US Platform to Measure Effectiveness of Seasonal Influenza, COVID-19 and other Respiratory Virus Vaccines for the Prevention of Acute Illness in Ambulatory Settings

Component D: Immunologic Study of Response to Influenza and COVID-19 Virus Vaccination

Randomized study of the immunogenicity and duration of antibody response against circulating SARS-CoV-2 variant and influenza viruses following concomitant versus sequential administration of mRNA COVID-19 vaccine and quadrivalent cell culture-based influenza vaccine among children and adults

Short Title: Comparative immunogenicity of concomitant vs sequential mRNA COVID-19 and influenza vaccinations

Statistical Analysis Plan

Version 1.0

NCT06020118

January 23, 2025

Table of Contents

LIST OF ABBREVIATIONS

ACE2	Angiotensin Converting Enzyme 2
ARI	Acute Respiratory Illness
BCR	B cell receptor
ccIIV4	Flucelvax Quadrivalent
GMT	Geometric Mean Titers
GMFR	Geometric Mean Fold Rise
HAI	Hemagglutination Inhibition
IRB	Internal Review Board
LAR	Legally Authorized Representative
MN	Microneutralization
PBMC	Peripheral Blood Mononuclear Cells
RT-PCR	Reverse-Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
TCR	T cell receptor

1 INTRODUCTION

This document describes the statistical procedures that will be utilized for the study of Comparative Immunogenicity of Concomitant vs Sequential mRNA COVID-19 and Influenza Vaccinations. This statistical analysis plan (SAP) describes the methods of statistical analysis. In this study, participants will be randomly assigned to receive an approved quadrivalent cell culture-based influenza vaccine (ccIIV4) and mRNA COVID-19 vaccine (Moderna) either concomitantly or sequentially, 28 days apart. Participants (aged 6-11 years and 18-64 years) will be enrolled in the 2023-2024 influenza season. Enrolled participants will be randomized to one of the following interventions (2:1:1) using a permuted block method stratified by age group (aged 6-11 years and 18-64 years) and site: (1) concomitant administration of the mRNA COVID-19 vaccine (Moderna) and quadrivalent influenza vaccine (ccIIV4); (2) sequential administration of the quadrivalent influenza vaccine (ccIIV4) at Visit 1 (day 0) and the mRNA COVID-19 vaccine (Moderna) at Visit 2 (day 28); (3) sequential administration of the mRNA COVID-19 vaccine (Moderna) at Visit 1 (day 0) followed by the quadrivalent influenza vaccine (ccIIV4) at Visit 2 (day 28).

2 PROTOCOL OBJECTIVES

2.1 Primary

PO1: To determine baseline and post-vaccination influenza antibody titers by hemagglutination inhibition (HAI against ccIIV4 vaccine antigens following concomitant administration with mRNA vaccine or alone.

2.2 Exploratory

EO1: To determine baseline and post-vaccination influenza antibody titers by microneutralization (MN), in a subset of samples, against ccIIV4 vaccine antigens following concomitant administration with mRNA vaccine or alone.

EO2: To determine baseline and post-vaccination SARS-CoV-2 antibody binding titers and ACE2 binding inhibition and viral neutralization, on a subset of specimens, against Moderna mRNA COVID-19 vaccine antigens following concomitant administration with ccIIV4 or alone.

EO3: To determine duration and rate of waning of influenza HAI and MN antibody titers against ccIIV4 vaccine antigens and circulating influenza viruses.

EO4: To determine duration of and rate of waning of SARS-CoV-2 antibody titers and viral neutralization titers, on a subset of samples, against Moderna mRNA COVID-19 vaccine antigens and circulating SARS-CoV-2 variants.

EO5: To determine influenza HAI and MN antibody titers against circulating influenza viruses during baseline and post-vaccination or post-infection.

EO6: To determine SARS-CoV-2 antibody binding titers and viral neutralization titers (in a subset of samples) against circulating SARS-CoV-2 variants during baseline and post-vaccination.

EO7: To compare the frequencies of antigen-specific T and B cells utilizing conventional ELISPOT assays, high-dimensional flow cytometric analysis of innate and adaptive immune cell

phenotype characterization using fluorochrome-conjugated antigen tags, and intracellular cytokine staining to 2023-24 ccIIV4 and mRNA COVID-19 vaccines administered either concomitantly or sequentially, 28 days apart.

