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The RECOVAC IR study: the immune response and safety of the mRNA-1273 COVID-19 vaccine in patients with chronic kidney disease, on dialysis or living with a kidney transplant

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Coronavirus disease 2019 (COVID-19) is associated with severe morbidity and mortality in patients with chronic kidney disease (CKD), on dialysis and kidney transplant recipients [1, 2]. Although effective COVID-19 vaccination would lead to great clinical benefit, most studies with the presently available vaccines have excluded the aforementioned patients. The resulting lack of data is a problem, because vaccine efficacy is known to be considerably lower in patients with CKD and renal replacement therapy [3]. Recent reports have suggested that only a minority of kidney transplant recipients developed anti-severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) antibodies after messenger RNA (mRNA) COVID-19 vaccination [4, 5].

The REnal Patients COVID-19 VACcination Immune Response (RECOVAC IR) study (ClinicalTrials.gov NCT04741386) aims to assess the immunogenicity and safety of COVID-19 vaccination in these specific patient groups up to 12 months post-vaccination (Figure 1). This prospective, controlled, multicenter study includes four different cohorts: (A) 175 patients with CKD Stages 4/5 (CKD4/5) (estimated glomerular filtration rate <30 mL/min/1.73 m^2), (B) 175 patients on dialysis, (C) 300 kidney transplant recipients and (D) 200 controls (family or household members) in four university medical centres across The Netherlands. Included are people >18 years of age without previously known COVID-19, active malignancy or immune deficiency (Supplementary data, Table S1). Participants receive two doses of the mRNA-1273 COVID-19 vaccine (Moderna Biotech Spain, S.L.) with a 28-day interval.

The primary endpoint is the SARS-CoV-2 spike S1-specific immunoglobulin G (IgG) antibody concentration on day 28 after the second vaccination, measured by a validated fluorescent bead-based multiplex immunoassay [6]. Classification as responders or non-responders is based on seroconversion. The threshold for seropositivity based on receiver operating

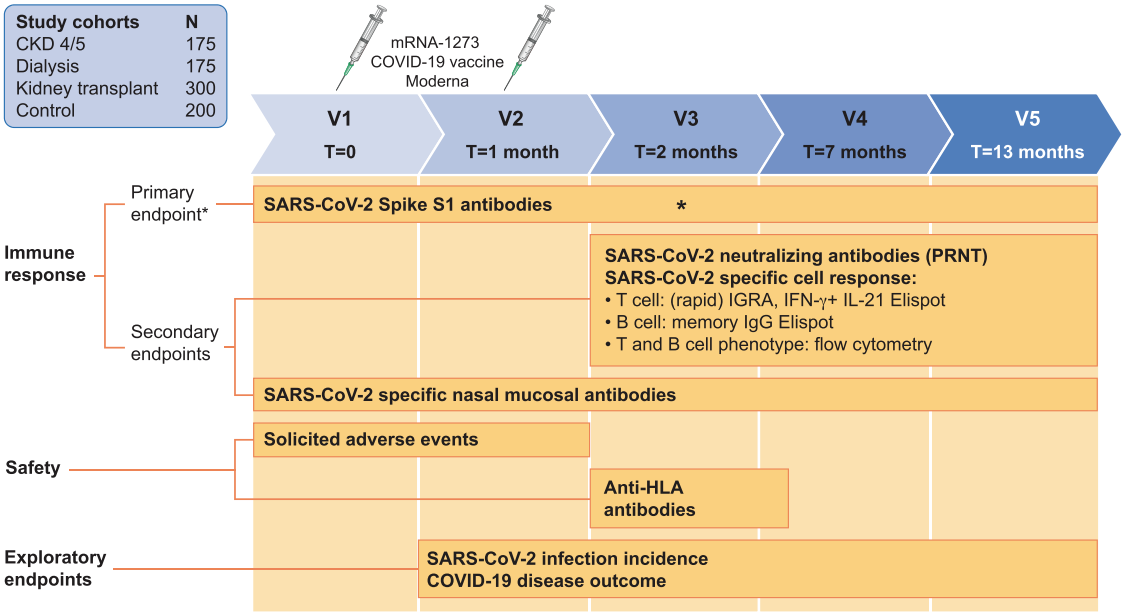

FIGURE 1: Four cohorts of study participants attend five study visits. At Visit 1 (V1) and V2, participants receive the mRNA-1273 COVID-19 vaccine (Moderna). SARS-CoV-2 spike S1 antibodies are measured at all time points, including baseline. The primary endpoint is the antibody response at V3. Secondary endpoints are SARS-CoV-2 neutralizing antibodies and specific T and B cell responses measured at V3–5 and SARS-CoV-2-specific nasal mucosal antibodies measured at all time points. Safety is monitored by questionnaires to register solicited adverse events during 7 days after every vaccination. In immunized patients, anti-HLA antibodies are monitored after vaccination. SARS-CoV-2 infection incidence and disease outcome from the first vaccination to the end of the study are exploratory endpoints. Visit 1, first vaccination; Visit 2, second vaccination; PRNT, plaque reduction neutralization assay; IL-21, interleukin-21; HLA, human leucocyte antigen.

