Title: Augmented humoral response to third and fourth dose of SARS-CoV-2 mRNA vaccines in lung transplant recipients

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Abstract

Background: Since lung transplant recipients (LTRs) exhibit low immunogenicity after two doses of SARS-CoV-2 mRNA vaccines, optimal vaccine strategies for SARS-CoV-2 are required in LTRs. This study aimed to investigate the efficacy and safety of the third and fourth doses of the SARS-CoV-2 mRNA vaccines in LTRs.

Methods: We conducted a single-center study of 73 LTRs and 23 healthy controls (HCs). Participants received two-to-four doses of SARS-CoV-2 mRNA vaccines. The LTRs were divided into three groups based on the number of vaccine dose. IgG titers against SARS-CoV-2 spike protein were measured, and adverse events were assessed. Factors associated with humoral response were analyzed using univariate and multivariate analyses.

Results: The Dose 4 group (n = 27) had a higher humoral response rate ($P = 0.018$) and higher levels of anti-SARS-CoV-2 IgG antibody ($P = 0.04$) than the Dose 2 group (n = 14). The Dose 3 group (n = 32) had lower humoral response rates ($P = 0.005$) and levels of anti-SARS-CoV-2 IgG antibody ($P = 0.0005$) than the HCs (n = 23) even after the same dose. Systemic adverse events were milder in the LTRs than in the HCs ($P < 0.05$). Increased number of vaccine dose was identified as a predictor of positive humoral response ($P = 0.021$).

Conclusion: Booster doses of SARS-CoV-2 mRNA vaccines may enhance humoral response with mild adverse events in LTRs. Repeated vaccination might be warranted for LTRs to prevent SARS-CoV-2 infection.

Key words: Adverse events, COVID-19, immunogenicity, lung transplantation, mRNA vaccine

Abbreviations:

HCs, healthy controls; IQR, interquartile range; LT, lung transplantation; LTRs, lung transplant recipients; MMF, mycophenolate mofetil; SOTRs, solid organ transplant recipients.

Introduction

The global SARS-CoV-2 pandemic continues to have significant impact on the survival of solid organ transplant recipients (SOTRs) due to their immunosuppressive state [1,2]. Among SOTRs, lung transplant recipients (LTRs) have the greatest risk for mortality resulting from COVID-19 infection [2,3]. The increased susceptibility to COVID-19 in LTRs might be attributed to their diminished immune reactivity to the SARS-CoV-2 mRNA vaccine. Although the vaccine efficacy rate ranges from 70% to 95% in the general population [4], the humoral response rate after the two doses of the vaccine varies from 18% to 64% in SOTRs [5–7]. Especially, LTRs exhibit decreased rates of humoral response, ranging from 0% to 40%, after receiving the two doses of the vaccine [8–14]. Moreover, the antibody titers to SARS-CoV-2 have been shown to decline over time after the two doses of the vaccine in SOTRs [15].

Additional booster doses of the vaccine might overcome the low immunogenicity in SOTRs. In fact, the booster doses of the vaccine in SOTRs have been shown to enhance humoral response rates of 56%–92% after the third dose [16–19] and 76%–94% after the fourth dose [20,21]. Despite the emerging evidence of booster doses of the vaccine in SOTRs, little is known about immune responses after the third or fourth dose of the vaccine in LTRs [22–26]. This study aimed to investigate the humoral responses after the third and fourth doses of the SARS-CoV-2 mRNA vaccine and assess the safety and feasibility of administering booster doses of the vaccine in LTRs.

Methods

Patients

We conducted a single-center prospective observational study among 97 LTRs who visited the

outpatient clinic or were hospitalized at Okayama University Hospital from April 15, 2022 to April 14, 2023 (**Figure 1**). Among these LTRs, 24 patients were excluded from this study due to prior history of COVID-19 infection (n =10), prior treatment with anti-SARS-CoV-2 neutralizing antibodies (n = 4), unknown doses of the SARS-CoV-2 mRNA vaccine (n = 4), no vaccination (n = 3), or five doses of the vaccine (n = 3). The remaining 73 patients were divided into three groups based on the number of doses received: 14 patients who had received two doses (the Dose 2 group), 32 patients who had received three doses (the Dose 3 group), and 27 patients who had received four doses (the Dose 4 group). Data on patient characteristics were collected from medical records. Additionally, 23 healthcare workers at Okayama University Hospital who had received four doses of the vaccine were included as healthy controls (HCs). The primary outcome of this study was to investigate the humoral responses after the third and fourth doses of the SARS-CoV-2 mRNA vaccine, and the secondary outcome was to assess the safety and feasibility of the booster doses of the vaccine in LTRs. The study protocol (No. 2111-048) was approved by the institutional review board of Okayama University Hospital on December 24, 2021, and written informed consent was obtained from each patient. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). All methods were performed in accordance with the relevant guidelines and regulations.

Anti-SARS-CoV-2 IgG antibody testing

All participants received two to four doses of mRNA SARS-CoV-2 vaccine (BNT162b2 vaccine, Pfizer Inc. and/or mRNA-1273, Takeda/Moderna) as per the recommendations of the Japanese Ministry of Health, Labour, and Welfare. The IgG antibody titers against the SARS-CoV-2 spike protein was tested with the Mokobio SARS-CoV-2 IgM & IgG Quantum Dot immunoassay

(Mokobio Biotechnology R&D center Inc., MD, USA). Responders were defined as individuals with antibody titers >250 U/mL, which corresponds to a concentration level two-fold higher than the LD_{50} *in vitro* [27]. The LTRs in the Dose 2–4 groups underwent the anti-SARS-CoV-2 IgG antibody testing, and the HCs underwent the antibody testing only after the third dose due to financial reasons.

Adverse events

Questionnaires were administered to all participants to report any adverse events experienced after each vaccine dose. The HCs reported adverse events after each dose, although the antibody testing was performed only after the third dose. Localized symptoms (pain, itch, redness/swelling) and systemic symptoms (fever, fatigue, headaches, chillness, nausea/vomiting, diarrhea, myalgias, arthralgias, rash) were categorized on an ordinal scale of none, mild, moderate, and severe. Mild, moderate, and severe symptoms were defined as those lasting less than a day, for two days, and more than three days, respectively.

Statistical analysis

All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical interface for R (The R Foundation for Statistical Computing, Vienna, Austria) [28]. Continuous data were presented as medians and interquartile range (IQR). Differences in continuous variables were assessed using the Kruskal-Wallis test, and the Mann-Whitney U test was used to compare responders and non-responders. Differences in categorical variables were examined using the Fisher's exact test, with Bonferroni correction applied for multiple testing when indicated. Logistic regression was used for univariate and

multivariate analyses to evaluate factors associated with humoral response to the vaccine. Based on previous reports, the continuous variables (age, trough levels of mycophenolate mofetil (MMF), time from lung transplantation (LT) to IgG testing, time from last vaccination to IgG testing, number of vaccine doses) and categorical variables (sex and type of vaccine) were selected from known risk factors for negative humoral response to the vaccine [8,9,14]. Statistical significance was considered at $P < 0.05$.

