Safety and Cross-Variant Immunogenicity of a Three-dose COVID-19 mRNA Vaccine Regimen in Kidney Transplant Recipients

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Summary

Background: The immunogenicity of a two-dose mRNA COVID-19 vaccine regimen is low in kidney transplant (KT) recipients. We report the first complete assessment of safety and cross-variant immunogenicity of a three-dose vaccine regimen in KT recipients.

Methods: We performed a prospective longitudinal study in sixty-one KT recipients given three doses of the BNT162b2 COVID-19 vaccine. We performed semi-structured pharmacovigilance interviews and monitored donor-specific antibodies and kidney function. We compared geometric mean titers (GMT) of anti-spike IgG, pseudo-neutralization activity against vaccine homologous and heterologous variants, frequency of spike-specific interferon (IFN)-γ-secreting cells, and antigen-induced cytokine production 28 days after the second and third doses. The immunoassays were also performed in non-transplanted individuals 28 days after the second dose to obtain reference values.

Findings: Reactions to vaccine were mild. One patient developed donor-specific anti-HLA antibodies after the second dose which could be explained by non-adherence to immunosuppressive therapy. Spike-specific IgG seroconversion raised from 44·3% (n=27) after the second dose to 62·3% (n=38) after the third dose (p<0·05). GMT increased from 528·3 (95% CI 300·0-930·1) to 2395 AU/ml (95% CI 1214-4724, p<0·0001). Serum neutralizing activity increased from 4·7% to 17·2% (Wuhan strain), 4·0% to 17·4% (alpha variant), 2·3% to 7·3% (beta variant), and 1·3% to 9·1% (gamma variant) after the third dose (p<0·0001), which remained lower than reference values. The mean frequency of IFN-γ-secreting cells increased from 19·9 (SD 56·0) to 64·0 (SD 76·8) cells/million PBMCs after the third dose (p<0·0001). A significant increase in IFN-γ responses was also observed in patients who remained seronegative after three doses (p<0·0001).

Interpretation: A third dose of the BNT162b2 vaccine increases both SARS-CoV-2-specific humoral and cellular responses in KT recipients with an acceptable tolerability profile. However, neutralizing antibody titres remain low after three doses, especially against variants of concern, and barrier measures and vaccination of the relatives remain essential.

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Introduction

Organ transplant recipients with SARS-CoV-2 infection are at increased risk of severe disease and death, and are therefore a priority for vaccination [1]. Messenger RNA (mRNA) COVID-19 vaccines are safe and effective at preventing COVID-19 in the general population [2]. In phase 3 trials, mRNA vaccines efficacy rates were higher than 90% after two doses across most demographic subgroups [3,4]. However, organ transplant recipients were excluded from these trials. Since these early reports, studies have shown that vaccine immunogenicity was severely reduced in this population as a consequence of immunosuppressive drugs. Several fatal cases of COVID-19 have been reported in organ transplant recipients who had received two doses of mRNA vaccines [5,6], the likely result of reduced vaccine immunogenicity. Indeed, less than 20% of transplant recipients developed IgG against the SARS-CoV-2 spike protein after a single vaccine dose [7] and only half of them after two doses [8]. Moreover only 35% of KT recipients generated spike-specific T Helper (Th) 1 cells after the second vaccine dose [9].

In April 2021, the French National Health Authority recommended administering a third dose of mRNA vaccine to be given four weeks after a second dose to improve vaccine responses in organ transplant recipients. In support of this recommendation, Kamar *et al.* have reported that a third dose of mRNA COVID-19 vaccine increased seroconversion rate in organ transplant recipients including KT recipients [10]. However, a thorough safety assessment and detailed immunogenicity data are still required to weigh up the risk-benefit value of a three-dose COVID-19 regimen in organ transplant recipients [11], amid the rapid surge of variants of concern. This prospective monocentric longitudinal study conducted in 61 KT recipients given three doses of the BNT162b2 COVID-19 vaccine demonstrates that such a regimen is safe and substantially improves vaccine immunogenicity.

Methods

Study design and participants

Sixty-one consecutive KT recipients were included in this prospective monocentric longitudinal study between March 24, 2021 and April 14, 2021 at the Nice University Hospital, Nice, France. All patients received three injections of the BNT162b2 mRNA COVID-19 vaccine (Pfizer-BioNTech) at day 0, day 21, and day 49 as recommended by the French National Authority for Health. Patients with a history of COVID-19 or a positive SARS-CoV-2 serology the day of inclusion were excluded. Blood samples were collected at baseline and 28 days after the second and the third vaccine dose. Blood samples were also collected in 12 non-transplanted healthy volunteers 28 days after the second vaccine dose to obtain reference values in the different immunoassays. All healthy volunteers were healthcare workers with no history of COVID-19 and negative SARS-CoV-2 serology. Clinical and laboratory data were collected using the observation booklet completed by the investigating physician in charge of the patient. The study protocol complies with the principles of the Declaration of Helsinki and was approved by our institutional committee. Written informed consent was obtained from all participants and all collected data and samples were anonymized and securely stored.

Adverse Events (AE) monitoring

In order to systematically identify eventual AE related to each dose, an advanced practice nurse conducted a telephone follow-up of every participant 72 hours after each dose. Local and systemic adverse reactions solicitation and assessment was based on a semi-structured interview with a questionnaire developed in collaboration with the local pharmacovigilance centre. For each reported AE, time-to-onset, duration, and evolution were recorded.

Classification of a serious AE included a report of one of the following: death or life-threatening illness, occurrence and duration of hospitalization, permanent disability, congenital anomaly or birth defect.

Patients sera were assessed for the presence of anti-HLA donor-specific antibodies before vaccination and 28 days after the second and the third injection by Luminex Single Antigen® (One Lambda) as performed routinely in organ transplant recipients. Kidney allograft function was assessed before and after vaccination.

SARS-CoV-2-specific IgG and IgA

We used a commercial ELISA (ABBOTT) validated for clinical use to assess patient serum IgG titers to SARS-CoV-2 spike. The assay detects IgG directed to the Receptor Binding Domain (RBD) of SARS-CoV-2 spike S1 subunit and was performed according to the manufacturer's instructions. Results were acquired on the Architect I1000 analyser (ABBOTT). Values higher than 50 AU/mL were considered positive as specified by the manufacturer.

