Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
mTOR inhibitors improve both humoral and cellular response to SARS-CoV-2 messenger RNA BNT16b2 vaccine in kidney transplant recipients

Giuseppe S. Netti1 | Barbara Infante2 | Dario Troise2 | Silvia Mercuri2 | Maddalena Panico2 | Federica Spadaccino1 | Valeria Catalano1 | Margherita Gigante1 | Simona Simone3 | Paola Pontrelli3 | Loreto Gesualdo3 | Elena Ranieri2 | Giuseppe Castellano4 | Giovanni Stallone2

1Clinical Pathology Unit, Department of Medical and Surgical Sciences, Advanced Research Center on Kidney Aging (A.R.K.A.), University of Foggia, Foggia, Italy
2Nephrology Dialysis and Transplantation Unit, Department of Medical and Surgical Sciences, Advanced Research Center on Kidney Aging (A.R.K.A.), University of Foggia, Foggia, Italy
3Nephrology Dialysis and Transplantation Unit, Department of Emergency and Organ Transplantation (DETO), University of Bari “Aldo Moro”, Bari, Italy
4Nephrology, Dialysis and Renal Transplant Unit, Department of Clinical Sciences and Community Health, Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, University of Milan, Milan, Italy

Correspondence
Giuseppe S. Netti, Clinical Pathology Unit and Center for Molecular Medicine, Department of Medical and Surgical Sciences, University of Foggia, Viale L. Pinto, 71122 Foggia, Italy.
Email: giuseppestefano.netti@unifg.it

Funding information
University of Foggia (Italy), Grant/Award Number: University Research Projects 2019 “PRA 2019”

Kidney transplant recipients (KTRs) have been considered as patients at higher risk of SARS-CoV-2-related disease severity, thus COVID-19 vaccination was highly recommended. However, possible interferences of different immunosuppression with development of both humoral and T cell–mediated immune response to COVID-19 vaccination have not been determined. Here we evaluated the association between mTOR-inhibitors (mTOR-I) and immune response to mRNA BNT162b2 (Pfizer-BioNTech) vaccine in KTR. To this aim 132 consecutive KTR vaccinated against COVID-19 in the early 2021 were enrolled, and humoral and T cell–mediated immune response were assessed after 4–5 weeks. Patients treated with mTOR-I showed significantly higher anti-SARS-CoV-2 IgG titer ($p = .003$) and higher percentages of anti-SARS-CoV-2 S1/RBD Ig ($p = .024$), than those without. Moreover, SARS-CoV-2-specific T cell–derived IFNγ release was significantly increased in patients treated with mTOR-I ($p < .001$), than in those without. Multivariate analysis confirmed that therapy with mTOR-I gained better humoral ($p = .005$) and T cell–mediated immune response ($p = .005$) in KTR. The presence of mTOR-I is associated with a better immune response to COVID-19 vaccine in KTR compared to therapy without mTOR-I, not only by increasing vaccine-induced antibodies but also by stimulating anti-SARS-CoV-2 T cell response. These findings are consistent with a potential beneficial role of mTOR-I as modulators of immune response to COVID-19 vaccine in KTR.

KEYWORDS
COVID-19, kidney transplantation, mTOR inhibitors, SARS-CoV-2 vaccine

Abbreviations: ATG, anti-thymoglobulin antibodies; COVID-19, coronavirus disease 2019; GFR, glomerular filtration rate; IFNγ, interferon gamma; IGRA, interferon gamma release assay; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; mTOR, mammalian target of rapamycin; Nab, neutralizing antibodies; NK, natural killer; PBMCs, peripheral blood mononuclear cells; Pred, prednisolone; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sVNT, surrogate virus neutralization test; Tac, tacrolimus.

Giuseppe Stefano Netti and Barbara Infante equally contributed to the present work.

[Correction added on May 14, 2022, after first online publication: CRUI-CARE funding statement has been added.]
1 | INTRODUCTION

During current pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), kidney transplant recipients (KTRs) have been considered as patients at higher risk of disease severity, morbidity, and mortality.1 To protect this population, SARS-CoV-2 vaccination was urged right away through international guidelines.2,3 Moreover, due to immunosuppressive therapy, KTRs are considered low responders to vaccines4 and were not included in pre-authorization clinical trials for SARS-CoV-2 vaccine.

Although transplant recipients were expected for reduced efficacy and immunogenicity of the vaccine, these patients were included in the prioritization groups for early vaccination and transplant societies, including the American Society of Transplantation and European Society of Organ Transplantation, recommended transplant recipients to get vaccinated as soon as vaccine was available.

