Alternative strategies to increase the immunogenicity of COVID-19 vaccines in kidney transplant recipients not responding to two or three doses of an mRNA vaccine (RECOVAC): a randomised clinical trial

Marcia M L Kho, A Lianne Messchendorp*, Sophie C Frölke, Celine Imhof, Vera JCH Koomen, S Reshwan K Malahe, Priya Vart, Daryl Geers, Rory D de Vries, Corine H GeurtsvanKessel, Carla C Baan, Renate G van der Molen, Dimitri A Diavatopoulos, Ester B M Remmerswaal, Debbie van Baarle, Rob van Binnendijk, Gerco den Hartog, Aiko P J de Vries, Ron T Gansevoort, Frederike J Bemelman, Marlies E J Reinders, Jan-Stephan F Sanders†, Luuk B Hilbrands†, RECOVAC collaborators‡*

Summary

Background An urgent need exists to improve the suboptimal COVID-19 vaccine response in kidney transplant recipients (KTRs). We aimed to compare three alternative strategies with a control single dose mRNA-1273 vaccination: a double vaccine dose, heterologous vaccination, and temporary discontinuation of mycophenolate mofetil or mycophenolic acid.

Methods This open-label randomised trial, done in four university medical centres in the Netherlands, enrolled KTRs without seroconversion after two or three doses of an mRNA vaccine. Between Oct 20, 2021, and Feb 2, 2022, 230 KTRs were randomly assigned block-wise per centre by a web-based system in a 1:1:1 manner to receive 100 µg mRNA-1273, 2×100 µg mRNA-1273, or Ad26.COV2-S vaccination. In addition, 103 KTRs receiving 100 µg mRNA-1273, were randomly assigned 1:1 to continue (mycophenolate mofetil+) or discontinue (mycophenolate mofetil–) mycophenolate mofetil or mycophenolic acid treatment for 2 weeks. The primary outcome was the percentage of participants with a spike protein (S1)-specific IgG concentration of at least 10 binding antibody units per mL at 28 days after vaccination, assessed in all participants who had a baseline measurement and who completed day 28 after vaccination without SARS-CoV-2 infection. Safety was assessed as a secondary outcome in all vaccinated patients by incidence of solicited adverse events, acute rejection or other serious adverse events. This trial is registered with ClinicalTrials.gov, NCT05030974 and is closed.

Findings Between April 23, 2021, and July 2, 2021, of 12 158 invited Dutch KTRs, 3828 with a functioning kidney transplant participated in a national survey for antibody measurement after COVID-19 vaccination. Of these patients, 1311 did not seroconvert after their second vaccination and another 761 not even after a third. From these seronegative patients, 345 agreed to participate in our repeated vaccination study. Vaccination with 2×mRNA-1273 or Ad26.COV2-S was not superior to single mRNA-1273, with seroresponse rates of 49 (68%) of 72 (95% CI 56–79), 46 (63%) of 73 (51–74), and 50 (68%) of 73 (57–79), respectively. The difference with single mRNA-1273 was –0·4% (–16 to 15; p=0·96) for 2×mRNA-1273 and –6% (–21 to 10; p=0·49) for Ad26.COV2-S. Mycophenolate mofetil– was also not superior to mycophenolate mofetil+, with seroresponse rates of 37 (80%) of 46 (66–91) and 31 (67%) of 46 (52–80), and a difference of 13% (–5 to 31; p=0·15). Local adverse events were more frequent after a single and double dose of mRNA-1273 than after Ad26.COV2-S (65 [92%] of 71, 67 [92%] of 73, and 38 [50%] of 76, respectively; p<0·0001). No acute rejection occurred. There were no serious adverse events related to vaccination.

Interpretation Repeated vaccination increases SARS-CoV-2-specific antibodies in KTRs, without further enhancement by use of a higher dose, a heterologous vaccine, or 2 weeks discontinuation of mycophenolate mofetil or mycophenolic acid. To achieve a stronger response, possibly required to neutralise new virus variants, repeated booster vaccination is needed.

Funding The Netherlands Organization for Health Research and Development and the Dutch Kidney Foundation.

Introduction

Kidney transplant recipients (KTRs) are at risk for a severe course of COVID-19 with a high mortality rate.[1] Although effective COVID-19 vaccination is therefore of great importance, the humoral and cellular immune response after two primary mRNA-based vaccinations is severely diminished in KTRs, especially when their immunosuppressive regimen contains mycophenolate mofetil or mycophenolic acid.[2] Consequently, administration of additional vaccine doses to KTRs has become

Lancet Infect Dis 2022

Published **Online** October 27, 2022 https://doi.org/10.1016/S1473-3099(22)00650-8

See **Online/Comment** https://doi.org/10.1016/S1473-3099(22)00666-1

*Contributed equally as first authors

†Contributed equally as last authors

‡A list of RECOVAC collaborators is added at the end of the article

Department of Internal Medicine, Nephrology and Transplantation (M M L Kho MD, S R K Malahe MD, Prof C C Baan PhD, Prof M E J Reinders PhD), **Department Viroscience** (D Geers MSc, R D de Vries PhD, C H GeurtsvanKessel PhD), **Erasmus MC Transplant Institute, Erasmus Medical Center, Rotterdam, Netherlands; Department of Internal Medicine, Division of Nephrology** (A L Messchendorp PhD, C Imhof MD, P Vart PhD, Prof R T Gansevoort PhD, J-S F Sanders, PhD), **Department of Medical Microbiology and Infection Prevention** (C Imhof, Prof D van Baarle PhD), **University of Groningen, University Medical Center Groningen, Groningen, Netherlands; Renal Transplant Unit, Amsterdam UMC** (S C Frölke MD, Prof F J Bemelman PhD), **Department of Experimental Immunology, Amsterdam Infection and Immunity Institute** (E B M Remmerswaal PhD), **University of Amsterdam, Amsterdam, Netherlands;**

Department of Nephrology (V J C H Koomen MSc, Prof L B Hilbrands, PhD), **Radboud Institute for Molecular Life Sciences, Department of Laboratory Medicine, Laboratory of Medical Immunology** (R G van der Molen PhD, D A Diavatopoulos PhD), **Radboud Center for Infectious Diseases** (D A Diavatopoulos), **Radboud University Medical Center Nijmegen, Nijmegen, Netherlands; Center for Infectious Disease Control, National Institute for Public Health and the Environment, Bilthoven, Netherlands** (Prof D van Baarle, R van Binnendijk PhD, G den Hartog PhD); **Department of Nephrology, Leiden University Medical Center, Leiden, Netherlands** (A P J de Vries PhD)

Corresponding author: Prof Luuk B Hilbrands Department of Nephrology, Radboud University Medical Center, Radboud Institute for Health Sciences, Geert Grooteplein 10, 6525GA Nijmegen, Netherlands
luuk.hilbrands@radboudumc.nl

Research in context

Evidence before this study

We searched PubMed for COVID-19 vaccination studies in kidney transplant recipients published between May and August, 2021 using terms such as "COVID", "vaccine*", "booster", "third dose", "immunogenicity", "humoral response", and "cellular response". We found observational studies and only one randomised trial reporting that a third dose of SARS-CoV-2 mRNA vaccine resulted in a seroconversion rate of only 25–44% in patients who were seronegative after two doses of an mRNA vaccine. Alternative vaccination strategies to increase the immunogenicity of COVID-19 vaccination are therefore needed. Although increased immunogenicity of higher doses has been shown for hepatitis B and influenza vaccination in immunocompromised patients, the effect of a higher SARS-CoV-2 vaccine dose has not been studied in such patients. There are conflicting results with respect to heterologous vector based–mRNA vaccination compared with homologous regimens with an observational study showing a higher T-cell response in healthy adults, whereas one clinical trial showed no advantage in kidney transplant recipients. Lastly, a strong association between reduced vaccination efficacy and the use of mycophenolate mofetil or mycophenolic acid has repeatedly been reported, suggesting that temporarily withdrawing this medication might increase the immunogenicity of COVID19 vaccination.

Added value of this study

In this prospective randomised trial we compared the immunogenicity of three alternative vaccination strategies to that of a control single dose of mRNA-1273 in kidney transplant recipients who remained seronegative after two or three previous mRNA-based vaccinations. Even with a broad spectrum of immunological parameters we did not find superiority of a double dose of mRNA-1273 at two anatomical locations, heterologous vaccination, or temporary withdrawal of mycophenolate mofetil or mycophenolic acid. To our knowledge, we are the first to report on the effect of different vaccination strategies in patients using immunosuppressive drugs in a randomised trial including a proper control group.