Results

A schematic diagram of the study cohort is shown in **Figure 1**. The clinical characteristics of participants are summarized in **Table 1**. The total IgG level was higher in the Dose 3 group than in the other groups ($P = 0.044$), but there was no significant correlation between the total IgG levels and the titer levels of anti-SARS-CoV-2 IgG antibody (**Supplementary Figure 1**). The Dose 2 group had a shorter time from LT to IgG testing ($P = 0.001$) and longer time from the last dose to IgG testing ($P < 0.001$) than the other groups. Additionally, the type of vaccine was different among the groups due to vaccine availability in Japan during the study period ($P = 0.022$).

As shown in **Figure 2A**, the humoral response rate after vaccination was higher in the Dose 4 group than in the Dose 2 group (0% vs. 44.4%, $P = 0.018$). However, the Dose 3 group had a lower response rate than the HCs even after the third dose of the vaccine (34.4% vs. 82.6%, $P = 0.005$). Similarly, whereas the titer levels of anti-SARS-CoV-2 IgG antibody were higher in the Dose 4 group than in the Dose 2 group (53.15 U/mL [0.01–4682.92] vs. 0.01 U/mL [IQR 0.01–15.81], $P = 0.04$), the titer levels of anti-SARS-CoV-2 IgG antibody in the Dose 3 group were lower than those in the HCs, even after the third dose of the vaccine (0.01 U/mL [IQR 0.01–1345.61] vs. 2035.96 U/mL [334–6422], $P = 0.0005$; **Figure 2B**). Of note, the antibody titer levels in the Dose

2 group peaked at late time points as compared to the other groups (**Figure 2C**), although the Dose 2 group had a longer time from the last dose to IgG testing than the other groups as described above.

The most common symptoms of localized and systemic adverse events reported by LTRs were pain at the injection site (62%–88%) and fatigue (10%–14%), respectively (**Figure 3**). No serious adverse events, such as acute allograft rejection and anaphylaxis, were reported during the study period. The localized symptoms were milder in the LTRs than in the HCs after Dose 2 (P = 0.002) and Dose 4 (P = 0.027; **Figure 4A**). Although not statistically significant, a similar trend was observed after Doses 1 and 3. Regarding systemic symptoms, severity was lower in the LTRs than in the HCs after each dose of vaccination (Dose 1, P = 0.003; Dose 2, P < 0.001; Dose 3, P < 0.001; Dose 4, P = 0.013; **Figure 4B**). No significant differences in adverse events were observed according to the number of vaccinations.

Furthermore, we assessed the factors associated with humoral response to the vaccine in the LTRs. **Table 2** shows the clinical characteristics of LTRs, as categorized by humoral responses to the vaccines. No significant differences in the known risk factors for negative humoral response to the vaccine [8,9,14], including age, MMF trough level, time from LT to IgG test, time from the last dose to IgG test, and the type of vaccine, were observed between responders and non-responders. However, only number of vaccine dose was different between responders and non-responders (P = 0.007). To explore the effect of repeating vaccine doses on the humoral response rate, logistic regression analyses were performed using the known risk factors and number of vaccine dose (**Table 3**). In the univariate analysis, the number of vaccine dose was identified as the only significant factor (P = 0.009). The multivariate analysis demonstrated that low MMF trough level (OR: 0.52 [95% CI: 0.28–0.95], P = 0.033) and increased number of vaccine dose (OR: 6.49 [95% CI: 1.33–31.7], P = 0.021) were significant and independent predictors of positive humoral

response. For further validation, the same analysis was performed using the Dose 3 and Dose 4 groups only. Although the number of vaccine doses was not found as a significant factor in this cohort (**Supplementary Table 1-2)**, the analysis using extracted samples with similar time periods from the last dose to IgG test in the Dose 3 and Dose 4 groups revealed that the number of vaccine doses, age, and MMF trough levels remained significant factors (**Supplementary Table 3–5**).

Discussion

In this study, the humoral response rates to the mRNA SARS-CoV-2 vaccine and levels of anti-SARS-CoV-2 IgG antibody were significantly higher after the fourth dose than after the second dose in the LTRs. However, even after the third dose, the humoral response rates and IgG antibody levels in the LTRs were significantly lower than those in the HCs. In contrast, adverse events, especially systemic adverse events, were significantly milder in the LTRs than in the HCs. The multivariate analysis revealed that increased number of vaccine dose was a positive predictor of humoral response to the vaccine. These findings suggest that administering the third and fourth doses of the vaccine might enhance humoral response to the vaccine with mild adverse events in LTRs. To the best of our knowledge, this report is the first to describe the humoral response after the fourth dose of the mRNA SARS-CoV-2 vaccine in the LTRs.

The humoral response rate after the third dose was 34.4% among LTRs in our study, similar to the results previously reported (16%–62%) [22–26]. Whereas the humoral response rate after the fourth dose of the vaccine has been shown to be 76%–92% in SOTRs [20,21], that in LTRs was 44.4% in our study. These findings might be explained by the fact that LTRs generally require a higher level of life-long immunosuppression to avoid allograft rejection than other SOTRs [29]. Since the total IgG levels did not correlate with the levels of anti-SARS-CoV-2 IgG antibody

despite the significant difference in total IgG levels between the groups, the total IgG levels did not appear to affect the antibody levels in our study. Although the Dose 2 group had a longer interval between the last vaccine dose and IgG testing than the other groups in our study, this longer interval would not lead to an underestimation of the antibody response in the Dose 2 group. This is because different from the healthy subjects, LTRs have been shown to exhibit delayed humoral responses to the initial two doses of the SARS-CoV-2 vaccine, and the antibody titers have not yet peaked 6 months after the vaccination [30]. Considering that humoral response to the SARS-CoV-2 vaccine has been extensively investigated only after the second dose in LTRs [8–14], our study may provide pertinent information about the efficacy of the third and fourth doses of the vaccine in LTRs.

Consistent with the results after the second and third doses of the vaccine previously described [14,26], our findings indicated that adverse events were significantly milder in the LTRs than in the HCs even after the third and fourth doses of the vaccine. Obviously, the immunosuppressed state in LTRs contributed to these outcomes. According to previous studies, the most frequent localized symptom is pain at the injection site (41.8%–76.0%), and the most common systemic symptom was fatigue (19%–56%) after the second and third doses [8,11,14]. Similar results for the most common adverse events after Doses 2–4 were observed in our study. Our results indicate that adverse events resulting from repeated vaccinations up to the fourth dose appear to be comparable to those of the second dose. Taken together, the third and fourth doses of the vaccine might not affect the severity and frequency of adverse events in LTRs.

