Centers for Disease Control and Prevention

Clinical Immunization Safety Assessment (CISA) Project

Safety of Simultaneous versus Sequential Administration of mRNA COVID-19 Vaccines and Quadrivalent Inactivated Influenza (IIV4) in Adults, Adolescents and Children: A Randomized Observer Blinded Study

Short Title: Simultaneous mRNA COVID-19 and IIV4 Vaccination Study

Statistical Analysis Plan

Version 1.0

March 2nd, 2023

1 INTRODUCTION

This document describes the statistical procedures that will be utilized for the CISA protocol Safety of Simultaneous versus Sequential Administration of mRNA COVID-19 Vaccines and Quadrivalent Inactivated Influenza (IIV4) in Adults, Adolescents and Children: A Randomized Observer Blinded Study that was approved on September 30, 2021. This statistical analysis plan (SAP) describes the methods of statistical analysis. The initial draft SAP (Version 0.1) was developed prior to any data being analyzed in order to avoid bias. Any subsequent changes that occur to the study protocol and requires changes to the analysis procedures will be documented in the SAP (both draft versions (0.X) and the final version (X.0)). Table 1 below will be used for tracking of changes to the SAP. In this study 450 persons age ≥5 years are randomized 1:1 to receive either IIV4 or saline placebo injection by an unblinded vaccinator at Visit 1. Those receiving quadrivalent inactivated influenza vaccine (IIV4) at visit 1 will receive saline placebo at Visit 2 and those receiving saline placebo at Visit 1 will receive IIV4 at Visit 2. Randomization will be stratified by age (5 to < 12 years of age, 12 to < 18 years of age, 18 to <65 years of age, and ≥ 65 years of age) and receipt of either a primary two-dose series or booster dose of mRNA COVID-19 vaccine (for the ≥18 age groups) using a permuted block randomization scheme stratified by Lead and Contributing Sites [Duke University (Lead); Johns Hopkins University (Contributing); Cincinnati Children's Hospital (Contributing)]. An interim data analysis will be conducted for data collected in the 2021-22 flu season. This interim data analysis will include blinded data from selected secondary and exploratory measures. This data will be presented to the study team and potentially the Safety Panel. More details will be provided in the Interim Data Analysis Plan.

Table 1. Statistical Analysis Plan Versions

Version	Date of Approval	Major Changes from Prior Version
0.1	TBD	NA
0.2	TBD	Addition of EO6: comparisons by vaccine product
0.3	TBD	Minor edits and modifications based on CDC review and discussion. Addition of Section 9 for a sensitivity analysis if a participant receives the incorrect study product of IIV4 and placebo.
0.4	TDB	Addition of EO8 SARS-CoV-2 serum antibody assessments. Minor edits to align with protocol amendment and comments CDC review.
1.0	3/2/2023	Minor edits provided by the CDC.

2 PROTOCOL OBJECTIVES

2.1 Primary

a) PO1: To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the group receiving IIV4 simultaneously with mRNA COVID-19 vaccine at Vaccination Visit 1 (Simultaneous group) with the group receiving IIV4 alone one to two weeks later at Vaccination Visit 2 (Sequential group) following both Vaccination Visit 1 and 2

Research hypothesis: The proportion of participants with moderate or more severe fever, chills, myalgia or arthralgia will be noninferior (not higher) in the Simultaneous group versus the Sequential group.

2.2 Secondary

a) SO1: To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus the Sequential Group following the first vaccination visit.

b) SO2: To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus Sequential Group following the second vaccination visit.

c) SO3: To describe the proportions of participants in the Simultaneous and Sequential vaccination groups with solicited local and systemic reactogenicity events according to severity grade after the first and second vaccination visit and third vaccination visit for those receiving two doses of mRNA COVID-19 vaccine.

d) SO4: To describe the proportions of participants in the Simultaneous and Sequential vaccination groups experiencing at least one serious adverse event and a description of these events.

