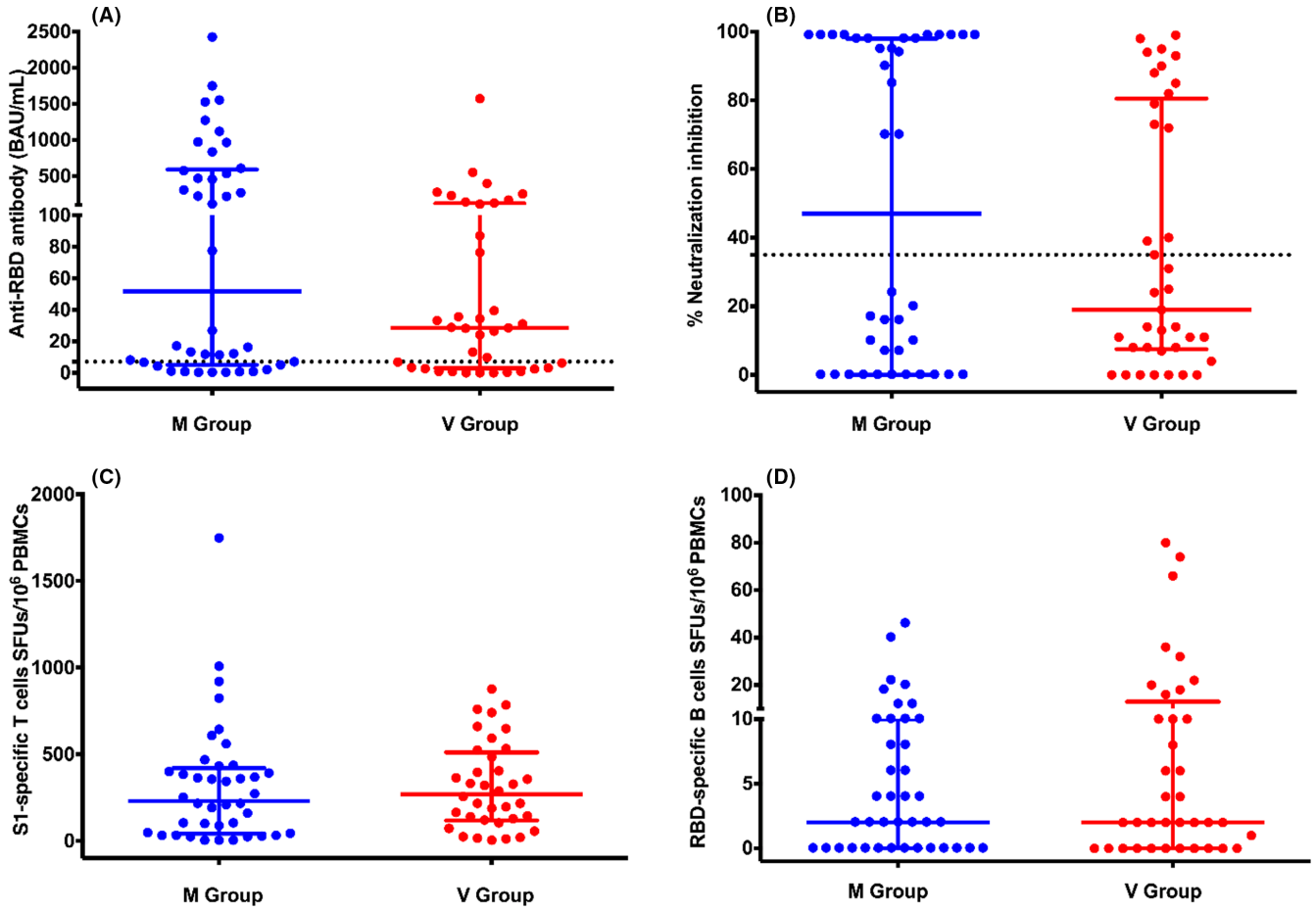

FIGURE 2 Immunogenicity at 2 weeks post-additional vaccine dose. Kidney transplant (KT) recipients received either an mRNA vaccine (BNT162b2 or mRNA-1273; M group) or a dose of ChAdOx1 nCoV-19 vaccine (AstraZeneca; V group). (A) Using scatter dot plots, anti-receptor-binding domain (RBD) antibody levels are presented in binding antibody units (BAUs)/ml. Each dot represents an individual participant, and horizontal lines indicate the median and interquartile range (IQR). The dotted line indicates the threshold value of 7.1 BAU/ml. (B) The percentages of neutralization inhibition are presented. The dotted line indicates the 35% threshold for neutralization positivity. Horizontal lines indicate the median and IQR. (C, D) SARS-CoV-2-specific, IFN-γ-producing T cell responses to the S1 protein (C) and SARS-CoV-2-specific, IFN-γ-producing B cell responses to the RBD (D) are presented in scatter dot plots. Horizontal lines indicate the median and IQR. IFN-γ, interferon-γ; SFU, spot-forming unit; PBMCs, peripheral blood mononuclear cells; S, spike glycoprotein; S1, S1 domain of spike protein

immunocompromised individuals. Compared with a placebo, a third homologous dose of mRNA COVID-19 vaccine was shown, in two randomized control trials, to significantly improve immunogenicity in SOT recipients who had previously received two doses of mRNA vaccine.[8,9] Furthermore, another randomized clinical trial found that a third homologous dose with mRNA COVID-19 vaccine could induce an immune response with the same seroconversion rate as a heterologous regimen with viral vector COVID-19 vaccine in the same population.[18]