Recently, it has been shown that only 54% of solid organ transplant recipients developed a positive antibody response after two doses of SARS-CoV-2 mRNA vaccine and, among KTR, antibody response was detected only in 48% of patients.5 Assessment of the humoral response to a vaccine usually provides a reliable evaluation of its efficacy. However, in a population characterized by lower seroconversion rates than the general non-immunosuppressed population, the evaluation of the cellular immune response could be particularly beneficial and relevant.6

Different immunosuppressive protocols represent a main factor of variability in the response to vaccines and as such need to be investigated.

In detail, mTOR has important roles in regulation of both innate and adaptive immunity and its inhibition, in combination with calcineurin inhibitors (CNIs), offers comparable efficacy and graft function as compared to standard-of-care (CNI only).7 However, whether and how mTOR affects humoral immune responses have yet to be fully understood. It has been described that in virus infections, the inhibition of mTOR, a kinase involved in several biological processes, improves the function and the response of memory CD8+ T cells8 and modulates antigen-specific humoral immune responses by differentially regulating B cell and CD4 T cell responses during acute viral infection.9

We thus aimed to explore if the presence of mTOR inhibitors in immunosuppressive regimens of KTR ameliorates the immunogenicity of mRNA BNT162b2 vaccine after two doses, by not only assessing vaccine-induced antibodies but also evaluating anti-SARS-CoV-2 spike-specific T cell response.

2 | METHODS

2.1 | Study population

A multicenter, observational, case-control study was performed, including 132 consecutive KTR (86 M, 46 F) actively followed at the Nephrology Units of Foggia and Bari (Italy), between March 2021 and June 2021. All the enrolled patients at time of transplantation received induction therapy with Basiliximab and after were treated with CNI-based maintenance therapy (Group A: Tacrolimus + MMF + Prednisolone) or with CNI/mTOR inhibitors (mTOR-I) based maintenance therapy (Group B: Tacrolimus + Everolimus + Prednisolone), according to the immunosuppressive policy of the Transplant Center. No changes in immunosuppressive therapy were done during the posttransplant follow-up and no patients were treated with belatacept.

Exclusion criteria for receiving the vaccine and entering the study included age <18 years, transplantation within the last 3 months, having received anti-thymocyte globulins (ATG) or rituximab in the last 3 months for rejection and active or previous SARS-CoV-2 infection. To this aim, all the eligible patients were assessed for both PCR nasal swab and detection of anti-SARS-CoV-2 IgM and IgG, both resulted negative, and were therefore considered as SARS-CoV-2 naïve.

Once written informed consent was collected, all the enrolled subjects received two doses of the anti-SARS-CoV-2 mRNA BNT16b2 Vaccine (Comirnaty, Pfizer-Biontech, USA). All the clinical data at enrolment were collected and recorded.

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review board (Decision no. 64711/COMET/2021).

2.2 | Sample collection

In all the enrolled subjects, serum samples were collected before vaccination (Time 0, T0) and 4–5 weeks after the second vaccine dose (Time 2, T2) and stored at −30°C, until analyzed. Moreover, whole blood (25 ml) was harvested from all patients at T0 and T2 and peripheral blood mononuclear cells (PBMCs) were isolated by density separation on SepMate™ (STEMCELL Technologies, Vancouver, Canada), according to manufacturer’s instructions, and stored at −80°C, until analyzed.

2.3 | Detection of anti-SARS-CoV-2 antibodies

Anti-SARS-CoV-2 IgG and IgM were analyzed by using a chemiluminescent analytical assay (CLIA) commercially available kit (New Industries Biomedical Engineering Co. Ltd, Shenzhen, China), as described in Supplementary Methods.

2.4 | Neutralizing antibody level assessment

Serum neutralizing antibody (NAb) levels were assayed in the entire study population, using a commercially available ELISA Kit, according to the manufacturer’s instructions (SARS-CoV-2 NeutralISA, EUROIMMUN Medizinische Labor diagnostika AG, Lübeck, Germany). This competitive semi-quantitative test allows to evaluate the ability...
of Nab to prevent the link between the S1/RBD domain and the ACE2 receptor. In detail, microplate was coated with recombinant S1/RBD domain of SARS-CoV-2. Sample and controls were diluted 1:5 in dilution buffer containing soluble ACE2 conjugated to biotin and incubated in the reaction wells. Both Nab and soluble ACE2 competed for the binding site on the antigen surface. The photometric measurement at 450 nm yielded the results as a percentage of inhibition (%IH). According to manufacturer instructions, 20%IH was considered negative, 20 to 35%IH borderline, and >35%IH positive.