2.3 Exploratory

a) EO1: To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus Sequential Group following the third vaccination visit for those receiving two doses of mRNA COVID-19 vaccine.

b) EO2: To further characterize and describe the proportion of participants in the Simultaneous and Sequential groups with local or systemic reactogenicity events of greater severity following each vaccination visit and cumulatively.

c) EO3: To describe the proportion of participants in Simultaneous and Sequential groups experiencing at least one unsolicited adverse event and one adverse event of special interest and to characterize these events.

d) EO4: To compare the change of health-related quality of life (HRQOL) from baseline in the Simultaneous versus Sequential groups following the Vaccination Visit 1.

e) EO5: To assess the safety profiles in the simultaneous and sequential group participants by baseline COVID-19 serostatus (positive versus negative). To be included, the blood needs to be collected within the protocol specified window and have a definitive result.

f) EO6: To assess the safety profiles in the simultaneous and sequential group participants by COVID-19 vaccine product (Pfizer or Moderna).

g) EO7: To assess the effect of simultaneous administration of IIV4 and COVID-19 vaccine on IIV4 immunogenicity as assessed by hemagglutination inhibition assay (HAI).

h) EO8: To assess serum antibody levels to SARS-CoV-2 antigens.

3 STUDY ENDPOINTS

3.1 Primary

a) POM1a: Comparison of the proportion of participants reporting at least one solicited reactogenicity event to include fever, chills, myalgia, or arthralgia of moderate or greater severity in the Simultaneous group with the Sequential group within 1-7 days following Vaccination Visit 1 visit during which participants receive either IIV4 or placebo with an mRNA COVID-19 vaccine.

3.2 Secondary

a) SOM1a: Comparison of the proportion of participants reporting at least one solicited reactogenicity event to include fever, chills, myalgia, or arthralgia of moderate or greater severity in the Simultaneous group with the Sequential group within 1-7 days following Vaccination Visit 1 visit during which participants receive either IIV4 or placebo with an mRNA COVID-19 vaccine.

b) SOM2a: Comparison of the proportion of participants reporting at least one solicited reactogenicity event to include fever, chills, myalgia, or arthralgia of moderate or greater severity in the Simultaneous group with the Sequential group within 1-7 days following Vaccination Visit 2 during which participants receive either IIV4 or placebo without an mRNA COVID-19 vaccine.

c) SOM3a: The proportion of participants in each vaccination group reporting specified solicited local and systemic reactogenicity events of any severity and by severity grade within 1-7 days following the first vaccination visit during which participants receive either IIV4 or placebo with an mRNA COVID-19 vaccine.

   SOM3b: The proportion of participants in each vaccination group reporting specified solicited local and systemic reactogenicity events of any severity and by severity grade within 1-7 days following the second vaccination visit during which participants receive either IIV4 or placebo.

   SOM3c: The proportion of participants in each vaccination group reporting specified solicited local and systemic reactogenicity events of any severity and by severity grade within 1-7 days following the third vaccination visit during which all participants receive an mRNA COVID-19 vaccine alone for those receiving two doses of mRNA COVID-19 vaccine.

d) SOM4a: The proportion of participants in Simultaneous and Sequential Groups with at least one serious adverse event occurring during the study period and a description of each event.

3.3 Exploratory

a) EOM1a: Comparison of the proportion of participants reporting at least one solicited reactogenicity event to include fever, chills, myalgia, or arthralgia of moderate or greater severity in the Simultaneous group with the Sequential group within 1-7 days following Vaccination Visit 3 during which all participants receive an mRNA COVID-19 vaccine alone for those receiving two doses of mRNA COVID-19 vaccine.

b) EOM2a. The proportion of participants reporting at least one moderate or greater or at least one severe or greater solicited local or systemic reactogenicity event in each

vaccination group within 1-7 days following the first vaccination during which participants receive either IIV4 or placebo with an mRNA COVID-19 vaccine.

EOM2b: The proportion of participants reporting at least one moderate or greater or at least one severe or greater solicited local or systemic reactogenicity event in each vaccination group within 1-7 days following the second vaccination visit which participants receive either IIV4 or placebo.

EOM2c: The proportion of participants reporting at least one moderate or greater or at least one severe or greater solicited local or systemic reactogenicity event in each vaccination group within 1-7 days following the third vaccination visit during which all participants receive an mRNA COVID-19 vaccine alone for those receiving two doses of mRNA COVID-19 vaccine.

EOM2d: The proportion of participants reporting at least one moderate or greater or at least one severe or greater solicited local or systemic reactogenicity event in each vaccination group within 1-7 days following all vaccination visits combined.

c) EOM3a: The proportion of participants in each vaccination group with an unsolicited adverse event occurring during the 7 days post each vaccination visit according to severity and system organ classification.

