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.
BRIEF COMMUNICATION

mTOR inhibitors, mycophenolates, and other immunosuppression regimens on antibody response to SARS-CoV-2 mRNA vaccines in solid organ transplant recipients

Sunjae Bae1,2 | Jennifer L. Alejo3 | Teresa P. Y. Chiang3 | William A. Werbel4 | Aaron A. R. Tobian5 | Linda W. Moore6,7 | Ashrith Guha7,8 | Howard J. Huang7,9 | Richard J. Knight6,7 | A. Osama Gaber6,7 | R. Mark Ghobrial6,7 | Mara A. McAdams-DeMarco1,2 | Dorry L. Segev1,2

1Department of Surgery, NYU Grossman School of Medicine, New York, New York, USA
2Department of Population Health, NYU Grossman School of Medicine, New York, New York, USA
3Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
4Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
5Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
6Department of Surgery, Houston Methodist Hospital, Houston, Texas, USA
7JC Walter Jr Transplant Center, Houston Methodist Hospital, Houston, Texas, USA
8Methodist DeBakey Heart & Vascular Center, Houston Methodist Hospital, Houston, Texas, USA
9Department of Medicine, Houston Methodist Hospital, Houston, Texas, USA

Correspondence
Dorry L. Segev, Department of Surgery, NYU Grossman School of Medicine, 1 Park Ave, 6-704, New York, NY 10016, USA. Email: Dorry.Segev@nyulangone.org

Funding information
Ben-Dov family; National Institute of Allergy and Infectious Diseases, Grant/Award Number: K24AI144954; National Institute of Diabetes and Digestive and Kidney Diseases, Grant/Award Number: R01DK120518 and T32DK007713

A recent study concluded that SARS-CoV-2 mRNA vaccine responses were improved among transplant patients taking mTOR inhibitors (mTORi). This could have profound implications for vaccine strategies in transplant patients; however, limitations in the study design raise concerns about the conclusions. To address this issue more robustly, in a large cohort with appropriate adjustment for confounders, we conducted various regression- and machine learning-based analyses to compare antibody responses by immunosuppressive agents in a national cohort (n = 1037). MMF was associated with significantly lower odds of positive antibody response (aOR = 0.090.130.18). Consistent with the recent mTORi study, the odds tended to be higher with mTORi (aOR = 1.001.452.13); however, importantly, this seemingly protective tendency disappeared (aOR = 0.470.731.12) after adjusting for MMF. We repeated this comparison by combinations of immunosuppression agents. Compared to MMF + tacrolimus, MMF-free regimens were associated with higher odds of positive antibody response (aOR = 2.394.267.92 for mTORi + tacrolimus; 2.345.5415.32 for mTORi-only; and 6.7810.2515.93 for tacrolimus-only), whereas MMF-including regimens were not, regardless of mTORi use (aOR = 0.811.542.98 for MMF + mTORi; and 0.811.512.87 for MMF-only). We repeated these analyses in an independent cohort (n = 512) and found similar results. Our study demonstrates that the recently reported findings were confounded by MMF, and that mTORi is not independently associated with improved vaccine responses.

KEYWORDS
clinical research/practice, immunosuppressant, immunosuppression/immune modulation, infection and infectious agents, infection and infectious agents—viral: SARS-CoV-2/COVID-19, infectious disease

Abbreviations: mTORi, mammalian target of rapamycin inhibitor; MMF, mycophenolates.

© 2022 The American Society of Transplantation and the American Society of Transplant Surgeons.
1 | INTRODUCTION

Antibody responses after SARS-CoV-2 mRNA vaccines are substantially attenuated in organ transplant recipients.1-6 The low vaccine efficacy in this population is primarily attributed to immunosuppression therapy.2,7,8 Given that several regimens with various levels of potency are used for post-transplant immunosuppression,9 understanding the impact of immunosuppressive regimens on the immunogenicity of SARS-CoV-2 mRNA vaccines could allow us to enhance vaccine efficacy via immunosuppression adjustments in this high-risk population.

Recently in the American Journal of Transplantation, Netti, and colleagues10 concluded that mTOR inhibitors (mTORi) provided a beneficial role in mRNA vaccine-induced immunogenicity. However, there were a number of limitations in the design of this study, including: the sample size was only 132, of whom only 28 received mTORi, which limits the ability to account for confounding, and2 the comparison groups were mTORi-based regimens (where no patients received MMF) versus MMF-based regimens (where no patients received mTORi), thereby fully confounding any conclusions about mTORi by the well-documented negative effects of MMF. This issue needs to be clarified because of its potential profound implications on immunosuppression strategies in trying to improve vaccine responses in transplant patients.