2.5 | Interferon gamma release assay (IGRA)

PBMCs isolated from patients were thawed, counted, and stimulated with SARS-CoV-2 IGRA stimulation tube set (EUROIMMUN Medizinische Labor diagnostika AG, Lübeck, Germany).

In details, 1 × 10^6 PBMCs were resuspended in PBS/EDTA anddispensed in each of the three stimulation tubes for 20 h: CoV-2 IGRA BLANK for the determination of the background concentration of interferon gamma (IFNy); CoV-2 IGRA STIM containing a mitogen causing nonspecific secretion of IFNy; CoV-2 IGRA TUBE containing SARS-CoV-2 S1 components for the determination of specific IFNy secretion.

After stimulation, samples were centrifuged and the supernatants used for subsequent quantitative assay using IFNy ELISA, according to the manufacturer instructions (EUROIMMUN Medizinische Labor diagnostika AG, Lübeck, Germany). Reaction wells were coated with anti-IFNy monoclonal antibody. Samples and controls were diluted 1:5 in a diluent buffer, incubated and processed according to manufacturer instructions. For IFNy quantification, a four-parameter logistic was applied.

2.6 | Statistical analysis

Statistical analysis was performed using SPSS 25.0 software (IBM Corp., Armonk, NY), as described in Supplementary Methods.

3 | RESULTS

Among 200 consecutive renal transplant patients actively followed at Nephrology Units, 132 met the including criteria and entered the study. The remaining 68 were excluded due to history of proved COVID-19 infection (n = 53) or recent acute rejection episode (n = 15) (Figure 1). The main clinical and laboratory features of all patients at baseline, as well as their immunosuppressive therapy are shown in Table 1.

After stratification in two groups according to the maintenance therapy with CNI (Group A, n = 104) or with CNI/m-Tor-I (Group B, n = 28), no significant differences were shown between two groups. All the patients completed the vaccine schedule and 28-35 days after the administration of the second doses, the total anti-SARS-CoV-2 IgG titer was assessed in the entire cohort. A positive antibody response was observed in 78.8% of renal transplant recipients. After stratification according to immunosuppressive therapy, patients treated with mTOR-I (Group B) showed higher proportion of antibody response, as compared with those without mTOR-I (Group A) (85.7% vs. 63.5%, p = .0439). Moreover, if the mean serum levels of anti-SARS-CoV-2 IgG were assessed, patients treated with mTOR-I showed significantly higher levels, as compared with those without mTOR-I (649.3 ± 173.6 vs. 350.3 ± 62.5 BAU/ml, p = .003; Figure 2A).

To assess the presence of neutralizing antibodies (NAb), all the sera were tested with an enzyme-linked immunosorbent assay (ELISA)-based surrogate virus neutralization test (sVNT) for the detection of anti-SARS-CoV-2 S1/RBD Ig. All the values above the manufacturer’s specified cutoff value of 35% were considered as positive for the ELISA-based surrogate assay. In our cohort study, a significantly higher proportion of patients treated with mTOR-I passed the positive cutoff value (>35%), as compared with those without mTOR-I (71.4% vs. 42.2%, p = .0113). As shown in Figure 2B, renal transplant patients treated with mTOR-I showed higher percentages of anti-SARS-CoV-2 S1/RBD Ig after a complete vaccine schedule, as compared with those without mTOR-I (55.8 ± 6.7% vs. 38.2 ± 4.0%, p = .024; Figure 2B). Moreover, results from both assays showed a strength correlation (R² = 0.8428, p < .001; Figure 2C).

Then we assessed the T cell response against COVID vaccine in our cohort. In detail, all the enrolled patients were propensity
The T cell reactivity to SARS-CoV-2-related S1/RBD was assessed in 40 renal transplant patients undergone COVID vaccination and vs. 44.0 ± 10.0 mUI/ml, with mTOR-I (78.1 ± 3.5%, p < .001; Figure 3A). Moreover, we assessed the T cell reactivity as a ratio (IFN-γ released after SARS-CoV-2-related S1/RBD-specific stimulus/IFN-γ release after nonspecific mitogen exposure). Renal transplant recipients treated with mTOR-I showed stronger capacity (%) to release IFN-γ, as compared with these not treated with mTOR-I (88.7 ± 8.9 vs. 44.0 ± 10.0 mUI/ml, p = .001; Figure 3A). Moreover, we assessed the T cell reactivity as a ratio (IFN-γ released after SARS-CoV-2-related S1/RBD-specific stimulus/IFN-γ release after nonspecific mitogen exposure). Renal transplant recipients treated with mTOR-I showed stronger capacity (%) to release IFN-γ after specific stimulus as compared to the maximum release induced by nonspecific mitogen, while this ratio was significantly lower in patients not treated with mTOR-I (78.1 ± 4.6% vs. 25.0 ± 3.5%, p < .001; Figure 3B).