Serum IgA titers to SARS-CoV-2 Spike protein were determined using the V-PLEX® SARS-CoV-2 Panel 2 (IgA) Kit (MSD), according to the manufacturer's instructions. Data were acquired on the V-PLEX® Sector Imager 2400 plate reader and analysed using the Discovery Workbench 3·0 software (MSD). Standard curves were generated using standards provided in the kits. Serial 4-fold dilutions of the standards were run to generate a 7-standard curve, and the diluent alone was used as a blank. IgA concentrations were determined by extrapolation from the standard curve using a 4-parameter logistic curve fit.

SARS-CoV-2 pseudo-neutralization assay

Serum samples were assessed for levels of antibodies inhibiting the binding of SARS-CoV-2 full-length Spike and RBD derived from the homologous vaccine (Wuhan) sequence and from the alpha (B1·1·7), beta (B·1·351) and gamma (P1) variants, to angiotensin-converting enzyme 2 (ACE2) receptor, using the multiplex neutralization assay V-PLEX® SARS-CoV-2 Panel 7 (ACE2) Kit (MSD). Assays were performed according to the manufacturer's instructions. Data were acquired on the V-PLEX® Sector Imager 2400 plate reader and analysed using the Discovery Workbench 3·0 software (MSD). Standard curves were generated using standards provided in this kit. Serial 4-fold dilutions of the standards were run to generate a 7-standard concentration set, and the diluent alone was used as a blank. The % inhibition was calculated according to the manufacturer's instructions.

SARS-CoV-2-specific T cell responses

For measuring the frequency of IFN-γ-secreting SARS-CoV-2-specific T cells, we used the ELISpot Path Human IFN-γ (SARS-CoV-2, S1scan+S2N+SNMO) ALP assay (MABTECH). Briefly, PBMCs (5 $X10^5$ to $1X10^6$ cells/well) were incubated for 24 hours

at 37°C in a 96-well ELISpot strip plate pre-coated with anti-IFN-γ monoclonal antibody (mAb), a mixture of three pools of SARS-CoV-2 synthetic peptides (SARS-CoV-2 S1, SARS-CoV-2 S2 N and the SARS-CoV-2 SNMO), and anti-CD28 mAbs (0·1 µg/ml). For measuring cytokine levels produced by SARS-CoV-2-specific T cells, we used the QuantiFERON® SARSCoV-2 Starter Set kit (Qiagen) in which 1 ml of blood was collected in tubes containing a mixture of SARS-CoV-2 peptides. Cells were incubated at 37°C for 24 hours and supernatants were assessed for IFN-γ and IL-2 levels using the V-PLEX® kit (MSD). All assays were performed according to the manufacturer's instructions. Data were acquired on the V-PLEX® Sector Imager 2400 plate reader and analysed using the Discovery Workbench 3·0 software (MSD). Standard curves for each cytokine were generated using standards provided in the kits. Serial 4-fold dilutions of the standards were run to generate a 7-standard concentration set, and the diluent alone was used as a blank. Cytokine concentrations were determined from the standard curve using a 4-parameter logistic curve fit to transform the mean luminescence intensities into concentrations. As the lower limit of detection (LLOD), we used the median LLOD compiled over multiple plates and corresponding to the concentration calculated based on the signal recorded for the blank plus 2·5 standard deviations. For each cytokine, samples that fell below the LLOD were assigned an arbitrary value equal to half the LLOD.

Statistical analyses

Data are presented as geometric mean and 95% confidence interval of geometric mean, mean with standard error of mean (SEM) and median with interquartile ranges (IQR) for quantitative variables, or as numbers and percentages for categorical variables. Comparison between time points (after the second and third dose) was performed with the Wilcoxon matched-pairs, two-tailed rank test. The Chi-square test was used for comparison of seroconversion rates. Statistical analyses were performed using GraphPad Prism 9·0 (GraphPad Software, Inc., San Diego, CA). Differences were considered significant when p-value was < 0·05. For multivariate analysis, a logistic regression using seroconversion as the outcome and risk factors as independent variables was performed. Logistic regressions analysis were conducted in R (R Core Team, 2020).

Role of the funding source

Funding sources played no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Results

Characteristics of the study population

Sixty-one KT recipients with no history of COVID-19 and a negative SARS-CoV-2 serology at the time of inclusion were enrolled. Patients' baseline characteristics are shown in Table 1. Seventeen (27·9%) participants were women and 44 (72·1%) were men. The median age was 58 years (IQR, 47·1-66·1), and the median time since transplantation was 4·5 years (IQR, 1·8-11·3). Thirteen (21·3%) patients had type 2 diabetes. Maintenance immunosuppressive therapy included calcineurin inhibitors (93·4%, 57 of 61 patients), corticosteroids (88·5%, 54 of 61 patients), and antimetabolites (62·3%, 38 of 61 patients). Baseline creatininemia was 145·0 µmol/L (IQR, 106·5-180·5). Thirteen (21·3%) patients had donor-specific antibodies before vaccination. All participants received the BNT162b2 vaccine at day 0, day 21, and day 49. Blood samples were collected at baseline and 28 days after the second and the third doses. For reference, a panel of sera was collected from 12 healthy adult subjects 28 days after the second dose of BNT162b2 COVID-19 vaccine.

Safety and reactogenicity of a three-Dose BNT162b2 vaccine regimen in KT patients

Every participant was contacted after each vaccine dose. The proportions of patients who reported at least one adverse drug reaction after solicitation were 73·8% (45 of 61 patients), 68·9% (42 of 61 patients), and 68·9% (42 of 61 patients) after the first, second, and third vaccine dose respectively (Table 2). Pain at the injection site was the most frequent AE reported in 60·7% (37 of 61 patients), 65·6% (40 of 61 patients), and 67·2% (41 of 61 patients) of patients after the first, second, and third vaccine doses respectively.

