Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Hall VG, Ferreira VH, Ku T, et al. Randomized trial of a third dose of mRNA-1273 vaccine in transplant recipients. N Engl J Med. DOI: 10.1056/NEJMc2111462

A Randomized Trial of Third Dose mRNA-1273 Vaccine in Transplant Recipients

This supplement contains the following items:

1. Original protocol, final protocol, summary of changes.

2. Original statistical analysis plan, final statistical analysis plan, summary of changes

A Randomized Trial of Third Dose mRNA-1273 Vaccine in Transplant Recipients

Table of Contents:

[¶]Version 1,2,4 were internal protocol drafts. Version 3 is the first one approved by the University Health Network Research Ethics Board on May 4, 2021.
*Version 5 was approved by the Ethics Board on May 20, 2021.
[§]The statistical analysis plan (Version 1) corresponds to first approved protocol version 3.

CONFIDENTIAL

Title

PREVent-COVID Sub-study: A randomised, double-blind, controlled trial of a third dose of Moderna vaccine versus placebo in solid organ transplant recipients

Protocol Number
UHN-TID-COVAX-2

Investigators

Deepali Kumar MD
Victoria Hall MBBS
Natalia Pinzon MSc
Victor Ferreira PhD
Atul Humar MD

Version 3
April 22, 2021

Institution

[1]Transplant Infectious Diseases, University Health Network, Toronto, Ontario, Canada

1. Background

This is a sub-study of an observational, comparative, cohort study "The PREVent-COVID" Study (CAPCR ID 20-6069).

There are now three COVID-19 vaccines with Health Canada approval that are currently being used for vaccination. The Moderna vaccine (mRNA-1273) has proven highly efficacious and safe in a phase III large, randomised control trial of 30,420 volunteers participants, and has been in use in Canada since December 2020.[1] Multiple other "real-world" studies post-approval have reflected these findings.

In solid organ transplant recipients (SOTr), there is still a paucity of data for the effectiveness of the available COVID-19 vaccines. However, Johns Hopkins University (JHU) has recently published their experience in 436 SOTr after one dose of either the Pfizer or Moderna COVID-19 vaccine; with an overall detectable antibody response in 76 out of 436 participants (17%, 95% CI, 14-21%).[2] A separate study in the same population confirmed safety and minimal adverse effects, with expected vaccine reactogenicity as experienced in the general population studied in the randomised phase III trial.[1,3] Data after two doses is expected to be published shortly.

In the PREVENT-COVID study, we have recruited 120 SOTr who have received both scheduled doses of the Moderna vaccine at 0 and 1 months. They have had blood work performed prior to vaccination, 3-4 weeks after the first dose, and 4-6 weeks after the second dose of vaccine to determine antibody and cell-mediated immunity. Side effects have been tracked with a vaccine diary and demographic data has been collected (age, sex, transplant organ, date of transplant, current immunosuppressive medications, recent rejection episodes).

Our preliminary analysis of 6 patients shows that there is no antibody response in any of these patients after the first dose of Moderna vaccine compared to 100% antibody response in healthy controls. Given the expected impaired immune response in terms of antibody titers and cell-mediated immunity, also based on data from JHU and previous experience with vaccination in this patient population; we propose that a third dose of the Moderna vaccine will act as a booster to pre-existing but blunted immunity.[2,4-6] This is expected to be safe with side effects as reflected by previously published data.[7]

This study will be a randomised, double-blind, placebo-controlled trial, with participants recruited, consented and then randomised from the main PREVent-COVID study who have already received both scheduled doses of the Moderna vaccine (0 and 1 months). Participants will be randomised to receive either a third dose of the Moderna vaccine or saline placebo at 3 months post initial vaccination.

2. Rationale

Pre-existing data suggest a reduction in the immune response to the available COVID-19 vaccines in SOTr.[2] There is previously published evidence for the safe administration of booster doses of vaccines for other infections such as influenza to achieve protective antibody titers in the immunocompromised host, including solid organ transplant recipients.[5,7] An

effective and durable vaccine response is desired in this immunocompromised population, who have expected worse outcomes if they develop COVID-19 infection due to their immunocompromised state, age and co-morbidities.[8,9] Therefore, it is paramount to investigate ways of optimising the vaccine response in this vulnerable population.

3. Study aim and hypothesis

3.1 Aim:

To investigate if a third dose of the Moderna vaccine at 3 months post initial vaccination will boost the antibody titer and immune response in solid organ transplant recipients.

3.2 Hypothesis:

A third dose of COVID-19 vaccine will provide a greater immune response compared to only two doses.

4. Study objectives

4.1 Primary objective

- To determine the immunogenicity of a third dose of Moderna vaccine given at 3 months post-initial vaccination in solid organ transplant recipients.

4.2 Secondary objectives

- Vaccine safety and side effects – patients will be asked to record side effects that occur daily up to 7 days after vaccination. Follow-up will occur for graft rejection and any health-care contact up to 3 months post-study vaccine.

5. Methods

5.1 Study design

This is a randomised, placebo-controlled, double blind study of transplant recipients already enrolled in the PREVENT-COVID Study. All 120 participants who have already received both doses of the Moderna vaccine at 0 and 1 months will be approached for the study. Consented patients will be randomized 1:1 to receive a third dose of the Moderna vaccine (0.5mL intramuscular injection of Moderna COVID-19 vaccine) or saline placebo (0.5mL intramuscular injection of normal saline) in the deltoid muscle of the arm. Both participant and healthcare provider who administers the vaccine would be blinded to intervention versus placebo; Blood work would then be performed 4-6 weeks after immunization to determine immunogenicity. All participants will provide 10mL of blood for serum. A subset of 50 participants will be asked to provide an additional 20mL of blood for cell-mediated immunity. These will be the same 50 participants that consented to the PBMC substudy in PREVenT-COVID.

5.1.2 Recruitment and consent process

- The study coordinator will contact all transplant recipients that are enrolled in the main PREVENT-COVID study and who have received both doses of the Moderna vaccine.

