COVID19-TB-03

A randomized study to evaluate antibody response to an additional dose of SARS-CoV-2 vaccination with and without immunosuppression reduction in kidney and liver transplant recipients

Version 8.0 / September 13, 2023
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Confidentiality Statement

SITE INVESTIGATOR SIGNATURE PAGE	
Protocol Number: COVID19-TB-03	**Version Number/Date: v8.0/September 13, 2023**
Protocol Title: A randomized study to evaluate antibody response to an additional dose of SARS-CoV-2 vaccination with and without immunosuppression reduction in kidney and liver transplant recipients	
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Return Signed Form to: The original signature page must be kept for your records. Return an electronic PDF copy of the signed signature page (*as described below) to the DAIT Regulatory Management Center via the applicable DAIT RMC email at [redacted]	

I confirm that I have read the above protocol in the latest version. I understand it, and I will work according to the principles of Good Clinical Practice (GCP) as described in the United States Code of Federal Regulations (CFR) – 45 CFR part 46 and 21 CFR parts 50, 56, and 312, 812 and in the International Conference for Harmonisation (ICH) document entitled *Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice E6(R2)*. Further, I will conduct the study in keeping with local legal and regulatory requirements.
As the site Principal Investigator, I agree to carry out the study by the criteria written in the protocol and understand that no changes can be made to this protocol without the written permission of the IRB and NIAID.

*[*The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).]*

_____
Site Principal Investigator (Print)

_____ _____
Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	A randomized study to evaluate antibody response to an additional dose of SARS-CoV-2 vaccination with and without immunosuppression reduction in kidney and liver transplant recipients
Short Title	COVID Protection After Transplant-Immunosuppression Reduction (CPAT-ISR)
Clinical Phase	Phase 2
Number of Sites	15 U.S. Transplant Centers
IND Sponsor/Number	IND Exempt
Study Objectives	To elicit a more robust antibody response to vaccination against SARS-CoV-2 in kidney and liver transplant recipients who have had a suboptimal (≤ 2500 U/mL) response to a completed primary series of an mRNA COVID-19 vaccine.
Study Design	A randomized, open-label multi-site trial designed to induce an enhanced antibody response to SARS-CoV-2 in kidney and liver transplant recipients who have ≤ 2500 U/mL anti-spike antibody (as measured by the Roche Elecsys® anti-SARS-CoV-2 S assay) after a minimum of three mRNA COVID-19 vaccine doses. Participants will be randomized to two 2 groups: Group One will undergo a temporary, prescribed reduction in their maintenance immunosuppressive regimen and receive a dose of mRNA-based COVID-19 vaccine while Group Two will receive a dose of mRNA-based COVID-19 vaccine with no change in their maintenance immunosuppressive regimen.
Primary Endpoint(s)	The primary endpoint is the -fold increase in antibody titer (using the Roche Elecsys® anti-SARS-CoV-2 S assay) from before receiving the study dose of vaccine to 30 days after the study dose.
Safety, Secondary, and Exploratory Endpoint(s)	**Secondary Safety Endpoints:** Local and systemic vaccine reactogenicity and/or allergy Serious adverse events occurring during the 30 days following the additional dose of vaccine Treated acute cell-mediated and/or antibody-mediated allograft rejection (clinical or biopsy-proven) within 60 days following the additional dose of vaccine Development of de novo donor-specific anti-HLA antibody within 90 days of the vaccine Graft loss within 60 days of the vaccine Death within 60 days of the vaccine **Secondary Efficacy Endpoints:** Duration of anti-SARS-CoV-2 S antibody titers and neutralizing capacity in the year following the additional dose of vaccine

	SARS-CoV-2 PCR positivity Symptomatic COVID-19 COVID-19 requiring hospitalization Log SARS-CoV-2 antibody titer at 30 days post-vaccine Longitudinal changes in log SARS-CoV-2 antibody titer during the study **Exploratory Mechanistic Endpoints:** Anti-S1 and anti-RBD serology Detection of SARS-CoV-2 spike protein in blood after the additional dose of vaccine Additional serological panels and correlates of neutralizing antibody SARS-CoV-2 virology, diagnostics, and sequencing, including novel variants of concern Oral fluid anti-N and anti-S immunoglobulin levels (IgA (total and secretory), IgM, IgG) Antigen-specific B cell response Antigen-specific T cell response Evidence of immune activation, transcriptomics and cytokine signaling Durability of de novo donor-specific anti-HLA antibody
Accrual Objective	400 transplant kidney and liver transplant recipients with negative or low (≤ 2500 U/mL) antibody titers using the Roche Elecsys® anti-SARS-CoV-2 S assay after an authorized or licensed mRNA based anti-COVID-19 vaccination regimen.
Study Duration	Accrual: 4 months Screening, Vaccination and Follow-up: 13 months Total Duration: 17 months
Treatment Description	Participants will be randomized to an additional dose of COVID-19 vaccine only OR immunosuppression (IS) reduction plus an additional dose of COVID-19 vaccine. IS reduction will be based on the participant's IS regimen upon study entry, as described in Section 6.3.
Inclusion Criteria	Individuals must meet all the following criteria to be eligible: 1. Able to understand and provide informed consent. 2. Individual ≥ 18 years of age. 3. Recipient of a kidney or liver transplant ≥12 months prior to enrollment, without allograft rejection in the 6 months preceding enrollment. 4. Negative for anti-donor HLA antibodies at screening (central lab). 5. Currently taking one of the following tacrolimus-based immunosuppressive regimens: Tacrolimus plus MMF or MPA, with or without a corticosteroid Tacrolimus with trough level ≥ 5 ng/mL , with or without ≤ 5 mg of prednisone or equivalent 6. Received a minimum of 3 doses of either the Moderna COVID-19 vaccine or Pfizer-BioNTech COVID-19 vaccine.

	7. Participant must be ≥ 60 days after completion of primary vaccination or receipt of the most recent booster dose with any authorized or approved monovalent or bivalent COVID-19 vaccine at the time of study vaccine. 8. Serum antibody negative or low (titer ≤ 2500 U/mL) at ≥ 30 days from the last dose of mRNA COVID-19 vaccine and ≥ 30 days following receipt of a monoclonal antibody product or convalescent plasma for COVID-19, measured using the Roche Elecsys® anti-SARS-CoV-2 S assay. 9. Participant's transplant physician must confirm the participant's eligibility based on medical history and concur with the plan for immunosuppression modification.
Exclusion Criteria	Individuals who meet any of the following criteria will not be eligible: 1. Currently on an IS regimen different from the two regimens described in the Inclusion Criteria, for example (but not limited to) those including sirolimus, everolimus, belatacept, or azathioprine. 2. Recipient of any allograft other than a kidney or liver. 3. Participant is pregnant. 4. Any past history of Donor Specific Antibody (DSA) by local site standards. 5. Currently taking any systemic immunosuppressive agent, other than their prescribed transplant immunosuppression. 6. Known history of severe allergic reaction to any component of an authorized or licensed COVID-19 vaccine. 7. Thrombotic events, myocarditis, or pericarditis temporally associated with a prior dose of COVID-19 vaccine. 8. History of heparin-induced thrombocytopenia. 9. Any change in transplant immunosuppression regimen (drug or dose) in response to suspected or proven rejection within the last 6 months. 10. Significant graft dysfunction (*see study definitions*). 11. Receipt of any cellular depleting agent (e.g. ATG, rituximab, alemtuzumab, Cyclophosphamide) within 12 months preceding enrollment. 12. Concurrent autoimmune disease at risk for exacerbation with immunosuppression reduction. 13. Any untreated active infection including BK viremia $>10^4$ copies. 14. Infection with HIV. 15. Recent (within one year) or ongoing treatment for malignancy with the exception of (1) non-melanomatous skin cancer definitively treated by local therapy, and (2) definitively treated carcinoma-in-situ of the cervix (Stage 0 cervical cancer) 16. Receipt of a monoclonal antibody product or convalescent plasma within the last 30 days. 17. Any past or current medical problems, treatments, or findings which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the candidate's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study.

Pausing Rules	**Pausing rules will include:** If the following occur, relevant study interventions will be temporarily halted pending a DSMB review and recommendation as to whether the study may proceed. 1. Any Grade 4 or 5 event that is definitely or possibly attributed to the study dose of vaccine or to immunosuppression reduction. 2. Any probable or confirmed case of either acute myocarditis or acute pericarditis that occurs within 6 weeks of the study vaccine. 3. Excess incidence of acute rejection (clinically treated or biopsy-proven). 4. Excess incidence of graft loss. 5. Excess mortality. ***See Section 11.5 for further details and specific event rates triggering a pause in enrollment.***

Table of Contents

Glossary of Abbreviations

ATG	Anti-thymocyte globulin
CFR	Code of Federal Regulations
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
dnDSA	De novo Donor Specific Antibody
DSA	Donor Specific Antibody
EUA	Emergency Use Authorization
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IND	Investigational New Drug
IRB	Institutional Review Board
IS	Immunosuppression
MOP	Manual of Procedures
NIAID	National Institute of Allergy and Infectious Diseases
PI	Principal Investigator
PrEP	Pre-Exposure Prophylaxis
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SOP	Standard Operating Procedure
SOT	Solid Organ Transplant
SUSAR	Serious Unexpected Suspected Adverse Reaction
ULN	Upper Limit of Normal

Study Definitions Page

Acute Rejection	**Acute Cell-Mediated Rejection, Biopsy Proven**: Rejection (including borderline) graded according to 2019 Banff Criteria. **Antibody-Mediated Rejection**: A clinical and histologic event that meets the Banff 2019 criteria for antibody mediated rejection. **Clinically Diagnosed and Treated Rejection**: Any clinical event, with or without supporting evidence from a biopsy, which the treating physician diagnoses as "rejection" AND for which the patient is treated with steroids, lytic therapy, or an increase in dose or number of immunosuppressive medications.
Acute Myocarditis / Acute Pericarditis	Myocarditis is inflammation of the heart muscle. Pericarditis is inflammation of the lining outside the heart. In this study, the definition of suspected or confirmed acute myocarditis or acute pericarditis is the Centers for Disease Control and Prevention (CDC) case definition criteria: https://www.cdc.gov/mmwr/volumes/70/wr/mm7027e2.htm
Basic Metabolic Panel	Includes sodium, potassium, chloride, carbon dioxide (CO2) total, blood urea nitrogen, creatinine, glucose, calcium, anion gap and BUN/creatinine ratio.
Comprehensive Metabolic Panel	Includes sodium, potassium, chloride, carbon dioxide (CO2) total, blood urea nitrogen, creatinine, glucose, calcium, total protein, albumin, total bilirubin, alkaline phosphatase (Alk Phos), aspartate aminotransferase (AST), alanine aminotransferase (ALT), anion gap, BUN/creatinine ratio, and AST/ALT ratio.
Donor Specific Antibodies (DSA)	Antibodies directed at donor human leukocyte antigens (HLA) by central lab assessment.
Graft Failure	Kidney: Any of the following events: renal dialysis of more than 3 months duration; listing for re-transplantation; death from kidney failure. Liver: Re-listing for transplantation or death from liver failure.
IS Reduction Failure	Participant resumes baseline IS per the criteria outlined in Section 6.7 *Premature Discontinuation of IS Reduction* but does not receive definitive treatment for rejection e.g. thymoglobulin, steroids, or a sustained increase in dose or number of immunosuppressive medications.
Lost to Follow-up	Missing primary endpoint assessment and/or final study visit.
Medical Monitor	The physician who is responsible for Sponsor oversight of safety aspects of the trial. The medical monitor will determine the attribution of Serious Adverse Events after considering all investigator input.
Significant Graft Dysfunction for Study Eligibility	**Kidney**: eGFR <35mL/min/1.73m^2. **Liver:** INR >ULN (in patients not on anticoagulation), direct bilirubin >1.
Negative or indeterminate Antibody Titer	A measurement of <0.8 U/mL using the Roche Elecsys® anti-SARS-CoV-2 S assay.