Then we aimed to assess the possible combined role of several factors with mTOR-I therapy onto the humoral and cellular response to the COVID-19 vaccine in KTR. In detail, the relative risk for a positive surrogate virus neutralization test was estimated and a Cox regression analysis was performed, using anti-SARS-CoV-2 S1/RBD IgG above or below the cutoff value (35%) as dependent variable, and patient’s age and gender, diabetes, donor type, time from transplant to vaccine, eGFR, lymphopenia, and therapy with mTOR-I as covariates (Table 2A). Univariate analysis showed that only time from transplant to vaccine (HR 1.919, 95% CI 1.308–2.817, p = .001) and therapy with mTOR-I (HR 3.547, 95% CI 1.430–8.794, p = .005) affected the anti-SARS-CoV-2 S1/RBD IgG positivity. Moreover, the results of the multivariate analysis confirmed a significant effect on test positivity of both time from transplant to vaccine (HR 2.288, 95% CI 1.440–3.637, p < .001) and therapy with mTOR-I (HR 4.254, 95% CI 1.440–11.816, p = .005).

Then, we evaluated the relative risk for a SARS-CoV-2-related S1/RBD-specific IFN-γ release above or below the 50th percentile (56.5 mUI/ml). Thus, a second Cox regression analysis was performed, using the IFN-γ release above or below the 50th percentile as dependent variable, and patient’s age, time from transplant to vaccine, lymphopenia and therapy with mTOR-I as covariates (Table 2B). In this model, univariate analysis showed that only time from transplant to vaccine (HR 2.449, 95% CI 1.208–4.969, p = .013) matched to two groups according to the type of maintenance therapy with nearest neighbor 1:1 matching (Group A [CNI], n = 20; Group B [CNI/m-Tor-I], n = 20). The two resulting groups showed no differences in age and gender distribution as well as in the main clinical and laboratory data. Then, a SARS-CoV-2 interferon gamma release assay (IGRA) was performed onto PBMC isolated from 40 renal transplant patients undergone COVID vaccination and the T cell reactivity to SARS-CoV-2-related S1/RBD was assessed. Patients treated with mTOR-I showed significantly higher release of IFN-γ, as compared with these not treated with mTOR-I (88.7 ± 8.9 vs. 44.0 ± 10.0 mUI/ml, p = .001; Figure 3A). Moreover, we assessed the T cell reactivity as a ratio (IFN-γ released after SARS-CoV-2-related S1/RBD-specific stimulus/IFN-γ release after nonspecific mitogen exposure). Renal transplant recipients treated with mTOR-I showed stronger capacity (%) to release IFN-γ after specific stimulus as compared to the maximum release induced by nonspecific mitogen, while this ratio was significantly lower in patients not treated with mTOR-I (78.1 ± 4.6% vs. 25.0 ± 3.5%, p < .001; Figure 3B).

Then we aimed to assess the possible combined role of several factors with mTOR-I therapy onto the humoral and cellular response to the COVID-19 vaccine in KTR. In detail, the relative risk for a positive surrogate virus neutralization test was estimated and a Cox regression analysis was performed, using anti-SARS-CoV-2 S1/RBD IgG above or below the cutoff value (35%) as dependent variable, and patient’s age and gender, diabetes, donor type, time from transplant to vaccine, eGFR, lymphopenia, and therapy with mTOR-I as covariates (Table 2A). Univariate analysis showed that only time from transplant to vaccine (HR 1.919, 95% CI 1.308–2.817, p = .001) and therapy with mTOR-I (HR 3.547, 95% CI 1.430–8.794, p = .005) affected the anti-SARS-CoV-2 S1/RBD IgG positivity. Moreover, the results of the multivariate analysis confirmed a significant effect on test positivity of both time from transplant to vaccine (HR 2.288, 95% CI 1.440–3.637, p < .001) and therapy with mTOR-I (HR 4.254, 95% CI 1.440–11.816, p = .005).