In this study, we aimed to dissect the respective impacts of mTORi, MMF, and other immunosuppressive agents in the immunogenicity of SARS-CoV-2 mRNA vaccines using two independent cohorts of solid organ transplant recipients (n = 1037 and 512).

2 | METHODS

2.1 | Study population

We recruited a prospective cohort (“primary cohort”) of solid organ transplant recipients with no known history of a positive PCR test for COVID-19 infection from across the United States through an online campaign. The participants reported two congruent SAR-CoV-2 mRNA vaccines, either BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna), between December 16, 2020 and May 21, 2021, and were followed up until July 6, 2021. The study was approved by the Johns Hopkins Medical Institutional Review Boards.

This study also included an independent cohort (“secondary cohort”) of solid organ transplant recipients at a tertiary transplant center who were vaccinated between January 4, 2021 and May 31, 2021. Those who were vaccinated prior to transplantation, had no medical records of vaccine type or antibody results after the second dose of vaccine, or history of a positive test for COVID-19 infection were excluded from this cohort. Data were collected with a waiver of informed consent approved by the Houston Methodist Research Institute IRB. De-identified data were shared between institutions after approval from both IRBs.

In both cohorts, we excluded those who used belatacept as belatacept use was rare and associated with a substantially lower chance of positive antibody response.11 A comparison of these two cohorts is presented in Table 1.

2.2 | Antibody response

Antibodies were measured one month after the second dose (median [interquartile range]; 30 [28–33] days) via an electrochemiluminescence immunoassay for antibodies to SARS-CoV-2 spike protein receptor binding domain (Elecsys® Anti-SARS-CoV-2 S, Roche) or an enzyme-linked immunosorbent assay for antibodies to SARS-CoV-2 spike protein (S1 subunit) (EUROIMMUN Anti-SARS-CoV-2 ELISA IgG, EUROIMMUN). We used the manufacturer-suggested thresholds (0.8 U/ml for Elecsys and ≤1.1 AU for EUROIMMUN) to determine positive antibody responses.

2.3 | Antibody response by immunosuppressive agents

We quantified the association between individual immunosuppressive agents with antibody response after adjusting for age, time since transplant, and vaccine type (mRNA-1273 vs. BNT162b2) using logistic regression. We conducted stepwise model building in which the immunosuppressive agents were gradually included in the model.

2.4 | Antibody response by immunosuppressive regimens

Post-transplant immunosuppression regimen is typically planned as combination therapy of multiple agents, resulting in a strong correlation between the use of each agent. To account for this correlation in a more clinically relevant and methodologically robust way, we repeated the comparison by regimens, that is, the combinations of the immunosuppressive agents. The cohort was divided into seven categories: single therapy (MMF only, mTORi only, and tacrolimus only), double therapy (MMF+tacrolimus, MMF+mTORi, and mTORi+tacrolimus), and others. We simply adjusted for steroids, rather than creating more categories by steroids because the association of steroids with antibody response was consistent even after adjusting for other agents in our previous analysis, implying minimal impacts from correlation (Table 2). A full analysis including steroids as a separate category is presented in the Appendix S1.

2.5 | Variable importance of immunosuppressive agents

We used gradient boosting, a general-purpose machine learning algorithm, to create a prediction model for positive antibody response
based on immunosuppressive agents and other clinical factors. During this procedure, the association of each immunosuppressive agent with a positive antibody response is characterized in a flexible and entirely data-driven manner. We then examined these associations using variable importance, a metric for assessing how much contribution each variable had made to the overall prediction ability of the machine learning model. Variables included in the models were immunosuppressive regimen use (MMF, mTORi, tacrolimus, steroids, and azathioprine), age, sex, race, organ(s) received (kidney, pancreas, liver, heart, and lung), time since transplant, number of transplants, and vaccine type (BNT162b2 vs. mRNA-1273).

### 2.6 | Sensitivity analysis

As antibody titer may wane over time after vaccination, the time interval from vaccination to antibody testing may confound the association of immunosuppressive regimens with antibody response. To assess the impact of this potential confounding, we conducted sensitivity analyses where we repeated the analyses described above after including the time interval from vaccination to antibody testing as an additional covariable.

### 2.7 | Statistical analysis

We described the population characteristics using median [interquartile range] for continuous variables and N (%) for categorical variables. We used a significance level of 0.05 for all statistical testing. Confidence intervals are reported as per the method of Louis and Zeger. All analyses were performed using R version 4.1.2.

