Statistical Analysis Plan

Seqirus

V130_10

A Phase 3, Randomized, Observer-Blind, Multicenter, Noninferiority Study to Evaluate Safety and Immunogenicity of a Cell-Based Quadrivalent Subunit Influenza Virus Vaccine (QIVc) and a United States-licensed Quadrivalent Influenza Virus Vaccine (QIV) in Healthy Subjects aged 6 Months through 47 Months

[redacted]

Document Version : Final Version 3.0
Document Date : 13 May 2020

[redacted]

Clinical Development Commercialization Services

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Approvals

The undersigned agree that all required reviews of this document are complete, and approve this Statistical Analysis Plan as final. Programming of the tables, figures and listings based upon the specifications within this document can proceed.

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Version History

Version #	Description of Changes	Version Date
Version 1.0 Final	Final version	02OCT2019
Version 2.0 Final	Final version according to Protocol version 4	10DEC2019
Version 3.0 Final	Final version including minor edits and addition of sensitivity analysis	13MAY2020

Glossary of Abbreviations

Abbreviation	Term
AE	Adverse event
CBER	Center for Biologics Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CMI	Cell Mediated Immunity
CRF	Case Report Form
CSR	Clinical Study Report
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GLM	Generalized Linear Model
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
ID	Identification
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IRT	Interactive Response Technology
LAR	Legally Acceptable Representative(s)
LLOQ	Lower Limit Of Quantification
LQ	Limit of Quantification
MCAR	Missing Completely At Random
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliters
MN	Microneutralization
NOCD	New Onset of Chronic Disease
PD	Protocol Deviation
PFS	Pre-filled syringes
PPS	Per Protocol Set
PT	Preferred Term
QIVc	Cell-derived Quadrivalent Influenza Vaccine
QIV	Quadrivalent Influenza Vaccine
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SD	Standard Deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TIVc	Cell-based Trivalent Influenza Vaccine
US	United States of America
WHO	World Health Organization

1. Source Documents

The Statistical Analysis Plan (SAP) was written based on the following documentation:

Document	Date	Version
Protocol	17MAY2019	Final Version 3.0
eCRF	01Aug2018	Version 0.4

This document presents the SAP for Seqirus, Protocol No. V130_10: Phase 3, Randomized, Observer-Blind, Multicenter, Noninferiority Study to Evaluate Safety and Immunogenicity of a Cell-Based Quadrivalent Subunit Influenza Virus Vaccine (QIVc) and a United States-licensed Quadrivalent Influenza Virus Vaccine (QIV) in Healthy Subjects 6 Months Through 47 Months.

The purpose of this study is to demonstrate that vaccination with QIVc elicits an immune response that is not inferior to that of a United States (US)-licensed QIV containing the recommended strains for the season, in children 6 months through 47 months of age.

It describes the data and variables to be summarized and analyzed, including specifics of the statistical analyses to be performed. This analysis plan is based on the protocol Version 4.0, dated 9 DEC 19 and is compliant with ICH Harmonized Tripartite Guideline, 5 February, 1998, Statistical Principles for Clinical Trials, E9; World Health Organization, WHO Technical Report, Series No. 924. 2004, Annex 1: Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations; and FDA Center for Biologics Evaluation and Research (CBER) Guidance for Industry, May 2007, Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines.

The SAP provides the description of the analysis for the active study period and safety data through to the final evaluation (6 months following last study vaccination dose).

The data from this study will be used to support the licensure of QIVc for use in children 6 months through 47 months of age.

In this SAP the final analysis of the primary and secondary immunogenicity endpoints will be conducted on cleaned and locked data once all subjects have completed all immunogenicity assessments (end of treatment period, i.e. up to 28 days following the last vaccination dose). At this time, the analysis of all solicited adverse events and of unsolicited adverse events reported during the treatment period will also be conducted. These results will be used to prepare a Clinical Study Report (CSR) with treatment period data. No individual unblinded listings will be generated at this stage.

A final CSR will present all clinical study data collected up to 180 days following the last vaccination dose, including safety data collected during the follow up period.

2. Protocol Details

2.1.1 Primary Immunogenicity Objectives

1. To demonstrate that vaccination with QIVc elicits an immune response that is not inferior to that of an US-licensed QIV containing the recommended strains for the season, in subjects 6 months through 47 months of age, as measured by hemagglutination inhibition (HAI) assay for A/H1N1, B/Yamagata and B/Victoria strains and by microneutralization (MN) assay for A/H3N2 strain, using cell-derived target viruses.