As for serious adverse events (SAEs), no clinical rejection or kidney graft failure episodes had occurred by the end of the follow-up period (Table 2). None but one of the participants did develop *de novo* donor-specific antibodies (Table 2). He developed a donor-specific anti-HLA class II (DQB1*06:03) antibody 28 days after the second vaccine dose (Mean Fluorescence Intensity: 5209, which increased to 11318 after the third dose, not shown). This 19-year-old patient retrospectively admitted non-adherence to his immunosuppressive medication during the vaccination period. A

kidney graft biopsy did not show any sign of humoral rejection and creatininemia or proteinuria levels had not increased at the time of submission of this article.

Seroconversion after a third dose of BNT162b2 COVID-19 vaccine

Serum spike-specific IgG and IgA antibody titers were measured 28 days after the second and third doses. Spike-specific IgG seroconversion rate raised from 44·3% (27 of 61 patients) after the second dose to 62·3% (38 of 61 patients) after the third dose ($p<0·05$, Figure 1A). Among the 34 (55·7%) patients who had failed to seroconvert after the second dose, 11 (32·3%) seroconverted after the third dose. In patients who had seroconverted after the second dose, GMT of IgG to spike (Wuhan) increased from 528·3 AU/ml (95%CI 300·0-930·1) to 2395 AU/ml (95%CI 1214-4724) after the third dose ($p<0·0001$, Figure 1B). Of note, GMT of anti-spike IgG after the third dose was much lower in KT patients than the reference GMT of a set of 12 non-transplanted individuals sampled 28 days after the second dose (represented by the dotted line, Figure 1B). Likewise, GMTs of spike-specific IgA and IgG after the third dose were highly correlated ($r = 0·87$; $p<0·0001$, Supplemental Figure S1).

The risk factors for failing to seroconvert after a third dose were antiproliferative drugs and lymphopenia in multivariate analysis (OR 12·27 (95% CI, 2·08-111·42), p=0·01 and OR 0·16 (95% CI, 0·04-0·51, p=0·01) respectively, Table 3).

Neutralizing antibody responses after a third dose of BNT162b2 vaccine

Sera from KT patients and the reference group of 12 healthy individuals were assayed for levels of SARS-CoV-2 neutralizing antibodies by means of a pseudo-neutralizing assay based on inhibition of binding of SARS-CoV-2 spike protein to its ACE2 receptor using full-length spike protein and truncated RBD homologous to the vaccine sequence (Wuhan strain) and corresponding proteins derived from alpha, beta, and gamma variants.

As seen in Figure 1B, the mean neutralizing (inhibitory binding) activity of sera from KT patients increased after the third dose for all strains tested ($p<0·0001$, Figure 2A). Similar results were obtained when analysing the inhibition of spike RBD binding to ACE2 (Figure 2B). Overall, serum neutralizing activities to vaccine homologous and variant proteins increased by two to three fold but remained markedly lower in KT recipients even after the third dose compared to non-transplanted healthy individuals after the second dose.

T cell responses after a three-dose BNT162b2 vaccine regimen

The frequency of blood IFN-γ-secreting cells increased from 19·9 (SD 56·0) cells per million PBMCs 28 days after the second dose to 64·0 (SD 76·8) 28 days after the third dose ($p<0·0001$, Figure 3A). Similar results were obtained when measuring cell-free IFN-γ levels in supernatants from whole blood cultures of PBMCs stimulated with a SARS-CoV-2 peptide cocktail. In addition to IFN-γ responses, IL-2 levels also increased between the second and the third vaccine dose. However, these remained lower than corresponding values calculated in non-transplanted individuals (Figure 3B and 3C).

T cell responses in KT recipients who remained seronegative after the third vaccine dose

Because repetitive antigen stimulation and complete blockade of the effector immune response may induce antigen-specific tolerance [12,13], we analysed the production of inhibitory cytokines by PBMCs after stimulation with spike peptides in patients who remained seronegative after the third vaccine dose. The levels of IL-10 and TGF-β after spike-specific stimulation did not differ between the second and the third dose in these patients suggesting that the third dose did not induce spike-specific tolerance (Supplemental Figure S2). Inhibitory cytokine levels in these patients were also comparable to those of patients who seroconverted (Supplemental Figure S2). We then compared the frequency of spike-specific IFN-γ-secreting T cells after the second and the third dose in patients who failed to seroconvert after the third dose (Figure 4). This frequency increased by over four-fold between the second and the third dose ($p<0·001$, Figure 4).

Discussion

In March 2021, the French national health authorities recommended a three-dose regimen of mRNA COVID-19 vaccines in severely immunocompromised individuals amid a reportedly low immunogenicity of these vaccines in solid organ transplant recipients [7,8]. At that time, no data were available on the risks and benefits of an additional vaccine injection in solid-organ transplant recipients. Health authorities in some other countries are currently waiting for more safety and immunogenicity data before issuing such a recommendation [11]. We provide here support to this recommendation based on a thorough comparative safety assessment and comprehensive immunogenicity data in 61 KT recipients given two and three doses of mRNA BNT162b2 COVID-19 vaccine.

Overall, the reactogenicity profile of the three-dose vaccine regimen was manageable. The third dose of vaccine did not increase the risk of local or systemic adverse reactions. In one patient who developed a *de novo* donor-specific antibody during vaccination, the occurrence of a donor-specific humoral response was most likely associated with the patient's non-adherence to immunosuppressive therapy rather than vaccination, in keeping with the WHO-UMC causality assessment system.

In this series of KT recipients, antibody titers, serum neutralizing activity and T cell responses to SARS-CoV-2 increased after the third vaccine dose. While these results are encouraging and suggest that a three-dose regimen improves vaccine immunogenicity, they should be interpreted with caution. First, the magnitude of antibody and T cell responses in KT recipients were markedly lower than those seen in healthy individuals. Second, neutralizing antibody responses after a third vaccine dose remained very low, especially against variants of concern. Increased production of inhibitory cytokines such as IL-10 and TGF-β by SARS-CoV-2 spike reactive T cells could explain such weak responses. However, analyses of SARS-CoV-2-stimulated PBMCs did not disclose any appreciable differences between the second versus third dose of vaccine in KT patients who had seroconverted or not. The relatively small number of patients who were followed in this study and its short duration leave open questions regarding the long-term safety and duration of immunological responses induced by a third dose of mRNA COVID-19 vaccine. It is also important to bear in mind that the clinical efficacy of COVID-19 vaccines, including mRNA vaccines, currently approved for Emergency Used and based on two-dose regimens in organ transplant recipients is unknown at that stage. Furthermore, the cross-protective

efficacy of COVID vaccines against infection by certain newly emerging SARS-CoV-2 variants has been shown to decrease although its impact on disease severity has not been fully assessed at that stage. The foregoing considerations underline the limitations of results from phase III efficacy trials which have by and large excluded immunocompromised patients.