Then, we evaluated the relative risk for a SARS-CoV-2-related S1/RBD-specific IFN-γ release above or below the 50th percentile (56.5 mUI/ml). Thus, a second Cox regression analysis was performed, using the IFN-γ release above or below the 50th percentile as dependent variable, and patient’s age, time from transplant to vaccine, lymphopenia and therapy with mTOR-I as covariates (Table 2B). In this model, univariate analysis showed that only time from transplant to vaccine (HR 2.449, 95% CI 1.208–4.969, p = .013)
and therapy with mTOR-I (HR 9.333, 95% CI 1.193–72.991, \( p = .033 \)) affected the IFN\(\gamma\) release above or below the 50° percentile, while in the multivariate analysis only therapy with mTOR-I reached the statistical significance (HR 15.362, 95% CI 2.304–102.436, \( p = .005 \)).

Finally, all the 40 patients tested for both surrogate virus neutralization test (sVNT) and interferon gamma (IFN\(\gamma\)) release assay (IGRA) were assigned to three groups, depending on the quality of immune response to BNT16b2 vaccine: Group 1 (sVNT < 35% AND specific IFN\(\gamma\) release <50° percentile), Group 2 (sVNT > 35% OR specific IFN\(\gamma\) release >50° percentile), and Group 3 (sVNT > 35% AND specific IFN\(\gamma\) release >50° percentile). As shown in Figure S1, none of the patients belonging to the Group 1 was treated with mTOR-I, while 57.1% of patients belonging to the Group 2 and as many as 83.3% of patients belonging to the Group 2 were both treated with mTOR-I (\( p < .001 \)).

4 | DISCUSSION

Our data should be examined in the light of the broad debate on the quantitative and qualitative humoral immune response to mRNA vaccines and, more generally, the protective efficacy of these vaccines to COVID-19 in solid organ transplantation recipients. It is well known that mRNA vaccines administered in a two-dose series have been shown to be more than 94% effective in preventing COVID-19 in clinical trials, without safety concerns identified,\(^{10,11}\) while solid organ transplant recipients were not included in that studies, due to the less intensive response to viral vaccines in patients with immunosuppression.\(^{12,13}\)

To date, the antibody response rate to mRNA vaccines in kidney transplant recipients is lower than in general population, ranging between 37.5% and 54%, as reported by recent reports.\(^{5,14,15}\) However, the humoral response alterations in renal transplant recipients encompassed not only the quantity but also the functionality, as reflected by significantly lower frequency of neutralizing anti-S1/RBD Ig, being suggestive of impaired virus neutralization in these patients as compared with other subjects.\(^{16}\)

Our data show a higher overall proportion of patient with positive humoral response to mRNA vaccine, but also a significantly higher serum levels of total anti-SARS-CoV-2 IgG and proportion of neutralizing anti-SARS-CoV-2 S1/RBD Ig in patients treated with mTOR-I. These observations suggest a possible enhancement of mTOR-I on the immune response to mRNA vaccines. To date, limited reports suggest that inhibition of mTOR could restore B cell homeostasis and functions in autoimmune diseases.\(^{17}\)

Moreover, the strength correlation between total anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 S1/RBD Ig observed in our study, although worthy of confirmation in future studies, suggest the employ of total IgG serum level as a surrogate marker of vaccine response and could
facilitate the evaluation of possible waning protection of vaccine in long term and the allocation of booster doses.

Limited data are currently available on the elicited virus-specific T cell responses. As a matter of fact, assessment alone of humoral response may underestimate the vaccine immunogenicity, so that additional evaluation of cell-mediated immunity is crucial to estimate the response to the vaccine.

To this aim, we performed a SARS-CoV-2 interferon gamma (IFNγ) release assay (IGRA) on PBMC from renal transplant patients. In this test the source of antigen-specific IFNγ production was mostly CD4 and sometimes CD8 T cells, which is consistent with previous reports. CD3 negative cells did not produce antigen-specific IFNγ.

The result indicated that patients treated with mTOR-I showed significantly higher T cell reactivity to SARS-CoV-2-related S1/RBD, as compared with those without mTOR-I. With regards to these data, it is important to underline that the presence of post-vaccine anti-spike T cells, thus in the presence of reduced specific antibodies, could suggest a protective effect from future SARS-CoV-2 infection, by limiting the extent of viral replication, as reported in the setting of CMV infection in kidney transplant recipients.

Taken together, our results seem to suggest that the immune response to BNT262b2 vaccine in renal transplant recipient is strongly influenced by the immunosuppressive protocol. In fact, as further underlined by both univariate and multivariate analysis, mTOR inhibition has been confirmed as independent factor affecting two major surrogate endpoints of COVID-19 vaccine: an anti-SARS-CoV-2 S1/RBD Ig above the cutoff value (35%) and a SARS-CoV-2-related S1/RBD-specific IFNγ release above or below the 50° percentile (56.5 mUI/ml). Among the remaining covariates, only the time of vaccination from transplantation positively affected the neutralizing anti-S1/RBD Ig rate as significant independent factor in the multivariate analysis. This observation is consistent with the evidence that vaccine response is expected to be impaired when immunosuppressive therapy is particularly stronger, such as early post-transplantation.