2.1.2 Secondary Immunogenicity Objectives

1. To describe the immunogenicity of QIVc and US-licensed QIV by HAI assay for A/H1N1, B/Victoria, and B/Yamagata strains, and by MN assay for A/H3N2 strain, using egg-derived target viruses.
2. To describe the immunogenicity of QIVc and US-licensed QIV by HAI assay for A/H1N1, B/Victoria, and B/Yamagata strains, and by MN assay for A/H3N2 strain, using cell-derived target viruses.
3. To describe the immunogenicity of QIVc and US-licensed QIV by MN assay for A/H1N1, B/Victoria, and B/Yamagata, in a subset of subjects

2.1.3 Secondary Safety Objectives

1. To evaluate the safety and reactogenicity of QIVc and US-licensed QIV

2.1.4 Exploratory Immunogenicity Objective

1. To evaluate the homologous cell-mediated immunity (CMI) response, pre-vaccination and post-vaccination, in a small population of subjects.
2. To further describe the immune response to vaccination, additional immunogenicity analyses may be conducted such as HAI assay for A/H3N2 using cell- and egg-derived target virus.

2.2 Overall Study Design

This multicenter, phase 3 clinical study evaluates the immunogenicity and safety of a cell-based Quadrivalent subunit influenza virus-vaccine (QIVc) compared to US-licensed QIV in children 6 months through 47 months of age. Study features an observer blind, comparator-controlled design and 2:1 randomization between QIVc and US-licensed QIV.

Subjects who meet the eligibility criteria will be enrolled into the study and randomized to one of the two treatment groups using a 2:1 allocation ratio to receive either Seqirus QIVc or US-licensed QIV.

The study has a treatment period and a follow-up period.

Subjects with a previous influenza vaccination history:

- The treatment period begins at the time of vaccination and ends 28 days after vaccination and will consist of 2 clinical visits and one reminder call to complete the Subject Diary Card.
- The follow up period begins 28 days after vaccination and ends at the time of study completion visit.

Subjects without or unknown previous influenza vaccination history:

- The treatment period begins at the time of first vaccination and ends 28 days after the second vaccination and will consist of 3 clinical visits and two reminder calls to complete the Subject diary card, one after each vaccination.
- The follow-up period begins 28 days after second vaccination and ends at the time of study completion visit.

All subjects, irrespective of previous influenza vaccination history, will receive 1 safety assessment call 90 days after last vaccination during the follow up period and the follow-up period will conclude with a study completion visit (call).

Currently available influenza vaccines are not licensed below age of 6 months, and protocol will exclude any subjects who have received influenza vaccine in the past 6 months. Therefore, all eligible subjects below 12 months of age will be considered as not previously influenza vaccinated for enrolment in the study.

The timing and frequency of the study visits are described in Schedule of Assessments (Appendix A).

The treatment arms of Study Vaccine comprise:

Study treatment:

Seqirus' Quadrivalent (QIVc): Flucelvax Quadrivalent

The dose to be administrated 0.5 mL of QIVc (cell-derived seasonal Quadrivalent influenza vaccine) contains nominally 15 µg of hemagglutinin (HA) of each of the 2 influenza type A strains and each of the 2 influenza type B strains for a total of 60 µg of HA in the vaccine. The strain composition will be that recommended by the World Health Organization (WHO) for Quadrivalent influenza vaccines contemporaneous to the timing of the study.

Comparator treatment:

US-licensed Quadrivalent: Afluria® Quadrivalent

Afluria® Quadrivalent is an inactivated influenza virus vaccine for intramuscular injection. Afluria® Quadrivalent is supplied in a 0.25 mL and a 0.5 mL pre-filled syringe (PFS). For children 6 months through 35 months of age, 0.25 mL dose of vaccine will be administered. For children 36 months through 47 months of age, 0.5 mL will be administered.

Withdrawn Subjects

Subjects may withdraw from the study at any time at their own request or request of the parent or legally acceptable representative (LAR) or they may be withdrawn at

any time at the discretion of the investigator or Seqirus for safety or administrative reasons.