Implications of all the available evidence

Repeated vaccinations are the most successful strategy to achieve a humoral immune response in kidney transplant recipients. We think that our results are directly useful for doctors caring not only for kidney transplant recipients but also for other patients on immunosuppressive drugs. Additionally, these data are important for the design of future vaccination strategies for immunosuppressed patients against other pathogens.

common practice. However, even after a third or fourth vaccination, a considerable proportion of organ transplant recipients remains a serological non-responder.[3] It is therefore imperative to investigate whether alternative vaccination strategies could be more immunogenic.[4]

A potential option to increase immunogenicity of repeated COVID-19 vaccination is to increase vaccine dose, as is also applied for hepatitis B vaccination in patients receiving haemodialysis and for influenza vaccination in organ transplant recipients.[5] Applying a multisite injection regimen could provide additional stimulation of the immune system.[6] A second option could be to use different combinations of vaccines, so-called heterologous vaccination. Studies have suggested that heterologous prime-boost vaccination regimes (vector-based followed by mRNA) could result in a stronger immune response compared with homologous regimes.[7] Finally, the strong negative association between the use of mycophenolate mofetil–mycophenolic acid and vaccine immunogenicity[2] suggests that temporary discontinuation of the use of these drugs might improve the immune response to vaccination.

Based on these considerations, we designed a randomised clinical trial to compare the immunogenicity of a double dose of the mRNA-1273 vaccine, heterologous vaccination with Ad26.COV2-S, and temporary discontinuation of mycophenolate mofetil or mycophenolic acid to the immunogenicity of a control single dose mRNA-1273 vaccination. This trial was done in KTRs who were serological non-responders after two or three doses of an mRNA-based vaccine.

Methods

Study design

This prospective, open-label, randomised, controlled trial was done between Oct 20, 2021, and Feb 5, 2022, in four university medical centres in the Netherlands (Amsterdam UMC, UMC Groningen, Radboudumc Nijmegen, and Erasmus MC Rotterdam), as part of the Dutch Renal patients COVID-19 VACcination (RECOVAC) study. Ethical approval was obtained from the Dutch Central Committee on Research Involving Human Subjects, the central ethics committee at the UMC Groningen, and the local ethics committees of the participating centres.

Patients

Between April 23, 2021, and July 2, 2021, all adult patients with a functioning kidney transplant in the Netherlands were asked to participate in a study for antibody measurement after COVID-19 vaccination. Patients who had given informed consent, either electronically or in writing, were sent a finger prick package to collect a blood sample at home between 14 and 56 days after COVID-19 vaccination.[8] A central laboratory did the anti-SARS-CoV-2 RBD IgG ELISA assay. For this assay, which was used to identify seronegative patients from our national survey, the validated cutoff concentration for seropositivity is ≥50 binding antibodies units (BAU)/mL.[8,9]

For the present study, we invited patients without seroconversion at 14–56 days after the second or third dose of an mRNA-based COVID-19 vaccine, either mRNA-1273 (Moderna Biotech Spain, Madrid, Spain) or BNT162b2 (BioNTech/Pfizer, Mainz, Germany), or a combination of both (figure 1). Patients who had COVID-19 (defined as a reported positive SARS-CoV-2 PCR-test or presence of nucleocapsid-specific antibodies) before or during this study were excluded. Detailed inclusion and exclusion criteria are provided in the appendix (p 3).

Randomisation

The study was done in two different cohorts. In cohort one, KTRs receiving any combination of immunosuppressive drugs were included. These patients were randomly assigned in a 1:1:1 manner to receive either a single dose of the mRNA-1273 vaccine (100 µg, intramuscularly), two doses of mRNA-1273 simultaneously in both upper arms (2×100 µg, intramuscularly), or the Ad26.COV2-S vaccine (Janssen Biologics, Leiden, The Netherlands; 5×10^{10} viral particles, intramuscularly). This cohort is referred to as the alternative vaccination study group. In cohort two, only patients receiving triple immunosuppressive therapy consisting of a calcineurin inhibitor, mycophenolate mofetil or mycophenolic acid, and steroids were included. These patients were randomly assigned to either continuation of mycophenolate mofetil or mycophenolic acid (mycophenolate mofetil+) or discontinuation of mycophenolate mofetil or mycophenolic acid (mycophenolate mofetil–) from 1 week before until 1 week after vaccination with a single 100 µg intramuscular dose of the mRNA-1273 vaccine. This cohort is referred to as the mycophenolate mofetil–mycophenolic acid discontinuation study group. In both study groups, randomisation was done block-wise per centre, by means of the web-based randomisation system ALEA (FormsVision, Abcoude, Netherlands). Patients could only participate in one cohort. Masking was infeasable as a proportion of patients was assigned to receive 2 × mRNA-1273 in both upper arms or to temporarily discontinue mycophenolate mofetil or mycophenolic acid.

Procedures

In both study groups, blood samples were collected at baseline (ie, before vaccination) and at 28 days after vaccination. In cohort two, an additional blood sample was collected at 1 and 2 weeks after discontinuing mycophenolate mofetil or mycophenolic acid mainly to monitor kidney transplant function. Questionnaires were used to report solicited local and systemic adverse events for 7 days after vaccination and to monitor occurrence of SARS-CoV-2 infections. A detailed overview of study visits and assessments is provided in the appendix (p 4).

Outcomes

Primary outcome was the percentage of participants with a spike protein (S1)-specific IgG concentration of at least 10 BAU/mL at 28 days after vaccination, assessed in all participants who had a baseline measurement and who completed day 28 after vaccination without SARS-CoV-2 infection. As a post-hoc sensitivity analysis, we also assessed the percentage of responders to vaccination after exclusion of the patients who appeared to be anti-S1 IgG positive at time of repeated vaccination. Secondary outcomes were the serum concentration of S1-specific IgG, the presence of virus neutralising antibodies, SARS-CoV-2 specific T-cell response and safety, all collected at 28 days after vaccination. Exploratory outcomes were the association between baseline clinical and immunological parameters on the one hand and the primary outcome on the other. Post-hoc added exploratory outcomes were the correlation between neutralising activity against the ancestral, delta, and omicron strains and S1-specific IgG concentration, the correlation between S1-specific IFN-γ spot-forming cells (SFCs) and the concentration of S1-specific antibodies, and the correlation between the results of both T-cell assays. The prespecified outcomes anti-SARS-CoV-2 antibodies in nasal fluid (secondary) and SARS-CoV-2 reactive $CD4^+$ and $CD8^+$ cells and RNA-seq analysis (exploratory) will be reported separately.

See Online for appendix

S1-specific IgG was measured in serum samples by a validated fluorescent bead-based multiplex-immunoassay as described previously[10,11] and expressed as BAU/mL. Patients were classified as seropositive or seronegative based on a threshold for seropositivity for this specific assay, defined by a receiver operator curve analysis at a S1-specific IgG concentration of at least 10 BAU/mL.[11,12]

To identify patients who had a SARS-CoV-2 infection before study entry, nucleocapsid-specific antibodies were measured at baseline by multiplex immunoassay, as previously described,[10] and classified as positive or negative (cutoff for positivity set at ≥22 arbitrary units per mL).[13]

Plaque reduction neutralisation tests against the ancestral, delta, and omicron SARS-CoV-2 variants were done as previously described.[2,12,14] For feasibility, it was a priori decided to measure neutralising antibodies only in a random sample of 25 KTRs in each study group.

SARS-CoV-2-specific T-cell responses were measured in subsets of patients by means of an interferon-gamma (IFN-γ) ELIspot assay and a commercially available IFN-γ release assay (IGRA) as previously described.[12,15] The ELISpot assay was done in the same random sample of patients selected for the measurement of neutralising antibodies. IGRA was done in 95 KTRs included in the alternative vaccination study group at one participating centre (Erasmus MC).

Safety was assessed in all vaccinated patients in terms of incidence of solicited local and systemic adverse events within 1 week after vaccine administration graded according to severity. Participants reported these adverse