- A telephone script will be used to introduce this study and initiate the consent process.
- Questions about the study can be answered over the phone at this time.
- The study investigator(s) will be available in case of questions.
- Consent will then be finalised via email / file share; or if blood collection occurs at UHN, this can be re-iterated in person and a printed and signed consent form collected at this time, as an in-person consent.
- After the consent process is satisfied, study eligibility and screening for inclusion and exclusion criteria will take place.
- The participant will then be enrolled with their pre-existing unique study identifier from the main study, and then be randomised to the Moderna vaccine vs placebo.
- The first study visit where the participant receives the third dose of Moderna vaccine or placebo then occurs after the participant has consented to take part in the study. At the first study visit, eligibility will again be reviewed.
- After they have had the vaccine or placebo, blood work will be organised within 4-6 weeks.

5.2 Study setting and vaccine or placebo administration

- Vaccine (or placebo) will be administered in clinic and the patient will be asked to wait 15 minutes as per standard procedure. Vaccine doses will be obtained from UHN vaccine supply.

5.3 Patient population

Inclusion criteria:

- Adult SOTr that are stable outpatients and enrolled in the PREVENT-COVID main study
- Already received two doses of vaccine at 0 and 1 months respectively of the Moderna vaccine
- Comply with blood collection 4-6 weeks after vaccination or placebo dose
- Able to provide informed consent

Exclusion criteria:

- Previous documented COVID-19 infection
- Febrile illness in the past one week
- Active therapy for Cytomegalovirus viremia
- Use of Rituximab in the past 6 months
- Ongoing or recent (in past 30 days) therapy for acute rejection
- Receipt of intravenous immunoglobulin (IVIG) in the past 30 days or planning to receive IVIG in the next 4 weeks
- Anaphylaxis or allergic reaction to initial Moderna vaccine

5.4 Follow-up

Patients will be followed every 2 weeks for 3 months after the third Moderna vaccine (or placebo) dose. This is 6 months after enrolment in the main PREVenT-COVID study. They will be telephoned every 2 weeks to determine whether they were diagnosed with COVID or if they have any COVID-related symptoms (fever, cough, myalgias, sore throat, runny nose, diarrhea, shortness of breath). We will also be reminding participants to let the study team

know immediately if they have been diagnosed with COVID. We will also record all adverse events including serious adverse events. Non-routine health-care contacts including hospitalization and the reasons for these healthcare visits will be recorded every two weeks up to 3 months post-vaccination. These will be judged as related or unrelated to vaccine according to investigator judgement. We will specifically note if the patients have had any rejection – whether a biopsy was done, and whether rejection treatment was started. The final phone contact will be similar to the other calls. We will re-iterate COVID precautions and ask patients to let their transplant physician know if they are diagnosed with COVID up to 9 months after study vaccine. Thus, passive follow-up will occur from 3 to 9 months after the 3rd dose of Moderna vaccine (or placebo). This will correspond to between 6 and 12 months of the initial vaccine dose.

Type of contact	**Consent**	**COVID-19 Vaccination**	**Visit 1 (Blood collection)**	**Phone calls Every Two weeks**	**Passive follow-up**
Time points	**Day 0**	**Day 7**	**4- 6 weeks post vaccination**	**Up to 3 months post-vaccination**	**Up to 9 months post-vaccination**
Eligibility review	●				
Review of signed consent form	●				
Blood draw			●		
Follow up calls				●	
Patient reporting of COVID diagnosis			●	●	●

5.5 Patient Confidentiality

Participants already have a unique study numerical identifier from the main study. Initially a separate list with participant identifier and study number will be kept in order to contact the participant arrange for blood collection post vaccination and for regular follow-up to ascertain if they developed COVID-19 infection despite vaccination. However, when the study is complete, this will be destroyed. Samples will only contain study number and date. Samples will be kept until the study is complete.

All records and documents pertaining to the study will be retained by the study trial site at UHN for up to 25 years from the completion of the study. Paper records will be stored in a secure filing cabinet in a locked office on UHN grounds. Baseline demographic data will be collected and then stored in an electronic database. The database will be stored on the UHN network accessed from a password protected computer located in a secure location in the hospital.

5.7 LABORATORY METHODS

5.7.1 Laboratory Methods:

Serology:

- Peripheral blood will be collected in red-top tubes and serum will be isolated via centrifugation. Measurement of geometric titres of COVID-19 Ab against the appropriate vaccine antigen (e.g. spike glycoprotein or nucleoprotein) will be performed using internally validated ELISA or chemiluminescent assays.

Cell-mediated Immunity:

PBMCs will be isolated in a subset of 50 patients. These will be analysed via flow cytometry for cytokine production after stimulation with COVID antigens.

5.8 OUTCOME MEASUREMENTS

5.8.1 Primary outcome:

The primary outcome will be the assessment of vaccine immunogenicity measured by antibody titre quantification in both arms of the study at 4-6 weeks post third dose of Moderna vaccine versus placebo

5.8.2 Secondary outcome:

- Safety and tolerability of vaccine (see analysis below)
- Cell-mediated immune response to COVID-19 antigens in a subset of patients
- Documented COVID-19 infection (i.e. microbiology proven by PCR from appropriate clinical specimen and compatible clinical syndrome) in the 6 months following vaccination.

6 STATISTICAL CONSIDERATIONS

6.1 SAMPLE SIZE JUSTIFICATION

We hypothesize that at least 33% of participants who are randomized to receive a third dose of the Moderna vaccine will have an antibody titer >100 AU/mL when measured at 4-6 weeks post vaccination compared to the expected 10% that have received two doses of vaccine.

For two independent study groups, to achieve a power of 80% and alpha level 0.05; at 1:1 randomisation, a sample size of 98 patients is required. We will aim to enrol all 120 patients to account for any loss to followup.