The reasons for premature withdrawal from the study include the following:

- Adverse event (AE)
- Death
- Withdrawal of consent
- Loss to follow-up
- Other
- Protocol deviation
- Study terminated by Sponsor

In accordance with International Conference on Hamonization (ICH) principles of Good Clinical Practice (GCP) the investigator always has the option to advise a subject to withdraw from the study if the subject's safety or wellbeing is compromised by his or her further participation in the study. Concern for the interests of the subject must always prevail over the interests of the study.

If a subject is withdrawn from the study or further participation is declined, they will continue to have access to medical care and will be treated as per routine medical practice.

Randomization

Enrolled subjects will be randomized in the Interactive Response Technology (IRT) system by a 2:1 ratio and automatically assigned a unique Subject ID. Subjects participating in the CMI subset (approximately 84 subjects) will be enrolled in a limited number of sites through sequential allocation. The Subject ID will be the subject’s unique identification number for all eCRFs and associated study documentation that will be used for duration of the study. After randomization, the Screening Number ceases to be used and remains in the Screening and Enrolment Log only. The list of randomization assignments is produced by the EDC/IRT service provider and approved by Seqirus according to applicable Seqirus Standard Operating Procedure (SOP).

Stratification through IRT will be used to ensure a balanced distribution among the age groups, at least 30% of the subjects should be 6 through 23 months and at least 30% of the subjects should be from 24 through 47 months of age.

For A/H1N1, B/Victoria, and B/Yamagata strains, immunogenicity of QIVc and US-licensed QIV will be evaluated by MN assay in a 20% subset of subjects. After blood specimens have been collected a randomly selected subset (20%) of these samples will be analyzed.

If for any reason, after signing the Informed Consent Form (ICF), the subject who is eligible and enrolled fails to be randomized, this is called a randomization failure. The reason for all randomization failures will be recorded in the Screening and Enrollment Log.

If for any reason, after randomization the subject fails to undergo treatment, this is an Early Termination and the reason should be recorded in source document.

The information on these Early Termination subjects should be kept distinct in the source documentation from randomization failures.

Blinding

The trial is designed as an observer-blind study. During the treatment period of the study designated and trained unblinded nurse(s), physician(s), or other qualified health care professional will be responsible for administering the study vaccines to the subjects. They will be instructed not to reveal the identity of the study vaccines either to the subject's parent(s)/LAR/delegate or to the investigative site personnel (i.e., investigator and study nurse) involved in the monitoring of conduct of the trial, except in an emergency if unblinding in IRT is not possible. Vaccine administration should be shielded from the subject's parent(s)/LAR(s) and blinded study personnel. The unblinded personnel should not be involved in data collection or data review such as safety assessments and/or collect study data after the vaccinations. Study vaccines will be assigned through an Interactive Response technology system.

Except in the case of medical necessity, a subject's treatment should not be unblinded without the approval of the Sponsor. In such instance, every effort should be made to contact the Sponsor prior to unblinding. If unblinding occurs, by either accidental unblinding or emergency unblinding for a serious adverse event, prior to completion of the study, the investigator must promptly contact the Sponsor and document the circumstances on appropriate forms. In case of an emergency, the information can be retrieved by the Investigator from the IRT system either via web or phone (a 24/7 backup service). If the subject unblinded by the Investigator, the subject could be removed from an Analysis Set.

Investigators, Seqirus study team members, and all External Service Providers (ESPs)s who are directly involved in the conduct of the trial or in the analysis of the study results, or have contact with study centers, will remain blinded to the treatment codes until the final database lock at the end of the study. Only biostatisticians conducting the statistical analysis for the treatment period, will have access to the individual treatment codes. No unblinded individual subject level information will be shared with Seqirus and ESP personnel conducting the study until the final database lock. Seqirus study team members preparing the draft CSR with treatment period data will remain blinded to the individual treatment codes.

If a subject is unblinded during the study, it is to be reported as major Protocol Deviation (PD), except for subjects unblinded by Pharmacovigilance due to suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately. The unblinded subject(s) are excluded from the Per Protocol Set (PPS).