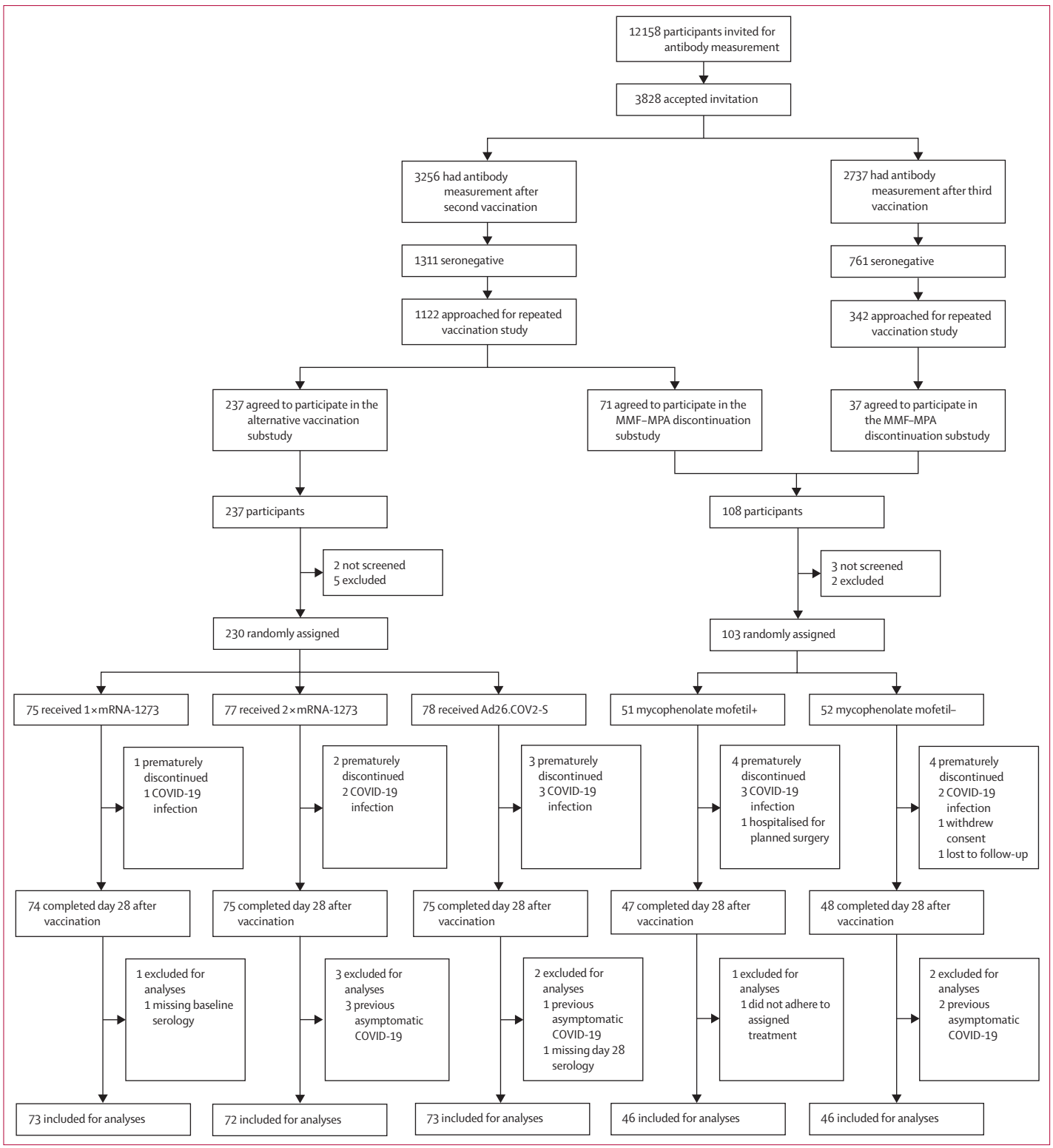

Figure 1: **Flowchart of the vaccination study**
Kidney transplant recipients with available antibody measurements after COVID-19 vaccination; study enrolment and outcomes in alternative vaccination study group; study enrolment and outcomes in the MMF/MPA discontinuation study group. MMF/MPA=mycophenolate mofetil–mycophenolic acid.

	Alternative vaccination study group			Mycophenolate mofetil–mycophenolic acid discontinuation study group	
	1×mRNA-1273 (n=73)	2×mRNA-1273 (n=72)	Ad26.COV2-S (n=73)	Mycophenolate mofetil+ (n=46)	Mycophenolate mofetil– (n=46)
Female	25 (34%)	27 (38%)	25 (34%)	24 (52%)	17 (37%)
Ethnicity					
White	68 (93%)	68 (94%)	65 (89%)	46 (100%)	45 (98%)
Asian	5 (7%)	1 (1%)	7 (10)	0	1 (2)
Black	0	2 (3%)	1 (1%)	0	0
Age, years	57·3 (13·5)	58·5 (11·6)	60·1 (12·4)	59·0 (11·8)	60·5 (12·0)
Body-mass index, kg/m²	26·7 (5·64)	26·0 (3·90)	26·6 (4·97)	26·4 (4·72)	26·6 (3·75)
Systolic blood pressure, mm Hg	149 (24)	145 (18)	146 (22)	141 (14)	145 (20)
Diastolic blood pressure, mm Hg	85 (11)	84 (11)	83 (12)	85 (9)	84 (11)
Number of comorbidities	2 (1–2)	1 (1–2)	1 (1–2·5)	1 (1–2)	1 (1–2)
Comorbidities					
Hypertension	65 (89%)	58 (81%)	64 (88%)	36 (78%)	35 (76%)
Diabetes	25 (34%)	16 (22%)	22 (30%)	9 (20%)	11 (24%)
History of coronary artery disease	9 (12%)	5 (7%)	14 (19%)	4 (9%)	4 (9%)
Heart failure	0	2 (3%)	5 (7%)	2 (4%)	1 (2%)
Chronic lung disease	3 (4%)	8 (11%)	8 (11%)	3 (7%)	2 (4%)
History of malignancy*	11 (15%)	15 (21%)	7 (10%)	3 (7%)	10 (22%)
Auto-immune disease	2 (3)	5 (7)	3 (4%)	6 (13%)	3 (7%)
Lymphocytes, 10^9/L	1·4 (1·1–2·1)	1·5 (1·0–1·9)	1·3 (0·8–1·6)	1·3 (0·9–1·5)	1·2 (1·0–1·6)
eGFR, mL/min per 1·73 m²	49·7 (18·8)	48·9 (18·8)	49·0 (19·1)	48·4 (16·0)	50·4 (19·0)
Primary renal diagnosis					
Primary glomerulonephritis	11 (15%)	12 (17%)	11 (15%)	8 (17%)	4 (9%)
Pyelonephritis	2 (3%)	3 (4%)	0	0	0
Interstitial nephritis	1 (1%)	3 (4%)	4 (5%)	1 (2%)	1 (2%)
Familial–hereditary renal diseases	15 (21%)	20 (28%)	13 (18%)	7 (15%)	8 (17%)
Congenital diseases	8 (11%)	2 (3%)	5 (7%)	1 (2%)	2 (4%)
Vascular diseases	6 (8%)	5 (7%)	8 (11%)	6 (13%)	2 (4%)
Secondary glomerular–systemic disease	8 (11%)	9 (13%)	9 (12%)	0	1 (2%)
Diabetic kidney disease	7 (10%)	1 (1%)	4 (5%)	3 (7%)	10 (22)
Other	5 (7%)	4 (6%)	6 (8%)	14 (30%)	14 (30%)
Unknown	10 (14%)	13 (18%)	13 (18%)	6 (13%)	9 (20%)
Transplant characteristics					
First kidney transplant	64 (88%)	55 (76%)	58 (80%)	40 (87%)	39 (85%)
Time after last transplantation, years	5·8 (3·0–10·5)	7·3 (2·7–12·5)	6·9 (2·5–12·2)	4·1 (2·0–8·0)	4·5 (1·9–7·3)
Last transplant					
Living donor	51 (70%)	54 (75%)	55 (75%)	37 (80%)	30 (65%)
Pre-emptive	31 (42%)	33 (46%)	28 (38%)	27 (59%)	16 (35%)
Number of immunosuppressive agents	2 (2–3)	2 (2–3)	2 (2–3)	3 (3–3)	3 (3–3)

(Table 1 continues on next page)

events daily on a specific form. The incidence of acute rejection and other serious adverse events was monitored until 28 days after vaccine administration. Information on SARS-CoV-2 infection and outcome of COVID-19 was collected by means of a questionnaire, completed at 28 days after vaccination.

Statistical analyses

The sample size was established to test the superiority of alternative vaccination strategies. In cohort one, assuming a response rate of 45% with the two alternative strategies (ie, 2×mRNA-1273 and Ad26.COV2.S) compared with the 20% that was expected with a single dose of 1×mRNA-1273, and a superiority margin of 5%, a group size of 89 was required to achieve a power of 80% and a level of significance of 2·5% (corrected from 5% because of multiple testing). To account for dropouts, we aimed to include 100 patients in each group. In cohort two assuming a superior response rate of 45% in patients with temporary discontinuation of mycophenolate mofetil or mycophenolic acid compared with the 20% that was expected with continuation of mycophenolate mofetil