The multivariate analysis revealed that the number of vaccine dose and MMF trough level were significant predictors of humoral response to the vaccine, whereas the univariate analysis showed that number of vaccine dose was only significant in our study. Currently, several predictors

of humoral response after vaccination, including age [8,12,13,25], time from transplantation to IgG test [8,25], MMF trough level [8,12,14,23,25], and type of vaccine (i.e. vaccine manufacturer), have been reported in LTRs [9,23]. Especially, MMF trough level is known to be a negative predictor of positive humoral response in LTRs [8,12,14,23,25], and cessation or reduction in MMF is recommended for the treatment of COVID-19 in LTRs [31]. Since the titers of anti-SARS-CoV-2 IgG antibody after vaccination decline over time in SOTRs [15,32], the positive effect of the number of vaccine dose on humoral response can be beneficial for LTRs in preventing COVID-19, as for other SOTRs [20,21]. With regard to the multivariate analysis, three variables, including time from LT to IgG test, time from the last dose to IgG test, and type of vaccine, were included to adjust the background biases due to the significant differences in clinical characteristics among the groups in our study.

This study had several limitations. First, our study was conducted at a single transplant center with a small sample size. Therefore, findings from the multivariate analysis should be interpreted accordingly. Second, the threshold of the anti-SARS-CoV-2 IgG antibody titer was not based on the neutralizing activity against the Omicron strain and its subvariants, although major strains of the current epidemic are subvariants of the Omicron strain [33]. Third, the titer of anti-SARS-CoV-2 IgG antibody was used as a surrogate marker of clinical efficacy of the vaccine [34]. Further studies are needed to investigate its clinical efficacy for the development and severity of COVID-19 infection and mortality due to COVID-19 in vaccinated LTRs. Fourth, antibody testing was performed only after the third dose in the HCs due to financial reason. Since the antibody titer levels were high enough even after the third dose in the HCs, antibody testing might not be necessary after the fourth dose in the HCs. Despite these limitations, our study provides valuable information on humoral response to the booster doses of the vaccine among LTRs.

234

235 **Conclusions**

236 Administration of booster doses of the SARS-CoV-2 mRNA vaccine proved effective in
237 augmenting humoral response to the vaccine in LTRs. While LTRs demonstrated a lower
238 immunogenicity than HCs, even after the booster doses, LTRs showed milder adverse events than
239 HCs without critical events during the study period. Repeated administration of vaccines may be
240 necessary for LTRs to prevent COVID-19 infection.

241

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245

246 **Author contributions**

247 S.K. and S.S. designed the study. S.K. analyzed the data and performed statistical analyses. S.K.
248 and S.S. drafted and revised the manuscript. S.K., S.S., K.M., H.C., and M.I. contributed to the
249 data acquisition. All authors critically edited and approved the final version of the manuscript.

250

251 **Data Availability**

252 The datasets used and/or analyzed during the current study are available from the corresponding
253 author on reasonable request.

254

255 **Conflict of Interest**

256 The authors have no conflicts of interest.

257

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Table 1. Clinical characteristics of participants

Variables	Dose 2 *n* = 14	Dose 3 *n* = 32	Dose 4 *n* = 27	Healthy controls *n* = 23	*P* value
Age, median	45 [31.25–58.5]	47 [32–56.5]	49 [41.5–59.5]	48 [35.5–57.5]	0.711
Sex, female	5 (35.7)	21 (65.6)	18 (66.7)	9 (39.1)	0.06
CNIs, Tac/CsA	13 (92.9)/1 (7.1)	29 (90.6)/3 (9.4)	20 (74.1)/7 (25.9)	NA	0.136
Tac trough level (ng/mL)	7.60 [7.00–8.50]	6.90 [5.70–9.20]	6.45 [5.00–7.85]	NA	0.432
Antimetabolite, MMF/AZ	14 (100.0)/0 (0.0)	29 (90.6)/1 (3.1)	25 (92.6)/0 (0.0)	NA	0.67
MMF dose (mg/day), median	1000 [500–1500]	500 [500–1000]	500 [500–1000]	NA	0.108
MMF trough level (ng/mL)	2.10 [1.10–3.15]	1.90 [0.90–2.50]	1.75 [0.88–2.92]	NA	0.824
mTOR inhibitor	1 (7.1)	4 (12.5)	4 (14.8)	NA	0.777
Prednisone (mg/day)	5.0 [5.0–6.0]	5.0 [5.0–5.0]	5.0 [5.0–5.5]	NA	0.31
Total IgG level (mg/dL)	802.80 [682.30–949.70]	939.15 [842.22–1180.95]	844.70 [711.48–1009.78]	NA	0.044
Time from LT to IgG test (d)	908 [210.5–2270.25]	3125 [1658–4116.5]	3514 [2927–4756]	NA	0.001
Time from the last dose to IgG test (d)	313.5 [202.25–365.75]	104 [74.5–131.5]	71 [30–107]	177[142.5–195.5]	<0.001
Type of vaccine					0.022
BNT162b2 (Pfizer)	13 (92.9)	16 (57.1)	12 (57.1)	17 (94.4)	
mRNA-1273 (Moderna)	1 (7.1)	3 (10.3)	1 (4.5)	0 (0.0)	
Mixed	0 (0.0)	9 (31.0)	8 (36.4)	1 (5.6)	

Data are shown as median [interquartile range] or n (%). AZ, azathioprine; CNIs, calcineurin inhibitors; CsA, cyclosporine A; LT, lung transplantation; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; Tac, tacrolimus.

Table 2. Clinical characteristics of LTRs stratified by humoral response

Variables	Non-responders *n* = 50	Responders *n* = 23	*P* value
Age	50 [37–58.75]	45 [23.5–56.5]	0.242
Sex, female	29 (58.0)	15 (65.2)	0.615
CNIs, Tac/CsA	44 (88.0)/ 6 (12.0)	18 (78.3)/ 5 (21.7)	0.306
Tac trough level (ng/mL)	6.90 [5.00–8.50]	7.55 [6.32–9.50]	0.187
CsA trough level (ng/mL)	143.4 [124.9–161.2]	138.2 [129.8–183.4]	0.754
Antimetabolite, MMF/AZ	2 (4.0)/ 48 (96.0)	2 (8.7)/ 20 (87.0)	0.247
MMF trough level (ng/mL)	1.85 [1.10–3.12]	1.10 [0.65–2.10]	0.052
Prednisone (mg/day)	5.0 [5.0–5.0]	5.0 [4.0–5.5]	0.232
mTOR inhibitor	8 (16.0)	1 (4.3)	0.257
Total IgG level (mg/dL)	845.90 [753.20–972.05]	985.15 [860.65–1177.12]	0.052
Time from LT to IgG test (d)	2874 [1374–3976.5]	3200 [1832.75–4141]	0.511
Time from the last dose to IgG test (d)	108.5 [72.25–199]	84 [51.75–127.5]	0.137
Type of vaccine			1
BNT162b2 (Pfizer)	27 (64.3)	14 (66.7)	
mRNA-1273 (Moderna)	3 (7.1)	2 (9.5)	
Mixed	12 (28.6)	5 (23.8)	
Number of vaccine dose, n (%)			0.007
Dose 2	14 (28.0)	0 (0.0)	
Dose 3	21 (42.0)	11 (47.8)	
Dose 4	15 (30.0)	12 (52.2)	

Data are shown as median [interquartile range] or n (%). AZ, azathioprine; CNIs, calcineurin inhibitors; CsA, cyclosporine A; LT, lung transplantation; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; Tac, tacrolimus.