Planned Unblinding procedures

The relevant portions of the clinical database will be cleaned and locked after collection of safety and immunogenicity data has been completed for all subjects during the treatment period of the study, i.e. up to 28 days following the last vaccination dose (final treatment period data). Only personnel of the ESP and Seqirus conducting the statistical analysis will have access to the individual treatment codes. This information will not be shared with Seqirus and ESP personnel conducting the study until the follow up safety data has been locked for final analysis. Seqirus study

team members preparing the CSR with treatment period data will remain blinded to the individual treatment codes. The results will not be shared with investigators and other site staff.
Safety follow up data will be locked when all subjects have completed all clinical data collected up to 180 days following the last vaccination dose (end of study). The Seqirus Benefit Risk Physician responsible for Sponsor's assessment of adverse events during the follow up phase will remain blinded at individual and group level through the follow up period, until final database lock at study completion. A final clinical study report will present all clinical study data collected up to 180 days following the last vaccination dose, including safety follow up data.

2.3 Sample Size and Power

Seqirus QIVc will be tested against comparator, i.e. US-licensed comparator QIV. The treatment randomization ratio is 2:1 (Seqirus QIVc: Comparator QIV).

This study is designed to achieve at least 90% power to demonstrate noninferiority for all of the 8 endpoints: seroconversion rates for 4 strains and Geometric Mean Titers (GMTs) for 4 strains using a one-sided alpha of 0.025 for each comparison. No alpha adjustment for multiple endpoints will be made. For comparisons of Seroconversion rate (SCR) a noninferiority margin of 10% (Comparator QIV-Seqirus QIVc) will be employed. It is assumed that the SCRs for A/H1N1, B strains and A/H3N2 based on Cell-based Trivalent Influenza Vaccine (TIVc) are 81%, 69% and 85% respectively These estimates are based on the estimated SCR rates of historical data, namely from study *V58P16*.

It is also assumed that the expected difference between antibody titers (QIV minus QIVc) is consistent with the SCR differences reported as per V58P16, namely 7% for A/H1N1 and 5% for A/H3N2 (based on the MN assay).

It is assumed that there is no difference between Seqirus QIVc and the comparator QIV for the B/strain.

For comparison of GMT ratio, a noninferiority margin of 1.5 (Comparator QIV/ Seqirus QIVc, equivalent to a difference on the log scale of 0.405465108) will be employed for the assessment for the A/H1N1, A/H3N2 and B strains. It is assumed that the GMT ratios for A/H1N1, A/H3N2 and B strains for QIVc are 1.49 for the A/H1N1, 1.00 for the A/H3N2 (based on the MN assay) and 0.84 for the B-strains. These GMT ratio estimates are consistent to those observed from protocol *V58P16*. It is assumed that the standard deviation of loge(titer)is 1.3 across all strains.

Under these assumptions and with n evaluable = 1450 in the Seqirus QIVc group and 725 in the Comparator QIV the power for 4 GMT ratio endpoints is 94.90% and the power for 4 SCR endpoints is 99.40%. The overall global power of the 8 endpoints is then 94.9%*99.40% = 94.33%.

This provides a total N evaluable = 2175. Allowing for a 10% drop-out, 2418 subjects will be recruited for immunogenicity assessment. Approximately 84 additional subjects between 24 through 47 months of age will be enrolled to study CMI

response, in order to have approximately 50 and 25 evaluable subjects in the QIVc and Comparator QIV groups, respectively. Thus, approximately 2502 subjects will be enrolled into study.

Sample size calculations were performed using PASS v12.0.02.

Table 2.3-1 Summarize the list of all co-primary endpoints with strains, the planned noninferiority margin and the underlying assumptions used for the sample size computations.

Table 2.3-1: Summary of Assumptions Used for Sample Size Calculations

NI Comparison	**A/H1N1**	**A/H3N2**	**B strains**
Test significance level, alpha (1-sided)	2.50%	2.50%	2.50%
Noninferiority Margin for the SCR comparison (%)	10	10	10
Assumed true SCR	81%	85%	69%
Power for SCR comparison tests for each strain (%)	100%	100%	99.7%
Global Power for 4 SCR endpoints	**99.40%**		
Noninferiority Margin for the GMT ratio	1.5	1.5	1.5
Common Standard Deviation of $\log_e$(titer)	1.3	1.3	1.3
Power for GMT ratio tests for each strain (%)	100%	100%	97.42%
Global Power for 4 GMT ratio Endpoints	**94.90%**		
Global Power for 8 co-primary Endpoints	**94.33%**		