EOM3b: The proportion of participants in each vaccination group with an adverse event of special interest occurring during the study period according to event type.

d) EOM4a: Comparison between vaccination groups of the mean maximal change in score from baseline on the EuroQOL 5 dimensions-5 level (EQ-5D-5L) and EuroQOL visual analogue scale (EQ VAS) within 1-7 days following the first vaccination visit.

e) EOM5a: To compare secondary outcomes 3a, 3b, 3c and 4a according to baseline COVID-19 serostatus.

f) EOM6a: To compare secondary outcomes 3a, 3b, 3c and 4a according to COVID-19 vaccine product (Pfizer or Moderna).

g) EOM7a. The proportion of participants in each vaccination group with a seroprotective HAI titer (≥ 1:40) pre- and post-IIV4 immunization for each IIV4 antigen.

EOM7b: The proportion of participants in each vaccination group achieving seroconversion following IIV4 (an HAI titer > 1:40 following IIV4 if the baseline titer is < 1:10 or a four-fold rise in HAI titer if the baseline titer is > 1:10) for each IIV4 antigen.

EOM7c: The geometric mean HAI titer (GMT) for each IIV4 antigen pre- and post-IIV4 in each vaccination group.

EOM7d: The geometric mean fold rise (GMFR) in HAI titer for each vaccination group.

h) EOM8a: The proportion of participants in each vaccination group seropositive to SARS-CoV-2 variants tested prior to and following mRNA COVID-19 vaccine.

EOM8b: The geometric mean ID_{50} and ID_{80} titers of neutralizing antibodies for each SARS-CoV-2 variant tested pre- and post-mRNA COVID-19 vaccine in each vaccination group.

4 STUDY DESIGN

4.1 Study Description

This study is a prospective, randomized, clinical trial to assess the safety of simultaneous versus sequential administration of mRNA COVID-19 and IIV4 vaccines in 450 persons age ≥5 years enrolled at Duke University Medical Center (Lead Contractor), Johns Hopkins University (Contributing Contractor), and Cincinnati Children's Hospital Medical Center (Contributing Contractor). Participants will be enrolled in the 2021-2022 and 2022-2023 influenza seasons. Individuals will be enrolled who have not received their influenza vaccine during the influenza season during which they are recruited and are either receiving a booster dose of mRNA COVID-19 vaccine (12 years of age or older) or are receiving a primary two-dose mRNA COVID-19 vaccine series (5 years of age or older). Health, demographic and health-related quality of life (HRQOL) data will be collected from study participants at baseline for participants aged ≥12 years. All participants receiving an mRNA COVID-19 vaccine on Day 1 (Visit 1) will be randomized to receive quadrivalent inactivated influenza vaccine (IIV4) or placebo injection. Persons aged <65 years will receive standard dose IIV4 (SD-IIV4) or saline placebo injection. Children < 9 years of age must only need to receive a single dose of IIV4 per ACIP recommendations for the 2022-2023 season. Persons aged ≥65 years will receive high-dose IIV4 (HD-IIV4) or saline placebo injection. Participants will receive the vaccines in an observer-blinded manner on the same day as they receive mRNA COVID-19 vaccine. Seven to fourteen days later (Visit 2) those who received IIV4 at Visit 1 will receive placebo and those who received placebo on at Visit 1 will receive IIV4 (SD-IIV4 or HD-IIV4, depending upon age). Participants will then receive a second mRNA COVID-19 vaccine (Visit 3), if applicable, and according to the recommended schedule for vaccination for the respective mRNA COVID-19 vaccine product used.

4.2 Laboratory

4.2.1 Influenza Hemagglutination Inhibition Assay

mRNA COVID-19 vaccine naïve participants will have blood draws on Day 1 (before vaccination) and Day 29 (Pfizer/BioNTech mRNA vaccine) and (Moderna mRNA vaccine) post-IIV4 vaccination to be stored for serum hemagglutination inhibition (HAI) antibody titers. Those receiving a booster dose of mRNA COVID-19 vaccine will have blood draws on Day 1 (before vaccination) and Day 29. HAI antibody titers will be compared between vaccination groups receiving COVID-19 and IIV4 simultaneously or sequentially for each of the four influenza vaccine strains contained in the respective vaccines for that season. Participants will not receive individual HAI antibody titer results; these are not routinely used in clinical practice.