NIAID Project Manager	NIAID assigned project manager who is responsible for all day to day administrative protocol-related issues, including version control, consent review, etc.
Principal Investigator	The physician responsible for supervising the conduct of the clinical investigation and for protecting the rights, safety, and welfare of participants consistent with 21 CFR Part 312.
Participant Premature Termination	Participants who are lost to follow up, withdraw consent, or die during the study. Data and specimens will no longer be expected from participants who are terminated from the study.
Program Officer	NIAID official who oversees the programmatic and budgetary aspects of the grant.
Protocol Mandated Procedures	A procedure or intervention that is a study requirement at the specified time point in the protocol.
Regulatory Affairs Officer	NIAID-assigned officer responsible for regulatory aspects of the study.
Site Principal Investigator	Lead investigator listed on the Investigator of Record Agreement at a particular clinical site who is responsible for the conduct of the study at that site.
Investigational Product	The investigational product is an additional dose of the Moderna or Pfizer-BioNTech COVID-19 vaccine
Immunosuppression Reduction	Protocol-mandated reduction in immunosuppression, determined by the participant's baseline regimen. See Section 6.3.

1. Background and Rationale

1.1 Background and Scientific Rationale

COVID-19 disproportionately affects vulnerable populations, including those with impaired immune defenses. Organ transplant recipients are particularly at risk as a result of both the need for life-long immunosuppressive therapy to prevent rejection and a high prevalence of other risk factors for severe COVID-19 including cardiovascular disease, hypertension, and diabetes. The consequences of COVID-19 are severe in organ transplant recipients; in one study of a large (n=482) cohort of patients who had a confirmed diagnosis of COVID-19 by polymerase chain reaction, 78% were hospitalized, and of those, 39% required ICU care and 31% required mechanical ventilation. Mortality at 28 days from diagnosis was 17.8% overall and 20.5% among those who were hospitalized. Risk factors for mortality included age >65 years, congestive heart failure, chronic lung disease and obesity.[1-5]

SARS-CoV-2 vaccines are highly immunogenic and effective in the general population. Transplant recipients were excluded from landmark trials, but it has become evident during the post-Emergency Use Authorization (EUA) experience that the response to vaccination is substantially lower among organ transplant recipients as compared to the general population: in a survey of 436 transplant recipients who had received two doses of either the Moderna COVID-19 vaccine or the Pfizer-BioNTech COVID-19 Vaccine, a positive anti-SARS-CoV-2 S response was detectable in only 17% after the first dose and 30-60% after two doses in multiple studies. Associations with suboptimal vaccine response include treatment with antimetabolite therapies such as mycophenolate or azathioprine, which are known to impair lymphocyte function and response to new antigens (such as influenza vaccination).[10-14]

It is critical to understand risk stratification for vaccine failure given mounting reports of serious breakthrough infections in fully vaccinated SOT recipients.[35] Persistent knowledge gaps include characterization of humoral vaccine responses (e.g., level and durability of neutralizing antibody, including versus novel variants), B cell memory responses, and antigen-specific T cell responses in transplant recipients.[15,16] Strategies to improve vaccine response to viral antigens in immunocompromised persons are largely based on experiences with influenza and hepatitis B vaccines; these include administration of additional vaccinations, higher antigen dosing, and mixing of vaccine platforms such as those containing immunostimulatory adjuvants.[17,18] Development of a strategy for eliciting an antibody response to COVID-19 vaccines in transplant recipients is complex as the pharmacologic immunosuppression that prevents response to the vaccine also prevents rejection of a life-sustaining allograft. Potential interventions include administration of an additional dose of vaccine; alternate vaccine strategies including higher doses of vaccine or the use of adjuvanted vaccines; and, most high risk, transient modification of a patient's immunosuppressive regimen before and after vaccine administration. A decision algorithm for an individual patient must consider the likelihood of success of a low risk intervention (i.e. a third dose of vaccine), the type of transplant (e.g. kidney, heart or lung), the likelihood and consequences of allograft rejection if the patient's immunosuppressive regimen is modified (e.g. the low likelihood of permanent injury after an episode of liver rejection versus the high likelihood of permanent injury after an episode of lung rejection), and the individual's risk for severe disease or death from COVID-19 if they remain without antibody protection.

This study will investigate the effect of an additional dose of either the Moderna or Pfizer-BioNTech COVID-19 vaccine, with or without a reduction in immunosuppression, in participants who demonstrate a negative or low (≤ 2500 U/mL) anti-RBD titer after a completed mRNA COVID-19 vaccine series. Response will be assessed using the Roche Elecsys® anti-SARS-CoV-2 S assay which has an approximate 1:1 conversion to World Health Organization binding antibody units (BAU) Anti-S1/RBD levels are well correlated with neutralizing antibody against SARS-CoV-2 and with vaccine sero-protection in the general population.[37,38] The precise cutoff that connotes protection from infection is not established for immunocompromised persons. Early studies, however, indicated anti-S1/RBD levels of 15-144 BAU[40] are correlated with live virus neutralization of ancestral variants in convalescent persons. Subsequent analyses of vaccine efficacy across trials indicated a level 2-4-fold higher for vaccine-evoked neutralization (compared to convalescents) best correlates with vaccine efficacy.[41] Consistent with this, several studies in transplant recipients have suggested anti-S1/RBD levels of 100-250 BAU after vaccination as associated with in vitro neutralization; below this level, neutralizing capacity against SARS-CoV-2 variants is not reliable and suggests higher risk of infection. Notably, variants of concern, particularly the Omicron variant and its sublineages demonstrate significant immune evasion and need for 10-20-fold higher levels of antibody to neutralize than required for ancestral variants.[52-55] Version 6.0 of the protocol increases the SARS-CoV-2 S titer for eligibility from 250 U/mL to 2500 U/mL to account for the findings that suggest the antibody levels needed to neutralize Omicron are significantly higher.[58]

Rationale for Selection of Investigational Product or Intervention

This study will utilize the mRNA-based Moderna and Pfizer-BioNTech COVID-19 Vaccines and will enroll kidney or liver recipients who demonstrate a persistently low anti-SARS-CoV-2 S titer after completing at least three doses of an mRNA based primary vaccine series for immunocompromised patients. Participants will receive a dose of COVID-19 vaccine, with or without concurrent reduction of their transplant immunosuppression regimen. These vaccines have been shown to be safe and highly immunogenic in the general population, with publication of large Phase III trials data supporting FDA EUA and/or licensure.[19,20] The additional dose of vaccine was chosen due to demonstrated safety of the authorized or licensed COVID-19 vaccines and experience with the efficacy of additional dosing strategies of other vaccines (e.g., hepatitis and influenza vaccines)[17,18] in immunocompromised people.[21-23]

1.2 Preclinical Experience

Studies performed in murine models in the 1970s and 1980s showed that the repeated immunization of up to 4 doses continues to have a positive impact on the titer, isotype, and affinity of the induced antibody responses.[24,25] Similarly, preclinical murine data indicate that immunization schedules that include third doses markedly increase the frequency and functional properties of antigen specific T cell immune responses.[26]

There are no specific preclinical studies addressing whether and how these effects of immunization schedule on vaccine-induced immunity are affected by the immunosuppressant medications used in transplantation.

1.3 Clinical Studies

The Moderna and Pfizer-BioNTech COVID-19 Vaccines are known to have excellent immunogenicity in the general adult population, even against some of the recently identified SARS-CoV-2 variants.[27,28] These

vaccines are well-tolerated, with mostly mild-to-moderate local and systemic reactogenicity that is more pronounced after the second dose.[29,30] However, organ transplant recipients have recently been shown to have much less reliable and robust responses to these vaccines.[10,12] In a large (N=658) prospective cohort study of U.S. transplant recipients, only 15% of patients had a positive anti-SARS-CoV-2 S response after a first dose of either the Moderna or the Pfizer vaccine, with improvement to only 54% by four weeks after the second dose of vaccine. Patients who had a positive response after the first dose of vaccine had higher titers of anti-SARS-CoV-2 S after dose two as compared with those who had a negative or indeterminate response after dose one but did have a measurable response after dose two. In those with a positive anti-SARS-CoV-2 S response after dose two, antibody levels were substantially lower than in immunocompetent vaccine recipients. Associations with lack of vaccine response included older age, use of antimetabolite therapies, history of kidney transplantation (versus other organ transplant), more recent transplantation, and receipt of the Pfizer-BioNTech vaccine (versus the Moderna mRNA-1273 vaccine). In a study of 101 kidney transplant recipients maintained on belatacept-based immunosuppression, only 6% of patients had measurable anti-SARS-CoV-2 S after 2 doses of the Pfizer-BioNTech vaccine. In that study, SARS-CoV-2-specific T-cell responses by EliSpot were present in 30% of those tested (N=23) after two doses of vaccine.[15]

Several observational series and one randomized trial have evaluated antibody response to a third dose of SARS-CoV-2 vaccination in solid organ transplant (SOT) recipients, predominately following a third mRNA vaccination, using a variety of anti-spike antibody assays.[42-46]

In observational series focusing on those with negative antibody levels after a two-dose mRNA series, seroconversion rates were 27-44%, generally to lower titers. Additionally, preliminary data from Johns Hopkins indicate that fully one third of SOT recipients who receive a third vaccine dose continue to demonstrate zero neutralizing antibody versus the delta variant.[47] In contrast, among those with low, but detectable antibody after a two-dose series, seroconversion rates above threshold were 80-100% and to a level approximately 5-fold higher than that observed in seronegative participants.[42-45] Notably, one cohort series of patients on maintenance belatacept therapy showed poor seroconversion rates after a third mRNA dose (increasing from 6% to 37%), noting a 5% clinical COVID-19 breakthrough rate with 50% mortality.

In the randomized trial of a third dose of Moderna vaccine given to 60 SOT recipients (20 kidney recipients), irrespective of anti-RBD antibody level, median fold-change in anti-RBD antibody level of approximately 40x in the vaccination group, and 75x higher than seen in the placebo group (median post intervention level 314 vs 1.2 u/mL). Additionally, surrogate plasma neutralization capacity versus “wildtype”/vaccine strain SARS-CoV-2 increased from 37% at baseline to 60% after a third dose. SARS-CoV-2-specific, polyfunctional CD4 and CD8 T cell responses were assessed via intracellular cytokine staining (IFN-γ and IL-2) in a subset of patients demonstrating higher median counts (432 vs 67 cells/10^6 CD4+ T cells).[45] In this trial, however, <20% of participants had any neutralizing capacity versus the Omicron variant following a third dose. This emphasizes the need to increase peak binding antibody responses in vulnerable transplant recipients, including those with low-level, positive antibody after standard vaccination series, in an effort to reduce the risk period for infection during expected antibody wane.[56,57]

Reassuringly, in these collective series with limited follow up, reactogenicity was similar after a third dose as that described in two-dose mRNA series, with no serious or medically-attended adverse events reported.