Table 3. Univariate and multivariate analyses for positive humoral response to the vaccine after LT

	Univariable Logistic Regression		Multivariable Logistic Regression	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age, 10 years*	0.79 (0.57–1.08)	0.138		
Sex, male	0.74 (0.26–2.05)	0.559		
MMF trough level*	0.58 (0.34–1.01)	0.053	0.52 (0.27–0.98)	0.033
Total IgG level, 100 mg/dL*	1.10 (0.95-1.28)	0.212		
Time from the last dose to IgG test*	0.99 (0.99–1.00)	0.059	1.00 (0.99–1.01)	0.31
Time from LT to IgG test*	1.02 (0.93–1.12)	0.666	0.93 (0.78–1.11)	0.753
Vaccine, mRNA-1273 (Moderna)	0.90 (0.30–2.72)	0.852	0.71 (0.11–4.71)	0.32
Number of vaccine dose*	2.93 (1.31–6.55)	0.009	7.64 (1.38–42.2)	0.021

CI, confidence interval; LT, lung transplantation; MMF, mycophenolate mofetil; OR, odds ratio. * Continuous variables .

371 **Figure legends**

372 **Figure 1.** Patient flowchart. Among the 97 lung transplant recipients, 24 patients were excluded.
373 They included 10 patients with prior COVID-19 history, four patients with prior anti-SARS-CoV-
374 2 neutralizing antibody treatment, four patients with unknown number of vaccine dose, three
375 patients with no vaccination, and three patients with five or more vaccine doses. The remaining 73
376 recipients were categorized according to number of vaccine dose: 14 patients with two doses, 32
377 patients with three doses, and 27 patients with four doses. Additionally, 23 healthcare workers at
378 Okayama University Hospital constituted the healthy control group.

379

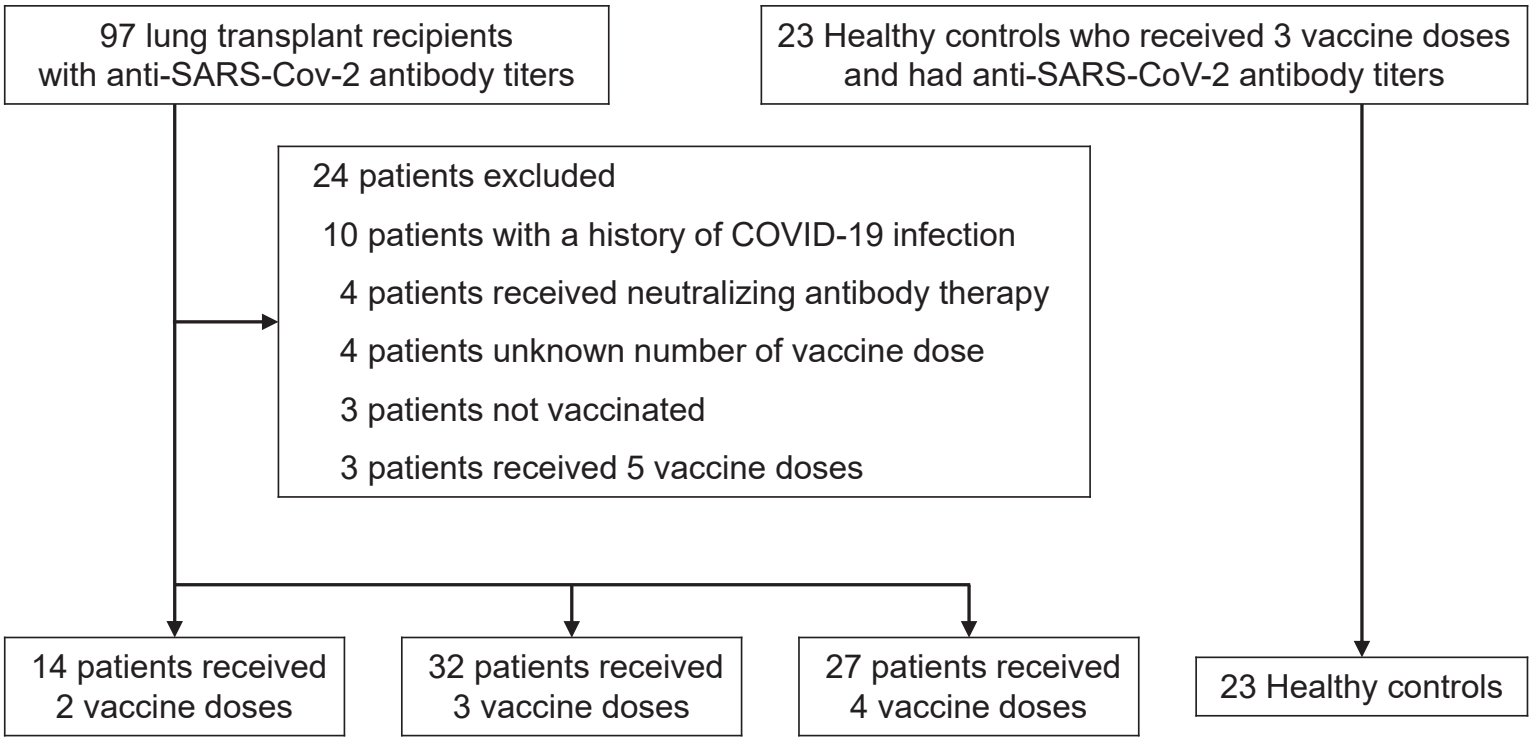
380 **Figure 2.** Humoral response in lung transplant recipients (LTRs) and healthy controls (HCs) after
381 each dose of mRNA SARS-CoV-2 vaccine. (A) Humoral response rate. (B) Median anti-SARS-
382 CoV-2 IgG antibody titers. The horizontal dashed line indicates the threshold for positive humoral
383 response (250 U/mL). CI, confidence interval; IQR, interquartile ranges. * $P < 0.05$, ** $P < 0.01$,
384 *** $P < 0.001$. (C) The relationship between the titer levels of anti-SARS-CoV-2 IgG antibody and
385 the timing of the antibody measurement. Lines indicate the geometric means for each group.