3. Immunogenicity and Safety Variables

3.1 Co-Primary Immunogenicity Endpoints

Serum HAI antibody titer against A/H1N1, B/Victoria and B/Yamagata vaccine strains at Day 29/57, using cell-derived target virus:

- GMT by HAI assay
- SCR defined as the percentage of subjects with either a pre-vaccination HAI titer < 1:10 and a post-vaccination HAI titer ≥ 1:40, or a pre vaccination HAI titer ≥ 1:10 and a ≥ 4-fold increase in post vaccination HAI titer

Serum neutralizing antibody titer against A/H3N2 vaccine strain at Day 29/57, using cell-derived target virus:

- GMT by MN assay
- SCR defined as the percentage of subjects with either a pre-vaccination MN titer < 1:10 and a post-vaccination MN titer ≥ 1:40, or a pre vaccination MN titer ≥ 1:10 and a ≥ 4-fold increase in post vaccination MN titer

Derived Variables:

- The GMT ratio (QIV/QIVc) for each strain
- The inter-group difference in the SCRs (QIV minus QIVc) for each strain

The noninferiority of QIVc compared to US-licensed QIV will be assessed for the eight co-primary endpoints of GMT and SCR for each cell-derived target virus strain included in QIVc as follows:

- The GMT ratio for the A/H1N1 strain (HAI assay)
- The GMT ratio for the A/H3N2 strain (MN assay)
- The GMT ratio for the B Yamagata strain (HAI assay)
- The GMT ratio for the B Victoria strain (HAI assay)
- The difference between the SCRs for the A/H1N1 strain (HAI assay)
- The difference between the SCRs for the A/H3N2 strain (MN assay)
- The difference between the SCRs for the B Yamagata strain (HAI assay)
- The difference between the SCRs for the B Victoria strain (HAI assay)

3.2 Secondary Endpoints

3.2.1 Secondary Safety Endpoints

The measures for assessing safety and reactogenicity are as follows:

- Percentage of subjects with solicited AEs will be assessed for 7 days after each study vaccination
- Percentage of subjects with any unsolicited AEs from Day 1 to Day 29 (in previously vaccinated subjects) and from Day 1 to Day 57 (in not previously vaccinated subjects)
- Percentage of subjects with any Serious Adverse Events (SAEs), New Onset of Chronic Diseases (NOCDs), AEs leading to withdrawal from during the entire study period (i.e., from Day 1 to Day 181 for Previously vaccinated subjects or from Day 1 to Day 209 for not previously vaccinated subjects).

3.2.2 Secondary Immunogenicity Endpoints

Humoral immune response in term of HAI antibodies against A/H1N1, B/Victoria and B/Yamagata strains, using cell- and egg- derived target virus:

- GMT by HAI assay at Days 1 and 29/57
- Geometric Mean Ratio (GMR), defined as the fold increase in serum HAI GMT post vaccination (Day 29/57) compared to prevaccination (Day 1)
- Seropositivity rates (percentages of subjects with HAI titer $\geq$ 1:10) at Days 1 and 29/57
- Percentages of subjects with HAI titer $\geq$ 1:40 at Days 1 and 29/57
- SCR by HAI assay

Neutralizing antibody titers against A/H3N2 vaccine strains, using cell- and egg-derived target virus:

- GMT by MN assay at Days 1 and 29/57
- Geometric Mean Ratio (GMR), defined as the fold increase in serum MN GMT post vaccination (Day 29/57) compared to prevaccination (Day 1)
- Seropositivity rates (percentages of subjects with MN titer $\geq$ 1:10) at Days 1 and 29/57

- SCR by MN assay, where SCR for MN is defined as the percentage of subjects with either a prevaccination MN titer < 1: 10 and a post vaccination MN titer ≥1:40, or a prevaccination MN titer ≥ 1: 10 (LLOQ) and a ≥4-fold increase in post vaccination MN titer

Neutralizing antibody titers against A/H1N1, B/Victoria and B/Yamagata vaccine strains, in a subset of subjects:

- GMT by MN assay at Days 1 and 29/57
- GMR, defined as the fold increase in serum MN GMT post vaccination (Day 29/57) compared to prevaccination (Day 1)
- Seropositivity rates (percentages of subjects with MN titer ≥ 1:10(Lower Limit of Quantification (LLOQ))) at Days 1 and 29/57
- SCR by MN assay

3.3 Exploratory Immunogenicity Endpoints

The CMI responses to vaccination are considered exploratory endpoints in the study. CD4+ T-cell / CD8+ T-cell responses to antigen stimulation in vitro will be indicated by expression of activation markers (CD154, IL2, IL13, IFNγ, and TNFα), as measured by immunostaining and flow cytometry at baseline (day 1) and Day 29 (previously vaccinated subjects) or at baseline (Day 1) and Day 57 (not previously vaccinated subjects), in approximately 84 subjects (2:1 ratio, 56 subjects in QIVc and 28 subjects in licensed QIV group).