One case of mild rejection was noted in a heart transplant recipient after a third dose of Pfizer-BioNTech vaccine (negative DSA), uncertain if related to vaccination.[42-46]

Given a significant subset of SOT recipients, particularly kidney recipients on multiple immunosuppressive agents, maintain negative or low sero-response after three vaccine doses, alternative strategies to augment immune response are needed. In particular, temporary immunosuppressive reduction peri-vaccination, particularly of anti-metabolite medications, is attractive potential intervention for those with low alloimmune risk. In persons with autoimmune disease, temporary hold of immunosuppressives does increase vaccine responses;[48,49] this approach is currently recommended by the American College of Rheumatology surrounding COVID-19 vaccination in select persons.[50] It is not known, however, the degree to which short-term immunosuppressive reduction will improve sero-response in transplant recipients and whether it is associated with events such as rise in donor-specific antibody or rejection. In light of the above data, withholding mycophenolate mofetil around the time of Pfizer-BioNTech COVID-19 vaccination is actively being pursued in the randomized trial setting by international investigators.[51]

2. Study Hypotheses/Objectives

2.1 Hypotheses

Kidney and liver transplant recipients who had a negative or low (≤ 2500 U/mL) anti-SARS-CoV-2 S response (using the Roche Elecsys® assay) to a completed primary series of the Moderna COVID-19 Vaccine or the Pfizer-BioNTech COVID-19 Vaccine will develop an enhanced SARS-CoV-2 antibody response after receiving a dose of study vaccine, with or without reduction of their maintenance transplant immunosuppression regimen.

Temporary reduction of maintenance immunosuppression as directed in this protocol will result in higher fold increase in antibody titer than achieved in those who do not have IS reduction, without an increased risk of alloimmune events (rejection or development of dnDSA).

2.2 Primary Objective(s)

The primary objective of this study is to elicit a more robust anti-SARS-CoV-2 S level in kidney and liver transplant recipients who had an undetectable or low (≤ 2500 U/mL) level of antibody after completing a primary series of mRNA COVID-19 vaccine. Antibody titers in response to the study dose of vaccine will be evaluated 30 days following vaccine administration and will be measured using the Roche Elecsys® anti-SARS-CoV-2 S. All participants with measurable SARS-CoV-2 antibody titers will also have neutralizing antibody titers and further quantification and characterization of antibody using the best available assay(s).

2.3 Secondary Safety Objective(s)

To determine the incidence of adverse events (AEs) and serious adverse events (SAEs), including reactogenicity, following the additional dose of vaccine.

To determine the incidence of alloimmune events (e.g., allograft rejection and development of de novo DSA) following the immunosuppression reduction and/or the additional dose of vaccine.

2.4 Secondary Efficacy Objectives

To determine the incidence of symptomatic or asymptomatic SARS-CoV-2 infection in the 12 months following the additional dose of vaccine.

2.5 Secondary Immunogenicity Objectives

To assess whether responses to additional doses of COVID-19 vaccine are improved by temporary reduction or withholding of immunosuppressive medications around the time of vaccine.

To assess whether response to an additional dose of COVID-19 vaccine is associated with clinical phenotype (e.g., age, organ type, time post-transplant, immunosuppressive regimen).

To evaluate durability of response over 1-year in those who have an antibody response to the additional dose of vaccine.

2.6 Exploratory Objectives

To identify immunologic correlates of vaccine response.

To evaluate immune activation in response to the vaccine.

To assess whether timing between vaccine doses is associated with differential antibody response to vaccination.

To assess whether there is a difference in antibody levels among those receiving additional doses of Pfizer versus Moderna vaccination.

3. Study Design

3.1 Description of Study Design

A randomized, open-label multi-site trial designed to induce an enhanced antibody response to SARS-CoV-2 in kidney and liver transplant recipients who have a negative or low (≤ 2500 U/mL) antibody response (using the Roche Elecsys® anti-SARS-CoV-2 S) following a completed primary series of mRNA COVID-19 vaccine. Participants will be randomized to receive a dose of COVID-19 vaccine (Moderna or Pfizer-BioNTech), with or without immunosuppression (IS) reduction.

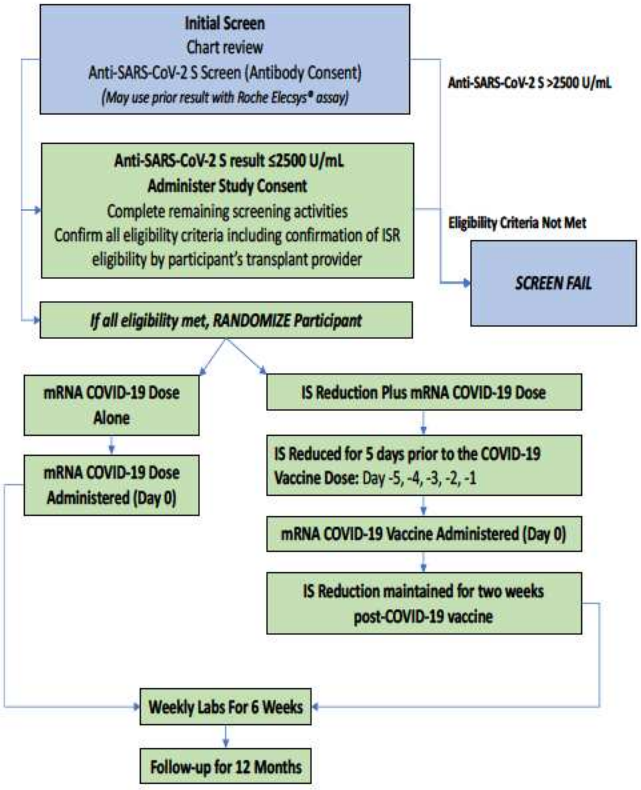


Figure 1. Study Design Diagram

3.2 Primary Endpoint

The primary endpoint is the -fold increase in antibody titer (using the Roche Elecsys® anti-SARS-CoV-2 S assay) from before receiving the study dose of vaccine to 30 days after the study dose of vaccine.

3.3 Secondary Endpoints

3.3.1 Secondary Safety Endpoints

Local and systemic vaccine reactogenicity and/or allergy

Serious adverse events occurring during the 30 days following the additional dose of vaccine

Treated acute cell-mediated and/or antibody-mediated allograft rejection (clinical or biopsy-proven) within 60 days following the additional dose of vaccine

Development of de novo donor-specific anti-HLA antibody within 90 days of the vaccine

Graft loss within 60 days of the vaccine

Death within 60 days of the vaccine

3.3.2 Secondary Efficacy Endpoints

Duration of anti-SARS-CoV-2 S antibody titers and antibody neutralization in the year following the additional dose of vaccine

SARS-CoV-2 PCR positivity

Symptomatic COVID-19

COVID-19 requiring hospitalization

Longitudinal changes in log SARS-CoV-2 antibody titer during the study

3.3.3 Exploratory Endpoints

Anti-S1 and anti-RBD serology

Detection of SARS-CoV-2 spike protein in blood after the additional dose of vaccine

Additional serological panels and correlates of neutralizing antibody

SARS-CoV-2 virology, diagnostics, and sequencing, including novel variants of concern

Oral fluid anti-N and anti-S immunoglobulin levels (IgA, IgM, IgG)

Antigen-specific B cell response

Antigen-specific T cell response

Evidence of immune activation, transcriptomics and cytokine signaling

Durability of de novo donor-specific anti-HLA antibody

3.4 Stratification, Randomization, and Blinding/Masking

This is an open-label study.

4. Selection of Participants and Clinical Sites/Laboratories

4.1 Rationale for Study Population

This study will enroll individuals who have completed primary series of mRNA COVID-19 vaccine and have a negative or low (≤ 2500 U/mL) antibody response measured at least 30 days after the last dose of vaccine. This group of patients is at high risk for severe COVID-19 disease due to pharmacologic immunosuppression and a high prevalence of non-transplant risk factors such as obesity and diabetes.

4.2 Inclusion Criteria

Individuals who meet all the following criteria are eligible for enrollment as study participants:

1. Able to understand and provide informed consent.
2. Individual ≥ 18 years of age.
3. Recipient of a kidney or liver transplant ≥ 12 months prior to enrollment, without allograft rejection in the 6 months preceding enrollment.
4. Negative for anti-donor HLA antibodies at screening (central lab).
5. Currently taking one of the following CNI-based immunosuppressive regimens:
   Tacrolimus plus MMF or MPA, with or without a corticosteroid
   Tacrolimus with trough ≥ 5ng/mL) with or without ≤ 5 mg of prednisone or equivalent
6. Received a minimum of 3 doses of either the Moderna COVID-19 Vaccine or Pfizer-BioNTech COVID-19 Vaccine.
7. Participant must be ≥ 60 days after completion of primary vaccination or receipt of the most recent booster dose with any authorized or approved monovalent or bivalent COVID-19 vaccine at the time of study vaccine.
8. Serum antibody negative or low (titer ≤ 2500 U/mL) at ≥ 30 days from the last dose of mRNA COVID-19 vaccine and ≥30 days following receipt of a monoclonal antibody product or convalescent plasma for COVID-19, measured using the Roche Elecsys® anti-SARS-CoV-2 S assay.
9. Participant's transplant physician or midlevel practitioner who is clinically licensed to prescribe and manage immunosuppression must confirm the participant's eligibility based on medical history.

4.3 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

1. Currently on an IS regimen different from the two regimens described in the Inclusion Criteria, for example (but not limited to) those including sirolimus, everolimus, belatacept, or azathioprine.
2. Recipient of any allograft other than a kidney or liver.
3. Participant is pregnant.
4. Any past history of Donor Specific Antibody (DSA) using local site standards.
5. Currently taking any systemic immunosuppressive agent, other than their prescribed transplant immunosuppression.
6. Known history of severe allergic reaction to any component of an authorized or licensed COVID-19 vaccine.
7. Thrombotic events, myocarditis, or pericarditis temporally associated with a prior dose of COVID-19 vaccine.
8. History of heparin-induced thrombocytopenia.

9. Any change in transplant immunosuppression regimen (drug or dose) in response to suspected or proven rejection within the last 6 months.
10. Significant graft dysfunction (*see study definitions*).
11. Receipt of any cellular depleting agent (e.g. ATG, rituximab, alemtuzumab, Cyclophosphamide) within 12 months preceding enrollment.
12. Concurrent autoimmune disease at risk for exacerbation with immunosuppression reduction.
13. Any untreated active infection including BK viremia $>10^4$ copies.
14. Infection with HIV.
15. Recent (within one year) or ongoing treatment for malignancy with the exception of (1) non-melanomatous skin cancer definitively treated by local therapy, and (2) definitively treated carcinoma-in-situ of the cervix (Stage 0 cervical cancer)
16. Receipt of a monoclonal antibody product or convalescent plasma within the last 30 days.
17. Any past or current medical problems, treatments, or findings which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the candidate's ability to comply with study requirements or may impact the quality or interpretation of the data obtained from the study.