Alternative or complementary strategies should be developed to improve the immunogenicity of COVID-19 vaccines in organ transplant recipients. The dose or the route of vaccines could be modified as shown with other vaccines [14,15]. The time interval between doses has a known impact on vaccine immunogenicity [16] and a 4-weeks interval between the second and third doses may be too short. Vaccine strategies based on heterologous prime-boost, e.g. mRNA vaccine followed by adjuvanted protein subunit or *vice versa*, could improve vaccine immunogenicity as has been shown after sequential administration of heterologous viral vaccines [17-21]. Finally, short-term lowering of immunosuppressive drugs before vaccination could ameliorate vaccine performance but will have to be balanced against the risk of acute rejection or apparition of donor-specific antibodies. Predictive immunomonitoring tools to differentiate low from high responders before vaccination could be used to tailor immunosuppressive therapy [22].

In conclusion, a third dose of the BNT162b2 mRNA COVID-19 vaccine in KT recipients increases both spike-specific antibody-binding and T cell responses with an acceptable tolerability profile. However, homologous and especially variant-specific neutralizing antibody responses remained low after three doses and underline the importance of barrier measures and of vaccination of patients' relatives.

Contributors

AS designed the study. FM, MC, AG, HG, LR, MB, MR, EM, JF developed the study methods. FM, NB, GB, GF, PH, VE, BSP, NG, AS contributed to the implementation of the study or data collection. FM, SB, AS conducted the statistical analysis. BSP and AS were investigators. NG and AS ensured data accuracy. FM, MC, AG, CC, NG, AS contributed to the preparation of the report. All authors critically reviewed and approved the final version. All authors had full access to all the data in the study and accepted responsibility to submit for publication.

Declaration of interest

The authors declare no competing interests.

Data sharing

Anonymized participant data presented in this paper and statistical analysis can be accessed with publication on request to the corresponding author. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement. Data will be made available for a minimum of 5 years.

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Research in context

Evidence before this study

Three-dose COVID-19 vaccine regimens have been recommended by the French national health authorities in April 2021. Before submitting this article on July 20, 2021, we conducted a search in PubMed for any published article, using key terms “COVID-19” or “SARS-CoV-2” AND “Vaccine”, AND “3 doses” or “Third dose” AND “Kidney Transplant Recipients” or “Transplantation”, without restrictions. In July 2021, two letters reported the prevalence and titres of spike-specific antibodies after a second and third dose of SARS-CoV-2 vaccines in kidney transplant recipients. These reports were retrospective in design and used different vaccines or time intervals between doses and, most importantly did not measure the two main functional immunogenicity markers, i.e. vaccine-specific and cross-variant neutralizing antibody responses and T cell immunity.

Added value of this study

To our knowledge, this is the first prospective longitudinal assessment of the safety and immunogenicity of a three-dose COVID-19 vaccine regimen in kidney transplant recipients. This report demonstrates that a third COVID-19 vaccine dose moderately enhances both vaccine-specific and cross-variant serum neutralizing antibody responses as well as T cell responses in kidney transplant recipients.

Implications of all the available evidence

This study provides evidence that administration of a third dose of COVID-19 mRNA vaccine is safe and of benefit for solid organ transplant recipients, in terms of vaccine immunogenicity. While these findings support recommending a third dose of vaccine in this population, neutralizing antibody responses after three doses were still relatively low, especially against variants of concern, calling for improved vaccination strategies, including heterologous prime-boost regimens with optimized dosages and time intervals between doses.

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Figures Legends

Figure 1. Spike-specific IgG antibodies

Serum spike-specific IgG antibody titers were measured 28 days after the second and third dose of BNT162b2 vaccine in 61 KT recipients. (A) Spike-specific IgG seroconversion rate after the second and third dose; *, p<0·05. (B) GMT of spike-specific IgG after the second and third dose in patients who have seroconverted after the second dose. The dotted line shows the GMT measured in a panel of sera collected from 12 healthy adult subjects 28 days after the second dose of BNT162b2 vaccine. ****, p<0·0001 (paired comparison).

Figure 2. Neutralising antibody responses

Serum samples were assayed for SARS-CoV-2 neutralizing antibodies using a pseudo-neutralisation assay based on inhibition of binding of ACE2 receptor to the SARS-CoV-2 spike (A) or RBD (B) of the Wuhan strain or the alpha, beta and gamma variants. The dotted line shows the mean percentage of inhibition measured in a panel of sera collected from 12 healthy adult subjects 28 days after the second dose of vaccine. Data are shown as mean and SEM. ****p<0·0001 (paired comparisons).

Figure 3. Spike-specific T cell responses

(A) An ELISpot assay was used to measure the frequency of blood spike-specific IFN-γ-secreting cells 28 days after the second and third dose. ****p<0·0001 (paired comparison). Representative images are shown. Cell-free IFN-γ (A) and IL-2 (B) levels were measured in supernatants from whole blood cultures of PBMCs stimulated with a cocktail of SARS-CoV-2 spike-derived peptides. *p<0·05 (paired comparisons). Data show median and interquartile range. The dotted line shows the mean values measured in a panel of sera collected from 12 healthy adult subjects 28 days after the second dose of the BNT162b2 vaccine.

Figure 4. Spike-specific T cell response in seronegative KT recipients

The frequency of spike-specific IFN-γ-secreting T cells was compared after the second and the third dose of vaccine in KT recipients who have failed to seroconvert after the third dose. Data are shown as median and interquartile range. ***p<0·001, paired comparisons.