4.2.2 SARS-CoV-2 Antibody Assay

4.2.2.1 Qualitative baseline assessment

Participants will have blood draws on day 1 (before vaccination). Serum will be assayed for the presence of SARS-CoV-2 antibody using the AdviseDx SARS-CoV-2 IgG II assay and the Alinity I SARS-CoV-2 IgG assay. The assays are intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2 indicating

recent or prior infection. Serologic testing will be completed in periodic batches throughout the course of the study and will therefore not be available in real-time or for use in clinical decision-making. The current tests described above are allowed under the FDA's Emergency Use Authorization during the COVID-19 pandemic.

4.2.2.2 Quantitative SARS-CoV-2 assessments

Participants will have blood draws on Day 1 (before vaccination) and following each vaccination according to schedules outlined in Section 5.1 to be stored for serum COVID-19 neutralization assays. COVID-19 neutralizing antibody titers will be compared between groups receiving COVID-19 and IIV4 simultaneously or sequentially for each variant tested.

4.3 Sample Size and Power

Four reactogenicity events were included in the primary statistical endpoint: fever, chills, myalgia, or arthralgia of moderate or greater severity. These were considered clinically meaningful and were solicited in both mRNA COVID-19 vaccine trials. Headache and fatigue were also solicited reactogenicity in both trials, but each occurred at the moderate-severe grade in >10% of participants after dose 1 placebo (in the Pfizer- Pfizer-BioNTech trial) and were thought to be less specific. Based on data from prelicensure studies , we assume that 15% of participants will have at least one reaction of moderate severity or greater to include fever, chills, myalgia, or arthralgia reactogenicity event in the Sequential group. We have selected a clinically meaningful noninferiority margin of 10%. We plan to recruit a total of 450 participants and assume a 5% drop out rate, leaving N=428 or N=214 per vaccination group. Statistical calculations show that with a one-side alpha level of 0.025, and 214 subjects in each group across all study sites, there is 82.6% power to be able to demonstrate that the proportion of participants with at least one severe solicited local or systemic reactogenicity event in the Simultaneous group is noninferior to the Sequential group. Enrollment in this study shall occur during two influenza season (2021-22, 2022-23).

4.4 Randomization

Participants will be randomized (1:1) to receive either IIV4 or saline injection by an unblinded vaccinator at Visit 1. Those receiving IIV4 at visit 1 will receive saline placebo at Visit 2 and those receiving saline placebo at Visit 1 will receive IIV4 at Visit 2. Randomization will be stratified by age (5 to < 12 years of age, 12 to < 18 years of age, 18 to <65 years of age, and ≥ 65 years of age) and receipt of either a primary two-dose series or booster dose of mRNA COVID-19 vaccine (for the ≥18 age groups) using a permuted block randomization scheme stratified by Lead and Contributing Sites. The project statistician will generate permuted block 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 unblinded vaccinator CRF. In the event that REDCap is unavailable, manual randomization will occur through the use of envelopes. The project statistician will prepare 10 envelopes per age group and vaccine series per site (total of 60 per site) 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, he/she will pull the next envelope in order. In order to capture the allocation per subject, a separate form in REDCap will be used by the unblinded personnel to add the assignment. A log will need to be kept at the site capturing these instances.

4.5 Blinding

SD-IIV4, HD-IIV4, and/or placebo will be administered to blinded participants in the opposite deltoid muscle, to which the mRNA COVID-19 vaccine was administered at Visit 1. SD-IIV4 or HD-IIV4 will be administered by unblinded licensed staff. In order to keep the participant blinded, the vaccine administrator will keep the prefilled syringes out of view of the participant at all times and will instruct the participant to turn their head in the opposite direction of the arm in which the vaccine is being administered. Similar instructions will be given to any persons accompanying the patient in the room. After administration, used study syringes will be disposed of according to site-specific SOPs. A licensed provider (MD, DO, NP, PA, RN, LPN), who will be trained on treating adverse reactions, will be immediately available at the time of vaccine administration along with emergency management supplies available for initial treatment of an allergic reaction if needed. Additionally, clinical members of the blinded data collection team will be present to assist study subjects.

5 PARAMETERS OF ANALYSIS

5.1 Data Collection and Storage

Data will reside on a secure Duke server maintained by Duke Health Technology Solutions (DHTS). For the study, a database will be developed and a data set for the study without personal identifiers will be made available to the CDC upon request.