EO8: To compare transcriptome, B cell receptor (BCR) and T cell receptor (TCR) diversity of antigen-specific cells and assess serological antibody repertoire (clonotypes) using 10X genomics platform, and liquid chromatography/mass spectroscopy in a subset of participants.

EO9: To investigate the waning of antigen-specific B cells and antibody clonotypes in a subset of participants.

EO10: To describe safety of concomitant versus sequential administration of ccIIV4 and mRNA COVID-19 vaccination.

3 STUDY ENDPOINTS

3.1 Primary

i. Proportion of participants in each vaccination group with a seroprotective HAI (in a subset of samples) titer (≥1:40) pre- and post-ccIIV4 immunization for each 2023-24 influenza vaccine antigen
ii. Proportion of participants in each vaccination group achieving HAI seroconversion (a titer ≥1:40 following ccIIV4 if the baseline titer is <1:10 or a four-fold rise in titer if the baseline titer is >1:10) following ccIIV4 vaccination for each 2023-24 influenza vaccine antigen
iii. HAI GMT for each 2023-24 influenza vaccine antigen pre- and post-ccIIV4 in each vaccination group
iv. GMFR in HAI titers for each 2023-24 influenza vaccine antigen in each vaccination group

3.2 Exploratory

EO1:

I. Proportion of participants in each vaccination group with a seroprotective MN (in a subset of samples) titer (≥1:40) pre- and post-ccIIV4 immunization for each 2023-24 influenza vaccine antigen
II. Proportion of participants in each vaccination group achieving MN (in a subset of samples) seroconversion (a titer ≥1:40 following ccIIV4 if the baseline titer is <1:10 or a four-fold rise in titer if the baseline titer is >1:10) following ccIIV4 vaccination for each 2023-24 influenza vaccine antigen
III. HAI (in a subset of samples) MN for each 2023-24 influenza vaccine antigen pre- and post-ccIIV4 in each vaccination group
IV. GMFR in MN (in a subset of samples) titers for each 2023-24 influenza vaccine antigen in each vaccination group

EO2. GMT for SARS-CoV-2 binding antibody titer and viral neutralization titer (for a subset of samples) pre- and post-vaccination with mRNA COVID-19 vaccine

EO3:

I. HAI and MN GMT for each 2023-24 influenza vaccine and circulating influenza virus antigens at visit 1, visit 2, visit 3, and visit 4 for each vaccination group
II. Geometric mean fold change in HAI and MN titers for each vaccination group from visit 1 to visit 2, visit 3, and visit 4 for each 2023-24 influenza vaccine and circulating influenza virus antigen

EO4:

I. SARS-CoV-2 binding antibody and viral neutralization GMT for each mRNA COVID-19 vaccine and circulating SARS-CoV-2 virus antigens at visit 1, visit 2, visit 3, and visit 4 for each vaccination group
II. Geometric mean fold change in SARS-CoV-2 and viral neutralization (in a subset of samples) titers for each vaccination group from visit 1 to visit 2, visit 3, and visit 4 for each COVID-19 Moderna mRNA COVID-19 vaccine and circulating SARS-CoV-2 virus antigen

EO5

I. Proportion of participants in each vaccination group with a seroprotective titer (≥1:40) pre- and post-ccIIV4 immunization (visits 1-3) and/or pre- and post-influenza infection (acute and convalescent visits), if applicable, for each 2023-24 circulating influenza virus antigen
II. Proportion of participants in each vaccination group achieving seroconversion (a titer ≥1:40 following ccIIV4 if the baseline titer is <1:10 or a four-fold rise in titer if the baseline titer is >1:10) following ccIIV4 vaccination (visit 1 to visits 2-3) and/or influenza infection (acute to convalescent visits), if applicable, for each 2023-24 circulating influenza virus antigen
III. HAI and MN GMT for each 2023-24 circulating influenza virus antigen pre- and post-ccIIV4 (visits 1-3) and/or pre- and post-influenza infection (acute and convalescent visits), if applicable, in each vaccination group
IV. GMFR in HAI or MN titers for each vaccination group following ccIIV4 vaccination (visit 1 to visits 2-3) and/or influenza infection (acute to convalescent visits), if applicable, for each 2023-24 circulating influenza virus antigen

EO6:

I. SARS-CoV-2 binding antibody and viral neutralization (for a subset of samples) titers GMT at pre- and post-vaccination (visits 1-3) and/or pre- and post-SARS-CoV-2 infection (acute and convalescent visits), if applicable, against 2023-24 circulating SARS-CoV-2 virus antigens
II. GMFR in SARS-CoV-2 binding antibody and viral neutralization (for a subset of samples) titers following mRNA COVID-19 vaccination (visit 1 to visits 2-3) and/or SARS-CoV-2 infection (acute to convalescent visits), if applicable, against 2023-24 circulating SARS-CoV-2 virus antigens

EO7:

I. The frequency of antigen-specific T and B cells to influenza and SARS-CoV-2 at visit 1, visit 2, visit 3, visit 4, and acute and convalescent visits, if applicable
II. High-dimensional flowcytometric analyses of innate and adaptive immune cells
III. ELISPOT evaluation for SARS-CoV-2 memory B cells

EO8: BCR and TCR diversity and clonal dominance (antibody clonotypes) of serological repertoire in a subset of participants

EO9: The frequency of antigen-specific B cells and clonal dominance (antibody clonotypes) to influenza and SARS-CoV-2 at visit 1, visit 2, visit 3, visit 4, and acute and convalescent visits (if applicable) in a subset of participants

E10:

I. Number and percent of participants reporting fever or injection site and/or systemic reactions during the first 3 days following each vaccination visit stratified by severity and, for those reporting symptoms, their impact on health
II. Number and percent of participants reporting a change in health status requiring hospital admission (including a description of the event) during the study period

4 STUDY DESIGN

4.1 Study Description

This study is a randomized immunogenicity study in an enrolled cohort with active surveillance for acute respiratory illness (ARI). During this study, participants will be randomly assigned to receive an approved quadrivalent influenza vaccine (Flucelvax [ccIIV4]) and mRNA COVID-19 vaccine (Moderna) either concomitantly (given in contralateral arms) or sequentially (28 days apart).

Blood samples from participants will be collected for measurement of biomarkers of immune response at baseline (visit 1; day 0), post-vaccination 1 (visit 2; day 28), post-vaccination 2 (visit 3; day 56), and post-season (visit 4; day 180). Serum and peripheral blood mononuclear cells (PBMC) and plasma (target to collect PBMC and plasma samples at every visit from a subset of approximately 250 participants; 200 adults and 50 children) samples will be isolated from whole blood and tested for biomarkers of vaccine immunogenicity, and duration of antibody responses.

Participants will receive electronic surveys via email or text message three days after each vaccination asking about vaccine reactogenicity and weekly asking about change in health status and new ARI symptoms; those reporting illness may be asked to provide a respiratory swab for laboratory testing for influenza and SARS-CoV-2 and up to 2 additional blood draws (acute [< 10 days after symptom onset] and convalescent [28 days after acute visit if lab-confirmed positive for influenza or SARS-CoV-2]).

4.2 Laboratory

Blood samples from participants will be collected for measurement of biomarkers of immune response at Visit 1 (day 0; baseline), Visit 2 (day 28; post-vaccination 1), Visit 3 (day 56; post-vaccination 2), and Visit 4 (day 180; post-season). Sera, PBMC, and plasma samples will be tested for biomarkers of vaccine immunogenicity, duration of antibody responses, and immune response to circulating SARS-CoV-2 variants and influenza virus strains.

4.3 Randomization

Permuted block randomization stratified by age group (children aged 6-11 years and adults aged 18-64 years) and site will be used at each site to ensure even allocation to each study arm. At each site, participants will be randomized 2:1:1 to receive: (i) concomitant administration of a mRNA COVID-19 vaccine and a quadrivalent influenza vaccine (ccIIV4), (ii) sequential administration of a quadrivalent influenza vaccine (ccIIV4) at Visit 1 (day 0) and a mRNA COVID-19 vaccine at Visit 2 (day 28), and (iii) sequential administration of a mRNA COVID-19 vaccine at Visit 1 (day 0) and a quadrivalent influenza vaccine (ccIIV4) at Visit 2 (day 28).

The project statistician will generate the randomization schemes which will be uploaded to REDCap. The randomization schedule will not be available to the study staff, so the next randomization allocation will not be known before randomization occurs. Following confirmation of study eligibility criteria during Visit 1, participant randomization will be through REDCap with treatment allocation recorded on the case report form (CRF).