4.4 Selection of Clinical Sites

This study will be conducted at approximately 15 clinical sites across the United States that have transplant programs with the population described in the protocol and which are able to comply with the requirements of the protocol. Sites may be added or withdrawn to meet the accrual goals outlined in the protocol.

5. Known and Potential Risks and Benefits to Participants

The risks cited below reflect the information currently included in the Moderna and Pfizer-BioNTech Package Inserts and Emergency Use Authorizations. The safety data accrued with the bivalent vaccines (Original and Omicron BA.1) and with the original COVID-19 vaccines are relevant to the updated 2023-2024 formulations because these vaccines are manufactured using the same process.

5.1 Risks of the Investigational Products as cited in the Full FDA EUA Prescribing Information

5.1.1 Risks of Moderna COVID-19 Vaccine (includes original, bivalent, and 2023-2024 formula)

The most common side effects reported include injection site reactions (pain, redness and swelling), axillary swelling/tenderness, fatigue, headache, myalgia, arthralgia, chills, and nausea/vomiting. Severe allergic reactions, including anaphylaxis, have been reported following the administration during mass vaccination outside of clinical trials. Severe allergic reactions included difficulty breathing, swelling of the face and throat, rash, dizziness, and weakness. Other risks include increased risks of myocarditis (inflammation of the heart muscle) and pericarditis (inflammation of the tissue surrounding the heart) following vaccination.

Additional adverse reactions, some of which may be serious, may become apparent with post-authorization use of the vaccine.

5.1.2 Risks of Pfizer-BioNTech COVID-19 Vaccine (includes original, bivalent, and 2023-2024 formula)

The most common side effects reported include non-severe allergic reactions (rash, itching, hives and facial swelling), injection site reactions (pain, redness and swelling), fatigue, headache, myalgia, arthralgia, chills, fever, lymphadenopathy, vomiting, diarrhea, arm pain and general malaise. Severe allergic reactions that include difficulty breathing, swelling of the face and throat, rash and dizziness or weakness have been reported post authorization but are less common. Other risks include increased risks of myocarditis (inflammation of the heart muscle) and pericarditis (inflammation of the tissue surrounding the heart) following vaccination.

Additional adverse reactions, some of which may be serious, may become apparent with post-authorization use of the vaccine.

5.2 Risks of Moderna COVID-19 Vaccine and Pfizer-BioNTech Vaccine in adults as cited in Medical Literature (includes original, bivalent, and 2023-2024 formulas)

The Moderna COVID-19 Vaccine and Pfizer-BioNTech COVID-19 Vaccine are being administered under an Emergency Use Authorization. Adverse Events that occur outside of clinical trials as a result of the mass vaccination effort are reported to each manufacturer and included in a Fact Sheet for Healthcare Providers.[33,34]

In a recent cohort of 741 organ transplant recipients who received two doses of either the Moderna or Pfizer-BioNTech vaccine, reactogenicity was similar to what has been reported in immunocompetent people: pain at injection site, fatigue, and headache were common. Severe reactogenicity (defined as preventing daily activity) occurred in 1-2% of study participants. Systemic reactogenicity was more common after the second dose of vaccine. There was an increased risk of local reactogenicity among recipients who

were maintained on an immunosuppressive regimen that included corticosteroids or mTOR inhibitors, and also among those who received the Moderna vaccine. There were no cases of anaphylaxis. There was one case of acute allograft rejection after the second dose of vaccine.[36]

Infrequent cases of myocarditis or pericarditis have been reported after the use of COVID-19 vaccines containing the SARS-CoV-2 S-antigen. Myocarditis and pericarditis have been reported in greatest numbers in males under the age of 40 years following a second dose of mRNA vaccines, but cases have been reported in older males and in females as well, and also following other doses, and other vaccine platforms, including after doses of an adjuvanted protein vaccine. The observed risk is highest in males 12 to 17 years of age. While some cases required intensive care support, available data from short-term follow-up suggest that symptoms resolve in most individuals with conservative management. Information is not yet available about potential long-term sequelae. The risk in children younger than 12 years old is currently being assessed, and both the size of the database and length of follow-up in this population are relatively smaller than that of those older than 12 years old. Therefore, the characterization of the risk in children younger than 12 years old is not as well-known as in adolescents and adults.

5.3 Risks Associated with IS Reduction

Any decrease in dose or frequency of immunosuppressive medications in a transplant recipient carries the risk of alloimmune activation leading to acute cellular rejection, subclinical rejection, acute or chronic antibody-mediated rejection, and/or the formation of de novo DSA. The inclusion criteria and the specific immunomodulatory strategies have been designed to minimize, but do not eliminate, this risk. Study participants undergoing IS reduction will be closely monitored for evidence of graft injury and will be returned to their baseline immunosuppressive regimen should graft injury be evident. In addition, subjects will be monitored for the development of de novo DSA throughout the study period.

5.4 Potential Risks to Study Population

There may be an increased risk of low-to-moderate reactogenicity to trial participants who receive an additional dose of vaccine. Based on currently available information, the risk of serious allergic events is likely to be extremely low, equal to or less than has been seen in the immunocompetent population. There is no evidence that the COVID-19 vaccine will result in new alloimmune events, and none are expected.

5.5 Risks of Other Protocol Specified Medications

Not Applicable

5.6 Risks of Study Procedures

5.6.1 Risk of Blood Draw

Collection of blood may cause slight discomfort, pain, bleeding or bruising at the injection site. Rarely, fainting or infection may occur.

5.6.2 Risks Associated with Nasal Mid-Turbinate Swab Collection

Nasal mid-turbinate swab collection may cause localized discomfort. Rarely, mild epistaxis may occur.

5.6.3 Risks Associated with Oral Fluid Collection

Oral fluid collection may result in mild irritation where the gums are swabbed.

5.6.4 Risk of Internet Based Data Collection

Data from this study will be entered into a computerized database through a secured web site. All information will be saved and transmitted in a coded form. Only authorized personnel requiring a password will be permitted to enter data. There is risk, although minimal, of unauthorized persons obtaining confidential information.

5.7 Potential Benefits

There might be no benefit to participants in this study. If a participant achieves an increased level of antibody against SARS-CoV-2 as a result of participating in this study, there might be associated benefit. However, whether any participant will achieve this is unknown.

6. Investigational Agents

The study products used in this protocol are the: Moderna COVID-19 Vaccine and the Pfizer-BioNTech COVID-19 Vaccine. The doses provided for the study will be according to the current CDC and FDA Emergency Use Authorizations. The current available CDC recommended vaccines are provided to the clinical sites.

6.1 Investigational Agent #1: Moderna COVID-19 Monovalent Vaccine (Protocol v1.0-5.0)

The Moderna COVID-19 monovalent vaccine will no longer be offered for the study.

6.2 Investigational Agent #2: Pfizer-BioNTech COVID-19 Monovalent Vaccine (Protocol v1.0-5.0)

The Pfizer-BioNTech COVID-19 monovalent vaccine will no longer be offered for the study.

6.3 Investigational Product #3: Moderna Bivalent COVID-19 Vaccine (Protocol v6.0-7.0)

Commented [MOU1]: I can either add sections addressing the adapted monovalent vaccines or we modify this section to be generic and refer to the most current vaccine?

The Moderna Bivalent COVID-19 Vaccine (Original and Omicron BA.4/BA.5), hereafter referred to as Moderna Bivalent COVID-19 Vaccine will no longer be offered for this study

6.4 Investigational Product #4: Pfizer-BioNTech Bivalent COVID-19 Vaccine (Protocol v6.0-7.0)

Pfizer-BioNTech Bivalent COVID-19 Vaccine (Original and Omicron BA.4/BA.5, hereafter referred to as Pfizer-BioNTech Bivalent COVID-19 Vaccine will no longer be offered for this study.

6.5 Investigational Product #5: Moderna COVID-19 Vaccine, Spikevax® 2023-2024 Formula (Protocol v8.0 and beyond)

The updated Moderna COVID-19 Vaccine, Spikevax® 2023-2024 Formula, hereafter referred to as Moderna COVID-19 Vaccine 2023-2024 is being used in the study to provide an FDA approved dose of vaccine to eligible participants.

6.5.1 Formulation, Packaging, and Labelling

Moderna COVID-19 Vaccine 2023-2024 is supplied as a frozen suspension in multiple-dose vials, single dose vials, and single dose pre-filled syringes that do not contain a preservative and must be thawed prior to administration. Clinical sites will be provided with the formulation available to the study.

Each 0.5 mL dose of the Moderna COVID 19 Vaccine 2023-2024 contains 50 mcg of nucleoside-modified messenger RNA (mRNA) encoding the pre-fusion stabilized S-glycoprotein of SARS-CoV-2 Omicron variant lineage XBB.1.5.

Each 0.5 mL dose also contains the following ingredients: a total lipid content of 1.01 mg (SM-102, polyethylene glycol [PEG] 2000 dimyristoyl glycerol [DMG], cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC]), 0.25 mg tromethamine, 1.2 mg tromethamine hydrochloride, 0.021 mg acetic acid, 0.10 mg sodium acetate trihydrate, and 43.5 mg sucrose.

6.5.2 Dosage, Preparation, and Administration

The Moderna COVID-19 Vaccine 2023-2024 dose used in the study will be a single dose of 50 mcg (0.5 mL) administered in the deltoid muscle.

Vaccine preparation and administration details will follow the package insert and be recorded on the appropriate eCRF.

6.6 Investigational Product #4: Pfizer-BioNTech COVID-19 Vaccine, Comirnaty® 2023-2024 Formula (Protocol v8.0 and beyond)

The updated Pfizer-BioNTech COVID-19 Vaccine, Comirnaty® 2023-2024 Formula, hereafter referred to as Pfizer-BioNTech COVID-19 Vaccine 2023-2024 is being used in the study to provide an FDA approved dose to eligible participants.

6.6.1 Formulation, Packaging, and Labelling

Pfizer-BioNTech COVID-19 Vaccine 2023-2024 is supplied in single dose vials and single dose prefilled syringes. It is supplied as a frozen suspension that does not contain preservative. Clinical sites will be provided with the formulation available to the study.

Each 0.3 mL dose of Pfizer-BioNTech COVID-19 Vaccine 2023-2024 is formulated to contain 30 mcg of a nucleoside modified messenger RNA (modRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2 Omicron variant lineage XBB.1.5 (Omicron XBB.1.5).

Each 0.3 mL dose of Pfizer-BioNTech COVID-19 Vaccine 2023-2024 also includes the following ingredients: lipids (0.43 mg ((4- hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 0.05 mg 2-(polyethylene glycol 2000)-N,N-ditetradecylacetamide, 0.09 mg 1,2-distearoyl-sn-glycero-3-phosphocholine, and 0.19 mg cholesterol), 0.06 mg tromethamine, 0.4 mg tromethamine hydrochloride, and 31 mg sucrose.

