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BRIEF COMMUNICATION

mTOR inhibitors improve both humoral and cellular response to SARS-CoV-2 messenger RNA BNT16b2 vaccine in kidney transplant recipients

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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 finding 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, inferferon 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 vaccines[4] 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 cells[8] 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 + Prednisolon) or with CNI/mTOR inhibitors (mTor-I) based maintenance therapy (Group B: Tacrolimus + Everolimus + Prednisolon), 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 and dispensed 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

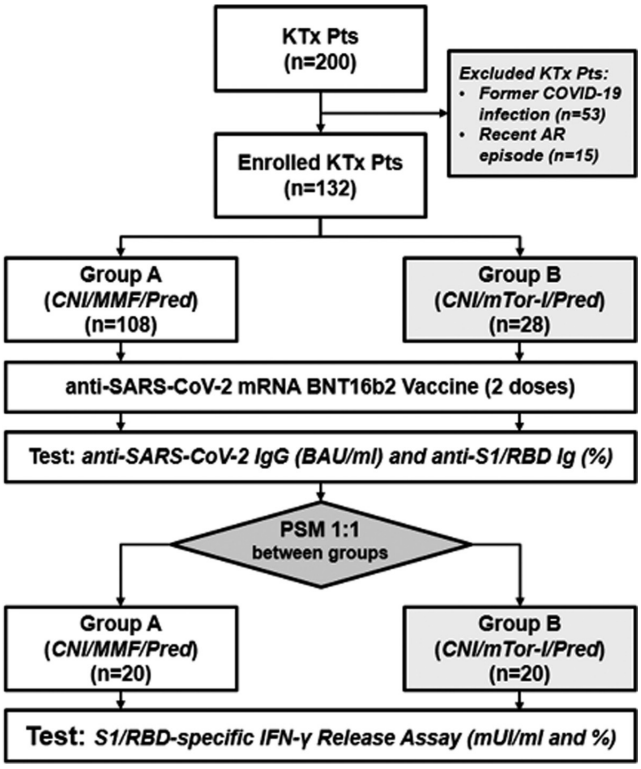

FIGURE 1 Algorithm of the study. Study design flow chart. KTx, kidney transplant recipients; PSM, propensity score matching

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 a 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 (R^2 = 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

TABLE 1 Demographic, clinical, and biochemistry data at baseline of renal transplant recipients enrolled in the study

	Total	Group A	Group B	*p* value
Number (*n*)	132	104	28	
Gender (% male)	65.1%	66.3%	60.7%	.579
Age (years)	54.8 ± 13.0	55.0 ± 12.9	54.2 ± 13.6	.784
Time of vaccination from transplantation (months)	117.1 ± 92.7	112.6 ± 81.2	133.9 ± 127.4	.408
Donor type (% living)	12.9%	14.4%	7.1%	.307
GFR (ml/min)	50.6 ± 22.6	51.5 ± 23.4	47.2 ± 19.8	.370
GFR < 60 ml/min (%)	68.2%	66.3%	75.0%	.383
Diabetes mellitus (%)	22.0%	22.1%	21.4%	.938
White blood cells (cells/μl)	10 220 ± 3360	10 460 ± 3340	9340 ± 3330	.121
Lymphocytes (cells/μl)	1595 ± 750	1520 ± 870	1890 ± 1450	.579
CD3+ T (cells/μl)	1495 ± 1230	1480 ± 1250	1545 ± 1195	.894
CD4+ Th (cells/μl)	620 ± 470	625 ± 455	595 ± 545	.773
CD8+ Ts (cells/μl)	620 ± 505	640 ± 534	545 ± 370	.274
CD19+ B (cells/μl)	125 ± 105	130 ± 110	95 ± 85	.082
NK (cells/μl)	360 ± 545	365 ± 595	355 ± 305	.903
Induction therapy				
Basiliximab (% yes)	100%	100%	100%	
Maintenance therapy				
Tac[a]/MMF[b]/Pred	104	104	0	
Tac[a]/mTOR-I[c]/Pred	28	0	28	

Note: Values are expressed as mean ± SD, counts (*n*), or percentages (%).

Abbreviations: GFR, glomerular filtration rate; MMF, mycophenolate mofetil; mTOR, mTOR inhibitors; NK, natural killer; Pred, prednisolon; Tac, tacrolimus.

[a]The trough level of tacrolimus during follow-up was 5.0–7.0 ng/ml.

[b]Mycophenolate mofetil (MMF) was administered at a standard dose of 500 mg twice daily.