6.2 STATISTICAL ANALYSIS

- This will be a randomised control trial, placebo vs third dose of Moderna vaccine.
- Study groups will be compared for the primary and secondary outcomes; with comparison of 1) percentage of patients that achieve 100 AU/mL on serology using chi-squared test and 2) vaccine immune response (humoral response at 4-6 weeks post 3rd dose with Mann-Whitney U test assuming non-parametric distribution.
- Safety of vaccination will be analysed amongst the two groups (vaccine vs. placebo). Local/systemic adverse events during the first 7 days will be reported using descriptive analysis. Rate of biopsy-proven or clinically treated rejection will be compared between the two groups using Chi-squared or Fisher's exact test.

All analyses will be performed using SPSS version 25.0 and Prism Graphpad version 8.

8 SIGNIFICANCE OF THE STUDY

The administration of a third dose of Moderna vaccine has not been investigated or published previously. We have a patient population already enrolled in a large observational cohort study. This will be the first published interventional vaccine study in COVID-19 literature, to help inform and generalise to other SOTr and immunocompromised populations globally. We expect this study to be potentially practice changing and inform transplant society and vaccination committee guidelines.

9 ETHICAL CONSIDERATIONS

Our own data (as well as worldwide data) has shown that transplant recipients are disproportionately impacted by COVID and have a 2-5x greater mortality than the general population. They are also hospitalized at a high rate (55% hospitalizations) and use significant hospital and ICU resources. Also, because of immunosuppression, they have the potential to become super-spreaders. Since they shed virus for a prolonged period, the virus can evolve and generate variants. The U.K. B.1.1.7 strain is thought to have emerged in an immunocompromised patient. We have now found that these patients don't mount adequate antibody responses to mRNA COVID vaccine compared to the general population. Their COVID antibody (anti-RBD) levels are about 3 logs (1000-fold) lower after the second dose compared to healthy controls. Therefore, it is of utmost importance at both the individual and societal level to study a booster dose in these patients not only to protect patients individually and their potential contacts, but also to save healthcare resources if we can prevent these patients from severe COVID. We will be using a minimal number of doses to demonstrate this booster effect (only 60 doses in the whole study vs. around 100,000 doses administered per day in Ontario). Moreover, the knowledge generated from this very novel study (not being done elsewhere in the world to our knowledge) will help transplant and perhaps other immunocompromised patients worldwide.

References

1. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med.* 2020.

2. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a Single Dose of SARS-CoV-2 Messenger RNA Vaccine in Solid Organ Transplant Recipients. *JAMA.* 2021.
3. Boyarsky BJ, Ou MT, Greenberg RS, et al. Safety of the First Dose of SARS-CoV-2 Vaccination in Solid Organ Transplant Recipients. *Transplantation.* 9000;Online First.
4. Danziger-Isakov L, Kumar D. Vaccination of solid organ transplant candidates and recipients: Guidelines from the American society of transplantation infectious diseases community of practice. *Clin Transplant.* 2019;33(9):e13563.
5. Hirzel C, Kumar D. Influenza vaccine strategies for solid organ transplant recipients. *Curr Opin Infect Dis.* 2018;31(4):309-315.
6. Dos Santos G, Haguinet F, Cohet C, et al. Risk of solid organ transplant rejection following vaccination with seasonal trivalent inactivated influenza vaccines in England: A self-controlled case-series. *Vaccine.* 2016;34(31):3598-3606.
7. Natori Y, Shiotsuka M, Slomovic J, et al. A Double-Blind, Randomized Trial of High-Dose vs Standard-Dose Influenza Vaccine in Adult Solid-Organ Transplant Recipients. *Clin Infect Dis.* 2018;66(11):1698-1704.
8. Kates OS, Haydel BM, Florman SS, et al. COVID-19 in solid organ transplant: A multi-center cohort study. *Clin Infect Dis.* 2020.
9. Pereira MR, Mohan S, Cohen DJ, et al. COVID-19 in solid organ transplant recipients: Initial report from the US epicenter. *Am J Transplant.* 2020;20(7):1800-1808.

CONFIDENTIAL

Title

PREVent-COVID Sub-study: A randomised, double-blind, controlled trial of a third dose of Moderna vaccine versus placebo in solid organ transplant recipients

Protocol Number
UHN-TID-COVAX-2

Investigators

Deepali Kumar MD
Victoria Hall MBBS
Natalia Pinzon MSc
Victor Ferreira PhD
Atul Humar MD

Version 5
May 20, 2021

Institution

[1]Transplant Infectious Diseases, University Health Network, Toronto, Ontario, Canada

1. Background

This is a sub-study of an observational, comparative, cohort study "The PREVent-COVID" Study (CAPCR ID 20-6069) with additional participant recruitment from the multi-organ transplant (MOT) program vaccine clinic that ran from March-April 2021. .

There are now three COVID-19 vaccines with Health Canada approval that are currently being used for vaccination. The Moderna vaccine (mRNA-1273) has proven highly efficacious and safe in a phase III large, randomised control trial of 30,420 volunteers participants, and has been in use in Canada since December 2020.[1] Multiple other "real-world" studies post-approval have reflected these findings.

In solid organ transplant recipients (SOTr), there is still a paucity of data for the effectiveness of the available COVID-19 vaccines. However, Johns Hopkins University (JHU) has recently published their experience in 436 SOTr after one dose of either the Pfizer or Moderna COVID-19 vaccine; with an overall detectable antibody response in 76 out of 436 participants (17%, 95% CI, 14-21%).[2] A separate study in the same population confirmed safety and minimal adverse effects, with expected vaccine reactogenicity as experienced in the general population studied in the randomised phase III trial.[1,3] Data after two doses is expected to be published shortly.

In the PREVENT-COVID study, we have recruited 120 SOTr who have received both scheduled doses of the Moderna vaccine at 0 and 1 months. They have had blood work performed prior to vaccination, 3-4 weeks after the first dose, and 4-6 weeks after the second dose of vaccine to determine antibody and cell-mediated immunity. Side effects have been tracked with a vaccine diary and demographic data has been collected (age, sex, transplant organ, date of transplant, current immunosuppressive medications, recent rejection episodes).