4. Analysis populations

There will be five analysis populations defined for the study analyses:

4.1 All Enrolled Set

All screened subjects who provide informed consent, receive subject ID, and provide demographic and/or baseline screening information, regardless of the subject's randomization and treatment status in the study.

4.2 Full Analysis Set (FAS)

All subjects in the All Enrolled Set who are randomized and receive a study vaccination. The FAS will be used to produce summaries and listings of subject characteristics.

In case of misrandomization with regard to treatment arm, subjects in the FAS will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

4.3 Safety Set

Overall Safety Set

All subjects who are in the solicited safety set and/or in the unsolicited safety set.

In case of vaccination error, subjects will be analyzed as "treated" (i.e., according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized).

If a subject received the correct study vaccine (dose, batch) but from another ongoing study at the site then the subject's safety data should be included in the safety analysis.

If a subject is unblinded during the study, he/she will be included in all safety sets.

The Overall Safety Set will be used to produce summaries and listings of all overall and unsolicited safety data after any vaccination. Subjects will be analysed according to the treatment they received.

Solicited Safety Set

All subjects in the Exposed Set with any solicited AE data.

Unsolicited Safety Set

All subjects in the Exposed Set with unsolicited AE data.

4.4 Full Analysis Set – Immunogenicity

The FAS for immunogenicity analyses will comprise all subjects in the FAS who:

- receive vaccine on Day 1
- provide serology specimens which yield valid serology assay results from both Day 1 and Day 29 (Previously vaccinated subjects) or Day 1 and Day 57 (Not previously vaccinated subjects)

The FAS will be used to produce summaries and listings of subject characteristics.

In case of vaccination error, subjects in the FAS Immunogenicity will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

If a subject is unblinded during the study, he/she will be included in the FAS Immunogenicity.

4.5 Per Protocol Set (PPS)

The PPS will comprise all subjects in the FAS Immunogencity who do not have any protocol deviations that are medically assessed as potentially impacting on immunogenicity results.

The PPS will be the primary set of interest for the primary/secondary immunogenicity analysis and a supporting analysis will be performed using the FAS Immunogenicity.

Membership of the PPS will be determined prior to unblinding the study.

Duplicate tables of primary and secondary immunogenicity analyses may also be produced based on the FAS Immunogencity population if there is >5% difference in the total number of subjects between the Per-Protocol Population and the FAS Immunogencity The decision to produce tables based on the FAS Immunogencity Population will be made by Seqirus after population sets are finalized and prior to unblinding.

Examples for subjects excluded due to other reasons than protocol deviations are subjects who withdrew informed consent.

In case of misrandomization with regard to treatment arm, the subject is excluded from the PPS.

If a subject is unblinded during the study, except for SUSAR, he/she will be excluded from the PPS.

If the serum sample was collected outside the time window of 21 days, the subject did not comply with the blood draw schedule and will be excluded from the PPS population. Hence, subjects will be excluded from PPS if blood draws >21 days after any vaccination.

4.5.1 Important Protocol Deviations Leading to Exclusion from the PPS Analysis

A protocol deviation (PD) is any change, divergence, or departure from the study design or procedures of a study protocol. A protocol deviation may be a reason to remove data from an analysis set at the time of analysis. PDs can be either observable or programmable. Programmable PDs are those PDs that can be programmed from the data recorded in the clinical database. Observable PDs are PDs identified by CRAs or other team members.

PDs will be classified as major and minor using a prespecified list of types of deviations. Major PDs are defined in accordance with ICH E3 as important PDs related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment resulting in the potential to jeopardize the safety or rights of the trial subjects or the scientific value of the trial.