	Alternative vaccination study group			Mycophenolate mofetil–mycophenolic acid discontinuation study group	
	1 × mRNA-1273 (n=73)	2 × mRNA-1273 (n=72)	Ad26.COV2-S (n=73)	Mycophenolate mofetil+ (n=46)	Mycophenolate mofetil– (n=46)
(Continued from previous page)					
Immunosuppressive treatment					
Steroids	42 (58%)	38 (53%)	46 (63%)	46 (100%)	46 (100%)
Azathioprine	4 (5%)	4 (6%)	1 (1%)	0	0
Mycophenolate mofetil or mycophenolic acid	57 (78%)	60 (83%)	58 (79%)	46 (100%)	46 (100%)
Calcineurin inhibitor	61 (84%)	60 (83%)	60 (82)	46 (100%)	46 (100%)
mTor inhibitor	3 (4%)	2 (3%)	1 (1%)	0	0
Other	0	2 (3%)	2 (3%)	0	1 (2%)
Induction therapy					
Basiliximab	52 (71%)	54 (75%)	45 (62%)	42 (91%)	37 (80%)
Alemtuzumab	1 (1%)	1 (1%)	2 (3%)	1 (2%)	1 (2%)
Antithymocyte globulin	1 (1%)	0	3 (4%)	1 (2%)	2 (4%)
Rituximab	12 (16%)	8 (11%)	12 (16%)	1 (2%)	1 (2%)
None	7 (10%)	7 (10%)	11 (15%)	0	2 (4%)
Unknown	1 (1%)	2 (3%)	2 (3%)	2 (4%)	4 (9%)
Number of previous SARS-CoV-2 vaccinations					
2	73 (100%)	72 (100%)	73 (100%)	33 (72%)	31 (67%)
3	0	0	0	13 (28%)	15 (33%)
Time since last SARS-CoV-2 vaccination, days	198 (189–205)	198 (187–217)	198 (194–220)	180 (115–193)	179 (109–195)
Seropositive at baseline†	20 (27%)	16 (22%)	11 (15%)	14 (30%)	14 (30%)

Data are n (%), mean (SD), or median (IQR). eGFR, estimated glomerular filtration rate. *Including melanomas, excluding all other skin malignancies. †Seropositivity was defined as S1-specific IgG ≥ 10 BAU/mL.

Table 1: **Baseline characteristics**

or mycophenolic, and a superiority margin of 5%, a group size of 71 was required to achieve a power of 80% and a level of significance of 5%. To account for dropouts, we aimed to include 80 patients in each group.

Continuous data are presented as mean SD or as median IQR in case of non-normal distribution. Categorical data are presented as percentages. Differences between groups were tested by means of an independent *t* test, Mann-Whitney-U test, Wilcoxon Singed Rank test (for within-group comparisons), or Pearson χ^2 test, depending on data type and distribution. Correlations were tested by means of the Pearson correlation with log transformation of data in case of non-normal distribution. In post-hoc subgroup analyses, the effect of vaccination strategies was compared after participants were stratified for sex (male or female), age (≥60 or <60 years), estimated glomerular filtration rate (eGFR; ≥45 or <45 mL/min per 1·73 m²), time after last kidney transplantation (≥6·5 or <6·5 years), first kidney transplantation (yes or no), and in the alternative vaccination study group, the use of mycophenolate mofetil or mycophenolic acid (yes or no). The association between baseline clinical parameters and the seroresponse at 28 days after vaccination was assessed by means of multivariable logistic regression analyses. All analyses were done with IBM SPSS statistics version 23·0 (SPSS, Chicago, IL). Figures were created with GraphPad Prism version 5·00 (GraphPad Software, San Diego, CA). A two-sided p value of less than 0·05 was adopted to denote significance, and corrected in case of multiple testing by means of Bonferroni correction unless stated otherwise. The study is funded by The Netherlands Organization for Health Research and Development and the Dutch Kidney Foundation, and is registered with www.ClinicalTrials.gov, NCT05030974.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From April 23, 2021, until July 2, 2021, of 12 158 invited Dutch KTRs, 3828 with a functioning kidney transplant were included in a national survey for antibody measurement after COVID-19 vaccination. Of these patients, 1311 did not seroconvert after their second vaccination and another 761 not even after a third. From these seronegative patients, 345 participated in our repeated vaccination study. A detailed flow chart is provided as figure 1A.

In the alternative vaccination study group, 230 patients were randomly assigned and in 218 patients, analysis of

S1-specific antibody concentrations was done at 28 days after vaccination: 73 received a regular single dose mRNA-1273 (control group), 72 received double dose mRNA-1273 and 73 received Ad26.COV2-S (figure 1).

In the mycophenolate mofetil–mycophenolic discontinuation study group, 103 patients were randomly assigned and in 92 patients analysis of S1-specific antibody concentrations was done at 28 days after vaccination: 46 continued mycophenolate mofetil or mycophenolic acid (mycophenolate mofetil+), and 46 discontinued mycophenolate mofetil or mycophenolic acid from 1 week before to 1 week after vaccination (mycophenolate mofetil–; figure 1). Baseline characteristics of all participants were similar between the groups (table 1).

In the alternative vaccination study group, the differences in seropositivity rate at day 28 after vaccination were –0·4% (95% CI–16 to 15; p=0·96) for the 2×mRNA-1273 group and –6% (–21 to 10; p=0·49) for the Ad26.COV2-S group compared with the 1×mRNA-1273 group. The corresponding seropositivity rates were 50 (68%) of 73 (57 to 79) in the 1×mRNA-1273 control group, 49 (68%) of 72 (56 to 79) in the 2×mRNA-1273 group, and 46 (63%) of 73 (51 to 74) in the Ad26.COV2-S group (figure 2A, left panel). The median concentration of S1-specific antibodies at day 28 after vaccination was not significantly different: 156 BAU/mL (2·47 to 797) in the 1×mRNA-1273 control group, 92·2 BAU/mL (1·77 to 648; p=0·94) in the 2×mRNA-1273 group, and 74·7 BAU/mL (1·60 to 250; p=0·10) in the Ad26.COV2-S group (figure 2B, left panel). The increase from baseline in antibody concentration was significant in each of the three groups, and these increases did not differ between groups (p=0·85 and p=0·11 *vs* control, respectively). 20 patients in the 1×mRNA-1273 control group, 16 in the 2×mRNA-1273 group, and 11 in the Ad26.COV2-S group had S1-specific antibodies of at least 10 BAU/mL at baseline. When these patients were excluded in a sensitivity analysis, seroconversion rate was 31 (58%) of 53 (44 to 72) in the 1×mRNA-1273 control group, 33 (59%) of 56 (45 to 72) in the 2×mRNA-1273 group, and 35 (56%) of 62 (43 to 69) in the Ad26.COV2-S group, again not significantly different (p=0·96 and p=0·83 *vs* control, respectively; appendix p 6). S1-specific antibody concentration at day 28 also did not significantly differ between these groups (p=0·88 and p=0·76; appendix p 6).

In the mycophenolate mofetil–mycophenolic acid discontinuation study group, the difference in seropositivity rate at day 28 after vaccination was 13% (–5 to 31) for the mycophenolate mofetil– group compared with the mycophenolate mofetil+ group (p=0·15). The corresponding seropositivity rates were 31 (67%) of 46 (52 to 80) in the mycophenolate mofetil+ group and 37 (80%) of 46 (66 to 91) in the mycophenolate mofetil– group (figure 2A, right panel). The median concentration of S1-specific antibodies at day 28 after vaccination was 143 (4·58–966) BAU/mL and 119 (23·0–1279) BAU/mL, respectively (p=0·29; figure 2B, right panel). The increase in antibody concentration did not differ between the two groups (p=0·24). Fourteen patients in the mycophenolate mofetil+ group and 14 in the mycophenolate mofetil– group had S1-specific antibodies of at least 10 BAU/mL at baseline. When these patients were excluded, seroconversion rate was 17 (53%) of 32 (95% CI 35 to 71) in the mycophenolate mofetil+ group and 23 (72%) of 32 (53 to 86) in the mycophenolate mofetil– group (p=0·12; appendix p 7) and again, also the median concentration of S1-specific antibodies at day 28 was not significantly different (p=0·17; appendix p 7).

In a random selection of 25 patients per group from each study group, the neutralising activity of serum against the ancestral SARS-CoV-2 and the delta and omicron (BA.1) variants was assessed. In both the