Our preliminary analysis of 6 patients shows that there is no antibody response in any of these patients after the first dose of Moderna vaccine compared to 100% antibody response in healthy controls. Given the expected impaired immune response in terms of antibody titers and cell-mediated immunity, also based on data from JHU and previous experience with vaccination in this patient population; we propose that a third dose of the Moderna vaccine will act as a booster to pre-existing but blunted immunity.[2,4-6] This is expected to be safe with side effects as reflected by previously published data.[7]

This study will be a randomised, double-blind, placebo-controlled trial, with participants recruited, consented and then randomised from both the main PREVent-COVID study as well as those who have already received both scheduled doses of the Moderna vaccine (0 and 1 months) through the Multi-Organ Transplant vaccine clinic. Participants will be randomised to receive either a third dose of the Moderna vaccine or saline placebo at 3 months post initial vaccination.

2. Rationale

Pre-existing data suggest a reduction in the immune response to the available COVID-19 vaccines in SOTr.[2] There is previously published evidence for the safe administration of

booster doses of vaccines for other infections such as influenza to achieve protective antibody titers in the immunocompromised host, including solid organ transplant recipients.[5,7] An effective and durable vaccine response is desired in this immunocompromised population, who have expected worse outcomes if they develop COVID-19 infection due to their immunocompromised state, age and co-morbidities.[8,9] Therefore, it is paramount to investigate ways of optimising the vaccine response in this vulnerable population.

3. Study aim and hypothesis

3.1 Aim:

To investigate if a third dose of the Moderna vaccine at 3 months post initial vaccination will boost the antibody titer and immune response in solid organ transplant recipients.

3.2 Hypothesis:

A third dose of COVID-19 vaccine will provide a greater immune response compared to only two doses.

4. Study objectives

4.1 Primary objective

- To determine the immunogenicity of a third dose of Moderna vaccine given at 3 months post-initial vaccination in solid organ transplant recipients.

4.2 Secondary objectives

- Vaccine safety and side effects – patients will be asked to record side effects that occur daily up to 7 days after vaccination. Follow-up will occur for graft rejection and any health-care contact up to 3 months post-study vaccine.

5. Methods

5.1 Study design

This is a randomised, placebo-controlled, double blind study of transplant recipients who have previously received two doses of the Moderna COVID-19 vaccine.

We will enrol 120 participants who have already received both doses of the Moderna vaccine at 0 and 1 months. Participants will be either a) patients enrolled in the PREVENT-COVID study, who have received their second dose of Moderna vaccine from March-April 2021 or b) others that are not enrolled in PREVENT-COVID but have also received Moderna vaccine with the last dose during March and April 2021.

Consented patients will be randomized 1:1 to receive a third dose of the Moderna vaccine (0.5mL intramuscular injection of Moderna COVID-19 vaccine) or saline placebo (0.5mL intramuscular injection of normal saline) in the deltoid muscle of the arm. Both participant and healthcare provider who administers the vaccine would be blinded to intervention versus placebo; Blood work would then be performed 4-6 weeks after immunization to determine immunogenicity.

Participants who have not previously been enrolled in the PREVENT-COVID study will have blood work performed pre-vaccination on the day of their vaccination visit.

At 4-6 weeks post intervention, all participants will provide 10mL of blood for serum. A subset of 50 participants will be asked to provide an additional 20mL of blood for cell-mediated immunity. These will be the same 50 participants that consented to the PBMC substudy in PREVenT-COVID.

5.1.2 Recruitment and consent process

- The study coordinator will contact all transplant recipients that are enrolled in the main PREVENT-COVID study and who have received both doses of the Moderna vaccine.
- For patients who received Moderna vaccine but are not enrolled in PREVENT-COVID, the initial contact will be through their clinical care team (transplant coordinator), who will let us know if the patient is interested in hearing more about the study. If this is the case, the study coordinator will then contact the patient.
- A telephone script will be used to introduce this study and initiate the consent process.
- Scenario A and B will be detailed in the telephone script if they are already enrolled in the PREVENT-COVID study or if they are not already enrolled.
- Questions about the study can be answered over the phone at this time.
- The study investigator(s) will be available in case of questions.
- Consent will then be finalised via email / file share; or if blood collection occurs at UHN, this can be re-iterated in person and a printed and signed consent form collected at this time, as an in-person consent.
- After the consent process is satisfied, study eligibility and screening for inclusion and exclusion criteria will take place.
- The participant will then be enrolled with their pre-existing unique study identifier from the main study, and then be randomised to the Moderna vaccine vs placebo.
- A new unique study identifier will be created if this is a new participant enrolment. For these patients, baseline and transplant demographics will need to be collected.
- The first study visit where the participant receives the third dose of Moderna vaccine or placebo then occurs after the participant has consented to take part in the study. At the first study visit, eligibility will again be reviewed.
- For those participants that have not been enrolled in the main PREVENT COVID study, pre-vaccination blood work will be performed at the first study visit.
- After they have had the vaccine or placebo, blood work will be organised within 4-6 weeks for all participants.

5.2 Study setting and vaccine or placebo administration

- Vaccine (or placebo) will be administered in clinic and the patient will be asked to wait 15 minutes as per standard procedure. Vaccine doses will be obtained from UHN vaccine supply.