Supplemental Figure S1. Correlation between spike-specific IgG and IgA antibody titers 28 days after the third dose of the BNT162b2 vaccine. Pearson's r coefficient and p-value are shown.

Supplemental Figure S2. Production of inhibitory cytokines after the second and the third vaccine dose

Cell-free levels of IL-10 (**A**) and TGF-β (**B**) were measured in supernatants from whole blood cultures of PBMCs stimulated with a cocktail of spike-derived peptides. Cytokine levels were compared after the second and the third dose in patients who have not seroconverted, and after the third dose in patients who have seroconverted. Data show median and interquartile range. ns, not significant.

Tables

Table 1. Baseline characteristics of the study population

Baseline characteristics	n=61
Males	44 (72·1)
Age, years	58·0 [47·1-66·1]
Retransplantation	7 (11·5)
Time post-transplantation, years	4·5 [1·8-11·3]
Cause of ESKD	
Diabetes	5 (8·2)
Vascular	7 (11·5)
Glomerular	10 (16·4)
Polycystic kidney disease	15 (24·6)
Others and unknown	24 (39·3)
Diabetes	13 (21·3)
Obesity (BMI>30kg/m²)	8 (13·1)
Maintenance immunosuppressive therapy	
Corticosteroïds	54 (88·5)
Antimetabolites	38 (62·3)
Calcineurin inhibitors	57 (93·4)
mTOR inhibitors	6 (9·8)
Belatacept	1 (1·6)
Laboratory values	
Creatininemia, µmol/L	145·0 [106·5-180·5]
White blood cell count, $\times 10^9$/l	6·6 [5·2-8·4]
Lymphocytes count, $\times 10^9$/l	1·3 [0·8-1·7]
Donor-speciifc antibodies	13 (21·3)

Data are shown as number and percentage, n (%) or median and interquartile range, m (IQR); ESKD: End-Stage Kidney Disease; BMI : Body Mass Index

Table 2. Frequency of adverse events after each dose

Adverse Events	Dose 1	Dose 2	Dose 3
Adverse Drug Reactions	45 (73·8)	42 (68.9)	42 (68.9)
Injection-site pain	37 (60·7)	40 (65·6)	41 (67·2)
Fatigue	11 (18·0)	13 (21·3)	13 (21·3)
Headache	4 (6·6)	5 (8·2)	7 (11·5)
Diarrhea	4 (6·6)	3 (4·9)	7 (11·5)
Fever	3 (4·9)	3 (4·9)	4 (6·6)
Myalgia	2 (3·3)	3 (4·9)	2 (3·3)
Rhinorrhea	1 (1·6)	3 (4·9)	2 (3·3)
Nausea and vomiting	1 (1·6)	2 (3·3)	1 (1·6)
Cough	0	4 (6·6)	0
Hypertension	1 (1·6)	1 (1·6)	1 (1·6)
Anorexia	1 (1·6)	0	1 (1·6)
Vertigo	1 (1·6)	0	1 (1·6)
Local paresthesia	0	2 (3·3)	0
Abdominal pain	0	2 (3·3)	0
Rash	0	0	1 (1·6)
Insomnia	1 (1·6)	0	0
Serious Adverse Events	0	1 (1·6)	1 (1·6)
De novo donor-specific antibody	0	1 (1·6)	0
Acute rejection	0	0	0
Kidney allograft failure	0	0	0

Data are shown as number and percentage, n(%).

Table 3. Multivariate regression of risk factors for anti-spike IgG seronegativity after three doses

Variables	OR (95% CI)	p-value
Sexe (male)	0·53 (0·10 to 2·48)	0·42
Age (per 1-year increment)	1·03 (0·97 to 1·09)	0·36
Retransplantation	0·47 (0·04 to 4·43)	0·51
Time post-transplantation (per 1-year increment)	1·01 (0·914 to 1·058)	0·82
Diabetes	0·64 (0·08 to 4·23)	0·65
Maintenance immunosuppressive therapy		
Steroïds	2·29 (0·26 to 23·01)	0·45
Antiproliferatives	12·27 (2·08 to 111·42)	**0·01**
Calcineurins inhibitors	0·65 (0·02 to 26·40)	0·81
mTOR inhibitors	0·9 (0·19 to 17·72)	0·95
Creatininemia (µmol/l)	1·01 (0·99 to 1·02)	0·22
Lymphocytes count, $\times 10^9$/l	0·16 (0·04 to 0·51)	**0·01**

Odds Ratio (OR), 95% confidence intervals (CI) and p-values are shown; mammalian target of rapamycin (mTOR). Significant associations are highlighted.