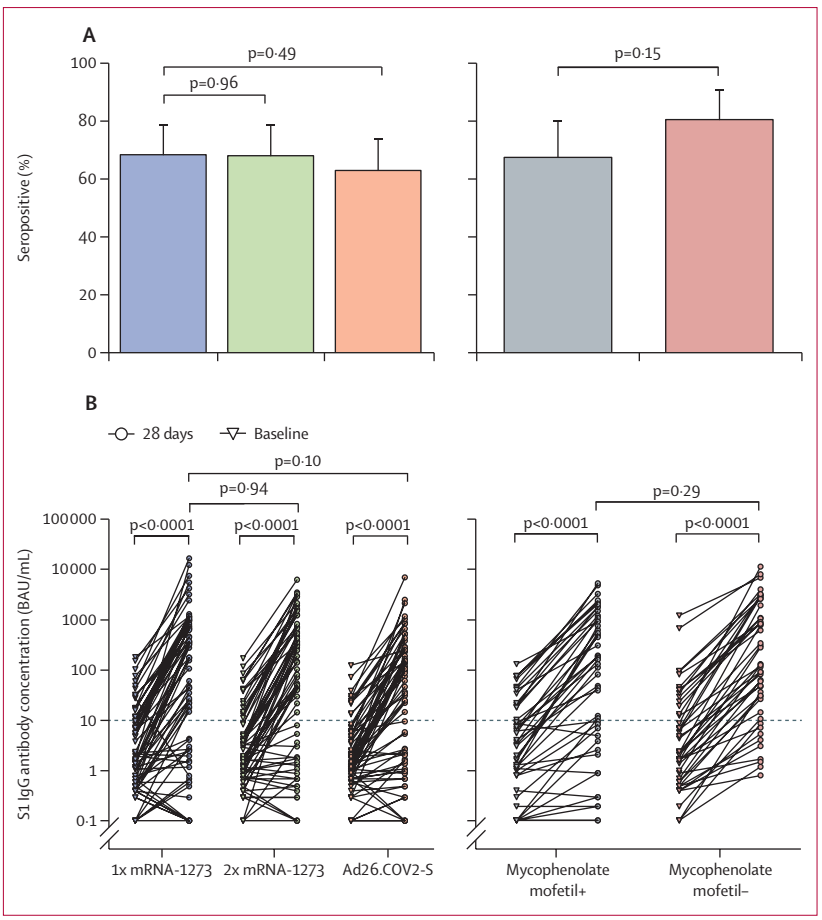

Figure 2: **Serological response in the alternative vaccination study group (left panel) and the mycophenolate mofetil–mycophenolic acid discontinuation study group (right panel)**
Proportion (95% CI) of seroresponders per randomisation group at 28 days after vaccination; responders were defined as participants with a S1-specific IgG antibody concentration ≥10 BAU/mL after vaccination; p values were calculated by means of the χ^2 test (A). SARS-CoV-2 Spike S1-specific serum IgG concentrations at baseline and 28 days after vaccination; depicted are dots representing each patient; dotted line indicates cutoff value for seropositivity; p values between groups were calculated by means of the Mann-Whitney U test and within groups with the Wilcoxon Signed Rank test (B). BAU= binding antibody units.

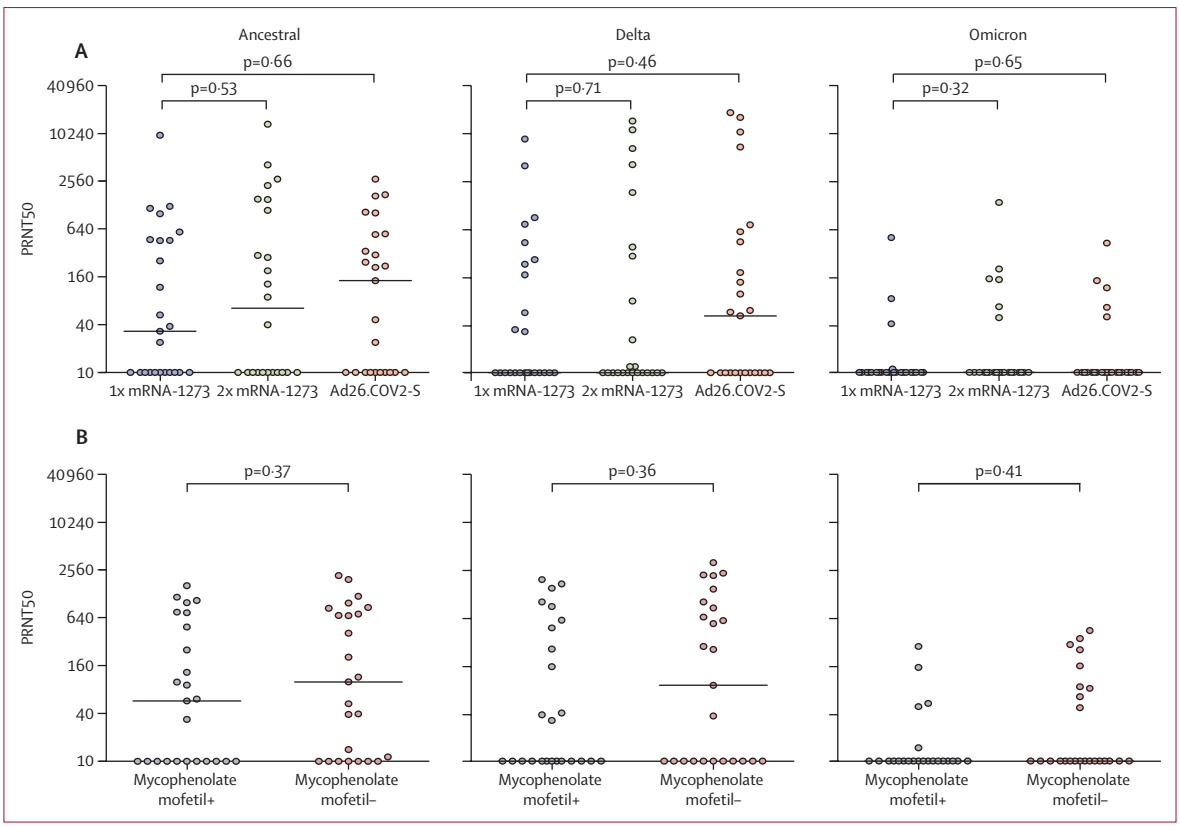

Figure 3: **Neutralising antibody titres for the ancestral, delta, and omicron (BA.1) strain of SARS-CoV-2 at 28 days after vaccination in the alternative vaccination study group (A) and the mycophenolate mofetil– mycophenolic acid discontinuation study group (B)**
p values were calculated by means of the Mann-Whitney U test. PRNT50=50% plaque reduction neutralisation test.

alternative vaccination study group and the mycophenolate mofetil–mycophenolic acid discontinuation study group, neutralising antibody concentrations at day 28 after vaccination were not significantly different between the groups (figures 3A and 3B). Neutralising activity against the delta and especially against the omicron variant was lower than against the ancestral variant.

In the alternative vaccination study group, the proportion of patients with a positive response in the ELISpot assay at 28 days after vaccination was 11 (52%) of 21 (95% CI 30–74) in the 1×mRNA-1273 control group, 11 (52%) of 21 (30–74) in the 2×mRNA-1273 group, and six (29%) of 21 (11–52) in the Ad26.COV2-S group (p=0·99 and p=0·12 *vs* control, respectively; figure 4A, left panel). Median S1-specific IFN-γ SFCs/10^6 peripheral blood mononuclear cells (PBMCs) at 28 days did not differ between the three study groups (figure 4B, left panel). At baseline, T-cell reactivity was found in a proportion of patients in the three groups: ten (45%) of 22 (24–68), seven (37%) of 19 (16–62), and nine (47%) of 19 (24–71), respectively (not significant). After exclusion of these patients, the proportion of patients with a positive response was five (42%) of 12 (15–72) in the 1×mRNA-1273 control group, six (50%) of 12 (21–79) in the 2×mRNA-1273 and 0 of nine in the Ad26.COV2-S group (p=0·68 and p=0·03 *vs* control, respectively).

In the mycophenolate mofetil–mycophenolic acid discontinuation study group, a positive response in the ELISpot assay at 28 days after vaccination was observed in 16 (67%) of 24 (95% CI 45–84) and 13 (54%) of 24 (32–74) of the mycophenolate mofetil+ and mycophenolate mofetil– groups, respectively (figure 4A, right panel). Median S1-specific IFN-γ SFCs/10^6 PBMCs at 28 days did not differ between the mycophenolate mofetil+ and mycophenolate mofetil– groups (figure 4B, right panel). T-cell reactivity at baseline was found in 12 (50%) of 24 (29–71) of the mycophenolate mofetil+ group and in nine (36%) of 25 (18–57) of the mycophenolate mofetil– group. After exclusion of these patients, the proportion of positive response was six (50%) of 12 (21–79) in the mycophenolate mofetil+ and six (40%) of 15 (16–68) in the mycophenolate mofetil– group (p=0·60).

In participants of the alternative vaccination study group included in one of the centres (Erasmus MC), the T-cell response was also assessed by an IGRA assay. The proportion of patients with a SARS-CoV-2-specific T-cell response at 28 days was five (17%) of 30 (6–35) in the 1×mRNA-1273 control group, five (17%) of 29 (6–36) in

the 2×mRNA-1273 group, and five (17%) of 29 (6–36) in the Ad26.COV2-S group (p=0·95 and p=0·95 *vs* control, respectively; appendix p 8). Median IFN-γ concentration at 28 days was not different between the three groups (appendix p 8).