5.3 Patient population

Inclusion criteria:

- Adult SOTr that are stable outpatients

- Already received two doses of vaccine at 0 and 1 months respectively of the Moderna vaccine
- Comply with blood collection 4-6 weeks after vaccination or placebo dose
- Able to provide informed consent

Exclusion criteria:

- Previous documented COVID-19 infection
- Febrile illness in the past one week
- Active therapy for Cytomegalovirus viremia
- Use of Rituximab in the past 6 months
- Ongoing or recent (in past 30 days) therapy for acute rejection
- Receipt of intravenous immunoglobulin (IVIG) in the past 30 days or planning to receive IVIG in the next 4 weeks
- Anaphylaxis or allergic reaction to initial Moderna vaccine

5.4 Follow-up

Patients will be followed every 2 weeks for 3 months after the third Moderna vaccine (or placebo) dose. This is 6 months after enrolment in the main PREVenT-COVID study. They will be telephoned every 2 weeks to determine whether they were diagnosed with COVID or if they have any COVID-related symptoms (fever, cough, myalgias, sore throat, runny nose, diarrhea, shortness of breath). We will also be reminding participants to let the study team know immediately if they have been diagnosed with COVID. We will also record all adverse events including serious adverse events. Non-routine health-care contacts including hospitalization and the reasons for these healthcare visits will be recorded every two weeks up to 3 months post-vaccination. These will be judged as related or unrelated to vaccine according to investigator judgement. We will specifically note if the patients have had any rejection – whether a biopsy was done, and whether rejection treatment was started. The final phone contact will be similar to the other calls. We will re-iterate COVID precautions and ask patients to let their transplant physician know if they are diagnosed with COVID up to 9 months after study vaccine. Thus, passive follow-up will occur from 3 to 9 months after the 3rd dose of Moderna vaccine (or placebo). This will correspond to between 6 and 12 months of the initial vaccine dose.

Type of contact	**Consent**	**COVID-19 Vaccination**	**Visit 1 (Blood collection)**	**Phone calls Every Two weeks**	**Passive follow-up**
Time points	**Day 0**	**Day 7**	**4- 6 weeks post vaccination**	**Up to 3 months post-vaccination**	**Up to 9 months post-vaccination**
Eligibility review	●				
Review of signed consent form	●				
Blood draw		● For patients not already enrolled in main	●		

		PREVENT COVID Study			
Follow up calls				●	
Patient reporting of COVID diagnosis			●	●	●

5.5 Patient Confidentiality

Participants already have a unique study numerical identifier from the main study. If they are not enrolled in the main PREVENT COVID Study, then a unique study identifier will be created.

Initially a separate list with participant identifier and study number will be kept in order to contact the participant arrange for blood collection post vaccination and for regular follow-up to ascertain if they developed COVID-19 infection despite vaccination. However, when the study is complete, this will be destroyed. Samples will only contain study number and date. Samples will be kept until the study is complete.

All records and documents pertaining to the study will be retained by the study trial site at UHN for up to 25 years from the completion of the study. Paper records will be stored in a secure filing cabinet in a locked office on UHN grounds. Baseline demographic data will be collected and then stored in an electronic database. The database will be stored on the UHN network accessed from a password protected computer located in a secure location in the hospital.

5.7 LABORATORY METHODS

5.7.1 Laboratory Methods:

Serology:

- Peripheral blood will be collected in red-top tubes and serum will be isolated via centrifugation. Measurement of geometric titres of COVID-19 Ab against the appropriate vaccine antigen (e.g. spike glycoprotein or nucleoprotein) will be performed using internally validated ELISA or chemiluminescent assays.

Cell-mediated Immunity:
PBMCs will be isolated in a subset of 50 patients. These will be analysed via flow cytometry for cytokine production after stimulation with COVID antigens.

5.8 OUTCOME MEASUREMENTS

5.8.1 Primary outcome:

The primary outcome will be the assessment of vaccine immunogenicity measured by antibody titre quantification in both arms of the study at 4-6 weeks post third dose of Moderna vaccine versus placebo

5.8.2 Secondary outcome:

- Safety and tolerability of vaccine (see analysis below)
- Cell-mediated immune response to COVID-19 antigens in a subset of patients
- Documented COVID-19 infection (i.e. microbiology proven by PCR from appropriate clinical specimen and compatible clinical syndrome) in the 6 months following vaccination.

6 STATISTICAL CONSIDERATIONS

6.1 SAMPLE SIZE JUSTIFICATION

We hypothesize that at least 33% of participants who are randomized to receive a third dose of the Moderna vaccine will have an antibody titer >100 AU/mL when measured at 4-6 weeks post vaccination compared to the expected 10% that have received two doses of vaccine.

For two independent study groups, to achieve a power of 80% and alpha level 0.05; at 1:1 randomisation, a sample size of 98 patients is required. We will aim to enrol all 120 patients to account for any loss to follow-up.

Given that we may not be able to enrol all 120 patients from the main PREVENT-COVID study, we will contact as many participants as needed from the MOT vaccine clinic to fulfill this sample size calculation.

6.2 STATISTICAL ANALYSIS

- This will be a randomised control trial, placebo vs third dose of Moderna vaccine.
- Study groups will be compared for the primary and secondary outcomes; with comparison of 1) percentage of patients that achieve 100 AU/mL on serology using chi-squared test and 2) vaccine immune response (humoral response at 4-6 weeks post 3rd dose with Mann-Whitney U test assuming non-parametric distribution.
- Safety of vaccination will be analysed amongst the two groups (vaccine vs. placebo). Local/systemic adverse events during the first 7 days will be reported using descriptive analysis. Rate of biopsy-proven or clinically treated rejection will be compared between the two groups using Chi-squared or Fisher's exact test.

All analyses will be performed using SPSS version 25.0 and Prism Graphpad version 8.

8 SIGNIFICANCE OF THE STUDY

The administration of a third dose of Moderna vaccine has not been investigated or published previously. We have a patient population already enrolled in a large observational cohort study. This will be the first published interventional vaccine study in COVID-19 literature, to help inform and generalise to other SOTr and immunocompromised populations globally. We

expect this study to be potentially practice changing and inform transplant society and vaccination committee guidelines.