characteristics curve analysis was set at 1.04 AU/mL or 10.08 binding antibody units (BAU)/mL according to the recently adopted National Institute for Biological Standards and Control/World Health Organization COVID-19 reference serum 20/136 in individuals without measurable anti-S antibodies at baseline [7]. The percentages of responders in cohorts A–C are compared with cohort D as well as quantitative levels within and between cohorts to define groups that respond suboptimal to vaccination. Individuals who appear seropositive at baseline will be analysed separately.

Secondary endpoints are antibody longevity up to 1 year post-vaccination and SARS-CoV-2-specific T and B cell responses. Neutralizing capacity of SARS-CoV-2-specific antibodies is determined by a plaque reduction neutralization assay in a subgroup of participants, guided by S1-specific IgG level outcome [8]. SARS-CoV-2-specific T cell response is measured by an interferon γ (IFN-γ) release assay (IGRA) on freshly collected whole blood and IFN-γ enzyme-linked immunosorbent spot assay (ELISpot) on cryopreserved peripheral blood mononuclear cells (PBMCs; Mabtech IFN-γ antibody pairs with alkaline phosphatase development). Results are expressed as IU IFN-γ per millilitre plasma (IGRA) or the number of IFN-γ-producing SARS-CoV-2-specific T cells per million PBMCs. Any spot above the median control is considered positive. The number and phenotype of SARS-CoV-2-specific T cells will be studied by flow cytometry with human leucocyte antigen (HLA) class I tetramers, as previously described [9, 10]. In-depth flow cytometric analyses for functional and phenotypic characterization of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cell responses will be performed in a subset of patients by staining for typical phenotypic markers in combination with the assessment of activation-induced markers (AIMs) and cytokine production after specific stimulation with overlapping peptide pools from the complete SARS-CoV-2 protein divided over two subpools (S1 and S2) [11, 12]. SARS-CoV-2-specific B cells will be enumerated and phenotyped by flow cytometry as previously published [13]. The frequency of SARS-CoV-2-specific memory B cells will be determined by ELISpot [14]. Infection with SARS-CoV-2 occurs via the mucosal surface of the respiratory tract. To understand if and how antibody concentrations in serum correlate with those on the mucosal surface [15], nasal mucosal lining fluid is collected by non-invasive sampling (nasosorption) in a subset of patients. Induction, persistence and neutralizing capacity of mucosal antibodies against SARS-CoV-2 will be assessed and correlated to immune responses in the blood.

Solicited local and systemic adverse events are reported over 7 days after each vaccination (Supplementary data, Questionnaire S1). The incidence and severity of COVID-19 is monitored for 1 year. The number of participants who underwent diagnostic testing and the number and results of the tests are reported, as well as information about disease severity for participants with a positive test (Supplementary data, Questionnaire S2). In immunized patients, anti-HLA antibodies will be measured after vaccination.

Sample size calculation is based on the primary endpoint: induction and levels of SARS-CoV-2-specific antibodies. Based

on published data, we expect a vaccine efficacy of 90% seroconversion in controls, while we assume a lower efficacy rate of 80% in both CKD4/5 and dialysis patients and of 65% in kidney transplant recipients, due to use of immunosuppressive medication and impaired kidney function. With a non-inferiority limit of 20%, an α of 0.05 and a β of 0.2, 155 participants in the CKD4/5 and dialysis groups and 172 kidney transplant recipients are required. Assuming a dropout rate of ~10%, we include 200 participants in the control cohort and 175 participants in the CKD4/5 and dialysis cohorts each. To allow analyses of the effects of time after transplantation and type of immunosuppressive medication, the number of kidney transplant recipients is expanded to 300.

As mRNA vaccines lead to endogenous antigen production and presentation, they are expected to induce balanced immune responses. Previous trials showed that mRNA-1273 vaccination leads to neutralizing antibody responses and induction of S-specific T cells. However, the exact correlates of protection against COVID-19 are still unknown. Moreover, larger-scale and long-term measurements of both humoural and cellular immune responses to COVID-19 vaccination have not yet been performed in kidney disease patients.