Due to its pleiotropic effects, the mechanisms underlying the enhanced immune response to mRNA COVID-19 vaccine in renal transplant recipient treated with mTOR-I are likely to be multifactorial. A possible role may be linked to the immunomodulatory effect of mTOR-I on memory CD8+ and CD4+ T cells by promoting the enhancement of memory precursor effector cells that could differentiate into long-lived memory cells.

These observations, coupled with the strong activation of the PI3K/AKT/mTOR pathway during COVID infection support a possible beneficial effect of mTOR-I during COVID infection, although these drugs are associated with potential lung toxicity and their use in KTR with COVID should be carefully evaluated.

As evidences in the setting of COVID-19 infection are still lacking, it is very suggestive to examine the possible role of mTOR inhibition in other viral infections, such as influenza.

In a previous study in elderly subjects, Mannick et al. showed that mTOR inhibition reduced the percentage of exhausted programmed cell death protein 1 (PD1)-positive T cells that had a defective response to antigen. In a further study, he demonstrated that mTOR inhibition also up-regulated a subset of IFN-stimulated genes that act as key players in the innate immune response to pathogens, particularly viruses. One possibility is that mTORC1 inhibition reduces cholesterol synthesis within cells due to decreased SREBP2 activation. Reduced cholesterol biosynthesis after SREBP2 knockdown has been previously shown to stimulate the expression of a subset of antiviral IFN-stimulated genes and protect against viral infection.

Another study suggested that blockade of mTOR by rapamycin efficiently boosted TLR-induced antigen-specific T and B cell responses to HBV vaccines.
Our observations for the first time suggest the potential better modulation of the immune response to mRNA vaccines due to mTOR inhibition in kidney transplant recipient and might be of a direct clinical relevance during current pandemic.

Potential study limitations include its relatively small number of patients and its lack of serial assessments after vaccination and of a long-term follow-up (more than 6 months) with the aim to assess the differential rates of post-vaccination COVID-19 infection between groups of treatment.

In conclusion, this study underlines the potential beneficial role of mTOR inhibitors to enhance the immunogenicity of mRNA BNT162b2 vaccine in kidney transplant recipients, not only by increasing vaccine-induced antibodies but also by stimulating anti-SARS-CoV-2 spike-specific T cell response. The results here reported represent the first demonstration that it is possible to explore novel strategy to better stimulate specific immunogenicity also in immunosuppressed kidney transplant recipients, thus likely improving the clinical management of viral infections in this cohort of frail patients.

ACKNOWLEDGMENTS
The study was supported with grant funding from University of Foggia (University Research Projects 2019 “PRA 2019” granted to G.S.N., 2019).

The authors thank Mr Luigi Consagro and all the Nursing Staff at the Nephrology Outpatients Services of the Nephrology Units participating in this study for their invaluable collaboration. Open Access Funding provided by Universita degli Studi di Foggia within the CRUI-CARE Agreement.

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

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ORCID
Giuseppe S. Netti https://orcid.org/0000-0003-3495-2707
Federica Spadaccino https://orcid.org/0000-0002-4375-0560
Margherita Gigante https://orcid.org/0000-0001-8228-7639
Paola Pontrelli https://orcid.org/0000-0002-7654-8318
Loreto Gesualdo https://orcid.org/0000-0002-4861-0911
Elena Ranieri https://orcid.org/0000-0002-4996-3938
Giuseppe Castellano https://orcid.org/0000-0002-0153-3795