Safety analysis was done in all patients who received a vaccination. In the alternative vaccination study group, the percentage of patients who reported any solicited adverse event after vaccination was significantly lower in patients who received the Ad26.COV2-S vaccine than in patients who received a single dose of the mRNA-1273 vaccine (60 [79%] of 76 *vs* 68 [96%] of 71; p=0·0024). This difference was mainly due to a lower percentage of patients with pain at the injection site in the Ad26.COV2-S group (table 2). Only four serious adverse events (dehydration, diarrhoea, pneumonia, and COVID-19) were reported, three in the 1×mRNA-1273 group and one in the Ad26.COV2-S group (table 2). These serious adverse events were considered not related to vaccination. In the mycophenolate mofetil–mycophenolic acid discontinuation study group, the percentage of patients who reported any solicited adverse event after vaccination was not different between the mycophenolate mofetil+ and mycophenolate mofetil– groups (table 2). Only two serious adverse events (cellulitis and COVID-19) were reported, one in the mycophenolate mofetil+ group and one in the mycophenolate mofetil– group. Serum creatinine at baseline and 28 days after vaccination was 133 (SD 46) µmol/L and 136 (48) µmol/L in the mycophenolate mofetil+ group (p=0·23), and 138 (60) µmol/L and 142 (55) µmol/L in the MMF– group (p=0·076).

For the exploratory outcomes, we first analysed the correlation between neutralising activity and S1-specific IgG concentration in each treatment group from both study groups (n=123). Neutralising activity against the ancestral, delta, and omicron strains correlated well with the concentrations of S1-specific IgG antibodies at 28 days after vaccination (ancestral R=0·88, p<0·0001; delta R=0·78, p<0·0001; omicron R=0·62, p<0·0001; appendix p 9). Notably, much higher S1-specific IgG concentrations were required for neutralisation of the omicron variant as compared with the delta and ancestral variant (appendix p 9). Second, at 28 days there was a moderate correlation between S1-specific IFN-γ SFCs and the concentrations of S1-specific antibodies (R=0·37, p<0·0001; appendix p 10). Third, in 28 participants T-cell responses were measured both by ELISpot and IGRA. There was a significant correlation between the results of both assays, both at baseline and at 28 days (R=0·42, p=0·027 and R=0·40, p=0·042, respectively; appendix p 11). Fourth, also in subgroup analyses, the effect of the various vaccination strategies did not differ significantly in either study group (appendix p 12). Fifth, in multivariable stepwise backward logistic regression analysis, diabetes and lower eGFR were significantly associated with the risk of being a non-responder in the alternative vaccination study group. In the mycophenolate mofetil–mycophenolic acid discontinuation study group, continuing mycophenolate mofetil or mycophenolic acid, higher age, lower eGFR, lower lymphocyte count, and hypertension were associated with the risk of being a non-responder (appendix p 5). Lastly, we compared baseline characteristics between patients who previously received two versus three SARS-CoV-2 vaccinations in the mycophenolate mofetil–mycophenolic acid discontinuation study group (appendix p 6). There were no significant differences, except from a higher proportion of patients with a history of malignancy in those who had received three vaccinations (appendix p 6).

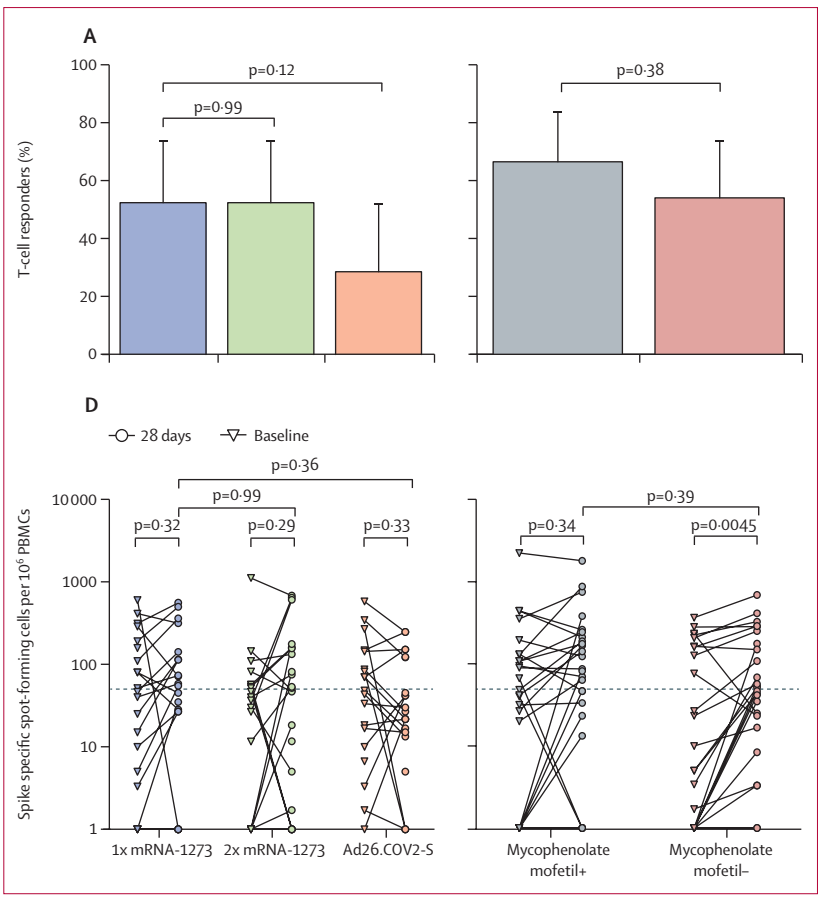

Figure 4: **T-cell response measured by ELISpot in the alternative vaccination study group (left panel) and the mycophenolate mofetil–mycophenolic acid discontinuation study group (right panel)**
Proportion (95% CI) of participants with response per randomisation group at 28 days after vaccination; p values were calculated by means of the χ^2 test (A). Spike specific IFN-γ SFCs/10^6 PBMCs at baseline and 28 days after vaccination; dotted line indicates threshold for T-cell response (≥50 spot forming cells/10^6 PBMCs); p values between groups were calculated by means of the Mann-Whitney U test and within groups with the Wilcoxon Signed Rank test (B). PBMCs=peripheral blood mononuclear cells.

Discussion

In this prospective, randomised trial we assessed the immunogenicity of a double dose of an mRNA vaccine, heterologous vaccination, or temporary discontinuation of mycophenolate mofetil or mycophenolic acid as

	Alternative vaccination study group					Mycophenolate mofetil–mycophenolic acid discontinuation study group		
	1 × mRNA-1273 (n=71)	2 × mRNA-1273 (n=73)	p value*	Ad26.COV2-S (n=76)	p value*	Mycophenolate mofetil+ (n=51)	Mofetil– (n=50)	p value
Any adverse event†	68 (96%)	72 (99%)	0·30	60 (79%)	0·0024	49 (96%)	47 (94%)	0·63
Any systemic symptom	48 (68%)	60 (82%)	0·043	54 (71%)	0·65	37 (73%)	40 (80%)	0·38
Arthralgia	21 (30%)	19 (26%)	0·63	24 (32%)	0·79	15 (29%)	15 (30%)	0·95
Fatigue	36 (51%)	44 (60%)	0·25	37 (49%)	0·81	28 (55%)	23 (46%)	0·37
Fever	2 (3%)	5 (7%)	0·26	1 (1%)	0·52	3 (6%)	3 (6%)	0·98
Chills	15 (21%)	27 (37%)	0·036	13 (17%)	0·54	18 (35%)	12 (24%)	0·21
Headache	25 (35%)	31 (42%)	0·37	40 (53%)	0·034	22 (43%)	20 (40%)	0·75
Myalgia	32 (45%)	43 (59%)	0·10	31 (41%)	0·60	19 (37%)	22 (44%)	0·49
Nausea	13 (18%)	16 (22%)	0·59	12 (16%)	0·68	10 (20%)	9 (18%)	0·84
Any local symptom	65 (92%)	67 (92%)	0·96	38 (50%)	<0·0001	49 (96%)	45 (90%)	0·23
Erythema	5 (7%)	10 (14%)	0·19	3 (4%)	0·41	12 (24%)	10 (20%)	0·67
Induration	8 (11%)	17 (23%)	0·057	5 (7%)	0·32	15 (29%)	15 (30%)	0·95
Pain at injection side	64 (90%)	67 (92%)	0·73	38 (50%)	<0·0001	47 (92%)	45 (90%)	0·70
Serious adverse events								
Number	n=75	n=77	..	n=78	..	n=51	n=51‡	..
Any serious adverse event	3 (4%)	0	0·076	1 (1%)	0·29	1 (2%)	1 (2%)	..
Related to vaccination	0	..	..	..	..	0	0	..
Not related to vaccination								
Total	3 (4%)	..	..	1 (1%)	..	1 (2%)	1 (2%)	..
Dehydration	1 (1%)	..	..	..	..	..	..	..
Diarrhoea	1 (1%)	..	..	..	..	..	..	..
Bacterial pneumonia	1 (1%)	..	..	..	..	..	..	..
COVID-19	..	..	..	1 (1%)	..	..	1 (2%)	..
Cellulitis	..	..	..	..	..	1 (2%)	..	..

Data n (%). Variables are given as number and percentage. p values were calculated by χ^2 test. *p values are given for the comparisons *vs* control groups. In case of multiple testing, a p value <0·025 was considered as significant. †Missing data for 11 subjects (n=4, 1 × mRNA-1273, n=4, 2 × mRNA-1273, n=2, Ad26.COV2-S and n=1, mycophenolate mofetil–). ‡Number not equal to number randomly assigned as one subject withdrew consent before receiving vaccination.