6.6.2 Dosage, Preparation, and Administration

The Pfizer-BioNTech COVID-19 Vaccine 2023-2024 dose used in this study will be a single dose of 30 mcg (0.3 mL) administered intramuscularly in the deltoid muscle.

Vaccine preparation and administration details will follow the package insert and be recorded on the appropriate eCRF.

6.7 Immunosuppression Reduction

6.7.1 Immunosuppression (IS) Reduction

Participants on a tacrolimus-based regimen that includes MMF (or equivalent), with or without corticosteroid:

MMF (or equivalent) will be withheld for five days prior to and 2 weeks following vaccine administration. During the interval that MMF is withheld, no adjustments will be made to the CNI or corticosteroid dose.

Participants on a tacrolimus based regimen with a trough level ≥ 5 ng/mL, with or without ≤5mg Prednisone (or equivalent) and no MMF or other antimetabolite

Reduce CNI by 25% of starting dose for 5 days prior to and 2 weeks following vaccine administration.

6.8 Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62) the investigator will maintain adequate records of the disposition of the investigational agent, including the date and quantity of the drug received, to whom the drug was dispensed (participant-by-participant accounting), and a detailed accounting of any drug accidentally or deliberately destroyed.

Records for receipt, storage, use, and disposition will be maintained by the study site. A drug-dispensing log will be kept current for each participant. This log will contain the identification of each participant and the date and quantity of drug dispensed.

All records regarding the disposition of the investigational product will be available for inspection.

6.9 Assessment of Participant Compliance with Investigational Agent

Not applicable due to the fact that the assigned COVID-19 vaccine will be a single dose administered by qualified personnel at the study site.

6.10 Toxicity Prevention and Management

The vaccine will be administered by qualified personnel in dedicated observation rooms. Participants will be monitored for allergic reactions or other intolerance for 30 minutes following vaccine administration. Appropriate medications to address allergic reactions will be supplied by the investigational pharmacy (i.e., epinephrine, anti-histaminergic medications) and there is access to local rapid response team support in case of serious adverse events.

6.11 Premature Discontinuation of Investigational Agent

Not applicable due to the fact that the additional dose of COVID-19 vaccine that will be administered under this protocol is a single dose.

6.12 Premature Discontinuation of IS Reduction

Monitoring for evidence of rejection during IS reduction is described in Section 8.3.3. A persistent increase in creatinine (for kidney recipients) or of ALT (for liver recipients) will be cause for return to baseline IS, as follows:

For kidney transplant recipients: A finding of a 0.3mg/dL or 25% elevation (whichever is higher) of serum creatinine over baseline (pre-reduction in IS) will be repeated in 1-3 days. If the elevation persists or increases, the subject will return to his/her baseline immunosuppression regimen. Further management will be determined by the treating transplant physician. If the serum creatinine returns to baseline without further treatment, the event will not be recorded as rejection. If the participant requires more than baseline levels of IS or receives specific treatment for rejection, the event will be recorded as rejection.

For liver transplant recipients: A finding of an elevation of an ALT >2X baseline (pre-reduction in IS) or >60 IU/L if the baseline ALT is <30 IU/L will be repeated in 1-3 days. If the elevation persists or increases, the subject will return to his/her baseline immunosuppression regimen. Further management will be determined by the treating transplant physician. If the ALT returns to baseline without further treatment,

the event will not be recorded as rejection. If the participant requires more than baseline levels of IS or receives specific treatment for rejection, the event will be recorded as rejection.

7. Other Medications

7.1 Concomitant Medications

7.1.1 Protocol-mandated
Not applicable

7.1.2 Other permitted concomitant medications
Not applicable

7.2 Prophylactic Medications
Infectious disease prophylaxis will be determined by the treating transplant physician.

7.3 Pre-Exposure Prophylaxis or Treatment of COVID-19 Infection
Per the Emergency Use Authorization, the participants in this study should not receive any pre-exposure prophylaxis (PrEP) e.g. tixagevimab plus cilgavimab (Evusheld™, AztraZeneca) during the 2-week period following any dose of COVID-19 vaccine. From a study perspective, it would be best to delay administration of any PrEP until after the Day 30 post-vaccine visit, when the primary endpoint is collected. If the participant or his/her treating physician feels it is in the patient's best interest to administer PrEP before Day 30 following the study dose of vaccine, we ask that the study team be informed since this will impact subsequent study evaluations, in particular antibody testing.

If a participant has a known exposure to COVID-19 and/or develops COVID-19 infection, treatment should be prescribed as clinically indicated by the participant's primary provider.

Administration of PrEP or any treatment of COVID-19 will be captured in the clinical database.

7.4 COVID-19 Vaccine Booster Doses
Participants should consult with the study team regarding non-study COVID-19 booster doses in order to maintain compliance with current CDC recommendations, while not compromising participation in the research study. Current CDC recommendations can be found at the following link:

https://www.cdc.gov/vaccines/covid-19/clinical-considerations/interim-considerations-us.html#immunocompromised

Current recommendations may change over the course of the CPAT-ISR study.

7.5 Rescue Medications
Not applicable

8. Study Procedures

8.1 Enrollment

Potential study participants may be identified by medical chart review and initially contacted by a member of the study team in person or over the phone. During this initial contact, the potential participant will be provided information on the study and asked about their interest in study participation. If they elect to continue, they will be consented to the SARS-CoV-2 S antibody testing using the Roche Elecsys® assay.

Potential participants with eligible SARS-CoV-2 antibody results will receive a concise and focused presentation of key information about the clinical trial, verbally and with a written consent form. The research study will be explained in lay terms to each potential research participant. The potential participant will sign an informed consent form before undergoing any study procedures.

8.2 Screening Period

The purpose of screening is to confirm eligibility prior to the study intervention.

Initial Screen

Chart review for eligibility
Blood drawn locally for anti-SARS-CoV-2 S (using Roche Elecsys® assay). A prior result may be used for eligibility if it was obtained at least 30 days following the last dose of mRNA COVID-19 vaccine administration and ≥ 30 days following receipt of a monoclonal antibody product or convalescent plasma for COVID-19.

Screening Procedures (Appendix 1)

The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility:

Review medical history and immunosuppression medications.
Blood drawn for detection of de novo DSA (central lab).
Research blood (central lab)
Serum creatinine, eGFR (for kidney recipients)
Hepatic panel, direct bilirubin, and INR (for liver recipients)
Confirm with the patient's transplant physician that they are eligible for IS reduction based on medical history, and that their physician concurs with the plan for IS reduction.

8.3 Study Visits or Study Assessments

8.3.1 Day -5 (IS Reduction Arm)

Participants randomized to the IS reduction arm will have a Day -5 visit. A study physician will discuss what IS medication will be withheld or reduced based on the participant's IS regimen at study entry (see Section 6.3 for further details).

8.3.2 Vaccination Visit: Day 0 (All Participants)

All participants will be scheduled for a vaccination visit.

Participants will have baseline research blood, oral fluid, and nasal samples collected on Day 0 per the Schedule of Events (Appendix 2 (COVID-19 Vaccine Dose Alone) and Appendix 3 (IS Reduction plus COVID-19 Vaccine Dose)). Following sample collection, the participant will proceed to the administration location and receive the assigned dose of COVID-19 vaccine (see Section 6 for vaccine details).

Participants will be observed for 30 minutes post vaccine administration.

8.3.3 Follow-up Visits (All Participants)

Follow-up will occur at weeks 1, 2, 3, 4, 5, 6 and Months 3, 6, 9, and 12 following vaccine administration. Participants will have additional blood tests to monitor their graft function during the time they are on a reduced level of immunosuppression and for 1 month following resumption of their original regimen of immunosuppression. Identical blood testing schedules will take place for both the COVID-19 vaccine dose group and the IS reduction plus COVID-19 vaccine dose group. Kidney recipients will have their serum creatinine levels measured at the weekly visits. Liver recipients will have a hepatic panel (AST, ALT, alk phos, bilirubin, direct bilirubin) at the weekly visits.

The Week 4 visit is the primary endpoint visit. During these visits, participants will be asked about their general health, any new events, immunosuppression medications and their transplanted organ per the Schedule of Events in Appendix 2.

Blood and nasal swabs will be collected for assessments to be run in the clinical and research laboratories per the Schedule of Events in Appendix 2 or Appendix 3 based on group assignment. Nasal mid-turbinate swabs for SARS-CoV-2 PCR testing will be collected at baseline and Months 1, 3, 6, 9 and 12 following vaccine administration.

Participants will be instructed to contact the study team if they receive any COVID-19 testing at an outside lab during the course of the study. All instances of COVID-19 infection will be reported on the applicable electronic case report form.

A subset of participants will have a study visit on Day 3 to have blood drawn for a research laboratory study to detect the presence of SARS-CoV-2 spike protein generated in response to the vaccine.

8.4 Unscheduled Visits

If there is a change in immunosuppressive medications, new medical events or a diagnosis of rejection between regularly scheduled visits, participants will be instructed to contact study personnel and may be asked to return to the study site for an “unscheduled” visit.

8.5 Suspected or Confirmed Myocarditis or Pericarditis

Participants will be instructed to seek prompt medical attention if they develop symptoms of myocarditis or pericarditis, such as including chest pain, shortness of breath, or palpitations. Participants will also be instructed to notify the study site team. Participants diagnosed with acute myocarditis or acute pericarditis within 6 weeks of any COVID-19 vaccine will be referred to a cardiologist for evaluation and management.

Cases of study-defined probable or definite acute myocarditis or acute pericarditis will be followed until resolution of symptoms and abnormal test findings.

8.6 Suspected or Confirmed Cases of COVID-19 Infection

If participants suspect they have contracted COVID-19 infection, they will be directed to follow-up with their local transplant physician or primary care provider to obtain the appropriate clinical care. Information related to their illness will be collected for the study and reported in the clinical database.

8.7 Visit Windows

Study visits should take place within the time limits specified below: the designated visit windows *(i.e. +/- n days)* for each scheduled visit are also indicated on the Table of Events.

Screening	-30 days for screening clinical laboratory assessments SARS-CoV-2 S antibody at least 30 days following last dose of COVID-19 vaccine -90 days for dnDSA
Day 3, Week 1	+/- 1 day
Weeks 2, 3, 4, 5, and 6	+/- 3 days
Months 3, 6, 9, and 12	+/- 2 weeks

9. Mechanistic Assays

9.1 Introduction

Effective vaccinations induce potent protective humoral and cellular immunity and promote development of robust T and B cell memory, together preventing infection and accelerating viral clearance following pathogen exposure. Current concepts are that vaccination results in activation of both T cells and B cells. Vaccine antigen-activated B cells can differentiate into low affinity IgG- or IgM-secreting plasma cells (PCs). Alternatively, vaccine antigen-activated B cells can interact with antigen-activated CD40+ CD4+ (Th1) helper T cells to undergo an isotype switch and enter germinal center (GC) reactions wherein they undergo affinity maturation. Positive selection of the highest affinity B cells in the GC requires cognate interactions with antigen specific IL-21-producing follicular helper CD4+ T cells (Tfh). The positively selected high affinity B cells then can differentiate into long lived antibody-secreting PCs, can become memory B cells (Bmem), or can reenter the GC to undergo further affinity maturation. Crucial features of the antibody repertoire relevant to pathogen clearance include antibody specificity, affinity, titer, isotype class, and glycosylation patterns, among others. Antigen-reactive CD4+ and CD8+ T cells that expand and differentiate following vaccination are also crucial mediators of pathogen clearance independent of their functions as helper cells for effective humoral immune responses. Important characteristics of these induced T cell responses include epitope specificity, cytokine secretion patterns, and cytotoxic function, among others. Effective anti-pathogen immunity also involves activation of innate immune cells, including dendritic cells, macrophages, and natural killer cells that can interact with an enhance the adaptive immune responses and have been recently shown to exhibit cellular and molecular features consistent with memory.