| TABLE 2 | Univariate and multivariate regression analyses of factors affecting vaccine response in renal transplant recipients |
|---------|--------------------------------------------------|
| **A. Factors affecting anti-SARS-CoV-2 S1/RBD IgG positivity (>35%) after COVID-19 vaccine (patients, n = 132)** |
| | Univariate analysis | Multivariate analysis |
| | HR | 95% CI | p value | HR | CI 95% | p value |
| Age | 0.850 | 0.601 | 1.201 | .356 | 0.973 | 0.940 | 1.007 | .116 |
| Gender | 0.761 | 0.371 | 1.559 | .455 | 0.732 | 0.321 | 1.667 | .458 |
| Diabetes | 1.466 | 0.641 | 3.357 | .365 | 1.930 | 0.727 | 5.123 | .187 |
| Donor type | 0.598 | 0.213 | 1.681 | .330 | 0.396 | 0.118 | 1.326 | .133 |
| Time from Tx | 1.919 | 1.308 | 2.817 | .001 | 2.288 | 1.440 | 3.637 | <.001 |
| eGFR (<60 ml/min) | 0.661 | 0.316 | 1.380 | .270 | 0.516 | 0.218 | 1.222 | .132 |
| Lymphopenia | 1.063 | 0.508 | 2.223 | .871 | 1.063 | 0.459 | 2.462 | .886 |
| mTor inhibitors | 3.547 | 1.430 | 8.794 | .006 | 4.254 | 1.531 | 11.816 | .005 |
| **B. Factors affecting S1/RBD-specific IFN gamma release assay response (>50° percentile) after COVID-19 vaccine (patients, n = 40)** |
| | Univariate analysis | Multivariate analysis |
| | HR | 95% CI | p value | HR | CI 95% | p value |
| Age | 1.000 | 1.000 | 0.446 | 2.241 | 0.701 | 0.213 | 2.303 | .558 |
| Time from Tx | 2.449 | 1.208 | 4.969 | .013 | 2.576 | 0.804 | 8.255 | .111 |
| Lymphopenia | 0.429 | 0.117 | 1.568 | .221 | 0.774 | 0.072 | 8.320 | .833 |
| mTor inhibitors | 9.333 | 1.193 | 72.991 | .033 | 15.362 | 2.304 | 102.436 | .005 |

Note: Age and time of vaccination from transplantation were entered as categorical variables (four groups and three groups, respectively), while the remaining factors (gender, diabetes, donor type, eGFR, lymphopenia) was entered as dichotomous variables. Significant variables are reported in bold, while p-values <.05 are in bold italics.

Abbreviations: CI, confidence interval; HR, hazard ratio.

aAge (<50, 50–60, 60–70, >70 years).
bTime from Tx (time of vaccination from transplantation) (<12, 12–60, >60 months).
cLymphopenia (<1000 vs. >1000/µl).
REFERENCES

1. Caillard S, Chavarot N, Francois H, et al. French SOT COVID Registry. Is COVID-19 infection more severe in kidney transplant recipients? Am J Transplant. 2021;21(3):1295-1303. 10.1111/ajt.16424

2. COVID-19 Vaccination Program Operational Guidance. https://www.cdc.gov/vaccines/covid-19/covid19-vaccination-guidance.html. Accessed October 29, 2021.

3. COVID-19 Vaccination and Prioritization Strategies in the EU/EEA. https://www.ecdc.europa.eu/sites/default/files/documents/COVID-19-vaccination-and-prioritisation-strategies.pdf. Accessed October 29, 2021.

4. Krueger KM, Ikizler MR, Sannella EC, et al. Decreased antibody response to influenza vaccination in kidney transplant recipients. Am J Kidney Dis. 2010;55(3):416-425. 10.1053/j.ajkd.2009.09.023

5. Boyarsky BJ, Werbel WA, Avery RK, et al. Antibody response to 2-dose SARS-CoV-2 mRNA vaccine series in solid organ transplant recipients. JAMA. 2021;325(21):2204-2206. 10.1001/jama.2021.7489

6. Bertrand D, Hamzaoui M, Lemée V, et al. Antibody and T cell response to SARS-CoV-2 messenger RNA BNT162b2 vaccine in kidney transplant recipients and hemodialysis patients. J Am Soc Nephrol. 2021;32(9):2147-2152. 10.1681/ASN.20211004480

7. Berger SP, Sommerer C, Witzke O, et al; TRANSFORM Investigators. Two-year outcomes in de novo renal transplant recipients receiving everolimus-facilitated calcineurin inhibitor reduction regimen from the TRANSFORM study. Am J Transplant. 2019;19(11):3038-3044. 10.1111/ajt.15480

8. Araki K, Turner AP, Shaffer VO, et al. mTOR regulates memory CD8 T-cell differentiation. Nature. 2009;460(7251):108-112. 10.1038/nature08155

9. Ye L, Lee J, Xu L, et al. mTOR promotes antiviral humoral immunity by differentially regulating CD4 helper T cell and B cell responses. J Virol. 2017;91(4):e01653-1716. 10.1128/JVI.01653-16

10. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the SARS-CoV-2 mRNA vaccine candidate BNT162b2 in adults at high risk for severe COVID-19. N Engl J Med. 2020;383(27):2603-2615. 10.1056/NEJMoa2034577

11. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384(5):403-416. 10.1056/NEJMoa2035389