386

387 **Figure 3.** Adverse events after each dose of SARS-CoV-2 mRNA vaccine in lung transplant
388 recipients. (A) Localized adverse events. (B) Systemic adverse events.

389

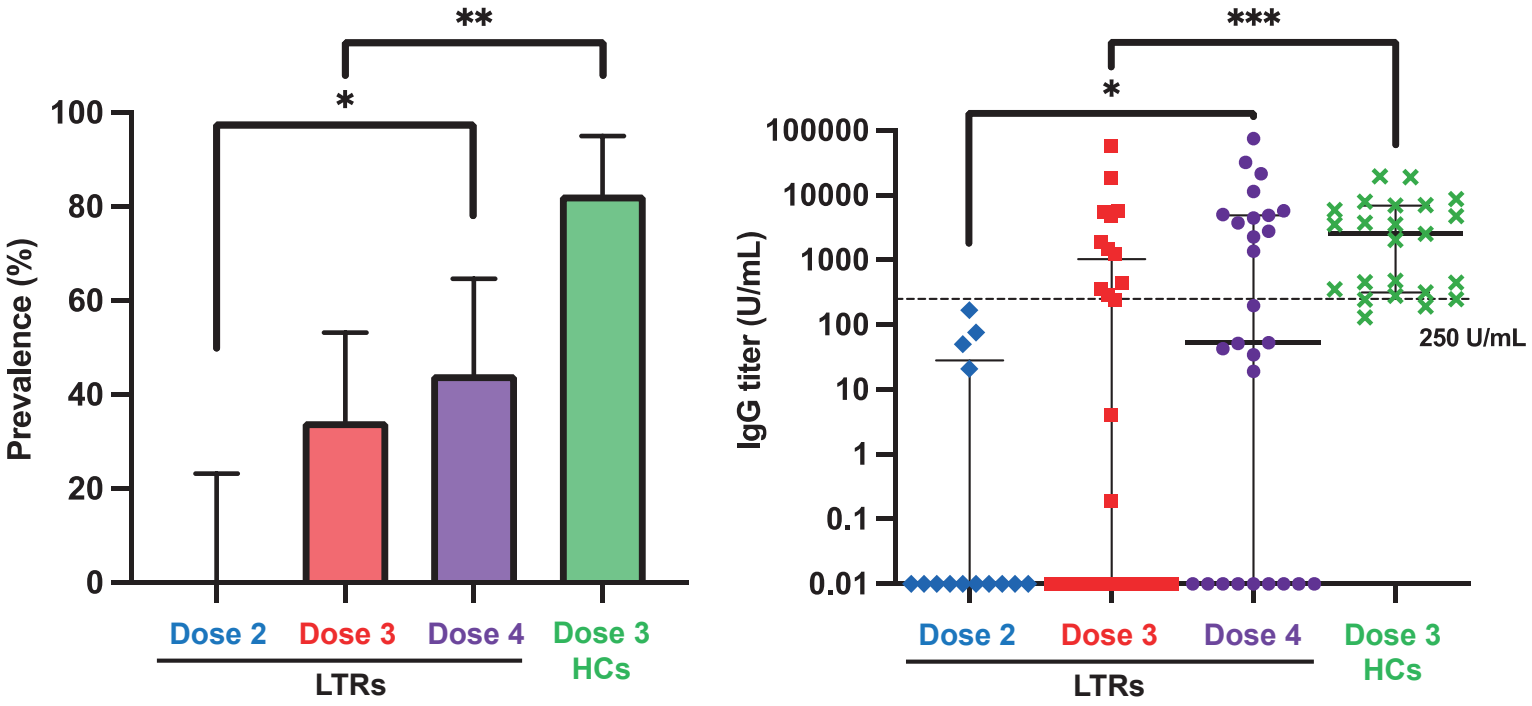
390 **Figure 4.** Adverse events in lung transplant recipients (LTRs) and healthy controls (HCs) after each
391 dose of mRNA SARS-CoV-2 vaccine. (C) Percentages of localized adverse events. (D) Percentages
392 of systemic adverse events. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.


97 lung transplant recipients with anti-SARS-Cov-2 antibody titers
23 Healthy controls who received 3 vaccine doses and had anti-SARS-CoV-2 antibody titers
24 patients excluded
10 patients with a history of COVID-19 infection
4 patients received neutralizing antibody therapy
4 patients unknown number of vaccine dose
3 patients not vaccinated
3 patients received 5 vaccine doses
14 patients received 2 vaccine doses
32 patients received 3 vaccine doses
27 patients received 4 vaccine doses
23 Healthy controls