5.2 Analytic Issues

There are three study sites participating in the study and analysis of the primary objective will be stratified by site (Duke, Boston, Cincinnati) to account for this unit of randomization. All objectives will be stratified by site when applicable. There is one primary objective being evaluated, using a noninferiority hypothesis at the one-sided alpha 0.025 level. Otherwise, the alpha level will be set at two-sided alpha 0.05 for the secondary objectives and all exploratory objectives.

6 ANALYSIS POPULATIONS

6.1 Full Analysis Population 1:

This population is defined as all subjects who are randomized, received at least one study vaccine at Visit 1, and provide at least one day of complete data on the symptom diary

6.2 Full Analysis Population 2:

This population is defined as all subjects who are randomized and vaccinated.

6.3 Immunogenicity Population

This population defined as subjects who received vaccine, provide baseline and Visit 3 and Visit 4 blood draws of acceptable volume and quality within the protocol-defined time frame for the respective vaccine with 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 -7/+14 days from the target date. Any subject with sufficient data for either or both of the influenza and SARS-CoV-2 analysis will be included in this population. Should 10% or more of the total study population be excluded from the Immunogenicity Population due to missing the visit window,

then an additional analysis will be performed including these subjects with the missing visit window exclusion.

The Full Analysis Population 1 is the primary population for analysis unless otherwise stated.

7 BASELINE DATA AND FLOW CHART

7.1 Presentation of Baseline Data

The following baseline information will be presented by site: vaccination group, vaccine product received, underlying medical conditions, age group, sex, ethnicity race and influenza season. Summary statistics (e.g., mean, standard deviation, median) will be presented for continuous variables. Categorical variables will be described with frequencies and percentages.

7.2 Flow Chart

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

8 ANALYSIS OF STUDY OBJECTIVES

8.1 Primary Objective

1. To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the group receiving IIV4 simultaneously with mRNA COVID-19 vaccine at Vaccination Visit 1 (Simultaneous group) with the group receiving IIV4 alone two weeks later at Vaccination Visit 2 (Sequential group) following both Vaccination Visit 1 and 2.
    - *Research hypothesis: The proportion of participants with moderate or more severe fever, chills, myalgia or arthralgia will be noninferior (not higher) in the Simultaneous group versus the Sequential group.*

      This objective will be assessed for visits 1 and 2 using a one-sided noninferiority test with the alpha level set at 0.025 and noninferiority margin of 10%. The null hypothesis is the Simultaneous group is inferior (i.e., Simultaneous group will have a higher proportion) to the Sequential group in regards to the proportion of participants with at least one moderate or severe fever, chills, myalgia, or arthralgia event after visits 1 and 2.

      Ho: Simultaneous group - Sequential group ≥ 0.10 (10%)

      The alternative hypothesis is the Simultaneous group is noninferior to the Sequential group in regards to the proportion of participants with at least one moderate or severe fever, chills, myalgia, or arthralgia event after visits 1 and 2.

      Ha: Simultaneous group - Sequential group < 0.10 (10%)

      The upper bound of a site-stratified Newcombe binomial confidence interval (Yan and Su 2010) with Cochran-Mantel-Haenszel (CMH) weighting of the difference will be used to make these assessments with no adjustment to the alpha level for multiple comparisons.

See Tables 1 and 2 below for details.

8.2 Secondary Objectives

1. SO1: To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus the Sequential Group following the first vaccination visit.

   This proportion will be compared between the Simultaneous versus the Sequential Group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The site adjusted odds ratio and corresponding 95% confidence interval for the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia following the first vaccination visit will also be calculated.

2. SO2: To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus Sequential Group following the second vaccination visit.

   These proportions will be compared between the Simultaneous versus the Sequential Group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The site adjusted odds ratio and corresponding 95% confidence interval for the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia following the second vaccination visit will also be calculated.

3. SO3: To describe the proportions of participants in the Simultaneous and Sequential vaccination groups with solicited local and systemic reactogenicity events according to severity grade after the first and second vaccination visit and third vaccination visit for those receiving two doses of mRNA COVID-19 vaccine.

   Tables (one for each visit 1, 2, and 3) will be produced that summarize each solicited local and systemic reactogenicity event by classification (grades 1-4 as well as grades 2- 4 combined), for each vaccination group. These tables will have the number and percentage for each classification by vaccination group and the 95% confidence interval of the difference between the vaccination groups for the percentage of grades 3 and 4 combined.