[c]The trough level of m-TOR-I (Everolimus) during follow-up was 3.0–5.0 ng/ml.

score 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 (IFNγ) 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 IFNγ, 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 (IFNγ released after SARS-CoV-2-related S1/RBD-specific stimulus/ IFNγ release after nonspecific mitogen exposure). Renal transplant recipients treated with mTOR-I showed stronger capacity (%) to release IFNγ 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, $p = .006$) 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 1.531–11.816, $p = .005$).

Then, we evaluated the relative risk for a SARS-CoV-2-related S1/RBD-specific IFNγ release above or below the 50° percentile (56.5 mUI/ml). Thus, a second Cox regression analysis was performed, using the IFNγ release above or below the 50° 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, $p = .013$)

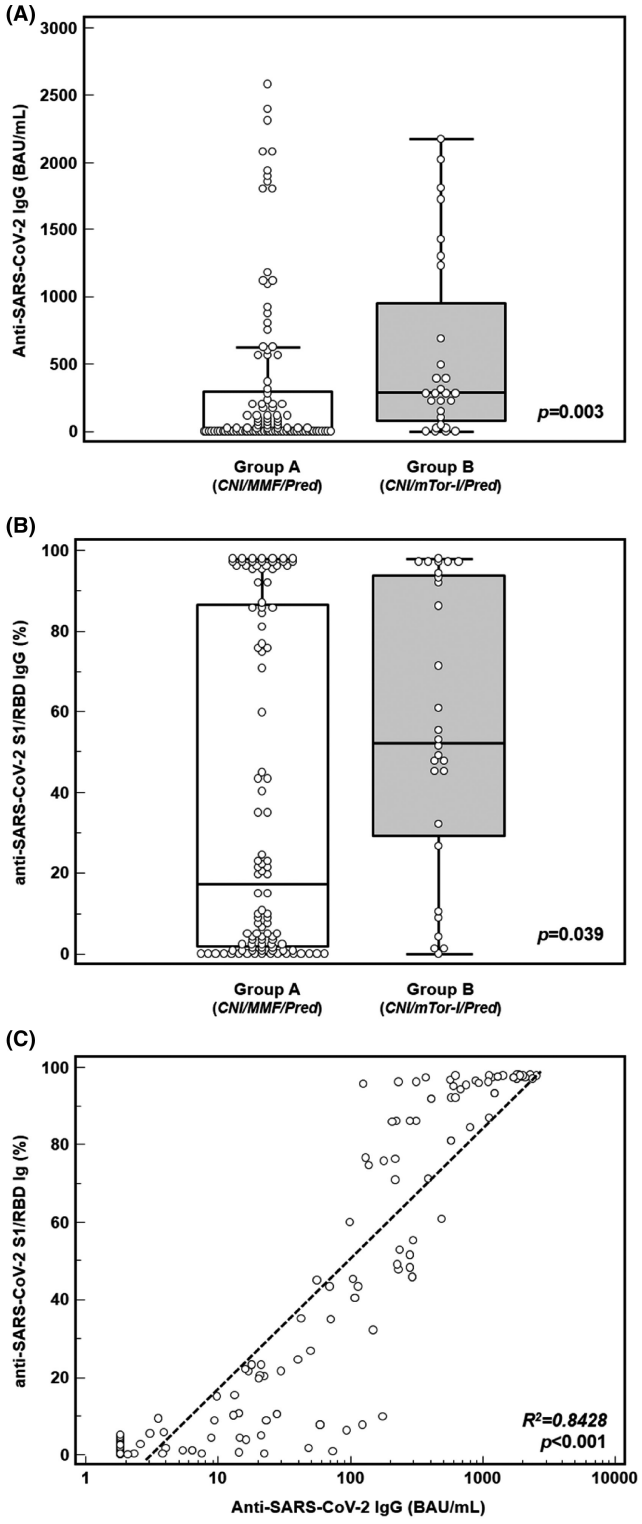

FIGURE 2 Anti-SARS-CoV-2 antibody response in renal transplant recipients after COVID-19 mRNA vaccine ($n = 132$). (A) Detection of total anti-SARS-CoV-2 IgG showing higher serum levels in patients treated with mTOR-I, as compared with those without mTOR-I (649.3 ± 173.6 vs. 350.3 ± 62.5 BAU/ml, $p = .003$). (B) Detection of total anti-SARS-CoV-2 S1/RBD Ig showing higher percentages in patients treated with mTOR-I, as compared with those without mTOR-I (55.8 ± 6.7% vs. 38.2 ± 4.0%, $p = .024$). (C) Correlation between total anti-SARS-CoV-2 IgG and anti-SARS-CoV-2 S1/RBD Ig in kidney transplant recipients ($R^2 = 0.8428$, $p < .001$)

and therapy with mTOR-I (HR 9.333, 95% CI 1.193–72.991, $p = .033$) affected the IFNγ 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 (IFNγ) release assay (IGRA) were assigned to three groups, depending on the quality of immune response to BNT16b2 vaccine: Group 1 (sVNT < 35% AND specific IFNγ release <50° percentile), Group 2 (sVNT > 35% OR specific IFNγ release >50° percentile), and Group 3 (sVNT > 35% AND specific IFNγ 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 and neutralizing anti-SARS-CoV-2 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