In settings where an inactivated or viral vector COVID-19 vaccine was initially implemented, inadequate immune responses were noted, particularly in KT recipients.[4,19] We finally reached close to 70% seroconversion in our cohort by achieving seroconversion in half of the previously non-responsive individuals. A study in immunocompetent individuals revealed that the use of an mRNA-based COVID-19 vaccine as a booster dose could induce stronger immune responses compared with the use of vaccines from other platforms in nations where the population was immunized with the standard two doses of ChAdOx1 nCoV-19 vaccine.[17] However, the difference in anti-RBD antibody levels induced by the mRNA COVID-19 vaccines in our population did not reach statistical significance compared to those induced by the viral vector COVID-19 vaccine. Additionally, the lack of statistical significance detected by our ITT analysis for the seroconversion rate was likely due to pre-existing anti-RBD antibody levels in some patients, particularly the few patients in the V group who received an mRNA vaccine. Our PP analysis likewise revealed a trend toward higher seroconversion rates in the M group, but this difference also failed to reach statistical significance.

Other studies on this topic have mainly investigated and compared T cell responses, while B cell responses, have not been explored much. Here, we observed low and comparable quantities of IFN-γ-producing T cells after stimulation with S1 antigen in both study groups, which is the same pattern as the previous result of an additional dose between viral vector and mRNA vaccine with