Table 1. Injection-site Reactogenicity Grading

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Noticeable but does not interfere with activity	Interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an emergency room (ER) visit or hospitalization

Table 1. Injection-site Reactogenicity Grading				
Local Reaction to Injectable Product	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Induration/Swelling (≥ 12 years of age)	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Requires an emergency room (ER) visit or hospitalization
Induration/Swelling (< 12 years of age)	0.5 – 2 cm	2.0 -7.0 cm	> 7 cm	Requires an emergency room (ER) visit or hospitalization
Erythema/Redness (≥ 12 years of age)	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Requires an emergency room (ER) visit or hospitalization
Erythema/Redness (< 12 years of age)	0.5 – 2 cm	2.0 -7.0 cm	> 7 cm	Requires an emergency room (ER) visit or hospitalization
Axillary (underarm) swelling or tenderness ipsilateral to side of injection	Noticeable but does not interfere with activity	Interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an emergency room (ER) visit or hospitalization

Table 2. Systemic Reactogenicity Grading (FDA modified)				
Systemic	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Fever (°C) **(°F)**	38.0 - 38.4 100.4 - 101.1	38.5 - 38.9 101.2 - 102.0	39.0 – 40.0 102.1-104.0	> 40.0 >104.0
Nausea/vomiting	Noticeable but does not interfere with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Significant; prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization
Diarrhea	Noticeable but does not interfere with activity or 2 – 3 loose stools/24 hours	Some interference with activity or 4-5 loose stools/24 hours	Significant; prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school] or 6 or more watery stools or > 24 hours	Requires an ER visit or hospitalization
Headache	Noticeable but does not interfere with activity	Some interference with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily routine activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization
Fatigue	Noticeable but does not interfere with activity	Some interference with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily routine activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization

Table 2. Systemic Reactogenicity Grading (FDA modified)				
Systemic	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Myalgia	Noticeable but does not interfere with activity	Some interference with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily routine activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization
Arthralgia	Noticeable but does not interfere with activity	Some interference with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily routine activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization
Chills	Noticeable but does not interfere with activity	Some interference with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily routine activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization

4. SO4: To describe the proportions of participants in the Simultaneous and Sequential vaccination groups experiencing at least one serious adverse event and a description of these events (tables 2 and 3 below).

   A table will be produced that summarize participants experiencing at least one serious adverse event during the study period by vaccination group. This table will have the number and percentage of participants experiencing at least one serious adverse event by vaccination group and the 95% confidence interval of the difference between the vaccination groups. Listings with the clinical narratives will also be provided. The primary analysis population will be the Full Analysis Population 2.

Serious adverse events (SAEs) will be collected and reported during the entire study period [i.e. from enrollment through 120 days following enrollment]

A SAE is defined as an AE that meets one of the following conditions:

- Results in death during the period of protocol-defined surveillance
- Is life-threatening (defined as immediate risk of death at the time of the event)
- Requires inpatient hospitalization or prolonged hospitalization during the period of protocol-defined surveillance
- Results in congenital anomaly or birth defect
- Results in a persistent or significant disability/incapacity
- Any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered an SAE when, based upon appropriate medical judgment, the event may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

SAEs will be graded as follows.

Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Noticeable but does not interfere with activity or measurement	Interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization

8.3 Exploratory Objectives

1. EO1: To compare the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia in the Simultaneous versus Sequential Group following the third vaccination visit for those receiving two doses of mRNA COVID-19 vaccine.

   This proportion will be compared between the Simultaneous versus the Sequential Group using an exact Mantel-Haenszel statistic (calculated in Proc Logistic in SAS) in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. The site adjusted odds ratio and corresponding 95% confidence interval for the proportion of participants with moderate or more severe fever, chills, myalgia, or arthralgia following the first vaccination visit will also be calculated.

2. EO2: To further characterize and describe the proportion of participants in the Simultaneous and Sequential groups with local or systemic reactogenicity events of greater severity following each vaccination visit and cumulatively.

   Tables for each visit 1, 2, and 3, as well as a combined visit table, will be produced that summarize the number of participants by vaccination group with at least one grade 1 or higher local or systemic reactogenicity event, as well as the number of participants by vaccination group with at least one grade 3 or higher local or systemic reactogenicity event. This table will have the number and percentage for each outcome by vaccination group and the 95% confidence interval of the difference between the vaccination groups will be presented.