9 ETHICAL CONSIDERATIONS

Our own data (as well as worldwide data) has shown that transplant recipients are disproportionately impacted by COVID and have a 2-5x greater mortality than the general population. They are also hospitalized at a high rate (55% hospitalizations) and use significant hospital and ICU resources. Also, because of immunosuppression, they have the potential to become super-spreaders. Since they shed virus for a prolonged period, the virus can evolve and generate variants. The U.K. B.1.1.7 strain is thought to have emerged in an immunocompromised patient. We have now found that these patients don't mount adequate antibody responses to mRNA COVID vaccine compared to the general population. Their COVID antibody (anti-RBD) levels are about 3 logs (1000-fold) lower after the second dose compared to healthy controls. Therefore, it is of utmost importance at both the individual and societal level to study a booster dose in these patients not only to protect patients individually and their potential contacts, but also to save healthcare resources if we can prevent these patients from severe COVID. We will be using a minimal number of doses to demonstrate this booster effect (only 60 doses in the whole study vs. around 100,000 doses administered per day in Ontario). Moreover, the knowledge generated from this very novel study (not being done elsewhere in the world to our knowledge) will help transplant and perhaps other immunocompromised patients worldwide.

References

1. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med.* 2020.
2. Boyarsky BJ, Werbel WA, Avery RK, et al. Immunogenicity of a Single Dose of SARS-CoV-2 Messenger RNA Vaccine in Solid Organ Transplant Recipients. *JAMA.* 2021.
3. Boyarsky BJ, Ou MT, Greenberg RS, et al. Safety of the First Dose of SARS-CoV-2 Vaccination in Solid Organ Transplant Recipients. *Transplantation.* 9000;Online First.
4. Danziger-Isakov L, Kumar D. Vaccination of solid organ transplant candidates and recipients: Guidelines from the American society of transplantation infectious diseases community of practice. *Clin Transplant.* 2019;33(9):e13563.
5. Hirzel C, Kumar D. Influenza vaccine strategies for solid organ transplant recipients. *Curr Opin Infect Dis.* 2018;31(4):309-315.
6. Dos Santos G, Haguinet F, Cohet C, et al. Risk of solid organ transplant rejection following vaccination with seasonal trivalent inactivated influenza vaccines in England: A self-controlled case-series. *Vaccine.* 2016;34(31):3598-3606.

7. Natori Y, Shiotsuka M, Slomovic J, et al. A Double-Blind, Randomized Trial of High-Dose vs Standard-Dose Influenza Vaccine in Adult Solid-Organ Transplant Recipients. *Clin Infect Dis.* 2018;66(11):1698-1704.
8. Kates OS, Haydel BM, Florman SS, et al. COVID-19 in solid organ transplant: A multi-center cohort study. *Clin Infect Dis.* 2020.
9. Pereira MR, Mohan S, Cohen DJ, et al. COVID-19 in solid organ transplant recipients: Initial report from the US epicenter. *Am J Transplant.* 2020;20(7):1800-1808.

Summary of Protocol Amendment:

Original Protocol Version: April 22, 2021

Final Protocol Version: May 20, 2021

Number of Amendments: 1

Type of Amendment: Modification to Recruitment

Description: In the original protocol, patients are recruited to participate in the randomized trial from those that are already enrolled an ongoing observational study of COVID-19 vaccine in organ transplant recipients (PREVENT-COVID). However, in order to ensure that sample size requirements are met, eligibility was expanded to also include transplant recipients that were not enrolled in PREVENT-COVID but received mRNA-1273 vaccine at 0,1 month with the last dose during March and April 2021.

Title

PREVent-COVID Sub-study: A randomised, double-blind, controlled trial of a third dose of Moderna vaccine versus placebo in solid organ transplant recipients

Protocol Number
UHN-TID-COVAX-2

Plan for Statistical analysis and other Statistical Considerations:

Version 1.0
April 22, 2021

Institution

[1]Transplant Infectious Diseases, University Health Network, Toronto, Ontario, Canada

STATISTICAL CONSIDERATIONS

PART A: SAMPLE SIZE JUSTIFICATION

Primary Hypothesis: We hypothesize that at least 33% of participants who are randomized to receive a third dose of the Moderna vaccine will have an antibody titer ≥100 units/mL when measured at 4-6 weeks post vaccination compared to the expected 10% that have received two doses of vaccine.

Sample Size: For two independent study groups, to achieve a power of 80% and alpha level 0.05; at 1:1 randomisation, a sample size of 98 patients is required. We will aim to enrol all 120 patients to account for any loss to follow-up.

Part B: Population definitions

Intention-to-treat population: Consented for the trial and assessments, and received the study vaccine (intervention).

Per-Protocol-population: provided follow blood at 4-6 weeks post-vaccine.

PART C: STATISTICAL ANALYSIS

1. Group Demographics

The following demographics will be ascertained for the whole cohort and then for the cohort receiving Moderna and the cohort receiving placebo. Group demographics will be ascertained using the intent-to-treat population. Descriptive statistics will be used for the following variables (includes number, percentage, median and IQR where applicable). No statistical comparison between groups is planned as groups will be randomized.

a) Type of organ transplant
b) Time from organ transplant. For patients with re-transplant, time from the first organ transplant will be used as the starting time. For example if a patient received their first kidney transplant on January 1st 2005 and their second kidney transplant on January 1st 2010, the time will be calculated from January 1st 2005. (median; IQR)
c) Sex of the patient
d) Age of the patient (median; IQR)
e) Immunosuppression: all antirejection drugs the patient is on at the time of the intervention (third dose booster). Includes but not limited to prednisone, tacrolimus, cyclosporine, mycophenolate (or derivatives), azathioprine, sirolimus. Current median daily dosage and range will be described. For drugs where TDM is performed, the closest level to the time

of the intervention will be used to analyze median blood level (median; IQR). Past receipt of thymoglobulin, and rituximab will be analyzed (prior 6 months).

f) Lymphocyte count in cells/µl (median; IQR).

2. Safety Evaluation (Secondary Endpoint)

Safety evaluation to be done in the intention-to-treat population using a participant-directed vaccine diary for local and systemic adverse events each day for the 7 days after injection plus contact patients every two weeks post intervention and chart review for episodes of acute organ rejection, hospitalization, other adverse events, or COVID-19 infection. Patients will continue to be followed passively for additional safety events out to 9 months post-intervention. Descriptive statistics will be used to describe safety events. No statistical comparison between placebo and Moderna are planned.