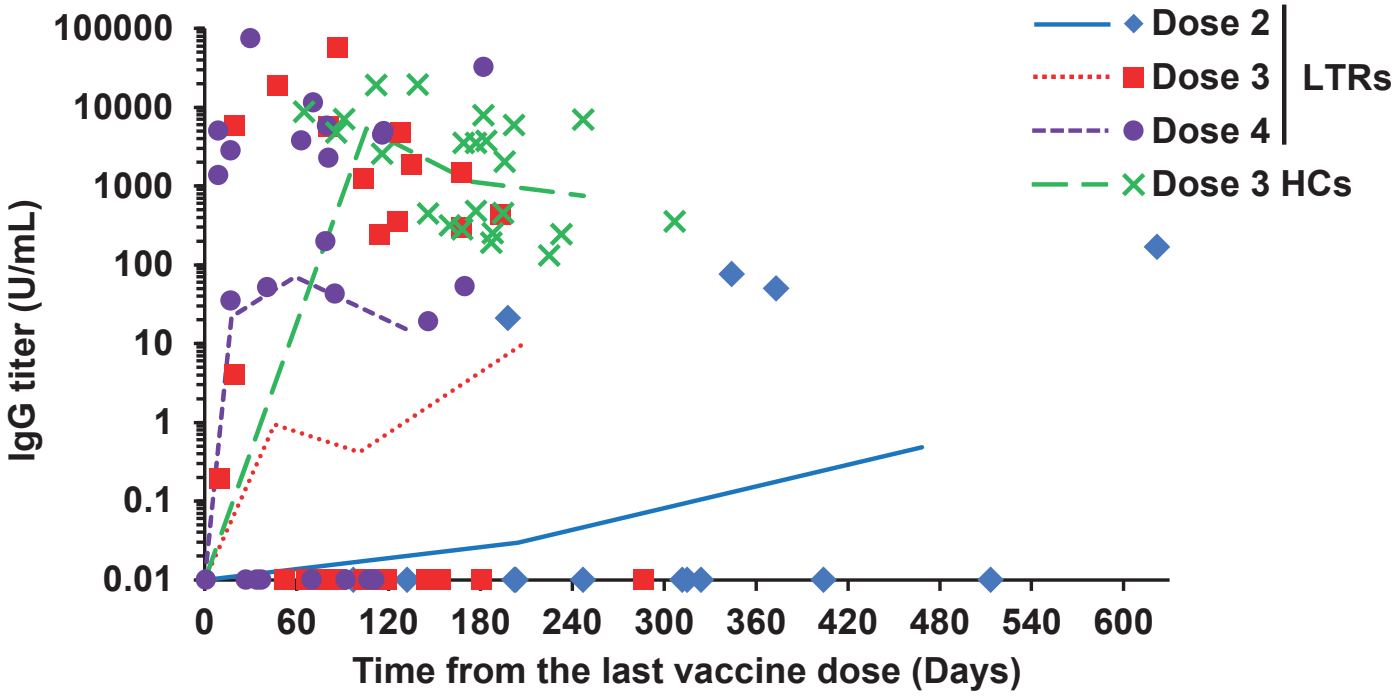
A

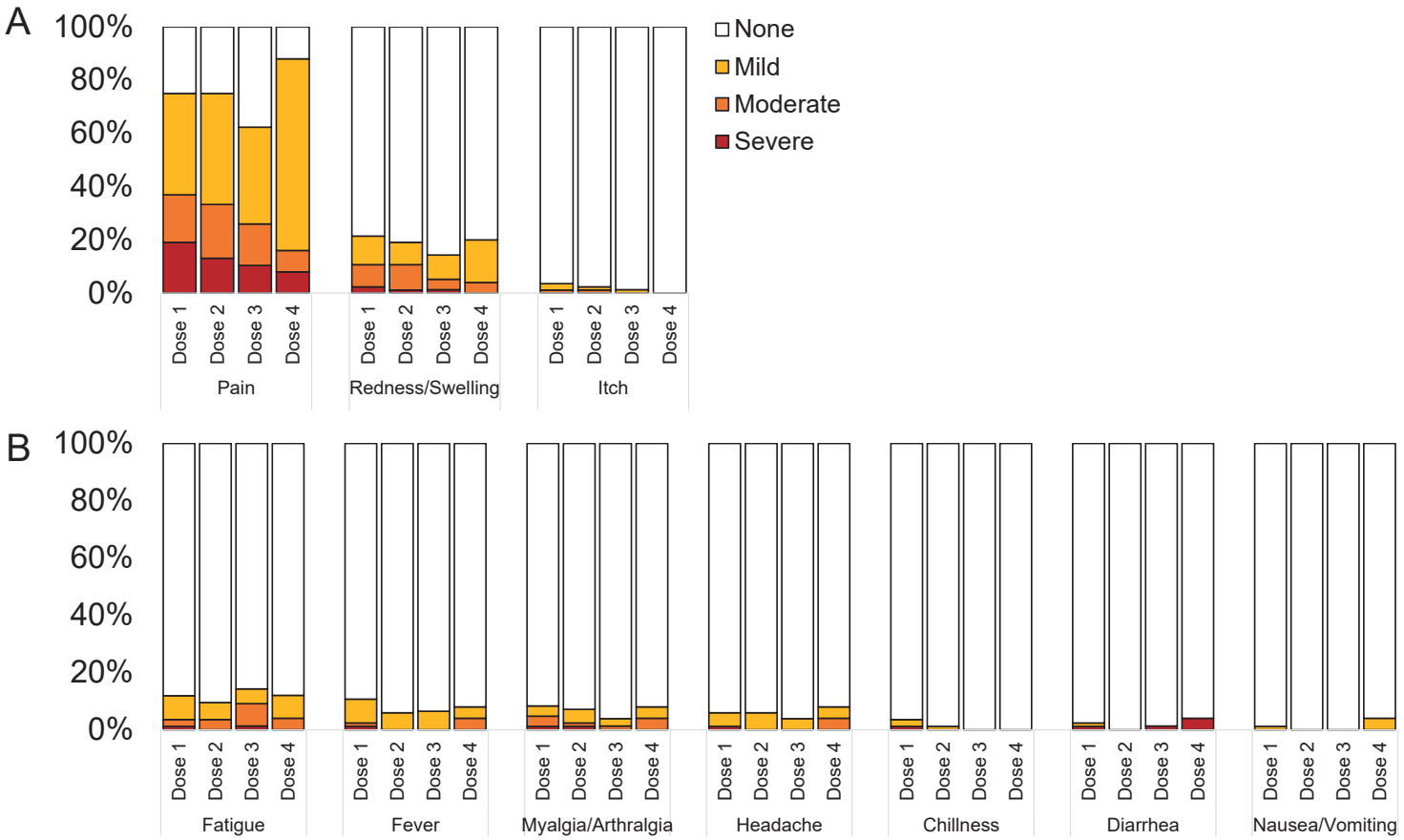
Response rate [95%CI]	0% [0-23.2%]	34.4% [18.6-53.2%]	44.4% [25.5-64.7%]	82.6% [61.2-95.0%]

Median (U/mL) [IQR]	0.01 [0.01-15.81]	0.01 [0.01-1345.61]	53.15 [0.01-4682.92]	2035.96 [334-6422]