Figure 1

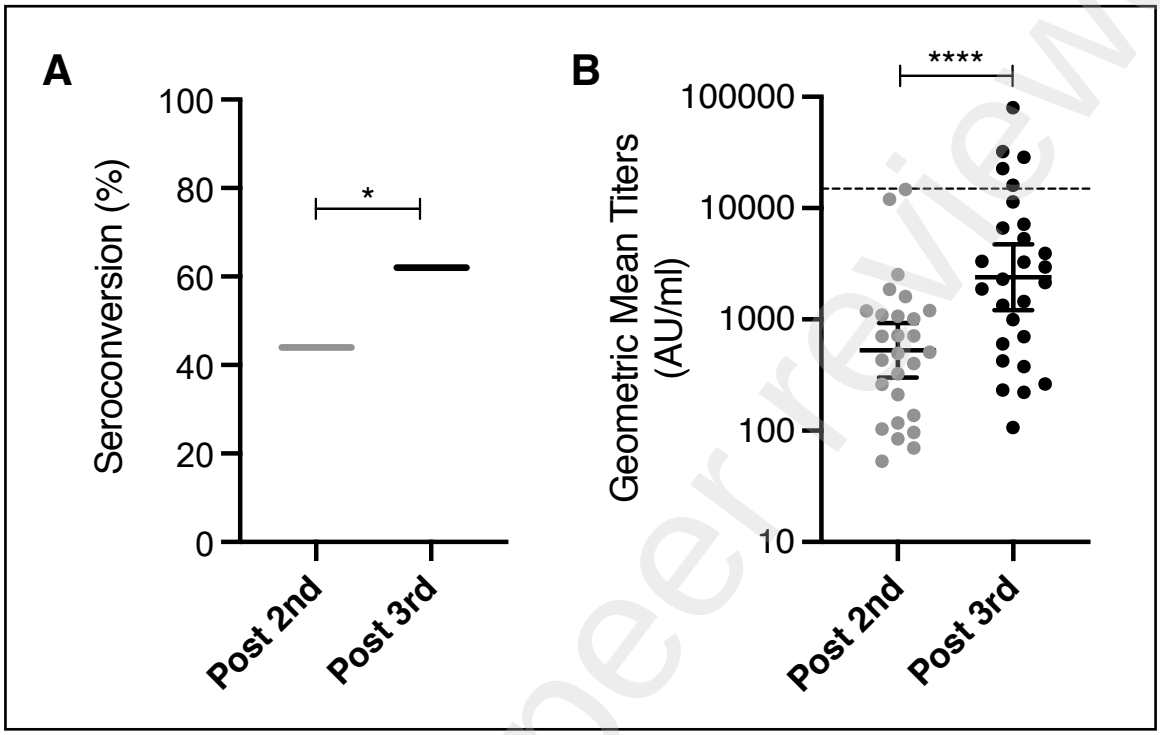


Figure 2

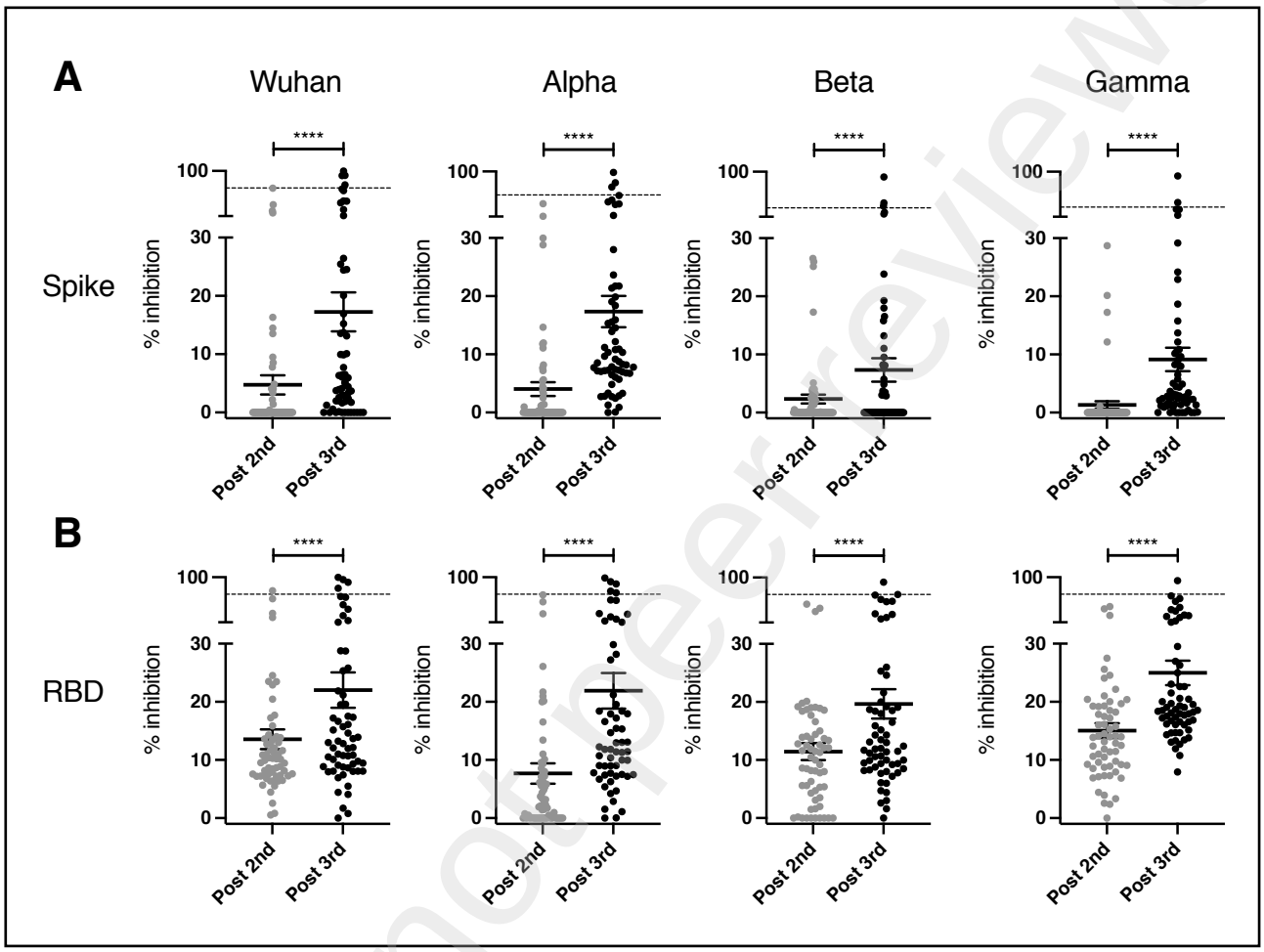


Figure 3