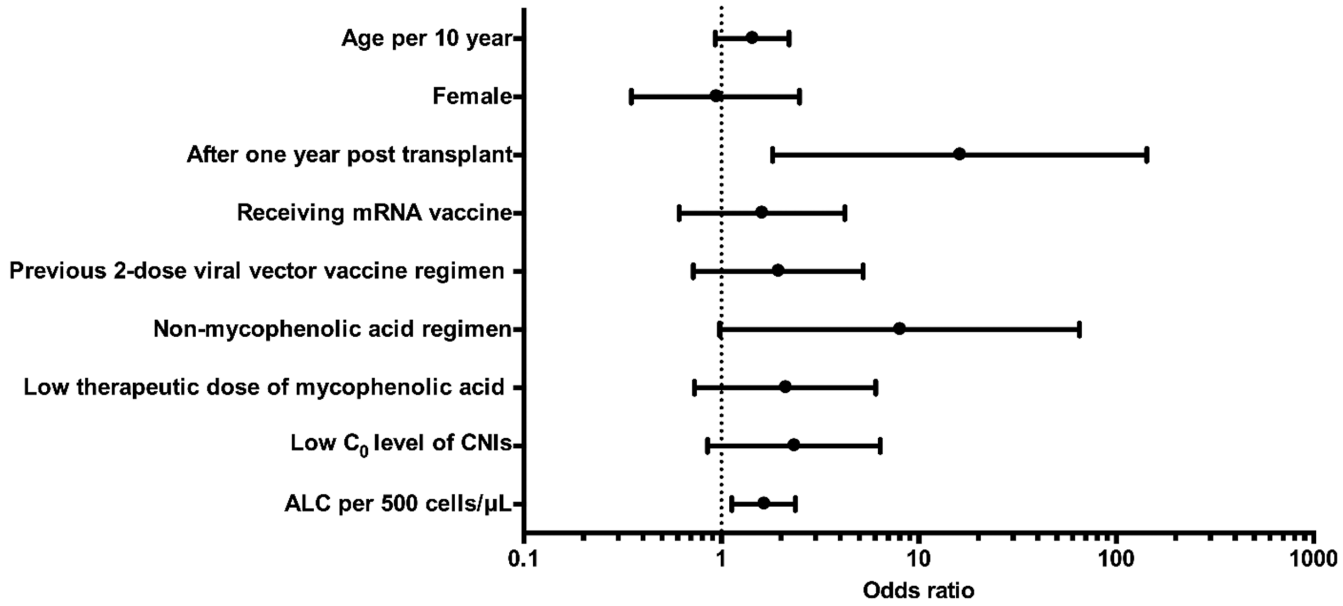

FIGURE 3 Forest plot of potential predictors of anti-RBD seroconversion after an additional COVID-19 vaccine dose. ALC, absolute lymphocyte count; CNIs, calcineurin inhibitors; mRNA, messenger ribonucleic acid

TABLE 3 Solicited adverse events in kidney transplant recipients in the mRNA vaccine and viral vector vaccine groups on day 3 and day 7 after receiving an additional COVID-19 vaccine dose

Solicited adverse events	mRNA vaccine group ($N = 40$)	Viral vector vaccine group ($N = 37$)	*p* value
Day 3			
Adverse events	37 (93)	31 (94)	.23
Grade 1	36 (97)	30 (97)	.89
Grade 2	1 (3)	1 (3)	
Grade 3	0 (0)	0 (0)	
Pain at the injection site	31 (78)	17 (46)	.02
Muscle aches	8 (20)	7 (19)	.90
Increased appetite	0 (0)	3 (8)	.11
Fever	6 (15)	5 (14)	.85
Sleepiness	3 (8)	6 (16)	.23
Others	9 (23)	9 (24)	.85
Day 7			
Adverse events	10 (25)	9 (24)	.94
Grade 1	10 (100)	9 (100)	1.00[a]
Grade 2	0 (0)	0 (0)	
Grade 3	0 (0)	0 (0)	
Pain at the injection site	5 (13)	3 (8)	.53
Muscle aches	2 (5)	3 (8)	.58
Increased appetite	0 (0)	1 (3)	.48
Fever	1 (3)	1 (3)	.96
Sleepiness	0 (0)	1 (3)	.48
Others	2 (5)	2 (5)	.94

[a]Fisher's exact test.

two-dose mRNA prime-boost vaccination among KT recipients.[15] Furthermore, the levels of IFN-γ-producing B cells, proposed to be memory B cells, which are believed to be a core immune component and marker for the sustainability of memory cells, were low in both groups which could be explained by a weak T cell responses upfront.[20]

Previous studies have shown that high therapeutic doses of MPA, defined as >1 g/day of MMF or >720 mg/day of MPS, can blunt the immune response induced by the primary series of inactivated, viral vector, or mRNA COVID-19 vaccines in KT recipients.[4,19,21] Here, we further explored potential predictors of failed seroconversion after an additional dose of COVID-19 vaccine. Although a high therapeutic dose of MPA was not associated with poor seroconversion in our study, a slight trend toward lower immune responses in those maintained on an MPA-based regimen was observed. Furthermore, shorter posttransplant duration was found to be an unfavorable predictor of seroconversion in our study, likely explained by the more profoundly immunosuppressive conditions in the aftermath of a transplant. KT recipients who were maintained on less than three immunosuppressive drugs are more likely to develop antibody responses, as are those with a longer posttransplant time.[16] In a French cohort study, lower posttransplant duration and high-maintenance immunosuppression were found to be unfavorable predictors for seroconversion in KT recipients.[22] These parameters could be used to create a COVID-19 immunization plan customized by individual immune status. The strategy of temporarily pausing immunosuppressant use has been proposed; however, allograft rejection is a barrier to implementing this practice, especially for those with high a immunologic risk or recent transplantation.[23] Although the mRNA COVID-19 vaccine was reported to induce more intense immune responses in both immunocompetent and immunocompromised patients,[8,9,24] when we specifically analyzed the magnitudes of the anti-RBD antibody level between subjects based on their actual type of vaccine immunization.