In the event that REDCap is unavailable, participants will be manually randomized through the use of sealed envelopes with randomization allocations enclosed. The project statistician will prepare envelopes that will use the same randomization strategy as the primary scheme embedded in REDCap. When an unblinded team member is informed of the age group, they will pull the next envelope in order. To capture the allocation per subject, a separate form in REDCap will be used by the unblinded study personnel to add the assignment. Sites will need to keep a log capturing these instances. The next available sequential study-number will be assigned to each enrolled participant upon study-entry.

4.4 Blinding

Participants will not be blinded to vaccine group and will receive two vaccines in separate limbs (for the concomitant vaccination [group i]) or one vaccine at Visit 1 (day 0) and a second vaccine at Visit 2 (day 28) (for sequential vaccination [groups ii and iii]) in an unblinded manner.

4.5 Data Collection and Storage

Data will be handled according to the Duke Vaccine and Trials Unit Standard Operation Procedure (SOP) (DVTU M010). Data will be captured electronically in a REDCap database. The enrollment questionnaire may be administered by research staff or completed online by the participant. Follow-up surveys will be completed online by the participant.

6 ANALYSIS POPULATIONS

6.1 Intent-to-Treat (ITT)

The ITT population will include any participant who was enrolled and randomized.

6.2 Modified Intent-to-Treat (mITT)

The mITT population will include any participant who was enrolled, randomized, and received both vaccines.

6.3 Influenza Immunogenicity Population

The Influenza Immunogenicity Population is a subset of the mITT Population that includes only subjects who received both vaccines, provide visit 1 and visit 2 blood draws available with HAI titer results for analysis within the protocol-defined time frame, did not have an influenza or SARS-CoV-2 infection between visits 1 and 2, and had no protocol violations affecting immunogenicity. Protocol violations affecting the immunogenicity analyses are defined in the Statistical Analysis Plan (SAP) Appendix 1. The acceptable visit window for sample analysis will be +14 days from the target date. Should 10% or more of the total study population be excluded from the Influenza Immunogenicity Population due to missing the visit window, then an additional analysis will be performed including these subjects with the missing visit window exclusion.

6.4 SARS-CoV-2 Immunogenicity Population

The SARS-CoV-2 Immunogenicity Population is a subset of the mITT Population that includes only subjects who received both vaccines, provide visit 1 and visit 2 blood draws available with MSD results for analysis within the protocol-defined time frame, did not have an influenza or SARS-CoV-2 infection between visits 1 and 2, and had no protocol violations affecting immunogenicity. Protocol violations affecting the immunogenicity analyses are defined in the Statistical Analysis Plan (SAP) Appendix 1. The acceptable visit window for sample analysis will be +14 days from the target date. Should 10% or more of the total study population be excluded from the SARS-CoV-2 Immunogenicity Population due to missing the visit window, then an additional analysis will be performed including these subjects with the missing visit window exclusion.

7 GENERAL ISSUES FOR STATISTICAL ANALYSIS

Data analysis will be performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC) or R software (R Core Team, Vienna, Austria).

7.1 Multicenter Studies

There are 7 sites participating in the study. Data will be pooled across investigational sites for analysis purposes. A fixed site effect will be included in all analyses of the primary objective.

7.2 Handling of Dropouts, Missing Data

Missing immunogenicity values are considered missing completely at random (MCAR) and imputation methods will not be used.

8 STUDY PARTICIPANTS

8.1 Disposition

The number of enrolled participants will be presented in a flow chart by age and randomization group. The number of visits completed and missed will be presented, along with a breakdown of the analysis populations.

8.2 Demographics

Demographic information including age, sex, race, ethnicity, and enrollment site will be presented by treatment group. Summary statistics (e.g., mean, standard deviation, median) will be presented for continuous variables. Categorical variables will be described with frequencies and percentages. Demographics for the ITT population will be presented.

9 ANALYSIS OF STUDY OBJECTIVES

9.1 Primary Objective

The primary objective will be analyzed using the immunogenicity population. All endpoints will be assessed with two-sided tests and alpha set to 0.05.

Endpoint i:

The proportion of participants with a seroprotective HAI before and 28-42 days after immunization will be compared between group i (concomitant administration) and ii (influenza administered first), using the following null (H_0) and alternative (H_1) hypotheses:

Ho: Simultaneous group – Sequential group = 0

$$H_0: \pi_1 - \pi_2 = 0$$

$$H_1: \pi_1 - \pi_2 \neq 0$$

where π is the seroprotective rate.