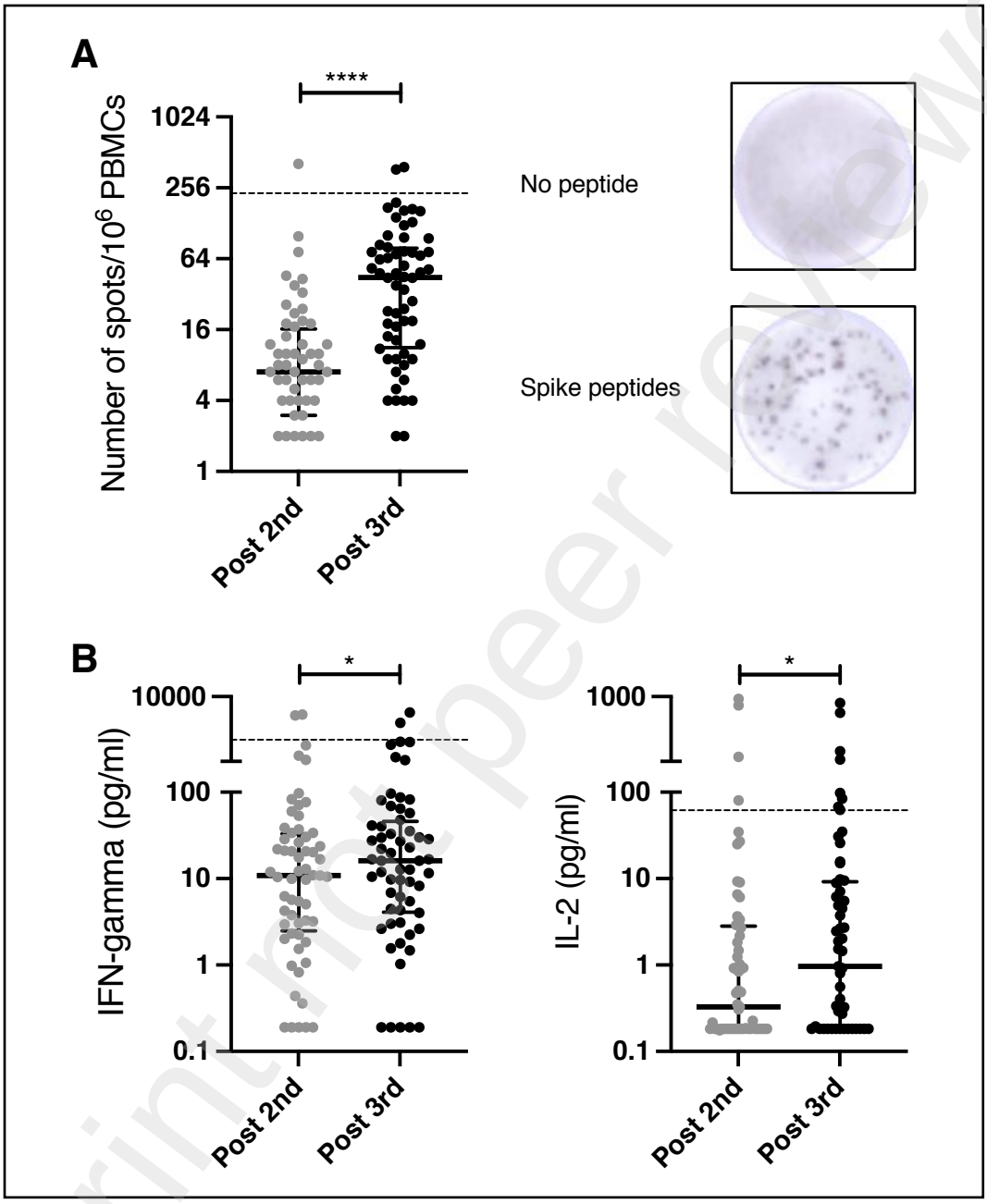
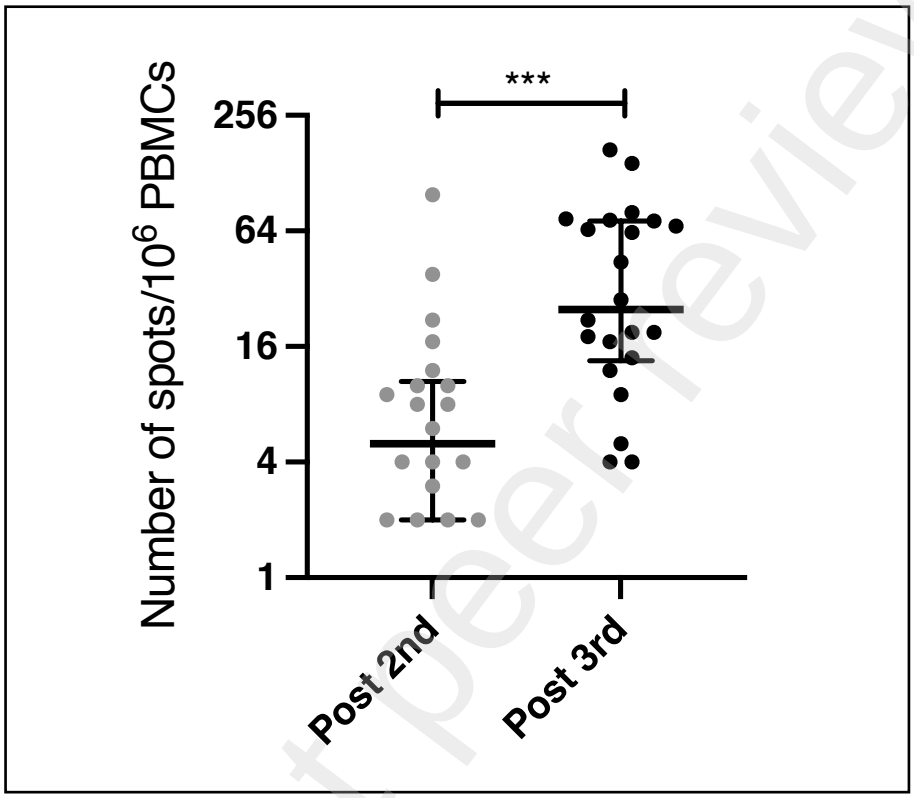
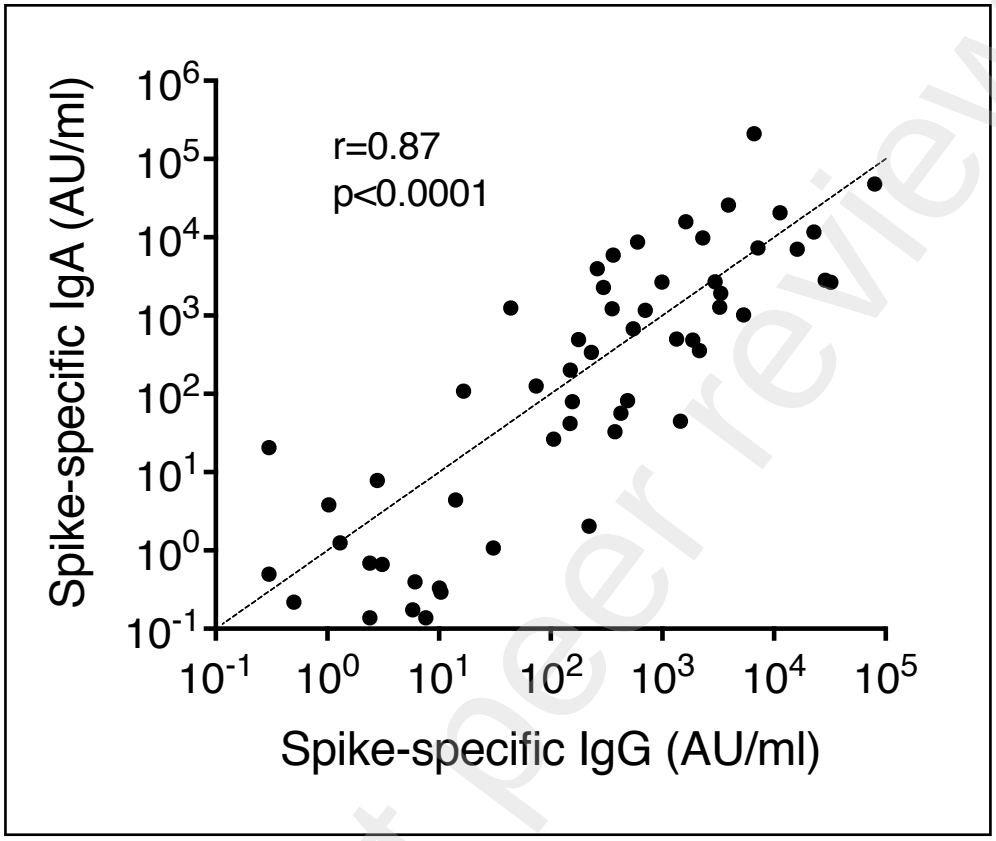