Even after receiving an extended COVID-19 vaccine series, a notable percentage (14%) of our study participants still had poor immune responses. An additional dose of BNT162b2 vaccine was found to improve the protection generated by primary immunization with two doses of BNT162b2 or ChAdOx1 vaccine, which may strengthen the protection against symptomatic disease with the Omicron variant.[25] Additionally, the sera of individuals vaccinated with three doses of homologous ChAdOx1 nCoV-19 vaccine were shown to neutralize omicron variants.[26] Our data could be helpful in settings where the primary COVID-19 vaccination regimen included a non-mRNA-based or viral vector vaccine. However, strategies to produce an immune response in these patients are needed. In the meantime, such individuals must rely on strict precautions against contracting COVID-19.

The assessment of potential safety issues following any vaccine regimens offered to KT patients is essential. Our data indicate that the majority of COVID-19 vaccine-associated AEs in the tested regimens are mild, suggesting there is no safety concern regarding their use. The observed AE profile is similar to those reported in previous third-dose COVID-19 vaccine studies.[8,9,18] Our data support the findings of the COV-BOOST study from England, in which immunocompetent individuals who were fully vaccinated with ChAdOx1 nCoV-19 or BNT162b2 were offered various COVID-19 vaccine types as a booster dose.[17] Some of our participants experienced more pain on the injected side after receiving the mRNA vaccine compared with the viral vector vaccine. Overall, our safety findings on an additional dose of COVID-19 vaccine for the immunocompromised subgroup of KT recipients are reassuring.

Several limitations of this study must be noted, such as its non-blind nature; a few patients requested to switch from one vaccine platform to another owing to concern about the weaker efficacy of a particular COVID-19 vaccine. This could affect the study's power due to an unexpectedly lower number of participants in the V group. Moreover, a lack of RT-PCR testing to exclude those who could have had an infection possibly resulted in the lack of difference in seroconversion rate. Due to a unique vaccine regimen previously received in our cohort and lacking the immunogenicity data on this particular regimen, the anticipated rate of seroconversion was postulated from our previous immunogenicity data (CVIM 2 and 3 studies). Therefore, an expected 30% absolute difference in seroconversion rate used for sample size calculation could limit a study's power. Additionally, a head-to-head comparison of immunogenicity may not elucidate due to variation in vaccine regimen, the duration between the vaccine and immune measurement (ranging from 2 to 4 weeks), and threshold to determine seroconversion.[27] Furthermore, it is not yet possible to assess the long-term immunogenicity and safety pattern of this multiple COVID-19 vaccine regimen in SOT recipients. Additionally, our data may not remain applicable to newly evolved circulating SARS-CoV-2 strains.

In conclusion, our study revealed comparable humoral and cell-mediated immune responses after an additional mRNA or viral vector vaccine dose in an extended COVID-19 vaccine series in KT recipients. Individuals with a greater lymphocyte count or a longer period since their transplant may have a higher chance of being seroconverted. Importantly, both COVID-19 vaccine types can be offered to KT recipients without concern regarding short-term adverse reactions.