12. Manuel O, Pascual M, Hoschler K, et al. Altered peripheral B lymphocyte homeostasis and functions mediated by IL-27 via activating the mammalian target of rapamycin signaling pathway in patients with rheumatoid arthritis. Clin Exp Immunol. 2021;206(3):354-365. 10.1111/cei.13663

13. Manuel O, Pascual M, Hoschler K, et al; Maricovid Research Group. Negative immune responses to two-dose mRNA COVID-19 vaccines in renal allograft recipients assessed with simple antibody and interferon gamma release assay cellular monitoring. Am J Transplant. 2021. 10.1111/ajt.16854

14. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell. 2020;183(1):158-168.e14. 10.1010/2020.06.29.174888

15. Liang Y, Huang L, Dedegolu B, Meijers RWJ, Kwekkeboom J, Betjes MGH. Protective cytomegalovirus (CMV)-specific T-cell immunity is frequent in kidney transplant patients without serum anti-CMV antibodies. Front Immunol. 2017;8:1137. 10.3389/fimmu.2017.01137

16. Liu Y, Lee J, Xu L, et al. mTOR promotes antiviral humoral immunity by differentially regulating CD4 helper T cell and B cell responses. J Virol. 2017;91(4):e01653-16. 10.1128/JVI.01653-16

17. Tang Y, Bai Z, Qi J, et al. Altered peripheral B lymphocyte homeostasis and functions mediated by IL-27 via activating the mammalian target of rapamycin signaling pathway in patients with rheumatoid arthritis. Clin Exp Immunol. 2021;206(3):354-365. 10.1111/cei.13663

18. Crespo M, Barrilado-Jackson A, Padilla E, et al; Maricovid Research Group. Negative immune responses to two-dose mRNA COVID-19 vaccines in renal allograft recipients assessed with simple antibody and interferon gamma release assay cellular monitoring. Am J Transplant. 2021. 10.1111/ajt.16854

19. Sekine T, Perez-Potti A, Rivera-Ballesteros O, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell. 2020;183(1):158-168.e14. 10.1010/2020.06.29.174888

20. Litjens NHR, Huang L, Dedegolu B, Meijers RWJ, Kwekkeboom J, Betjes MGH. Protective cytomegalovirus (CMV)-specific T-cell immunity is frequent in kidney transplant patients without serum anti-CMV antibodies. Front Immunol. 2017;8:1137. 10.3389/fimmu.2017.01137

21. Liu Y, Lee J, Xu L, et al. mTOR promotes antiviral humoral immunity by differentially regulating CD4 helper T cell and B cell responses. J Virol. 2017;91(4):e01653-16. 10.1128/JVI.01653-16

22. Granata S, Carratù P, Stallone G, Zaza G. mTOR-inhibition and control of cytokine release during severe COVID-19 in kidney transplant recipients: focus on pulmonary fibrosis. Front Pharmacol. 2021;12:710543. 10.3389/fphar.2021.710543

23. Mannick JB, Del Giudice G, Lattanzi M, et al. mTOR inhibition improves immune function in the elderly. Sci Transl Med. 2014;6(268):268ra179. 10.1126/scitranslmed.3009892

24. Mannick JB, Morris M, Hockey HP, et al. TORC1 inhibition enhances immune function and reduces infections in the elderly. Sci Transl Med. 2018;10(449):eaao1564. 10.1126/scitranslmed.aao1564

25. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;149(2):274-293. 10.1016/j.cell.2012.03.017

26. York AG, Williams KJ, Argus JP, et al. Limiting cholesterol biosynthesis via inhibiting the mammalian target of rapamycin signaling pathway improves immune function in the elderly. Sci Transl Med. 2015;7(301):301ra125. 10.1126/scitranslmed.aaa5894

27. Marinaki S, Adamopoulos S, Degeninis D, et al. Immunogenicity of SARS-CoV-2 BNT162b2 vaccine in solid organ transplant recipients. Am J Transplant. 2021;21(8):2719-2726. 10.1111/ajt.16615

28. Stumpf J, Siepmann T, Lindner T, et al. Humoral and cellular immunity to SARS-CoV-2 vaccination in renal transplant versus dialysis patients: a prospective, multicenter observational study using mRNA-1273 or BNT162b2 mRNA vaccine. Lancet Reg Health Eur. 2021;9:100178. 10.1016/j.lanepe.2021.100178

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Netti GS, Infante B, Troise D, et al. mTOR inhibitors improve both humoral and cellular response to SARS-CoV-2 messenger RNA BNT162b2 vaccine in kidney transplant recipients. Am J Transplant. 2022;22:1475-1482. doi:10.1111/ajt.16958