Figure 4

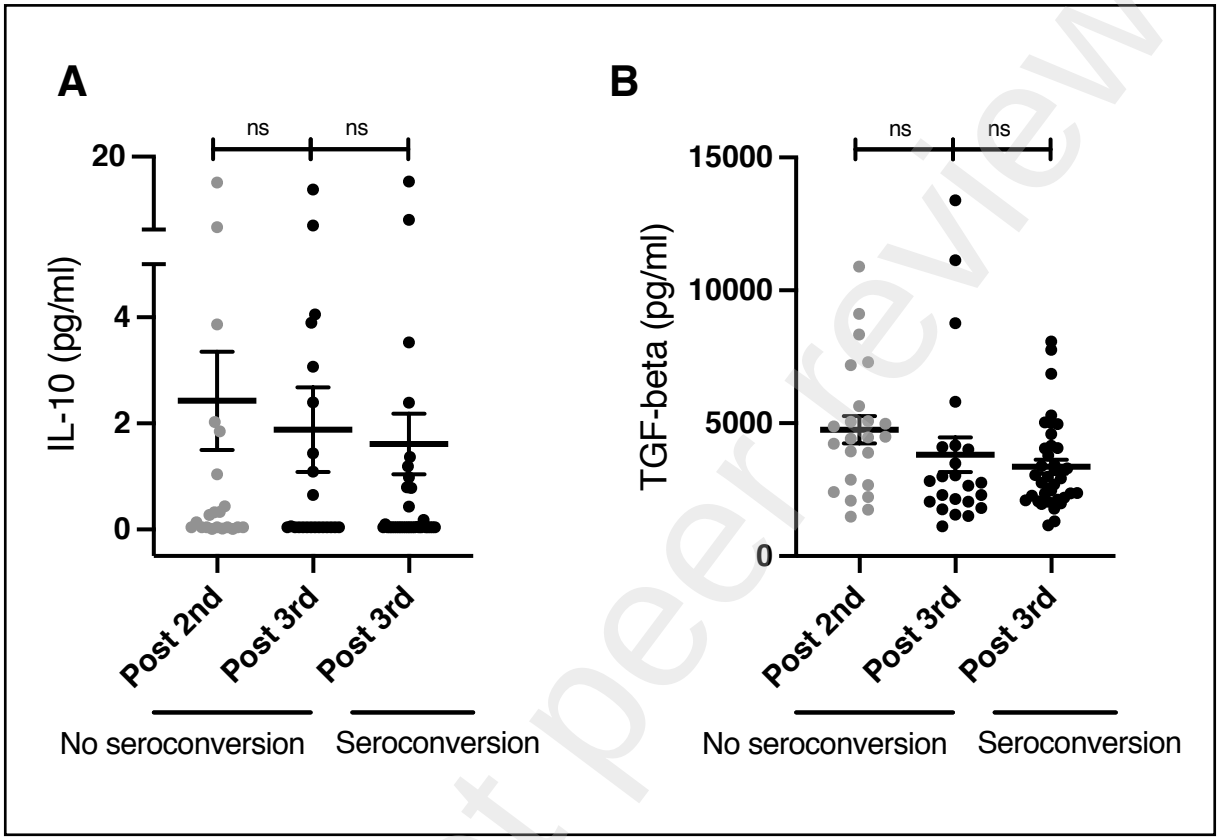


Supplemental Figure S1



Supplemental Figure S1. Correlation between spike-specific IgG and IgA antibody titers 28 days after the 3rd dose of the BNT162b2 vaccine. Pearson's r coefficient and p-value are shown.

Supplemental Figure S2



Supplemental Figure S2. Production of inhibitory cytokines after the 2nd and the 3rd vaccine dose

Cell-free levels of IL-10 (**A**) and TGF-β (**B**) were measured in supernatants from whole blood cultures of PBMCs stimulated with a cocktail of spike-derived peptides. Cytokine levels were compared after the 2nd and the 3rd dose in patients who have not seroconverted, and after the 3rd dose in patients who have seroconverted. Data are shown as median and interquartile range. ns, not significant.