### 3 | RESULTS

#### 3.1 | Study population

The primary cohort included 1037 solid organ transplant recipients. Median (interquartile range) antibody titers were 4.63 (<0.4 to 250) U/ml among 743 recipients with Elecsys and 1.56 (0.15–7.18) arbitrary units among 294 with EUROIMMUN (Figure S1). Using the manufacturer-suggested thresholds, 604 (58.2%) were classified as positive antibody responders. Compared to those with negative antibody responses, those with positive responses were more likely to have received mRNA-1273 (51.5% vs. 38.6%) and mTORi (17.4% vs. 11.3%), and less likely to be kidney transplant...
TABLE 2  Association of individual immunosuppressive agents with positive antibody response to the two-dose SARS-CoV-2 mRNA vaccine series in two independent cohorts of solid organ transplant recipients

|                          | MMF    | mTORi  | Tac    | Steroids |
|--------------------------|--------|--------|--------|----------|
| Primary cohort (n = 1037) |        |        |        |          |
| Model 1 (MMF only)       | 0.13 (0.09–0.18) |        |        |          |
| Model 2 (mTORi only)     |        | 1.45 (1.00–2.13) |        |          |
| Model 3 (Tac only)       |        |        | 0.70 (0.48–1.03) |          |
| Model 4 (Steroids only)  |        |        |        | 0.46 (0.35–0.60) |
| Model 5 (MMF and mTORi)  | 0.12 (0.09–0.17) | 0.73 (0.47–1.12) |        |          |
| Model 6 (MMF, mTORi, and Tac) | 0.12 (0.08–0.16) | 0.57 (0.35–0.92) | 0.58 (0.36–0.92) |          |
| Model 7 (MMF, mTORi, and Steroids) | 0.12 (0.09–0.17) | 0.74 (0.48–1.14) |        | 0.48 (0.35–0.64) |
| Model 8 (All four agents) | 0.12 (0.08–0.17) | 0.58 (0.36–0.94) | 0.58 (0.36–0.92) | 0.47 (0.35–0.64) |
| Secondary cohort (n = 512)|        |        |        |          |
| Model 1 (MMF only)       | 0.36 (0.23–0.57) |        |        |          |
| Model 2 (mTORi only)     |        | 2.14 (1.33–3.50) |        |          |
| Model 3 (Tac only)       |        |        | 1.31 (0.68–2.57) |          |
| Model 4 (Steroids only)  |        |        |        | 0.81 (0.49–1.35) |
| Model 5 (MMF and mTORi)  | 0.41 (0.24–0.70) | 1.32 (0.74–2.35) |        |          |
| Model 6 (MMF, mTORi, and Tac) | 0.41 (0.24–0.69) | 1.35 (0.75–2.39) | 1.48 (0.76–2.97) |          |
| Model 7 (MMF, mTORi, and Steroids) | 0.42 (0.24–0.70) | 1.32 (0.74–2.34) |        | 0.87 (0.52–1.45) |
| Model 8 (All four agents) | 0.41 (0.24–0.70) | 1.34 (0.75–2.38) | 1.47 (0.75–2.95) | 0.88 (0.52–1.47) |

Note: Estimates represent the adjusted odds ratio (95% confidence interval). Boldface indicates statistical significance. All models included age, time since transplant, and vaccine type (mRNA-1273 vs. BNT162b2) as covariable adjustments. Models 1–4 included each of the immunosuppressive regimens. Model 5 included MMF and mTORi in a bivariate manner. Model 6 included all four immunosuppressive regimens.

Abbreviations: MMF, mycophenolate mofetil; mTORi, mammalian target of rapamycin inhibitors; Tac, tacrolimus.

3.2 | Antibody response by immunosuppressive agents

When each immunosuppressive agent was modeled separately (Table 2; Model 1–4), MMF (aOR = 0.09, 0.13–0.18) and steroids (aOR = 0.46, 0.35–0.60) were significantly associated with lower odds of positive antibody response. On the contrary, mTORi showed a tendency toward higher odds of positive antibody response (aOR = 1.43, 1.05–1.95), although this tendency did not reach statistical significance. Nonetheless, this potentially protective tendency disappeared after adjusting for MMF (Model 5); in this bivariate approach, mTORi showed a tendency toward lower odds of positive antibody response (aOR = 0.47, 0.73–1.12) while MMF maintained a significant association with lower odds of positive antibody response (aOR = 0.09, 0.13–0.18). We observed similar associations after adding tacrolimus and steroids to the model (Model 6–8). Nonetheless, this potentially protective tendency disappeared after adjusting for MMF (Model 5); in this bivariate approach, mTORi showed a tendency toward lower odds of positive antibody response (aOR = 0.47, 0.73–1.12) while MMF maintained a significant association with lower odds of positive antibody response (aOR = 0.09, 0.13–0.18). We observed similar associations after adding tacrolimus and steroids to the model (Model 6–8).