As noted in the background section, emerging evidence indicates that the majority of transplant recipients taking immunosuppressant medications do not produce effective antibody responses to SARS-CoV-2 spike protein mRNA vaccinations. Whether these patients develop and maintain protective T cell responses after the same vaccines is not known. A detailed kinetic analysis of innate and adaptive immunity following SARS-CoV-2 mRNA vaccination, particularly comparing responders to non-responders, is thus essential in order to interpret the clinical trial outcome and to guide design of follow up studies aimed at overcoming identified defects. Moreover, if it were possible to identify an early post vaccine molecular signature that correlates with vaccine success vs failure, such a biomarker could be used to guide subsequent strategies aimed at enhancing induction protective immunity in each transplant patient.

Thus, the overall goals of the proposed mechanistic studies are to a) characterize the vaccine-induced innate and adaptive immune responses in each study subject, b) compare the induced immune responses among participants in the various treatment arms. c) define the durability of the responses over 1-year post vaccination, d) provide insight into immune mechanisms that prevent formation and/or durability of the induced responses within each and among treatment arms, d) identify molecular markers of successful vaccination.

To this end we will serially collect serum, plasma, PBMC, and oral fluid samples over the entire study period. Using these samples we will a) quantify spike protein production in serum, b) provide a detailed kinetic characterization of the vaccine induced antibody repertoire (specificity, isotype, titer, neutralization capacity), c) perform a kinetic phenotypic and functional analysis of antigen specific Bmem (frequency, antigen specificity, surface markers, metabolomics, gene expression patterns, single cell sequencing) and

PC, d) perform a kinetic, analysis of antigen specific CD4+ and CD8+ T cells (frequency, epitope specificity, surface markers, metabolomics, gene expression patterns single cell sequencing), e) assess phenotypic, functional and gene expression patterns of innate immune DC, monocyte/macrophage and NK cell subsets in peripheral blood, and f) perform genomic profiling of peripheral blood cells that will provide a biomarker for an effective response.

9.2 Objective 1: To quantify spike protein production in serum or plasma after vaccination.

Transient expression of a modified spike protein using mRNA vaccines (Moderna and Pfizer-BioNTech) has been crucial in the global effort to control the SARS-CoV-2 pandemic. This platform offers the flexibility for rapid modification to control variants. Given the requirement for effective protein expression of immunogen, the extremely poor responses of transplant recipients, particularly those on mycophenolate mofetil raises the question whether the anti-metabolite properties of MMF may impair the transient expression of spike protein thereby contributing to the impaired vaccine response of transplant patients. Using a recently developed ultrasensitive molecular assay to detect spike protein in plasma we will assess expression in a subset of antibody negative study participants at baseline (pre-vaccine) and 3 days post-vaccination.

9.3 Objective 2: To provide a detailed kinetic characterization of the vaccine-induced antibody repertoire (specificity, isotype, titer, neutralization capacity).

While relative contributions of vaccine induced humoral and T cell immunity in the protection from severe SARS-CoV-2 disease are not fully understood, the development of a critical level of SARS-CoV-2 Ab are emerging as a correlate of protection. A detailed understanding of the profile of Ab responses in transplant recipients after vaccination is crucial step toward understanding what population benefits from an additional dose of vaccine. This objective will provide a deeper mechanistic characterization of to support hypothesis generating observations to understand the mechanisms underlying vaccine failure and to identify candidate humoral biomarkers to predict vaccine response. This includes surrogate and live virus neutralization titers including versus novel variants of concern.

9.4 Objective 3: To perform a kinetic phenotypic and functional analysis of antigen-specific Bmem (frequency, antigen specificity, surface markers, metabolomics, gene expression patterns, single cell sequencing) and PC.

To identify and characterize SARS-CoV-2 memory B cells we will use fluorescently labeled multimerized probes for targets of the vaccine (spike receptor-binding and ectodomain) and as well as non-vaccine expressed SARS-CoV-2 antigens (e.g. Nucleocapsid). The magnitude and differentiation of anti-specific IgM+ and IgG+ memory B cells will be assessed at each time point. We will use flow cytometry, CyTOF, single B cell multi-omics (cell surface CITE-seq profiling using tagged antibody and multimerized probes for vaccine targets), transcriptomics, and B cell receptor VDJ repertoire analysis to characterize antigen specific B cells. We will perform in vitro ELISPOT assays to determine frequencies of antigen specific PCs in peripheral blood.

9.5 Objective 4: To perform a kinetic, analysis of antigen-specific CD4+ and CD8+ T cells (frequency, epitope specificity, surface markers, metabolomics, gene expression patterns single cell sequencing).

While the requirements for sustained protection against SARS-CoV-2 are not yet known, effective control of viral pathogens often depends on the development of both humoral and cellular immunity.

We will assess the strength, breadth and character of the anti-spike T cell response in study participants using flow-cytometry based, ELISPOT, and single cell-multi-omics assays. Antigen-specific cells will be interrogated using a rapidly evolving set of peptide and MHC multimer reagents using flow-cytometry, CyTOF, and or ELISPOT readouts. For flow-based analysis we will utilize 1) antigen specific functional assays (activation-induced marker (AIM), intracellular cytokine secretion assays and novel T cell metabolic profiling assays and, 2) phenotypic characterization of spike-specific T cells MHC-peptide multimer staining. We will use single T cell multi-omics (cell surface CITE-seq profiling using tagged antibody and tetramers, transcriptomics, and T cell receptor VDJ repertoire analysis.

Exploratory immune profiling assessments will include:

9.6 Objective 5: To assess phenotypic, functional and gene expression patterns of innate immune DC, monocyte/macrophage and NK cell subsets in peripheral blood.

We will characterize the kinetics of peripheral blood immune landscape of mononuclear leukocyte lineage and phenotypic markers to broadly assess the immunological cellular profile of transplant recipients before and after vaccination, focusing on the absolute and relative frequency of T, B, NK and APC subsets.

We will characterize these cells types in peripheral blood using flow cytometry and or CyTOF assays. We will test function using in vitro assays that include in vitro stimulation of DC subsets with TLR ligands (cytokine and surface marker readouts as measures of activation), in vitro stimulation of macrophages with LPS (cytokine readouts as a measure of trained immunity, which would then be followed up with epigenetic analyses), in vitro functional analyses of NK cells.

9.7 Objective 6: Exploration of molecular biomarkers of early responders to vaccination

We will collect serial blood samples for RNA profiling (RNAseq) and/or proteomics in addition to characterization of cytokine signaling early after vaccine intervention.

10. Biospecimen Storage

Biological specimens obtained under this protocol may be used in future assays to reevaluate biological responses as additional research tests are developed over time. These may include, but are not limited to, tests examining aspects of host immunology, cell biology, or human genetics as it relates to any of these aspects. Appropriate informed consent will be obtained for both the collection and storage of samples. The specimens from these evaluations will be labeled with a coded ID and may be stored beyond the funding period.

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1 Participant Completion

All participants will be followed for 12 months following the COVID-19 vaccine administration.

11.2 Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up" (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.

11.3 Participant Replacement

Participants who withdraw or are withdrawn will not be replaced.

11.4 Follow-up after Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant may be asked to complete a final visit and/or final assessments.

11.5 Study Pausing Rules

If the following occur, relevant study interventions will be temporarily halted pending a DSMB review and recommendation as to whether the study may proceed.

1. Any Grade 4 or 5 event that is definitely or possibly attributed to the study dose of vaccine or to immunosuppression reduction.
2. Any probable or confirmed case of either acute myocarditis or acute pericarditis that occurs within 6 weeks of the study vaccine.
3. **Excess incidence of acute rejection (clinically treated or biopsy-proven):** Based on registry data, approximately 5-6 rejection events would be expected over the course of the study, independent of any effect of the study interventions. A pausing rule has been constructed such that the study would be paused if there is a >50% chance that the observed event rate exceeds three times the expected rate. The pausing rule will be applied to the study by group: (1) COVID-19 vaccine dose alone (2) MMF withdrawal (3) CNI reduction. Event rates triggering a pause are shown below:

Number of participants	Number of events required to trigger a pause	Event rate required to trigger a pause
10	3	30%
20	3	15%
30	3	10%
40	4	10%
50	4	8%
75	4	5%
100	5	5%
150	6	4.0%

200	7	3.5%
250	8	3.2%
300	9	3.0%
350	10	2.9%
400	11	2.8%

4. **Excess incidence of graft loss**: Based on registry data, it is expected that there will be approximately 6-7 graft losses over the course of the study, independent of any effect of the study interventions. A pausing rule has been constructed such that the study would be paused if there is a >50% chance that the observed event rate exceeds three times the expected rate. The pausing rule will be applied to the study by group: (1) COVID-19 vaccine dose alone (2) MMF withdrawal (3) CNI reduction. Event rates triggering a pause are shown below:

Number of participants	Number of events required to trigger a pause	Event rate required to trigger a pause
10	3	30%
20	3	15%
30	3	10%
40	4	10%
50	4	8%
75	4	5%
100	5	5%
150	6	4.0%
200	7	3.5%
250	8	3.2%
300	9	3.0%
350	10	2.9%
400	11	2.8%

5. **Excess Mortality:** Based on registry data, it is expected that there will be approximately 5 deaths over the course of the study, independent of any effect of the study interventions. A pausing rule has been constructed such that the study would be paused if there is a >50% chance that the observed event rate exceeds three times the expected rate AND is determined to be possibly, probably or definitely related to a study intervention. The pausing rule will be applied to the study overall and by group: (1) COVID-19 vaccine dose alone (2) MMF withdrawal (3) CNI reduction. Event rates triggering a pause are shown below:

Number of participants	Number of events required to trigger a pause	Event rate required to trigger a pause
10	3	30%
20	3	15%
30	3	10%
40	4	10%
50	4	8%
75	4	5%
100	5	5%
150	5	3.3%
200	6	3.0%
250	7	2.8%
300	8	2.7%
350	9	2.6%
400	10	2.5%

12. Safety Monitoring and Reporting

12.1 Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5, Reporting of Serious Adverse Events and Adverse Events) to the sponsor, DAIT/NIAID. Appropriate notifications will also be made to site principal investigators, Institutional Review Boards (IRBs), and health authorities.

Information in this section complies with ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version [enter 5.0 or correct version applicable to trial] : http://ctep.cancer.gov/reporting/ctc.html.