Table 2: **Incidence of solicited adverse events* up to 7 days after vaccination and serious adverse events up to 28 days after vaccination**

compared with standard dose mRNA vaccination against COVID-19 in KTRs who were serological non-responders after two or three doses of an mRNA vaccine.

The major finding of our study is that none of the investigated alternative vaccination strategies was more immunogenic than administering a single dose of the mRNA-1273 vaccine. Notably, in the two study groups, 63 to 80% of patients were seropositive after a repeated single dose vaccination. These figures are higher than the seroconversion rates of 39 to 54% reported in other studies assessing the response to third vaccination in seronegative KTR.[16,17] This discrepancy might in part be related to the fact that 24% of all participants who were seronegative during screening, appeared to be seropositive at the time of repeated vaccination, which took place at a median interval of about 6 months after the preceding vaccination. Seroconversion due to COVID-19 was excluded as well as possible on the basis of the reporting of patients and the measurement of SARS-CoV-2 nucleocapsid-specific antibodies, but asymptomatic cases could have gone unnoticed since a regular screening with PCR tests was not done. Alternatively, vaccination induced seroconversion could have occurred later than the time of assessment (14–56 days) after the second (or third) vaccination. Such a delayed humoral response after COVID-19 mRNA vaccination in KTRs has been described previously.[18,19] After exclusion of patients who were seropositive at time of vaccination, the response rate after repeated vaccination in our control group was only slightly higher than described in the literature. In addition, the longer time interval between the repeated and preceding vaccination in our patients (median 196 days) as compared with that in other studies (median 80–109 days)[16,17] might also have contributed to a relatively high seroconversion rate.[20] In any case, the fact that multiple studies have reported a considerable increase in seroresponse rate after each additional booster vaccination[21] underscores the importance of a high uptake in new booster vaccination campaigns for all KTRs.

The presence of neutralising antibodies probably represents a major mechanism of protection against COVID-19.[22] We therefore also assessed serum neutralising activity against different SARS-CoV-2 variants in randomly selected subgroups of study

participants. Although increasing concentrations of S1-specific antibodies were required to achieve neutralisation of newer SARS-CoV-2 variants, there were no significant differences between the various vaccination strategies with regard to neutralising antibody titres.

It has been shown that organ transplant recipients in whom antibodies are not detectable can still have developed cellular immunity.[23] We therefore also evaluated T-cell responses, in particular IFN-γ production by T-cells, as assessed by ELISpot and IGRA. Again, no significant effect of the type of vaccination strategy was observed. Notably, in a considerable proportion of patients a T-cell response was already detectable at baseline, suggesting that the T-cells of these patients had been primed before. This confirms the observation that the humoral and cellular immune response after COVID-19 vaccination can be discordant.[24] Unexpectedly, we observed a decrease in T-cell response between baseline and 28 days after vaccination in some participants. This suggests that ex vivo measured reactivity of T-cells isolated from peripheral blood might vary over time and can be influenced by factors unrelated to vaccination. The fact that these variations in time were observed with both ELISpot and IGRA, as well as the observed correlation between the results of both assays, argues against a major technical issue with one or both of these assays.

Previously, a stronger effect of higher vaccine doses has been shown for influenza vaccination in elderly adults and organ transplant recipients, and for hepatitis B vaccination in patients infected with the human immunodeficiency virus.[5,25] Moreover, in a phase one study with the mRNA-1273 vaccine, a dose of 250 µg was associated with increased antibody titres at 1 month after vaccination compared with a dose of 100 µg.[26] However, our data indicate that in the context of repeated COVID-19 vaccination in patients using immunosuppressive drugs, increasing the dose of the mRNA-1273 vaccine has no beneficial effect.

Several studies have suggested a stronger or longer lasting immunogenic effect of heterologous versus homologous vaccination schedules.[7,27] However in this study, we could not show an advantage on antibody response or T-cell reactivity at 28 days after heterologous vaccination with Ad26.COV2-S, which was corroborated by another randomised clinical trial.[17] However, in a non-randomised cohort study in organ transplant recipients who remained seronegative after two mRNA vaccines, percentages of seropositive patients were similar at 1 month but higher at 3 months and 6 months after administration of Ad26.COV2-S as compared with an mRNA vaccine.[28] Although the percentage of seropositive patients at 28 days after Ad26.COV2-S vaccination in our RCT was 63%, similar to that in the observational study, the design of our study did not allow us to investigate the presence of a delayed beneficial effect of heterologous vaccination. A remarkable finding with administration of Ad26.COV2-S was the lower incidence of pain at the injection site, which was also observed in the earlier comparisons with the mRNA vaccines.[17,28]

Our rationale for temporary cessation of mycophenolate mofetil or mycophenolic acid around the time of vaccination was the strong negative association between immunogenicity of COVID-19 vaccination in KTRs and the use of these drugs in the current and previous studies.[2] Since interruption of treatment with mycophenolate mofetil or mycophenolic acid might increase the risk of graft rejection, we opted for a relatively short duration of discontinuation (2 weeks). Risks were furthermore mitigated by restricting this strategy to patients who used triple immunosuppressive therapy with sufficient exposure to the other two drugs, exclusion of patients with a higher immunological risk of rejection, and close monitoring of kidney function. We found no beneficial effect of suspending the use of mycophenolate mofetil or mycophenolic acid on the immunogenicity of repeated vaccination. Interestingly, it has been reported that a relatively high seroconversion rate (76%) was obtained after a fourth vaccine dose (BNT162b2) in 29 KTRs without a humoral immune response after previous vaccinations in whom mycophenolate mofetil, or azathioprine in one patient, was discontinued from 4–7 days before to 28–35 days after the fourth vaccination.[29] Unlike our study, this study did not include a control group, which hampers the interpretation of the results. Moreover, 20% of their patients were left on single immunosuppressive therapy during discontinuation of mycophenolate mofetil or mycophenolic acid whereas all our patients remained on double immunosuppressive therapy. Finally, the mean time since transplantation was longer than in our study (9.9 years *vs* 4.3 years). It remains therefore to be established whether longer duration of mycophenolate mofetil or mycophenolic acid discontinuation or replacement by another drug can be helpful, and if so, how this should be timed in relation to the repeated vaccination.

Since none of the approaches investigated here appeared to augment the response to vaccination, alternative strategies should be considered to protect immunocompromised patients who remain persistently seronegative against the consequences of COVID-19. One such strategy could be pre-exposure prophylaxis with monoclonal antibodies,[30] although the efficacy of this treatment might decline with the emergence of newer virus variants.

The main strength of this study is the prospective, randomised design. We evaluated three alternative vaccination strategies in KTRs who remained seronegative after two or three doses of a COVID-19 mRNA vaccine and included control groups that received a standard dose of mRNA vaccine. In addition to S1-specific IgG antibodies, we measured serum virus

neutralising activity and T-cell reactivity at 28 days after vaccination. Lastly, our findings are relevant for other patients using immunosuppressive drugs, and useful for the design of vaccination strategies against other viruses in immunosuppressed patients.

Our study also has limitations. First, the number of patients analysed was lower than the predefined sample size in both study groups (82% and 65% of targets achieved, respectively). When we started recruitment of patients, some patients had already accepted an invitation for a third vaccination via the national vaccination programme. Moreover, patients were often reluctant to discontinue mycophenolate mofetil or mycophenolic acid temporarily for fear of rejection. Although there was a slight trend for a higher seroconversion rate in patients who temporarily suspended the use of mycophenolate mofetil or mycophenolic acid, it remains a matter of speculation whether an increase of the sample size would have changed the results essentially. Second, the sample size and duration of follow-up do not allow any conclusion on clinical efficacy against infection or disease. Nonetheless, S1-specific IgG concentrations and neutralising activity are considered the best surrogate measure for clinical outcome. Finally, we studied only one of the two available mRNA vaccines. Although increasing the dose of the mRNA-173 vaccine did not enhance the immunogenicity of vaccination, this might be different for the BNT162b2 vaccine which appears to be somewhat less immunogenic than the mRNA-1723 vaccine in the currently used dosages.

In conclusion, administering a double dose of mRNA-1273, heterologous vaccination with Ad26.COV2-S, or 2 weeks discontinuation of mycophenolate mofetil or mycophenolic acid did not increase the immunogenicity as compared with a single dose of mRNA-1273 in KTRs who remained seronegative after two or three mRNA vaccinations. Repeated vaccinations are therefore the most successful strategy to achieve seropositivity.