3. EO3: To describe the proportion of participants in Simultaneous and Sequential groups experiencing at least one unsolicited adverse event and one adverse event of special interest and to characterize these events.

   Tables will be produced that summarize participants experiencing at least one unsolicited adverse event and summarize participants experiencing at least one adverse event of special interest during the study period by vaccination group. These tables will have the number and percentage of participants experiencing either outcome by vaccination group and the 95% confidence interval of the difference between the vaccination groups. Listings with the clinical narratives will also be provided. The primary analysis population will be the Full Analysis Population 2.

   Unsolicited adverse events (AEs) will be collected and reported for the 7 days following each vaccine visit. Adverse events of special interest (AESI) will be collected and reported during the entire study period [i.e. from enrollment through 120 days following enrollment]. Unsolicited AEs, SAEs, and AESIs will be graded as follows.

Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Noticeable but does not interfere with activity or measurement	Interferes with activity but did not need a medical visit or absenteeism [i.e. missing work or school]	Significant; prevents daily activity and/or resulted in medical visit and/or absenteeism [i.e. missing work or school]	Requires an ER visit or hospitalization

4. EO4: To compare the change of health-related quality of life (HRQOL) from baseline in the Simultaneous versus Sequential groups following the Vaccination Visit 1.

   EQ-5D-5L and Visual Analogue Scale (VAS)
   The EQ-5D-5L is a standardized, generic measure of health status that provides information on HRQOL and activities of daily living: mobility, self-care, usual activities, pain/discomfort and anxiety/depression (http://www.euroqol.org/) [42]. In addition, the instrument contains the EQ Visual Analogue Scale (EQ-VAS) which measures the respondent's self-rated health.

   The EQ-VAS records the respondent's self-rated health on a 20 cm vertical, visual analogue scale with endpoints labelled 'the best health you can imagine' (100) and 'the worst health you can imagine' (0). The respondent marks an 'X' on the scale number to indicate how their health is 'today.' The minimum clinically important difference on the VAS is 8.

   The changes from baseline will be assessed within vaccination group maximal change from baseline for each participant for the EQ-VAS and EQ-5D-5L. For the EQ-5D-5L the change between vaccination groups from baseline will be compared using a t-test or the Mann-Whitney U test (Wilcoxon rank-sum test) at the alpha 0.05 level. For the EQ-VAS, if the maximal change from baseline is 8 or greater, this will indicate a clinically relevant change. The vaccination group mean changes will be compared using a paired t-test or Wilcoxon signed-rank test at the alpha 0.05 level.

   Participants under the age of 12 will not be included in the analysis because the validated index scoring is only for people 12 and older.

5. EO5: To assess the safety profiles in the simultaneous and sequential group participants by baseline COVID-19 serostatus (positive versus negative).

   Secondary objectives 3 and 4 described above will be re-analyzed using the baseline COVID-19 serostatus as the group comparison within each treatment group. See the statistical methods above for details.

6. EO6: To assess the safety profiles in the simultaneous and sequential group participants by vaccine product (Pfizer or Moderna).

   Secondary objectives 3 and 4 described above will be re-analyzed using the vaccine product as the group comparison within each treatment group. See the statistical methods above for details.

7. EO7: To assess the effect of simultaneous administration of IIV4 and COVID-19 vaccine on IIV4 immunogenicity as assessed by hemagglutination inhibition assay (HAI).

   The proportion of participants in each vaccination group with a seroprotective HAI titer (≥ 1:40) pre- and post-IIV4 immunization for each IIV4 antigen will be compared using a Mantel-Haenszel statistic in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level.

   The proportion of participants in each vaccination group achieving seroconversion following IIV4 (an HAI titer > 1:40 following IIV4 if the baseline titer is < 1:10 or a four-fold rise in HAI titer if the baseline titer is > 1:10) for each IIV4 antigen will be compared using a Mantel-Haenszel statistic in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level.

   The geometric mean HAI titer (GMT) for each IIV4 antigen pre- and post-IIV4 in each vaccination group will be compared (within vaccine group pre versus post and between vaccination groups) using a regression model with the log transformed titer value at the two-sided alpha 0.05 level. The geometric mean fold rise (GMFR) in HAI titer for each vaccination group will be presented along with a 95% confidence interval.

   The Immunogenicity Population is the primary analysis population.