12.2 Definitions

12.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from OHRP "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2)

For this study, adverse events will be considered for association with the additional dose of COVID-19 vaccine and study mandated procedures as follows:

Vaccine Administration (Moderna COVID-19 Vaccine (monovalent, bivalent, and 2023-2024 formula as per protocol version), Pfizer-BioNTech COVID-19 Vaccine (monovalent, bivalent, and 2023-2024 formula as per protocol version)

All AEs occurring within 30 days following a single COVID-19 vaccine dose and SAEs up to 6 months post vaccine administration.

Study mandated procedures:

Any AE occurring within 24 hours of a protocol mandated blood draw
Any AE occurring within 24 hours of a protocol mandated nasal swab
Any AE occurring within 24 hours of an oral fluid collection
Any AE occurring during the period from the onset of immunosuppression reduction until 30 days after return to full immunosuppression and SAEs up to 6 months post return to full immunosuppression.

12.2.2 Solicited Adverse Events

For the purposes of this study, the following specific local and systemic reactogenicity events, as well as symptoms of a potential allergic reaction, will be solicited from the participant through a remote contact on Day 7 post-vaccination. Any other adverse events reported during the course of this remote contact will be reported on the AE eCRF. Study clinicians will follow all adverse events to resolution.

- Local reactions at the injection site including erythema/redness, swelling/induration (hardness), and pain.
- Systemic reactions including fever, myalgia, arthralgia, fatigue, headache, nausea, vomiting, diarrhea, and chills.
- Potential allergic reaction, which includes the following symptoms:
- Skin: hives, swelling other than injection site, itching, redness other than injection site, rash
- Respiratory: wheezing, shortness of breath, coughing, tightness in the throat or chest, sneezing, nasal stuffiness or congestion
- Gastrointestinal: trouble swallowing, abdominal cramps, diarrhea, nausea, vomiting
- Dizziness or lightheadedness

12.2.3 Unsolicited Adverse Events

Participants will be instructed to contact the study site and report any adverse events up to 30 days post vaccine administration. This will include any delayed onset local reactions.

12.2.4 Adverse Events of Special Interest (AESI)

Any case of myocarditis or pericarditis within 6 weeks after study vaccination will be reported as an AESI. Regardless of seriousness, these events will be reported to DAIT/NIAID within 24 hours of awareness on the Serious Adverse Event case report form.

For myocarditis and pericarditis events, any relevant laboratory information, imaging (echocardiography, cardiac MRI) and/or pathology (cardiac biopsy) results will be reported on case report forms. The DAIT medical monitor may require that appropriately deidentified copies of studies, including but not limited to EKG, imaging, cardiac biopsies digital images, or other follow-up results (stress tests results) be made available for medical monitor or ad hoc DSMB review.

12.2.5 Suspected Adverse Reaction (SAR)

Any adverse event for which there is a reasonable possibility that the SARS-CoV-2 vaccine caused the adverse event. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug (21 CFR 312.32(a)).

12.2.6 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not consistent with the risk information described in Vaccine-specific FDA EUA Full Prescribing Information) (21 CFR 312.32(a) and 310.305)

12.2.7 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor DAIT/NIAID, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or Sponsor [DAIT/NIAID], its occurrence places the subject at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5. Congenital anomaly or birth defect.
6. Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations or hospital admissions for the purpose of conduct of protocol mandated procedures are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.3 Grading and Attribution of Adverse Events

12.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all adverse events. The NCI-CTCAE has been reviewed by the NIAID Medical Monitor and Protocol Chair and has been deemed appropriate for the subject population to be studied in this protocol.

Adverse events will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 = Moderate; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL: Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.)
Grade 3 = Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (ADL: Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.)
Grade 4 = Life-threatening consequences; urgent intervention indicated.

Grade 5 = Death related to AE.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

12.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE/SAE eCRF. Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 12.3.2 Attribution of Adverse Events

Code	Descriptor	Relationship (to primary investigational product and/or other concurrent mandated study therapy or study procedure)
UNRELATED CATEGORY		
1	Not Related	The adverse event is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possibly Related	The adverse event has a reasonable possibility to be related; there is evidence to suggest a causal relationship.
3	Related	The adverse event is clearly related.

12.4 Collection and Recording of Adverse Events

12.4.1 Collection Period

Adverse Events temporally associated (24 hours) with the research blood draws and nasal swabs will be collected from the time of the screening visit until a subject completes study participation.

COVID-19 Vaccine Dose Alone: Adverse Events as defined in Section 12.2.1 will be collected for 30 days following COVID-19 vaccine administration. Serious Adverse Events will be collected for 6 months following vaccine administration.

COVID-19 Vaccine Dose plus IS Reduction: All Adverse Events will be collected in those who undergo immunosuppression reduction occurring from the onset of IS reduction until 30 days after return to full immunosuppression. Serious Adverse Events will be collected for 6 months post onset of IS reduction.

12.4.2 Collecting Adverse Events

Adverse events (including SAEs) may be discovered through any of these methods:

- Observing the subject.
- Interviewing the subject [e.g., using a checklist, structured questioning, diary, etc.].
- Receiving an unsolicited complaint from the subject.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 12.3, *Grading and Attribution of Adverse Events.*

12.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 12.2, *Definitions)* on the appropriate electronic case report form regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

12.5 Reporting of Serious Adverse Events and Adverse Events

12.5.1 Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report serious adverse events to the sponsor via the AE/SAE eCRF. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

Site investigators will report all serious adverse events (see Section 12.2.3, *Serious Adverse Event)*, regardless of relationship or expectedness within 24 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE eCRF provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE eCRF will be updated and submitted. Initial SAE eCRFs should include as much information as possible, but at a minimum:

- AE term
- Relationship to study vaccination
- Relationship to study procedure
- Reason why the event is serious

- Supplementary eCRF pages that are current at the time of the SAE reporting e.g. medical history and vaccine administration

12.5.2 Reporting of Adverse Events to IRBs/IECs

All investigators shall report adverse events, including expedited reports, in a timely fashion to their respective IRBs/IECs in accordance with applicable regulations and guidelines.

12.5.3 Mandatory reporting to Vaccine Adverse Event Reporting System

Per the FDA EUA for the Moderna COVID-19 Vaccine and Pfizer-BioNTech COVID-19 Vaccine, reporting of the following to the Vaccine Adverse Event Reporting System (VAERS):

vaccine administration errors whether or not associated with an adverse event,
serious adverse events (irrespective of attribution to vaccination),
cases of myocarditis,
cases of pericarditis,
cases of Multisystem Inflammatory Syndrome in adults, and
cases of COVID-19 that result in hospitalization or death.

The site investigator, or designee, is also responsible for recording vaccination information in the state/local jurisdiction's Immunization Surveillance System or other designated system.

12.6 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as the SACCC when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an adverse event.

12.7 Review of Safety Information

12.7.1 Medical Monitor Review

The NIAID Medical Monitor shall receive monthly reports from the SACCC compiling new and accumulating information on AEs and SAEs recorded by the study site(s) on appropriate eCRFs.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the SACCC (See Sections 12.5.1, Reporting of Serious Adverse Events to Sponsor, and 12.6, Pregnancy Reporting).

12.7.2 DSMB Review

12.7.2.1 Planned DSMB Reviews

The Data and Safety Monitoring Board (DSMB) shall review safety data one month after all study participants have received study vaccine, unless a safety event requires earlier review. They will review the study again when the database is locked after the last patient's last visit. Interim DSMB reviews may occur at any time at the discretion of the medical monitor or the protocol chair. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

12.7.2.2 Ad hoc DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID. In addition, the following events will trigger an *ad hoc* comprehensive DSMB Safety Review:

Any death that occurs in the study which is possibly or definitely related to study treatment regimen.
A Pausing Rule (outlined in Section 11.2) is met.

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

12.7.2.2.1 Temporary Suspension of enrollment for ad hoc DSMB Safety Review

See Section 11.2 for Participant Stopping Rules and Withdrawal Criteria

13. Statistical Considerations and Analytical Plan

13.1 Overview

This is a two-arm, open-label randomized trial with the goals of comparing efficacy of additional COVID-19 vaccine doses for solid organ transplant recipients with a prior weak immune response, with vs without immunoreduction. The study population will consist of solid organ transplant recipients who had a negative or low response to a licensed or authorized COVID-19 vaccine regimen, who otherwise meet inclusion criteria.

13.2 Endpoints/Outcomes

The primary endpoint is the proportional increase in antibody titer U/mL (using the Roche Elecsys® anti-RBD assay) at 30 days after the study dose of vaccine. Negative or very low antibody responses (<10 U/mL) will be imputed to 10 U/mL, to reduce the influence of measurement error on the proportional increase calculation among individuals with very low titers. Secondary safety endpoints include: local and systemic vaccine reactogenicity and/or allergy; serious adverse events occurring during the 30 days following the additional COVID-19 vaccine dose; treated acute cell-mediated and/or antibody-mediated allograft rejection (clinical or biopsy-proven) within 60 days following the COVID-19 vaccine dose; development of de novo donor-specific anti-HLA antibody within 90 days of the vaccine; graft loss within 60 days of the vaccine; and death within 60 days of the vaccine. Secondary efficacy endpoints include: SARS-CoV-2 PCR positivity; symptomatic COVID-19; COVID-19 requiring hospitalization; and longitudinal changes in SARS-CoV-2 antibody response during the study.

13.3 Measures to Minimize Bias

All participants will be randomized to receive immunoreduction or no immunoreduction prior to receipt of the vaccine. Randomization will be clustered by study site, and stratified by pre-dose titer (<50 vs ≥50 U/mL). Treatment group assignment will be unmasked to participants and clinical study staff.
All laboratory staff will be masked to treatment assignment.

13.4 Analysis Plan

13.4.1 Analysis Populations.

For the primary outcome and each secondary outcome, the analysis population will consist of all individuals with a measurement for that outcome, in an intent-to-treat framework.

13.4.2 Primary Analysis of Primary Endpoint(s)/Outcome(s)

The primary endpoint is a continuous measurement. All individuals whose primary endpoint is measured will be included in the analysis population.

For the comparison of immunoreduction vs no immunoreduction, we will reject the null hypothesis if the two-sided rank-sum p value comparing the -fold increase between the two groups falls below 0.05.

13.4.3 Analyses of Secondary and Other Endpoint(s)/Outcome(s)

All binary secondary endpoints will be analyzed using a Fisher exact test. Severe adverse events will be analyzed by calculating the incidence rate difference with a two-sided 95% confidence interval for immunoreduction vs no immunoreduction participants.

13.4.4 Analyses of Exploratory Objectives

To assess whether timing between vaccine doses is associated with differential antibody response to vaccination, we will calculate the median value of time from last pre-study vaccine dose to study dose, and then repeat the analysis of antibody response, stratified by the timing between doses (above vs below the median). To assess whether there is a difference in antibody levels among those receiving additional doses of Pfizer versus Moderna vaccination, we will repeat the analysis of antibody response, stratified by type of dose.

13.4.5 Descriptive Analyses

Baseline and demographic characteristics will be compared between groups using a ranksum test for continuous variables and a Fisher exact test for binary/categorical variables. The relationship between demographic and other characteristics and antibody levels will be explored.