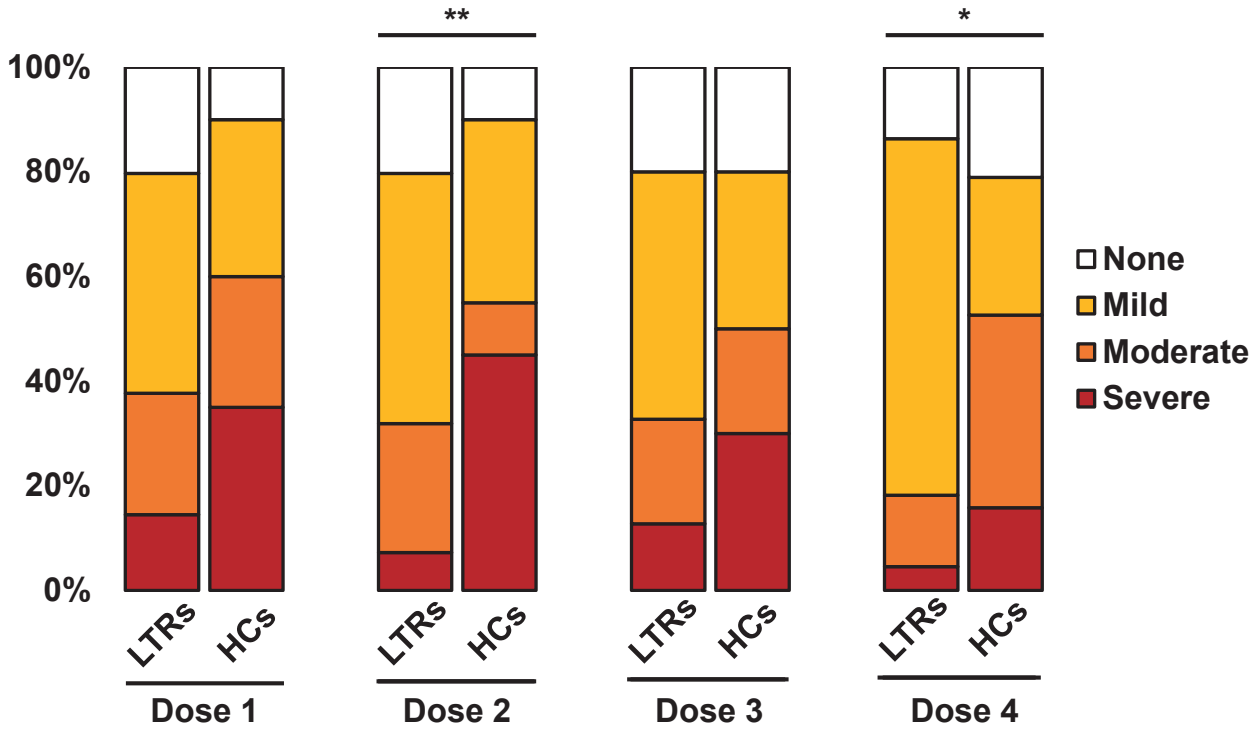


B

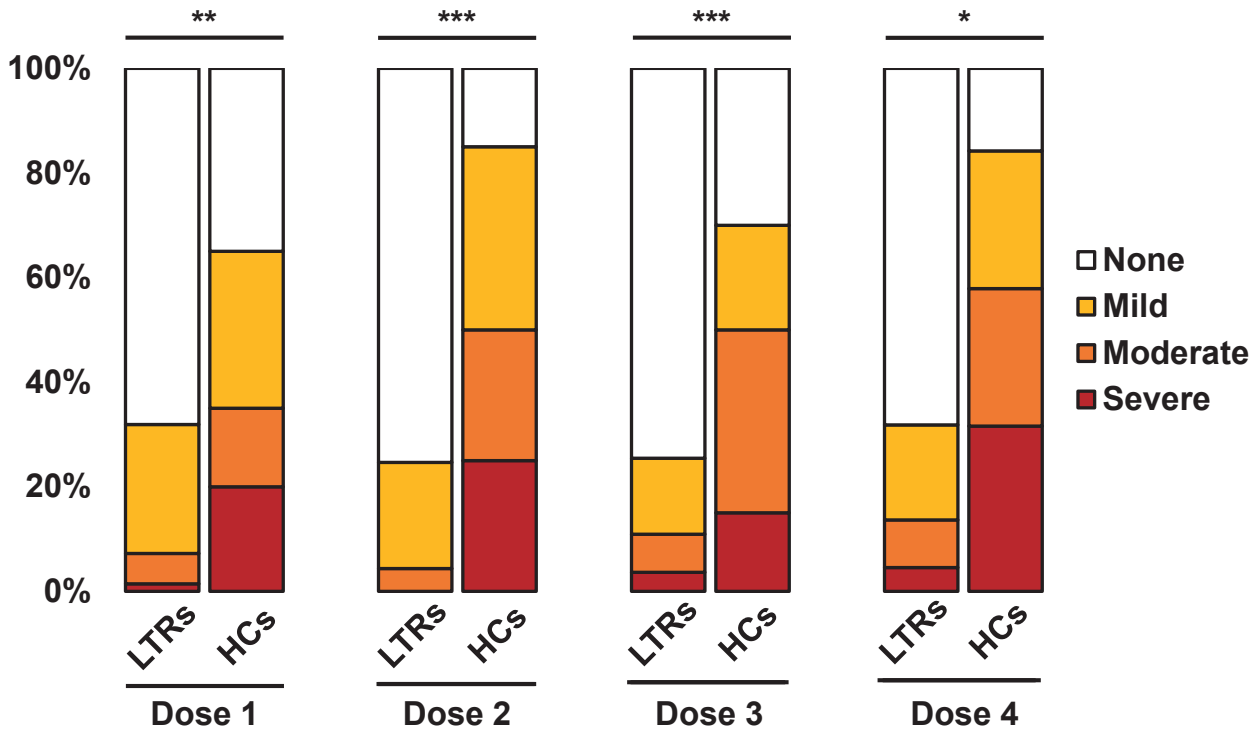



A
100%
80%
60%
40%
20%
0%
None
Mild
Moderate
Severe
Dose 1
Dose 2
Dose 3
Dose 4
Pain
Redness/Swelling
Itch
B
Fatigue
Fever
Myalgia/Arthralgia
Headache
Chillness
Diarrhea
Nausea/Vomiting

A


**
*
100%
80%
60%
40%
20%
0%
LTRs
HCs
Dose 1
Dose 2
Dose 3
Dose 4
None
Mild
Moderate
Severe

B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*
100%
80%
60%
40%
20%
0%
LTRs
HCs
Dose 1
Dose 2
Dose 3
Dose 4
None
Mild
Moderate
Severe

Supplementary Table 1. Clinical characteristics of LTRs after Dose 3 and 4 stratified by humoral response

Variables	Non-responders *n* = 36	Responders *n* = 23	*P* value
Age	50 [38.75–58.75]	45 [23.5–56.5]	0.15
Sex, female	24 (66.7)	15 (65.2)	1
CNIs, Tac/CsA	31 (86.1) / 5 (13.9%)	18 (78.3) / 5 (21.7)	0.49
Tac trough level (ng/mL)	6.30 [4.80–8.35]	7.55 [6.32–9.50]	0.079
CsA trough level (ng/mL)	134.15 [123.03–147.85]	138.2 [129.8–183.4]	0.327
Antimetabolite, MMF/AZ	34 (94.4) / 0 (0.0)	20 (87.0) / 1 (4.3)	0.423
MMF trough level (ng/mL)	1.80 [1.10–3.10]	1.10 [0.65–2.10]	0.065
Prednisone (mg/day)	5.0 [5.0–5.0]	5.0 [4.0–5.5]	0.413
mTOR inhibitor	7 (19.4)	1 (4.3)	0.133
Total IgG level (mg/dL)	871.6 [758.7–1004.02]	985.15 [860.65–1177.12]	0.123
Time from LT to IgG test (d)	3513.5 [1814.75–4585.25]	3256.5 [1898.5–4295.25]	0.524
Time from the last dose to IgG test (d)	84 [55.75–111]	84 [51.75–127.5]	0.718
Type of vaccine			0.373
BNT162b2 (Pfizer)	14 (50.0)	14 (66.7)	
mRNA-1273 (Moderna)	2 (7.1)	2 (9.5)	
Mixed	12 (42.9)	5 (23.8)	
Number of vaccine dose, n (%)			0.168
Dose 3	21 (58.3)	11 (47.8)	
Dose 4	15 (41.7)	12 (52.2)	

Data are shown as median [interquartile range] or n (%). AZ, azathioprine; CNIs, calcineurin inhibitors; CsA, cyclosporine A; LT, lung transplantation; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; Tac, tacrolimus.