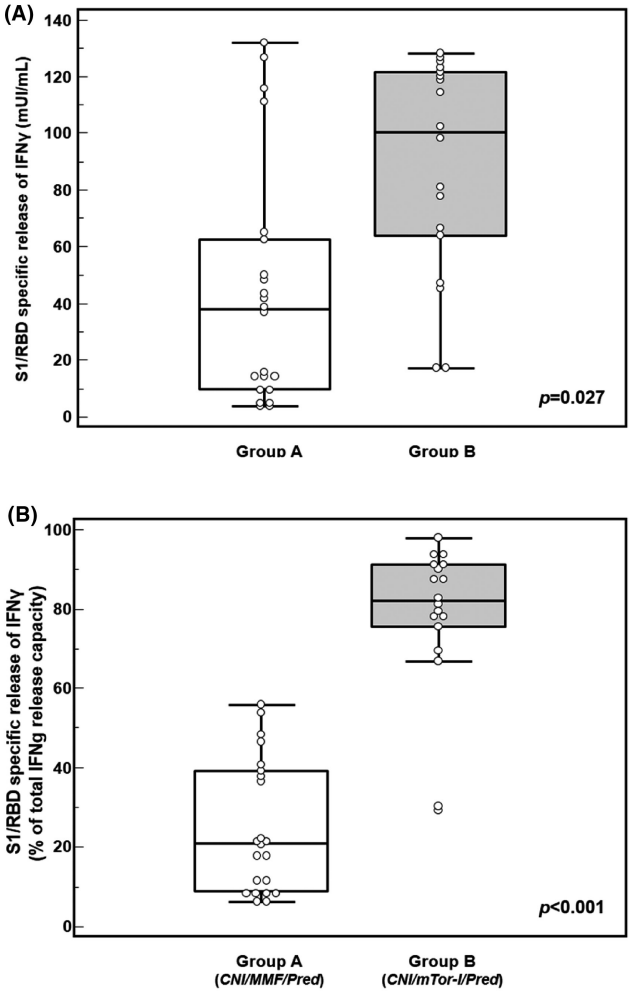

FIGURE 3 S1/RBD-specific IFN-γ release assay response in renal transplant recipients after COVID-19 mRNA vaccine ($n = 40$). (A) Release of IFNγ from PBMC stimulated with SARS-CoV-2 S1/RBD, showing higher titer in patients treated with mTOR-I, as compared with these not treated with mTOR-I (88.7 ± 8.9 vs. 44.0 ± 10.0 mUI/ml, $p = .001$). (B) Release of IFNγ from PBMC stimulated with SARS-CoV-2 S1/RBD, showing higher ratio (IFNγ released after SARS-CoV-2-related S1/RBD-specific stimulus/IFNγ release after a specific mitogen exposure) in patients treated with mTOR-I, as compared with these not treated with mTOR-I (78.1 ± 4.6% vs. 25.0 ± 3.5%, $p < .001$)

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.[18] 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.[19] 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.[20,21]

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.[22,23]

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.[7,24]

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.[25]

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.[26] 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.[27] One possibility is that mTORC1 inhibition reduces cholesterol synthesis within cells due to decreased SREBP2 activation.[28] 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.[29]

Another study suggested that blockade of mTOR by rapamycin efficiently boosted TLR-induced antigen-specific T and B cell responses to HBV vaccines.[30]

TABLE 2 Univariate and multivariate regression analyses of factors affecting vaccine response in renal transplant recipients

	Univariate analysis				Multivariate analysis			
		95% CI				CI 95%		
	HR	Lower	Higher	*p* value	HR	Lower	Higher	*p* value
A. Factors affecting anti-SARS-CoV-2 S1/RBD IgG positivity (>35%) after COVID-19 vaccine (patients, *n* = 132)								
Age[a]	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[b]	**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[c]	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)								
Age[a]	1.000	1.000	0.446	2.241	0.701	0.213	2.303	.558
Time from Tx[b]	**2.449**	**1.208**	**4.969**	***.013***	2.576	0.804	8.255	.111
Lymphopenia[c]	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.

[a]Age (<50, 50–60, 60–70, >70 years).

[b]Time from Tx (time of vaccination from transplantation) (<12, 12–60, >60 months).

[c]Lymphopenia (<1000 vs. >1000/μl).

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.

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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.

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SUPPORTING INFORMATION

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

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