13.5 Interim Analyses

13.5.1 Interim Analysis of Efficacy Data

An interim efficacy analysis is not planned for this trial, because (1) It is extremely unlikely that a definitive difference between arms would emerge at a time when it is still possible to stop enrollment, and (2) it is desirable to be able to evaluate safety outcomes in the full cohort, assuming that no safety stopping rules are met. However, at the time of the safety interim analyses, the DSMB will be provided concurrent SARS-CoV-2 antibody results in each arm to aid in evaluating the risk/benefit calculus.

13.5.2 Interim Analysis of Safety Data

Excess incidence of dnDSA in kidney transplant recipients: An interim analysis will be performed to look for evidence of an excess incidence of dnDSA in the IS reduction groups (those undergoing withdrawal of MMF; and those undergoing a decrease in CNI dose) as compared with those receiving an additional dose of vaccine alone. When fifteen subjects in each of these groups have reached day 30 in the study (study visit 4), the core HLA lab will determine whether de novo DSA is present. We will perform a similar analysis when fifty subjects in each group have reached day 30. At each of these two time points, the proportion with Class II dnDSA in each IS reduction group will be compared with the COVID-19 vaccine dose alone group using a one-sided Fisher exact test to determine if there is an excess incidence of Class II dnDSA, and the results presented to the DSMB for review

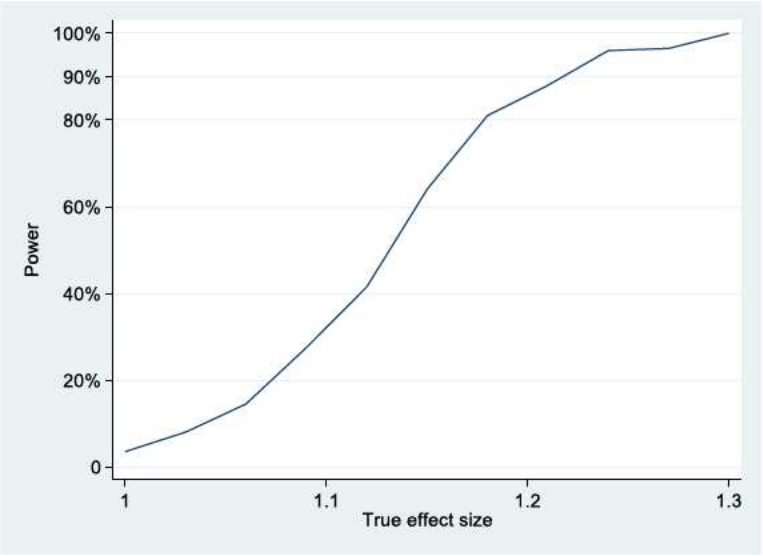
13.6 Statistical Hypotheses

All analyses are superiority framework; the null hypotheses are that there is no difference in outcome rates between participants who do / do not receive immunoreduction. The alternative hypotheses for both safety and efficacy outcomes is that the outcome occurs more frequently in the immunoreduction group.

13.7 Sample Size Considerations

Comparison of the primary endpoint will use a two-sided ranksum test. There will be 400 patients, 200 per treatment group. Power calculations assume an α of 0.05. The power of the study will depend on the distribution of -fold changes in each group.

We ran simulations In which -fold changes are log-normally distributed, and computed power based on the ratio of -fold change between the two groups. For example, if the average -fold increase in one group is a twofold increase and in the other group, a threefold increase, the ratio of -fold change is 3/2 or 1.5. (This does not mean that each individual in one group experienced a twofold increase; some individuals would experience a greater increase, and some a smaller increase.) In the simulation, with an N of 400, generally the study has 80% power to detect a difference of 20% (e.g. mean 2.5-fold increase in one group vs 3-fold increase in the other.)



14. Identification and Access to Source Data

14.1 Source Data

Source documents and source data are the original documentation where subject information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations, and other activities during a clinical trial.

14.2 Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID and their representatives as well as to relevant health authorities (Food and Drug Administration). Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. Quality Assurance and Quality Control

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements.

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

16. Protocol Deviations

16.1 Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance with the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human subject protection regulations, policies, or procedures.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the subject's rights, safety or well-being, or the completeness, accuracy, and reliability of the study data.

16.2 Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

17. Ethical Considerations and Compliance with Good Clinical Practice

17.1 Statement of Compliance

This clinical study will be conducted using good clinical practice (GCP), as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the Sponsor and Institutional Review Board (IRB). Any amendments to the protocol or to the consent materials will also be approved by the Sponsor and IRB before they are implemented.

17.2 Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or designee listed on the Investigator of Record Agreement will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in the participants' primary language. A copy of the signed consent form will be given to the participant.

The consent process will be ongoing, and any new findings will be communicated to the participants. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

17.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

18. Publication Policy

The publication guidelines and policies stipulated in the grant will apply to this protocol.

19. References

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Appendix 1: Screening (All Participants)

Initial Screening Assessment
• Chart review • Antibody consent • Anti-SARS-CoV-2 S (Roche Assay)
Final Screen
• Study consent form • Serum creatinine, eGFR (Kidney) • DSA (kidney only, sent to central lab, 90 day window) • Research blood (sent to central lab) • Hepatic panel, INR, direct bilirubin (liver) • Tacrolimus trough level • Pregnancy test (women of childbearing potential)
Randomization
1) Once the participant meets full eligibility, the participant may be randomized 2) Once the participant is randomized, the appropriate appendix should be followed based on the group assignment: o **Appendix 2**: COVID-19 Vaccine Dose Alone **OR** o **Appendix 3**: IS Reduction plus COVID-19 Vaccine Dose

Appendix 2: Schedule of Events (COVID-19 Vaccine Dose Alone)

Study Schedule	Days		Weeks						Months				
Time points	0	3[1]	1	2	3	4	5	6	3	6	9	12	For Cause[2]
Visit #	01	02	03	04	05	06	07	08	09	10	11	12	FC
Visit Windows (+/-)	-	1d	1d	3d	3d	3d	3d	3d	2w	2w	2w	2w	-
General Assessments													
Study Contact[3]	X		X	X	X	X	X	X	X	X		X	X
Medications (immunosuppression, PrEP, COVID-19 treatment)	X[4]	→	→	→	→	→	→	→	X	X[5]	X[5]	X[5]	X
Assessment of Events (AE, SAE, rejection, graft loss)	X	→	→	→	→	→	→	→	→	→	→	X	X
Clinical Laboratory Assessments													
Complete Blood Count with differential	X			X		X			X	X		X	
Metabolic Panel[6]	X								X	X		X	
eGFR, serum creatinine (kidney) Hepatic Panel including direct bilirubin (liver)	X		X	X	X	X	X	X					
Quantitative Immunoglobulins	X									X			
SARS-CoV-2 PCR (nasal swab, clinical)	X[7]					X			X	X	X	X	X
Investigational Intervention													
COVID-19 mRNA Vaccine	X												
Central Laboratory Assessments													
SARS-CoV-2 PCR (nasal swab, research)	X					X			X	X		X	X
Oral fluid anti-N and anti-S immunoglobulin levels	X					X			X	X		X	X
Anti-SARS-CoV-2 S (Roche Elecsys®)				X		X			X	X		X	X
Donor Specific Antibodies (kidney only)						X			X	X		X	X
Antibody/Neutralization assays	X			X		X			X	X		X	X
Spike Protein (***subset of sites***)	X	X											
Proteomics	X	X[8]				X							
Transcriptomics	X	X[7]				X							
T and B Cell Assays	X			X		X			X	X		X	X

[1] May be a home draw. The spike protein will only be performed at a subset of clinical sites.
[2] Unscheduled "for cause" visit for suspected rejection. Participants with confirmed COVID-19 will send in a nasal swab for research.
[3] Study contacts may be remote.
[4] Ensure the participant has not taken any medications since screening that would make them ineligible.
[5] Only PrEP and COVID-19 related treatment collected at these visits.
[6] Comprehensive metabolic panel at baseline, basic metabolic panel all other timepoints (see study definitions).
[7] If the Day 0 nasal swab is positive, the participant will be prematurely discontinued and followed until Day 30 for safety. This includes the Day 30 clinical labs, DSA and AE/SAE assessment. No further mechanistic blood will be collected.
[8] These Day 3 assessments will only be collected on participants included in the Day 3 spike protein.

Appendix 3: Schedule of Events (IS Reduction plus COVID-19 Vaccine Dose)

Study Schedule	Days			Weeks						Months				
Time points	-5	0	3[1]	1	2	3	4	5	6	3	6	9	12	For Cause[2]
Visit #	01	02	03	04	05	06	07	08	09	10	11	12	13	FC
Visit Windows (+/-)	-	-	1d	1d	3d	3d	3d	3d	3d	2w	2w	2w	2w	-
General Assessments														
Study Contact[3]	X	X		X	X	X	X	X	X	X	X		X	X
Medications (immunosuppression, PrEP, COVID-19 treatment)	X[4]	→	→	→	→	→	→	→	→	X	X[5]	X[5]	X[5]	X
Assessment of Events (AE, SAE, rejection, graft loss)	X	→	→	→	→	→	→	→	→	→	→	→	X	X
Clinical Laboratory Assessments														
Complete Blood Count with differential		X			X		X			X	X		X	
Metabolic Panel[6]		X								X	X		X	
eGFR, serum creatinine (kidney) Hepatic Panel including direct bilirubin (liver)		X		X	X	X	X	X	X					
Quantitative Immunoglobulins		X									X			
SARS-CoV-2 PCR (nasal swab, clinical)		X[7]					X			X	X	X	X	X
Investigational Intervention														
Immunosuppression Reduction	X	→	→	→	X[8]									
COVID-19 mRNA Vaccine		X												
Central Laboratory Assessments														
SARS-CoV-2 PCR (nasal swab, research)		X					X			X	X		X	X
Oral fluid anti-N and anti-S immunoglobulin levels		X					X			X	X		X	X
Anti-SARS-CoV-2 S (Roche Elecsys®)					X		X			X	X		X	X
Donor Specific Antibodies (kidney only)							X			X	X		X	
Antibody/Neutralization assays		X			X		X			X	X		X	X
Spike Protein (***subset of sites***)		X	X											
Proteomics		X	X[9]				X							
Transcriptomics		X	X[8]				X							
T and B Cell Assays		X			X		X			X	X		X	X

[1] May be a home draw. The spike protein will only be performed at a subset of clinical sites.
[2] Unscheduled "for cause" visit for suspected rejection. Participants with confirmed COVID-19 will send in a nasal swab for research.
[3] Study contacts may be remote.
[4] Ensure the participant has not taken any medications since screening that would make them ineligible.
[5] Only PrEP and COVID-19 related treatment collected at these visits.
[6] Comprehensive metabolic panel at baseline, basic metabolic panel all other timepoints (see study definitions).
[7] If the Day 0 nasal swab is positive, IS reduction will be prematurely discontinued and participants will be followed until Day 30 for safety. This includes the Day 30 clinical labs, DSA and AE/SAE assessment. No further mechanistic blood will be collected.
[8] Participant will be informed to restart full IS the following day.
[9] These Day 3 assessments will only be collected on participants included in the Day 3 spike protein.