Harmonization of methodology is crucial to enable the international scientific community to compare the efficacy of different SARS-CoV-2 vaccines. We hope that our study design can serve as a reference and model for other studies in specific risk populations.

To study the 'correlate of protection' of kidney disease patients after COVID-19 vaccination, as reflected by SARS-CoV-2 infection incidence and severity, additional large population-based studies are needed. Such studies should disclose which immunological test provides the best surrogate for protection against the presently most abundant variant and different variants of SARS-CoV-2.

In conclusion, the results of the RECOVAC-IR study will reveal whether CKD patients, those on dialysis and kidney transplant recipients can be adequately protected against COVID-19 by vaccination, or whether other measures, like booster vaccinations, are required.

ETHICS APPROVAL

Approval was obtained from the Dutch Central Committee on Research Involving Human Subjects (CCMO, NL76215.042.21) and the local ethics committees of the participating centres (University Medical Center Groningen, Radboud University Medical Center, Amsterdam University Medical Center and Erasmus Medical Center).

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AUTHORS' CONTRIBUTIONS

R.T.G. and J.-S.F.S. designed the study protocol. M.M.L.K., M.E.J.R., C.C.B., D.v.B., F.J.B., R.G.v.d.M., E.B.M.R., R.D.d.V. and L.B.H. contributed to the protocol design. D.A.D., F.R.M.v.d.K., M.P.G.K., A.L.M., N.R. and P.V. provided intellectual content of critical importance to the study. M.M.L.K., M.E.J.R., C.C.B., D.v.B., F.J.B., D.A.D., R.T.G., A.L.M., R.G.v.d.M., E.B.M.R., R.D.d.V., L.B.H. and J.-S.F.S. participated in preparation of the manuscript and implement the study. All authors revised and approved the final manuscript. The RECOVAC collaborators contributed to the design of the consortium or are involved in the implementation of the study.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Williamson EJ, Walker AJ, Bhaskaran K *et al.* Factors associated with COVID-19-related death using OpenSAFELY. *Nature* 2020; 584: 430–436
2. 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: 1973–1983
3. Reddy S, Chitturi C, Yee J. Vaccination in chronic kidney disease. *Adv Chronic Kidney Dis* 2019; 26: 72–78

4. Benotmane I, Gautier-Vargas G, Cognard N *et al.* Weak anti-SARS-CoV-2 antibody response after the first injection of an mRNA COVID-19 vaccine in kidney transplant recipients. *Kidney Int* 2021; 99: 1487–1489
5. Sattler A, Schrezenmeier E, Weber U *et al.* Impaired humoral and cellular immunity after SARS-CoV2 BNT162b2 (Tozinameran) prime-boost vaccination in kidney transplant recipients. *medRxiv* 2021; doi: 10.1101/2021.04.06.21254963
6. 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: 1452–1461
7. den Hartog G, Vos ERA, van den Hoogen LL *et al.* Persistence of antibodies to SARS-CoV-2 in relation to symptoms in a nationwide prospective study. *Clin Infect Dis* 2021; doi: 10.1093/cid/ciab172
8. Okba NMA, Muller MA, Li W *et al.* Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. *Emerg Infect Dis* 2020; 26: 1478–1488
9. Remmerswaal EBM, Hombrink P, Nota B *et al.* Expression of IL-7Rα and KLRG1 defines functionally distinct $CD8^+$ T-cell populations in humans. *Eur J Immunol* 2019; 49: 694–708
10. van der Putten C, Remmerswaal EBM, Terpstra ML *et al.* CD8 and CD4 T cell populations in human kidneys. *Cells* 2021; 10: 288
11. Grifoni A, Weiskopf D, Ramirez SI *et al.* Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* 2020; 181: 1489–1501.e15
12. Weiskopf D, Schmitz KS, Raadsen MP *et al.* Phenotype and kinetics of SARS-CoV-2-specific T cells in COVID-19 patients with acute respiratory distress syndrome. *Sci Immunol* 2020; 5: eabd2071
13. Brouwer PJM, Caniels TG, van der Straten K *et al.* Potent neutralizing antibodies from COVID-19 patients define multiple targets of vulnerability. *Science* 2020; 369: 643–650
14. van Besouw NM, Yan L, de Kuiper R *et al.* The number of donor-specific IL-21 producing cells before and after transplantation predicts kidney graft rejection. *Front Immunol* 2019; 10: 748
15. Cervia C, Nilsson J, Zurbuchen Y *et al.* Systemic and mucosal antibody responses specific to SARS-CoV-2 during mild versus severe COVID-19. *J Allergy Clin Immunol* 2021; 147: 545–557.e9

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