8. EO8: Assessments of the serum antibody levels to SARS-CoV-2 antigens.

   The proportion of participants in each vaccination group seropositive to SARS-CoV-2 variants tested prior to and following mRNA COVID-19 vaccine will be compared using a Mantel-Haenszel statistic in a stratified analysis by site to control for the randomization blocks at the two-sided alpha 0.05 level. Comparisons will be made pre- and post-vaccination for each variant tested. The definition of seropositive is as follows:
   - ID_{50} above Limit of Detection (LOD) at baseline
   - after vaccination and seronegative at baseline, ID_{50} above LOD is considered seropositive
   - after vaccination and seropositive at baseline, then an ID_{50} 3-fold over baseline ID_{50} is considered seropositive.

   The term ID_{50} refers to the inhibitory dilution where 50% neutralization occurs, and ID_{80} refers to the inhibitory dilution where 80% neutralization occurs. ID_{50} estimates are generally used for analysis purposes since they are in the linear part of the curve and more stable estimates.

   The geometric mean ID_{50} and ID_{80} titers pre- and post-vaccination with mRNA COVID-19 will be compared (within vaccine group pre versus post and between vaccination groups) using a regression model adjusting for vaccine platform (Pfizer or Moderna) and study site with the log transformed titer geometric mean ID_{50}/ID_{80} values at the two-sided alpha 0.05 level. Should there exist a substantial difference (based on a statistical evaluation of these data) in titer values between the vaccine platforms, these proposed analysis and tables will be done within vaccine platform type (i.e., separate analyses for Pfizer or Moderna). This decision will be made with the study team once all data have been collected and are ready for analysis. Tables will be generated to present the geometric mean raw ID_{50} and ID_{80} values pre- and post-vaccination by vaccination group.

9 SENSITIVITY ANALYSIS

If any participant in the Full Analysis Population 1 receives the incorrect study product (IIV4 and placebo switched), then a sensitivity analysis will be performed based on the actual study product received for Primary Objective and Secondary Objectives 1 and 2.

10 INTERIM SAFETY DATA REVIEW

The safety data review will provide the study the opportunity to identified unexpected safety concerns and make changes to the protocol if needed. The interim safety data review will be done by a safety monitoring panel with relevant expertise, comprised of experts who are not co-investigators on this study. The safety population for the interim safety review will include participants who were enrolled and randomized. The safety monitoring panel will review blinded group results (number and percent) of solicited local and systemic reactogenicity events as well as the number and percent of SAEs along with clinical narratives. If the CDC and study investigators determine additional analyses or reviews are needed, efforts will be made to conducted additional analysis or reviews that will not include analyzing the primary endpoint as a first step. This is to avoid introducing bias or increasing sample size needs for statistical power.

11 REFERENCES

Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. JAMA 2003;289(2):179-86. DOI: 10.1001/jama.289.2.179.

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APPENDIX 1

Major Protocol Violations for Influenza and SARS-CoV-2 Immunogenicity Analysis

1. Major Protocol Violations for Exclusion from Immunogenicity Populations:
   a. Not all study vaccines received
   b. No pre- and/or post-vaccination blood draw or insufficient volume for assay analysis for respective vaccine*
   c. Receipt of any other vaccine between the pre- and post-vaccination blood draws
   d. New immunosuppression disorders between the pre- and post-vaccination blood draws or receipt of immunosuppressive medication between the pre- and post-vaccination blood draws
   e. Subject who was inadvertently enrolled and randomized to the study, though they were later learned to have had met the following criteria (see f-k below) for study exclusion that would have affected immunogenicity
   f. Influenza vaccine receipt during the current influenza season prior to study enrollment
   g. COVID-19 vaccine prior to enrollment unless presenting for a booster dose
   h. Prior receipt of non-mRNA COVID-19 vaccine
   i. Use of oral or parenteral corticosteroids (≥ 20mg/day prednisone equivalent) or high-dose inhaled glucocorticoid for ≥ 14 consecutive days within the preceding 30 days
   j. Has an active neoplastic disease (excluding non-melanoma skin cancer), a history of any hematologic malignancy
   k. Has a history of receiving immunoglobulin or other blood product (with exception of Rh immunoglobulin) within the 3 months prior to study vaccination or during the study including Evushield or other COVID-19 monoclonal antibody.
   l. Subject who was enrolled for both the 2021-22 and 2022-23 flu seasons may be included in immunogenicity for the 2021-2022 season only.

   *Blood draws will not be considered out of window as long as blood was collected within -7/+14 days of the protocol window for the respective vaccine