Vaccine adverse events will be divided into local and systemic.
Adverse events toxicity scale (FDA) as follows (use the highest reported for the event during the 7-days for the analysis):

a) grade 1 (no interference in daily activities)
b) grade 2 (some interference in daily activities)
c) grade 3 (participants unable to perform daily activities)
d) grade 4 (potentially life threatening)

Overall percentage frequency of the following local and systemic events for placebo and Moderna

a) Pain
b) Erythema
c) Swelling
d) Fever
e) Chills
f) Fatigue
g) Myalgias
h) Arthralgias
i) Headache
j) Nausea/vomiting
k) Diarrhea
l) Rash
m) Other

3. Primary and Secondary Endpoints:

Primary endpoint: The percentage of patients that achieve an antibody level of ≥100 units/ml at the 4-6 week time-point post-intervention.

The percentage of patients in each arm that achieve the primary endpoint threshold at 4-6 weeks post intervention will be calculated (n (%)). The two arms will be compared using a corrected chi-squared test with a relative risk ratio along with 95% confidence interval.

Further details:
The primary endpoint will be based upon determination of antibody against the spike protein (receptor binding domain - RBD) at 4-6 weeks after the intervention. The primary endpoint is defined as the percentage of patients that achieve a threshold response (i.e. a positive response-see below). Antibody levels will also be determined prior to the intervention as a baseline (pre-intervention antibody level).

The threshold is based on a non-human primate study by McMahan et al. Nature 2021 and further by our own institutional data in transplant patients infected with COVID-19 (Marinelli et al; Transplantation).

The primary endpoint will be analyzed using the Per-Protocol-Population (patients who have provided follow-up blood at the 4-6 week post-intervention time point).

Definition of a positive antibody response: Threshold for defining a positive is 100 Units/ml; Use ≥ 100 U/ml as meaning a positive antibody response.

For secondary endpoints analysis will be as follows:

a) Assess pre-intervention antibody levels (i.e. baseline): absolute level (median; IQR) and percentage meeting threshold value (≥100) to ensure groups are balanced pre-intervention. Change in pre-post antibody level (paired data; Wilcoxon rank sum test)
b) Median levels of antibody post-intervention at the 4-6 week time period (median; IQR); compare using Mann-Whitney U test assuming non-parametric distribution.
c) Calculate fold-change in antibody level by using post-antibody divided by pre-antibody. If pre-or post intervention antibody is negative use a value of ½ the detection threshold for calculation. Compare fold-change difference in two groups (median and mean fold change).
d) Calculate T-cell response by using the number of SARS-CoV-2 specific CD4 and CD8 T-cells that produce both IFN-γ and IL-2 (defined as polyfunctional). The number is expressed as number of positive cells per 10^6 CD4 cells or CD8 cells respectively; (median; IQR with comparison between Placebo and Moderna post-third dose using Mann-Whitney U test). Baseline (pre-intervention) median number of positive cells to ensure balance of the groups pre-intervention (median; IQR).

Analytic tools:

All analyses will be performed using SPSS version 25.0 and Prism Graphpad version 8. Graphpad will be used to generate figures/graphs.

Title

PREVent-COVID Sub-study: A randomised, double-blind, controlled trial of a third dose of Moderna vaccine versus placebo in solid organ transplant recipients

Protocol Number
UHN-TID-COVAX-2

Plan for Statistical analysis and other Statistical Considerations:

Version 2.0
June 2nd, 2021

Institution

[1]Transplant Infectious Diseases, University Health Network, Toronto, Ontario, Canada

STATISTICAL CONSIDERATIONS

PART A: SAMPLE SIZE JUSTIFICATION

Primary Hypothesis: We hypothesize that at least 33% of participants who are randomized to receive a third dose of the Moderna vaccine will have an antibody titer ≥100 units/mL when measured at 4-6 weeks post vaccination compared to the expected 10% that have received two doses of vaccine.

Sample Size: For two independent study groups, to achieve a power of 80% and alpha level 0.05; at 1:1 randomisation, a sample size of 98 patients is required. We will aim to enrol all 120 patients to account for any loss to follow-up.

Part B: Population definitions

Intention-to-treat population: Consented for the trial and assessments, and received the study vaccine (intervention).

Per-Protocol-population: provided follow blood at 4-6 weeks post-vaccine.

PART C: STATISTICAL ANALYSIS

1. Group Demographics

The following demographics will be ascertained for the whole cohort and then for the cohort receiving Moderna and the cohort receiving placebo. Group demographics will be ascertained using the intent-to-treat population. Descriptive statistics will be used for the following variables (includes number, percentage, median and IQR where applicable). No statistical comparison between groups is planned as groups will be randomized.

a) Type of organ transplant
b) Time from organ transplant. For patients with re-transplant, time from the first organ transplant will be used as the starting time. For example if a patient received their first kidney transplant on January 1st 2005 and their second kidney transplant on January 1st 2010, the time will be calculated from January 1st 2005. (median; IQR)
c) Sex of the patient
d) Age of the patient (median; IQR)
e) Immunosuppression: all antirejection drugs the patient is on at the time of the intervention (third dose booster). Includes but not limited to prednisone, tacrolimus, cyclosporine, mycophenolate (or derivatives), azathioprine, sirolimus. Current median daily dosage and range will be described. For drugs where TDM is performed, the closest level to the time

of the intervention will be used to analyze median blood level (median; IQR). Past receipt of thymoglobulin, and rituximab will be analyzed (prior 6 months).

f) Lymphocyte count in cells/µl (median; IQR).

2. Safety Evaluation (Secondary Endpoint)

Safety evaluation to be done in the intention-to-treat population using a participant-directed vaccine diary for local and systemic adverse events each day for the 7 days after injection plus contact patients every two weeks post intervention and chart review for episodes of acute organ rejection, hospitalization, other adverse events, or COVID-19 infection. Patients will continue to be followed passively for additional safety events out to 9 months post-intervention. Descriptive statistics will be used to describe safety events. No statistical comparison between placebo and Moderna are planned.