3.3 | Antibody response by immunosuppressive regimens

The crude rate of positive antibody response tended to be higher in MMF-free regimens, including the mTORi only (83.3%), tacrolimus only (87.1%), and mTORi+tacrolimus (73.1%) groups, compared to MMF-including regimens, including the MMF only (58.5%), MMF+tacrolimus (38.4%), and MMF+mTORi (55.6%) groups (Table 3).

Our logistic regression showed similar trends. The MMF+tacrolimus group was the most common regimen (n = 528; 50.9%) and therefore used as the reference group. Compared to the MMF+tacrolimus group, the mTORi only (aOR = 2.43, 2.73–3.14), tacrolimus only...
(aOR = 6.78, 10.25, 15.93), and mTORi+ tacrolimus (aOR = 2.39, 4.26, 7.92) groups showed significantly higher odds of positive antibody response, whereas the MMF only (aOR = 0.81, 1.51, 2.87) and MMF + mTORi (aOR = 0.81, 1.54, 2.98) groups did not. We observed similar findings in the secondary cohort (Table 3) and in a sensitivity analysis that was restricted to kidney transplant recipients (Table S2). Lastly, we found congruent results in a full analysis that included steroids as a separate category (Table S3).

3.4 | Variable importance of immunosuppressive agents

MMF showed the highest variable importance (37.4%) among all variables that were used in the machine learning model to predict antibody response in the primary cohort. Steroids (2.8%), tacrolimus (1.2%), and mTORi (0.7%) contributed to much smaller extents to the prediction model.

3.5 | Sensitivity analysis

The time interval from vaccination to antibody testing was similar between those with positive and negative responses (median [interquartile range]: 30 [28–33] vs. 30 [28–32]), likely because the testing was conducted per protocol at one month after vaccination. Accordingly, the results largely remained the same after adding the time interval as a new covariable (Tables S4 and S5).

4 | DISCUSSION

In this analysis of 1549 solid organ transplant recipients, MMF showed a strong association with lower odds of positive antibody response after SARS-CoV-2 mRNA vaccines, which far outweighed the association of mTORi or other immunosuppressive agents with antibody response. While mTORi showed a tendency toward higher odds of positive antibody response (aOR = 1.45), this protective tendency disappeared after adjusting for MMF (aOR = 0.73). When compared by regimens (i.e., combinations of the agents), MMF-free regimens were associated with higher odds of positive antibody response, whereas MMF-including regimens were not, regardless of mTORi use. Lastly, MMF accounted for 37.4% of a machine learning model’s ability to predict antibody response, whereas no other immunosuppressive agents did for >3%. Our findings demonstrate that MMF avoidance, but not mTORi use, is independently associated with improved antibody responses to SARS-CoV-2 mRNA vaccines in transplant recipients.

Netti and colleagues recently concluded that mTORi was associated with improved antibody responses to BNT162b2. In this study, the authors compared 28 kidney transplant recipients who received everolimus + tacrolimus + prednisolone to 108 kidney transplant recipients who received MMF + tacrolimus + prednisolone and observed that the everolimus group was associated with a positive antibody response compared to the MMF group (e.g., aHR = 1.53, 4.25, 11.84). Based on these findings, the authors concluded that their result "underlines the potential beneficial role of mTOR inhibitors to enhance the immunogenicity of mRNA BNT162b2 vaccine in kidney transplant recipients".
On the one hand, our findings are highly congruent with those of Netti and colleagues. In our study, the recipients who received mTORi+tacrolimus had significantly higher odds of positive antibody response compared to the recipients who received MMF + tacrolimus (Table 3; aOR = 2.39\(\pm\)2.62). However, more importantly, we also found that the beneficial association of mTORi with antibody responses disappeared after adjusting for MMF, and MMF-free regimens showed superior antibody responses to MMF-including regimens regardless of mTORi use, and MMF had substantially greater variable importance than mTORi or any other agents had in a machine learning-based prediction model. These results were replicated in an independent secondary cohort. Our findings support that MMF avoidance, rather than mTORi use, would have been the primary factor that led to Netti and colleagues' results.

Due to the observational design of this study, the associations observed in our study do not warrant causal effects. Clinical factors that influence both the exposure (the selection of immunosuppressive regimen) and the outcome (vaccine efficacy) might have confounded the associations observed in our study. To mitigate this concern, we adjusted for age, time since transplant, and vaccine type, which were the major risk factors for negative antibody response.