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DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Bruminhent J, Ruangsubvilai N, Nabhindhakara J, Ingsathit A, Kiertiburanakul S. Clinical characteristics and risk factors for coronavirus disease 2019 (COVID-19) among patients under investigation in Thailand. *PloS One*. 2020;15(9):e0239250.
2. Fung M, Babik JM. COVID-19 in immunocompromised hosts: what we know so far. *Clin Infect Dis*. 2021;72(2):340-350.
3. Hadi YB, Naqvi SFZ, Kupec JT, Sofka S, Sarwari A. Outcomes of COVID-19 in solid organ transplant recipients: a propensity-matched analysis of a large research network. *Transplantation*. 2021;105(6):1365-1371.
4. Bruminhent J, Setthaudom C, Chaumdee P, et al. SARS-CoV-2-specific humoral and cell-mediated immune responses after immunization with inactivated COVID-19 vaccine in kidney transplant recipients (CVIM 1 study). *Am J Transplant*. 2022;22(3):813-822.
5. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384(5):403-416.
6. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;383(27):2603-2615.
7. World Health Organization. Interim recommendations for an extended primary series with an additional vaccine dose for COVID-19 vaccination in immunocompromised persons. 2021. 2022 [cited 2022 February 11, 2022]. Available from: https://apps.who.int/iris/bitstream/handle/10665/347079/WHO-2019-nCoV-Vaccination-SAGE-recommendation-Immunocompromised-persons-2021.1-eng.pdf?sequence=2.
8. Hall VG, Ferreira VH, Ku T, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. *N Engl J Med*. 2021;385(13):1244-1246.
9. Kamar N, Abravanel F, Marion O, Couat C, Izopet J, Del Bello A. Three doses of an mRNA Covid-19 vaccine in solid-organ transplant recipients. *N Engl J Med*. 2021;385(7):661-662.
10. Bruminhent J, Setthaudom C, Kitpermkiat R, et al. Immunogenicity of ChAdOx1 nCoV-19 vaccine after a two-dose inactivated SARS-CoV-2 vaccination of dialysis patients and kidney transplant recipients. *Sci Rep*. 2022;12(1):3587.
11. Azzi Y, Raees H, Wang T, et al. Risk factors associated with poor response to COVID-19 vaccination in kidney transplant recipients. *Kidney Int*. 2021;100(5):1127-1128.
12. Schiff J, Cole E, Cantarovich M. Therapeutic monitoring of calcineurin inhibitors for the nephrologist. *Clin J Am Soc Nephrol*. 2007;2(2):374-384.
13. Chew KL, Tan SS, Saw S, et al. Clinical evaluation of serological IgG antibody response on the Abbott architect for established SARS-CoV-2 infection. *Clin Microbiol Infect*. 2020;26:1256.e9-1256.e11.
14. SARS-CoV-2 NeutraLISA. 2022 [Cited February 13, 2022]. Available from: https://www.coronavirus-diagnostics.com/documents/Indications/Infections/Coronavirus/EI_2606_D_UK_F.pdf.
15. Sherina N, Piralla A, Du L, et al. Persistence of SARS-CoV-2-specific B and T cell responses in convalescent COVID-19 patients 6–8 months after the infection. *Med*. 2021;2(3):281-95.e4.
16. Reindl-Schwaighofer R, Heinzel A, Mayrdorfer M, et al. Comparison of SARS-CoV-2 antibody response 4 weeks after homologous vs heterologous third vaccine dose in kidney transplant recipients: a randomized clinical trial. *JAMA Intern Med*. 2022;182(2):165-171.
17. Munro APS, Janani L, Cornelius V, et al. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK(COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. *Lancet (London, England)*. 2021;398(10318):2258-2276.
18. Reindl-Schwaighofer R, Heinzel A, Mayrdorfer M, et al. Comparison of SARS-CoV-2 antibody response 4 weeks after homologous vs heterologous third vaccine dose in kidney transplant recipients: a randomized clinical trial. JAMA. *Intern Med*. 2021;182:165.
19. Watcharananan SP, Jaru-Ampornpan P, Sahawongcharoen S, et al. Comparison of the immunogenicity of ChAdOx1 nCoV-19 vaccine against the wild-type and delta variants in kidney transplant recipients and healthy volunteers. *Am J Transplant*. 2022;22:1459-1466.
20. Ochsenbein AF, Pinschewer DD, Sierro S, Horvath E, Hengartner H, Zinkernagel RM. Protective long-term antibody memory by antigen-driven and T help-dependent differentiation of long-lived memory B cells to short-lived plasma cells independent of secondary lymphoid organs. *Proc Natl Acad Sci U S A*. 2000;97(24):13263-13268.
21. Kantauskaite M, Müller L, Kolb T, et al. Intensity of mycophenolate mofetil treatment is associated with an impaired immune response to SARS-CoV-2 vaccination in kidney transplant recipients. *Am J Transplant*. 2022;22(2):634-639.
22. Charmetant X, Espi M, Barba T, et al. Predictive factors of a viral neutralizing humoral response after a third dose of COVID-19 mRNA vaccine in kidney transplant recipients. *Am J Transplant*. 2022;22(5):1442-1450.
23. Caillard S, Thaunat O. COVID-19 vaccination in kidney transplant recipients. *Nat Rev Nephrol*. 2021;17(12):785-787.
24. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19. *Vaccine*. 2020;383(27):2603-2615.
25. Andrews N, Stowe J, Kirsebom F, E, et al. Effectiveness of COVID-19 vaccines against the Omicron (B.1.1.529) variant of concern. 2021: 2021.12.14.21267615.
26. Dejnirattisai W, Huo J, Zhou D, et al. Omicron-B.1.1.529 leads to widespread escape from neutralizing antibody responses. 2021: 2021.12.03.471045.
27. Grupper A, Katchman H. SARS-CoV-2 vaccines: safety and immunogenicity in solid organ transplant recipients and strategies for improving vaccine responses. *Curr Transplant Rep*. 2022;9:1-13.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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