Vaccine adverse events will be divided into local and systemic.
Adverse events toxicity scale (FDA) as follows (use the highest reported for the event during the 7-days for the analysis):

a) grade 1 (no interference in daily activities)
b) grade 2 (some interference in daily activities)
c) grade 3 (participants unable to perform daily activities)
d) grade 4 (potentially life threatening)

Overall percentage frequency of the following local and systemic events for placebo and Moderna

a) Pain
b) Erythema
c) Swelling
d) Fever
e) Chills
f) Fatigue
g) Myalgias
h) Arthralgias
i) Headache
j) Nausea/vomiting
k) Diarrhea
l) Rash
m) Other

3. Primary and Secondary Endpoints:

Primary endpoint: The percentage of patients that achieve an antibody level of ≥100 units/ml at the 4-6 week time-point post-intervention.

The percentage of patients in each arm that achieve the primary endpoint threshold at 4-6 weeks post intervention will be calculated (n (%)). The two arms will be compared using a corrected chi-squared test with a relative risk along with 95% confidence interval.

Further details:
The primary endpoint will be based upon determination of antibody against the spike protein (receptor binding domain - RBD) at 4-6 weeks after the intervention using the Roche Elecsys anti-SARS-CoV-2 S enzyme immunoassay. The primary endpoint is defined as the percentage of patients that achieve a threshold response (i.e. a positive response-see below). Antibody levels will also be determined prior to the intervention as a baseline (pre-intervention antibody level). For the primary endpoint an adjusted RR estimate with 95% CI will also be calculated using a logistic regression model with baseline log(anti-RBD) titer as a covariate;

The threshold is based on a non-human primate study by McMahan et al. Nature 2021 and further by our own institutional data in transplant patients infected with COVID-19 (Marinelli et al; Transplantation). Additional corroboration is from Khoury et al. (Nature 2021) that looked at correlates of protection in a large cohort, validating the primary endpoint as the upper bound of the estimated 95% CI that correlates with 50% protective neutralization.

The primary endpoint will be analyzed using the Per-Protocol-Population (patients who have provided follow-up blood at the 4-6 week post-intervention time point).

Definition of a positive antibody response: Threshold for defining a positive is 100 Units/ml; Use ≥ 100 U/ml as meaning a positive antibody response.

For secondary endpoints analysis will be as follows:

a) Assess pre-intervention antibody levels (i.e. baseline): absolute level (median; IQR) and percentage meeting threshold value (≥100) to ensure groups are balanced pre-intervention. Change in pre-post antibody level (paired data; Wilcoxon rank sum test)
b) Median levels of antibody post-intervention at the 4-6 week time period (median; IQR); compare using Mann-Whitney U test assuming non-parametric distribution.
c) Calculate fold-change in antibody level by using post-antibody divided by pre-antibody. If pre-or post intervention antibody is negative use a value of ½ the detection threshold for calculation. Compare fold-change difference in two groups (median and mean fold change).
d) Calculate T-cell response by using the number of SARS-CoV-2 specific CD4 and CD8 T-cells that produce both IFN-γ and IL-2 (defined as polyfunctional). The number is expressed as number of positive cells per 10^6 CD4 cells or CD8 cells respectively; (median; IQR with comparison between Placebo and Moderna post-third dose using Mann-Whitney U test). Baseline (pre-intervention) median number of positive cells to ensure balance of the groups pre-intervention (median; IQR).
e) The Genscript SVNT (surrogate virus neutralization test) has become available and would provide additional insight into functional and neutralizing antibodies. The SVNT assay provides a neutralization percentage (0-100%). As per test manufacturer's instructions, a

threshold value of greater than 30% is considered positive for neutralizing antibody. Analysis plan will be both the absolute SVNT percentage value between Moderna and placebo (median percent neutralization) using a Mann-Whitney test and the percentage of patients that achieve >30% threshold value in the two study arms using a corrected Chi-square test.

Analytic tools:

Statistical analyses will be performed using 'R' Version 4.03, and Prism Graphpad version 9.1.1. Graphpad will be used to generate figures/graphs.

Summary of Statistical Analysis Plan Amendment:

Original SAP Version: April 22, 2021

Final SAP Version: June 2nd, 2021

Number of Amendments: 5
Timing of amendment in relation to enrollment: After commencement of enrolment but prior to collection of first follow-up blood.

Type of Amendment: (1) Addition of SVNT Neutralizing Antibody; (2) Statistical package change to R and update of Graphpad version 3) addition of adjusted RR (adjusted for baseline antibody level). 4) addition of manufacturer of the antibody test (anti-RBD) (Roche). 5)additional corroboration of primary endpoint based on recently published study

Description:

1. The Genscript SVNT (surrogate virus neutralization test) has become available and would provide additional insight into functional and neutralizing antibodies. The SVNT assay provides a neutralization percentage (0-100%). As per test manufacturer's instructions, a threshold value of greater than 30% are considered positive for neutralizing antibody. Analysis plan will be both the absolute SVNT percentage value between Moderna and placebo (median percent neutralization) using a Mann-Whitney test and the percentage of patients that achieve >30% threshold value in the two study arms using corrected Chi-square test.
2. Statistics will be performed using 'R' (Version 4.03)) and the Version of Prism Graphpad has been updated to Version 9.1.1 (from Version 8).
3. An additional adjusted RR estimate for the primary endpoint with 95% CI will be done using a logistic regression model with baseline anti-RBD as a co-variate.
4. The specific antibody kit for the study has been specified as the Roche Elecsys anti-SARS-CoV-2 S enzyme immunoassay.
5. Additional corroboration and justification of the pre-selected primary endpoint is from Khoury et al. (Nature 2021) that looked at correlates of protection in a large cohort, validating the primary endpoint as the upper bound of the estimated 95% CI that correlates with 50% protective neutralization.