Supplementary Table 2. Univariate and multivariate analyses for positive humoral response to Dose3 and 4

	Univariable Logistic Regression		Multivariable Logistic Regression	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age, 10 years*	0.71 (0.49–1.02)	0.064		
Sex, male	1.07 (0.35–3.21)	0.909		
MMF trough level*	0.57 (0.32–1.03)	0.062	0.48 (0.24-0.95)	0.036
Total IgG level, 100 mg/dL*	1.10 (0.92-1.30)	0.295		
Time from the last dose to IgG test*	1.00 (0.99–1.01)	0.852	1.01 (0.99-1.03)	0.211
Time from LT to IgG test*	1.00 (1.00–1.00)	0.454	1.00 (1.00-1.00)	0.229
Vaccine, mRNA-1273 (Moderna)	0.50 (0.16–1.61)	0.246	0.44 (0.09-2.25)	0.325
Number of vaccine dose*	1.53 (0.53–4.38)	0.43	4.03 (0.68-23.8)	0.124

CI, confidence interval; LT, lung transplantation; MMF, mycophenolate mofetil; OR, odds ratio. * Continuous variables .

Supplementary Table 3. Clinical characteristics of Dose 3 & 4 within 120 days from the last vaccination

Variables	Dose 3 *n* = 21	Dose 4 *n* = 22	*P* value
Age, median	46 [30–52]	49 [39.75–58.25]	0.355
Sex, female	14 (66.7)	16 (72.7)	0.747
CNIs, Tac/CsA	18 (85.7)/5 (23.8)	17 (77.3)/5 (22.7)	0.698
Tac trough level (ng/mL)	6.25 [5.78–9.05]	6.40 [5.00–7.40]	0.741
Antimetabolite, MMF/AZ	19 (90.5)/1 (4.8)	21 (95.5)/0 (0.0)	0.738
MMF trough level (ng/mL)	1.60 [0.85–2.70]	1.80 [0.90–3.00]	0.676
mTOR inhibitor	2 (9.5)	3 (13.6)	1
Prednisone (mg/day)	5.0 [5.0–5.0]	5.0 [5.0–5.0]	0.977
Total IgG level (mg/dL)	916.85 [834.65–1253.00]	854.55 [723.75–1009.78]	0.182
Time from LT to IgG test (d)	3020 [1839–4012]	3576 [2942.75–4721.75]	0.181
Time from the last dose to IgG test (d)	81 [67–104]	66.5 [27.75–84]	0.117
Type of vaccine			0.724
BNT162b2 (Pfizer)	11 (52.4)	11 (61.1)	
mRNA-1273 (Moderna)	3 (14.3)	1 (5.6)	
Mixed	7 (33.3)	6 (33.3)	

Data are shown as median [interquartile range] or n (%). AZ, azathioprine; CNIs, calcineurin inhibitors; CsA, cyclosporine A; LT, lung transplantation; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; Tac, tacrolimus.

Supplementary Table 4. Clinical characteristics of Dose 3 & 4 within 120 days from the last vaccination stratified by humoral response

Variables	Non-responders *n* = 28	Responders *n* = 15	*P* value
Age	50 [44.5–57.25]	38 [18.5–52.5]	0.047
Sex, female	19 (67.9)	11 (73.3)	1
CNIs, Tac/CsA	24 (85.7) / 4 (14.3%)	11 (73.3) / 4 (26.7)	0.419
Tac trough level (ng/mL)	6.25 [4.95–8.45]	6.40 [5.60–9.20]	0.683
CsA trough level (ng/mL)	134.15 [123.03–147.85]	156.6 [129.02–198.85]	0.248
Antimetabolite, MMF/AZ	27 (96.4)/0 (0.0)	13 (86.7)/1 (6.7)	0.428
MMF trough level (ng/mL)	1.80 [1.25–3.25]	0.90 [0.50–2.10]	0.021
Prednisone (mg/day)	5.0 [5.0–5.0]	5.0 [4.0–5.0]	0.193
mTOR inhibitor	4 (14.3)	1 (6.7)	0.643
Total IgG level (mg/dL)	840.2 [755.4–951.35]	975.9 [863.4–1154.65]	0.086
Time from LT to IgG test (d)	3513.5 [2370.25–4924.25]	3276 [2399.5–3585.5]	0.665
Time from the last dose to IgG test (d)	77.5 [40–95]	71 [25–84]	0.499
Type of vaccine			0.438
BNT162b2 (Pfizer)	12 (50.0)	10 (66.7)	
mRNA-1273 (Moderna)	2 (8.3)	2 (13.3)	
Mixed	10 (41.7)	3 (20.0)	
Number of vaccine dose, n (%)			0.203
Dose 3	16 (57.1)	5 (33.3)	
Dose 4	12 (42.9)	10 (66.7)	

Data are shown as median [interquartile range] or n (%). AZ, azathioprine; CNIs, calcineurin inhibitors; CsA, cyclosporine A; LT, lung transplantation; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; Tac, tacrolimus.

Supplementary Table 5. Univariate and multivariate analyses for positive humoral response to Dose3 & 4 within 120 days from the last vaccination

	Univariable Logistic Regression		Multivariable Logistic Regression	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Age, 10 years*	0.57 (0.36–0.92)	0.02	0.34 (0.15-0.77)	0.009
Sex, male	0.77 (0.19–3.09)	0.71		
MMF trough level*	0.45 (0.21–0.97)	0.042	0.37 (0.14-0.97)	0.042
Total IgG level, 100 mg/dL*	1.16 (0.93-1.45)	0.194		
Time from the last dose to IgG test*	0.99 (0.98–1.01)	0.486		
Time from LT to IgG test*	1.00 (1.00–1.00)	0.486		
Vaccine, mRNA-1273 (Moderna)	0.50 (0.13–1.91)	0.31		
Number of vaccine dose*	2.67 (0.72–9.87)	0.142	13.9 (1.14-170)	0.039

CI, confidence interval; LT, lung transplantation; MMF, mycophenolate mofetil; OR, odds ratio. * Continuous variables .