RECOVAC collaborators

Alferso C Abrahams (University Medical Center Utrecht, Utrecht, Netherlands), Marije C Baas (Radboud Institute for Health Sciences, Nijmegen, Netherlands), Pim Bouwmans (University of Maastricht, Maastricht, Netherlands), Marc A G J ten Dam (Nefrovisie, Utrecht, Netherlands), Lennert Gommers (Erasmus Medical Center, Rotterdam, Netherlands), Dorien Standaar (University of Amsterdam, Amsterdam, Netherlands), Marieke van der Heiden (University Medical Center Groningen, Groningen, Netherlands), Yvonne M R Adema (University of Groningen, University Medical Center Groningen, Groningen, Netherlands), Marieken J Boer-Verschragen (Erasmus Medical Center, Rotterdam, Netherlands), Wouter B Mattheussens (Radboud Institute for Health Sciences, Nijmegen, Netherlands), Ria H L A Philipsen (Radboud Institute for Health Sciences, Nijmegen, Netherlands), Djenolan van Mourik (Erasmus Medical Center, Rotterdam, Netherlands), Susanne Bogers (Erasmus Medical Center, Rotterdam, Netherlands), Laura L A van Dijk (Erasmus Medical Center, Rotterdam, Netherlands), Nynke Rots (National Institute for Public Health and the Environment, Bilthoven, Netherlands), Gaby Smits (National Institute for Public Health and the Environment, Bilthoven, Netherlands), Marjan Kuijer (National Institute for Public Health and the Environment, Bilthoven, Netherlands), Marc H Hemmelder (University of Maastricht, Maastricht, Netherlands).

Contributors

RTG, J-SFS, and LBH designed the study protocol. MMLK, ALM, PV, MEJR, DvB, FJB, DAD, RDdV, and CHG contributed to the protocol design. ALM, CCB, RvB, DAD, SCF, DG, CHG, GdH, VJCHK, CI, SRKM, APJdV, and RDdV were involved with data acquisition. MMLK, ALM, PV, MEJR, CCB, DvB, FJB, DAD, RTG, RGvdM, EBMR, RDdV, CHG, J-SFS, and LBH participated in the study, analysis of the data, and preparation of the article. ALM, MMLK, J-SFS, and LBH accessed and verified the data. LBH, J-SFS, RTG, ALM, and MMLK were responsible for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Data sharing

The data that support the findings of this study are available from the corresponding author, on reasonable request. Research proposals can be submitted to the consortium members via the corresponding author.

Acknowledgments

We thank our funding sources, The Netherlands Organization for Health Research and Development and the Dutch Kidney Foundation.

References

1 Hilbrands LB, Duivenvoorden R, Vart P, et al. COVID-19-related mortality in kidney transplant and dialysis patients: results of the ERACODA collaboration. *Nephrol Dial Transplant* 2020; **35:** 1973–83.

2 Sanders JF, Bemelman FJ, Messchendorp AL, et al. The RECOVAC immune-response study: the immunogenicity, tolerability, and safety of COVID-19 vaccination in patients with chronic kidney disease, on dialysis, or living with a kidney transplant. *Transplantation* 2022; **106:** 821–34.

3 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:** 1244–46.

4 Heeger PS, Larsen CP, Segev DL. Implications of defective immune responses in SARS-CoV-2 vaccinated organ transplant recipients. *Sci Immunol* 2021; **6:** eabj6513.

5 Mombelli M, Rettby N, Perreau M, Pascual M, Pantaleo G, Manuel O. Immunogenicity and safety of double versus standard dose of the seasonal influenza vaccine in solid-organ transplant recipients: a randomized controlled trial. *Vaccine* 2018; **36:** 6163–69.

6 Conference report. Rabies vaccines: WHO position paper—recommendations. *Vaccine* 2010; **28:** 7140–42.

7 Schmidt T, Klemis V, Schub D, et al. Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination. *Nat Med* 2021; **27:** 1530–35.

8 Bouwmans P, Messchendorp AL, Sanders JS, et al. Long-term efficacy and safety of SARS-CoV-2 vaccination in patients with chronic kidney disease, on dialysis or after kidney transplantation: a national prospective observational cohort study. *BMC Nephrol* 2022; **23:** 55.

9 Vogelzang EH, Loeff FC, Derksen NIL, et al. Development of a SARS-CoV-2 total antibody assay and the dynamics of antibody response over time in hospitalized and nonhospitalized patients with COVID-19. *J Immunol* 2020; **205:** 3491–99.

10 den Hartog G, Schepp RM, Kuijer M, et al. SARS-CoV-2-Specific antibody detection for seroepidemiology: a multiplex analysis approach accounting for accurate seroprevalence. *J Infect Dis* 2020; **222:** 1452–61.

11 den Hartog G, Vos ERA, van den Hoogen LL, et al. Persistence of antibodies to severe acute respiratory syndrome coronavirus 2 in relation to symptoms in a nationwide prospective study. *Clin Infect Dis* 2021; **73:** 2155–62.

12 Geers D, Shamier MC, Bogers S, et al. SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalescent donors and vaccinees. *Sci Immunol* 2021; **6:** eabj1750.

13 van den Hoogen LL, Smits G, van Hagen CCE, et al. Seropositivity to nucleoprotein to detect mild and asymptomatic SARS-CoV-2 infections: a complementary tool to detect breakthrough infections after COVID-19 vaccination? *Vaccine* 2022; **40:** 2251–57.

14 GeurtsvanKessel CH, Geers D, Schmitz KS, et al. Divergent SARS-CoV-2 omicron-reactive T and B cell responses in COVID-19 vaccine recipients. *Sci Immunol* 2022; **7:** eabo2202. https://doi.org/10.1126/sciimmunol.abo2202.

15 Oosting SF, van der Veldt AAM, GeurtsvanKessel CH, et al. mRNA-1273 COVID-19 vaccination in patients receiving chemotherapy, immunotherapy, or chemoimmunotherapy for solid tumours: a prospective, multicentre, non-inferiority trial. *Lancet Oncol* 2021; **22:** 1681–91.

16 Schimpf J, Davidovic T, Abbassi-Nik A, Sprenger-Mähr H, Lhotta K, Zitt E. Enhanced SARS-CoV-2 antibody response after a third heterologous vector vaccine Ad26COVS1 dose in mRNA vaccine-primed kidney transplant recipients. *Transpl Int* 2022; **36:** 10357.

17 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:** 165–71.

18 Georgery H, Devresse A, Saad Albichr I, et al. Delayed humoral response after 2 doses of the BNT162b2 vaccine in a Belgian kidney transplant cohort. *Transplantation* 2022; **106:** e192–93.

19 Sanders JSF, Messchendorp AL, de Vries RD, et al. Antibody and T-cell responses 6 months after COVID-19 mRNA-1273 vaccination in patients with chronic kidney disease, on dialysis, or living with a kidney transplant. *Clin Infect Dis* 2022; ciac557.

20 Amirthalingam G, Bernal JL, Andrews NJ, et al. Serological responses and vaccine effectiveness for extended COVID-19 vaccine schedules in England. *Nat Commun* 2021; **12:** 7217.

21 Osmanodja B, Ronicke S, Budde K, et al. Serological response to three, four and five doses of SARS-CoV-2 vaccine in kidney transplant recipients. *J Clin Med* 2022; **11:** 2565.

22 Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; **27:** 1205–11.

23 Schmidt T, Klemis V, Schub D, et al. Cellular immunity predominates over humoral immunity after homologous and heterologous mRNA and vector-based COVID-19 vaccine regimens in solid organ transplant recipients. *Am J Transplant* 2021; **21:** 3990–4002.

24 Fernández-Ruiz M, Almendro-Vázquez P, Carretero O, et al. Discordance between SARS-CoV-2-specific cell-mediated and antibody responses elicited by mRNA-1273 vaccine in kidney and liver transplant recipients. *Transplant Direct* 2021; **7:** e794.

25 Tian Y, Hua W, Wu Y, et al. Immune response to hepatitis B virus vaccine among people living with HIV: a meta-analysis. *Front Immunol* 2021; **12:** 745541.

26 Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 - preliminary report. *N Engl J Med* 2020; **383:** 1920–31.

27 Atmar RL, Lyke KE, Deming ME, et al. Homologous and heterologous Covid-19 booster vaccinations. *N Engl J Med* 2022; **386:** 1046–57.

28 Chiang TP, Alejo JL, Mitchell J, et al. Heterologous Ad.26.COV2.S versus homologous BNT162b2/mRNA-1273 as a third dose in solid organ transplant recipients seronegative after two-dose mRNA vaccination. *Am J Transplant* 2022; **22:** 2254–60.

29 Schrezenmeier E, Rincon-Arevalo H, Jens A, et al. Temporary antimetabolite treatment hold boosts SARS-CoV-2 vaccination-specific humoral and cellular immunity in kidney transplant recipients. *JCI Insight* 2022; **7:** e157836.

30 Levin MJ, Ustianowski A, De Wit S, et al. Intramuscular AZD7442 (tixagevimab-cilgavimab) for Prevention of Covid-19. *N Engl J Med* 2022; **386:** 2188–200.