P values and confidence intervals for the geometric mean titer ratio will be estimated using a logistic regression adjusted for site.

Endpoint ii:

The proportion of participants achieving seroconversion will be compared between group i (concomitant administration) and ii (influenza administered first), using the following null (H_0) and alternative (H_1) hypotheses:

Ho: Simultaneous group – Sequential group = 0

$$H_0: \pi_1 - \pi_2 = 0$$

$$H_1: \pi_1 - \pi_2 \neq 0$$

where π is the seroconversion rate.

P values and confidence intervals for the geometric mean titer ratio will be estimated using a logistic regression adjusted for site.

Endpoint iii:

GMTs before and 28-42 days after immunization will be compared between group i (concomitant administration) and ii (influenza administered first), using the following null (H_0) and alternative (H_1) hypotheses:

$$H_0: \mu_1/\mu_2 = 1$$

$$H_1: \mu_1/\mu_2 \neq 1$$

where μ is the geometric mean titer.

P values and confidence intervals for the geometric mean titer ratio will be estimated using a generalized estimating equation linear regression accounting for site clustering.

Endpoint iv:

Geometric mean fold rise will be compared between group i (concomitant administration) and ii (influenza administered first), using the following null (H_0) and alternative (H_1) hypotheses:

$$H_0: \mu_1/\mu_2 = 1$$

$$H_1: \mu_1/\mu_2 \neq 1$$

where μ is the geometric mean fold rise.

P values and confidence intervals for the geometric mean titer ratio will be estimated using a linear regression adjusted for site.

9.2 Exploratory Objectives

Serology and cell mediated immunity objectives will be analyzed using the immunogenicity population. All exploratory endpoints will be assessed with two-sided tests and alpha set to 0.05.

Serology

Microneutralization (MN), SARS-CoV-2 binding antibody and viral neutralization, and circulating virus objectives will all use the same statistical methods as the primary HAI objective. Waning objectives will use parametric models to estimate the rate of antibody decline by time since vaccination. Estimated GMTs and fold change will be presented for each time point with their associated 95% confidence intervals.

Cell Mediated Immunity

Assay results will be presented by time point and treatment group. Summary statistics (e.g., mean, standard deviation, median) will be presented for continuous outcomes. Categorical variables will be described with frequencies and percentages. Continuous outcomes will be log transformed ass appropriate for each assay.

Safety

Safety endpoints will be assessed using the mITT population.

Frequency and proportion of participants reporting injection site and/or systemic reactions during the first 3 days following each vaccination visit will be stratified by severity and their impact on health, and reported along with the difference in proportions reporting moderate/severe symptoms between groups and a 95% confidence interval for the difference.

Frequency and proportion of participants reporting a change in health status requiring hospital admission (including a description of the event) during the study period will be reported along with the difference in proportions between groups and a 95% confidence interval for the difference.

10 DERIVED AND TRANSFORMED DATA

Values below the limit of detection will be set to half the limit.

HAI and MN titers will log 2 transformed for calculation of GMT and GMFR.

Fold Rise or Fold Change is defined as a numeric variable for participants with non-missing values as:

post vaccination titer/pre vaccination titer

Geometric Mean Titer (GMT) is calculated by taking the anti-log of the mean of the log transformed titers

Geometric Mean Fold Rise/Change (GMFR) is calculated by taking the anti-log of the mean of the log transformed fold rise.

Seroconversion is defined as binary variable for participants with non-missing values as:

= 1, if achieving a HI antibody titer ≥1:40 following influenza vaccination if the baseline titer is <1:10 or a four-fold rise in titer if the baseline titer is >1:10

= 0, otherwise

Seroprotection is defined as binary variable for participants with non-missing values as:

= 1, if achieving a HI antibody titer ≥1:40

= 0, otherwise

APPENDIX 1

Protocol Violations for Exclusions from Immunogenicity Analysis

1. Missed visit/visit not conducted
2. Visit occurred outside of acceptable window
3. No pre- and/or post-vaccination blood draw or insufficient volume for assay analysis for respective vaccine
4. Did not meet inclusion
5. Met exclusion criteria
6. Delayed vaccine administration
7. Missed vaccine administration
8. Study product temperature excursion