In conclusion, our three analytical approaches to two independent cohorts of 1549 solid organ transplant recipients have consistently indicated that MMF avoidance, rather than mTORi use, would be the key determinant of the immunogenicity of SARS-CoV-2 mRNA vaccines in this population. As sustaining sufficiently high antibody titer is a central part of protection against SARS-CoV-2 and its variants, continued research on this topic is warranted.

ACKNOWLEDGMENTS
This research was made possible with the generous support of the Ben-Dov family. This work was supported by grants T32DK007713 (J.L.A.) and R01DK120518 (M.A.M.-D.) from the National Institute of Diabetes and Digestive and Kidney Diseases, and grant K24AI144954 (D.L.S.) from the National Institute of Allergy and Infectious Disease.

DISCLOSURE
The authors of this manuscript have conflicts of interest to disclose as described by the American Journal of Transplantation. D.L.S. has received consulting and speaking honoraria from Sanofi, Novartis, CLS Behring, Jazz Pharmaceuticals, Veloxis, Mallinckrodt, and Thermo Fisher Scientific. The other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data are not publicly available due to privacy or ethical restrictions.

ORCID
Sunjae Bae https://orcid.org/0000-0003-0098-8816
Jennifer L. Alejo https://orcid.org/0000-0003-3137-9271
Teresa P. Y. Chiang https://orcid.org/0000-0003-0601-7420
William A. Werbel https://orcid.org/0000-0003-2943-5895
Linda W. Moore https://orcid.org/0000-0003-2519-5150

REFERENCES
1. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a single dose of SARS-CoV-2 messenger RNA vaccine in solid organ transplant recipients. JAMA. 2021;325(17):1784-1786.
2. 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.
3. Werbel WA, Boyarsky BJ, Ou MT, et al. Safety and immunogenicity of a third dose of SARS-CoV-2 mRNA vaccine in solid organ transplant recipients: a case series. Ann Intern Med. 2021;174(9):1330-1332.
4. Alejo JL, Mitchell J, Chiang TP-Y, et al. Antibody response to a fourth dose of a SARS-CoV-2 vaccine in solid organ transplant recipients: a case series. Transplantation. 2021;105(12):e280-e281.
5. Grupper A, Rabinowich L, Schwartz D, et al. Reduced humoral response to mRNA SARS-CoV-2 BNT162b2 vaccine in kidney transplant recipients without prior exposure to the virus. Am J Transplant. 2021;21(8):2719-2726.
6. Cucciachi D, Egri N, Bodro M, et al. Cellular and humoral response after MRNA-1273 SARS-CoV-2 vaccine in kidney transplant recipients. Am J Transplant. 2021;21(8):2727-2739.
7. Kantauskaite M, Müller L, Kolb T, et al. Intensity of mycophenolate mofetil treatment is associated with an impaired immune response to SARS-CoV-2 vaccination in kidney transplant recipients. American J Transplantation. 2022;22(2):634-639.
8. Mitchell J, Chiang TP-Y, Alejo JL, et al. Effect of mycophenolate mofetil dosing on antibody response to SARS-CoV-2 vaccination in heart and lung transplant recipients. Transplantation. 2022;106:e269-e270.
9. Hart A, Smith JM, Skeans MA, et al. OPTN/SRTR 2018 annual data report: kidney. Am J Transplant. 2020;20(Suppl s1):20-130.
10. Netti GS, Infante B, Troise D, et al. mTOR inhibitors improve both humoral and cellular response to SARS-CoV-2 messenger RNA BNT16b2 vaccine in kidney transplant recipients. Am J Transplant. 2022;22:1475-1482.
11. Ou MT, Boyarsky BJ, Chiang TPY, et al. Immunogenicity and reactogenicity after SARS-CoV-2 mRNA vaccination in kidney transplant recipients taking Belatacept. Transplantation. 2021;105:2119-2123.
12. Alejo JL, Mitchell J, Chiang TP-Y, et al. Six-month antibody kinetics and durability in SARS-CoV-2 mRNA vaccinated solid organ transplant recipients. Transplantation. 2022;106(1):e109-e110.
13. Louis TA, Zeger SL. Effective communication of standard errors and confidence intervals. Biostatistics. 2009;10(1):1-2.

SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Bae S, Alejo JL, Chiang TP, et al. mTOR inhibitors, mycophenolates, and other immunosuppression regimens on antibody response to SARS-CoV-2 mRNA vaccines in solid organ transplant recipients. Am J Transplant. 2022;22:3137-3142. doi: 10.1111/ajt.17158