All PDs will be evaluated before unblinding and most will be classified into the following categories:

- Subject randomized and did not satisfy entry criteria
- Subject received the wrong treatment or incorrect dose
- Subject took an excluded concomitant medication
- Key study procedures missed or performed out of window, such as, for immunogenicity if the serum sample was collected outside the time window of 21 days the subject did not comply with the blood draw schedule and will be excluded from the PPS population. Hence, subjects will be excluded from PPS if blood draws >21 days after any vaccination.

The impact of major PDs on the efficacy, immunogenicity and/or safety results will be investigated by assessing the robustness of the study results.

Major PDs will lead to exclusion of the subject or part of the subject's data from at least one analysis set.

The number of subjects in any and by PD category will be summarized by treatment group, center and overall. Individual subject listings will be provided in an appendix, sorted by subject and by PD category.

Prior to unblinding the analysis, designated Seqirus staff will develop a memo that describes the PDs that led to exclusions from analysis sets. This memo will be signed off by at least the Biostatistician and the Clinical Scientist and will be included in the trial master file (Exclusion Memo).

Prematurely terminating study participation for reasons such as withdrawal of consent or occurrence of AEs (including death) is not considered as a PD. Any missing assessments that should have otherwise been collected for that subject later in the study is also not considered as a PD.

Deviations from the protocol will be documented on an ongoing basis by the study monitors and lead clinical research associate or designee throughout the study period.

At the time of database lock, prior to unblinding and while the major PDs are being reviewed, the project manager or designee will forward all relevant documentation highlighting PDs to the study statistician. These deviations will be included in the protocol deviation document for agreement and will be listed with the PDs in the clinical study report (CSR).

. PD listings will be reviewed by Seqirus prior to the finalization of the population datasets, which will occur prior to unblinding. The list will be used to determine which subjects should be excluded from either the FAS or the PPS.

4.6 Special Subpopulations

Not Applicable.

5. DATA Handling

5.1 Time points and Visit Windows

A summary of the assessments required at each study visit is described in (Appendix A).

Day 1 is defined as the day of first dose of the vaccination.

Relative days after Day 1 are calculated as (assessment date – Day 1 date) + 1. Relative days prior to Day 1 are calculated as (assessment date – Day 1 date). The day prior to Day 1 is Day -1

The following visit windows will be used.

Table 5.1-1: Visit windows for previously vaccinated subjects

Study part	Scheduled visit	Time interval (days)
Screening	Screening Visit	< 10 Days before Day 1
Treatment period	Clinic Visit V1	Day 1
	Reminder Phone V1	Day 3 (-1/+1 Day)
	Clinic Visit V2	Day 28 (+ 7 Days)
Follow-up Period	Safety Phone call V3	Day 90 (+ 7 Days)
	Safety Phone call V4	Day 180 (+ 14 Days)

Table 5.1-2: Visit windows for not previously vaccinated subjects

Study part	Scheduled visit	Time interval (days)
Screening	Screening Visit	< 10 Days before Day 1
Treatment period	Clinic Visit V1	Day 1
	Reminder Phone V1	Day 3 (-1/+1 Day)
	Clinic Visit V2	Day 28 (+ 7 Days)
	Reminder Phone	V2 + 2 Days (-1/+1 Day)
	Clinic Visit V3	V2 + 28 Days (+7 Days)
Follow-up Period	Safety Phone call V4	V2 + 90 Days (+7 Days)
	Safety Phone call V5	V2 + 180 Days (+14 Days)

Visit window deviations will be reviewed prior to unblinding.

5.2 Missing Values – Missing Visit.

Missing, unused and spurious data will be dealt with as such. There is no intention to implement any procedure for replacing missing data.

Titer values recorded as <1:10 (or < lower limit of quantification [LLOQ]) will be summarized as 1:5 (or ½* LLOQ).

No imputation of missing solicited or unsolicited AEs will be used but implausible measurement will be removed as described below. The percentage of subjects with missing solicited AE assessments (e.g. missing Patient Diary) and missing Safety Phone. Calls or Safety Assessments will be reported for each time period.

Missing immunogenicity values are considered missing completely at random (MCAR) and therefore will not contain information that impact the result of the analysis (i.e., not informative). Imputation methods will therefore not be used.

The PPS will be used for the primary immunogenicity analysis and a supporting analysis will be performed using the FAS Immunogenicity, as noted in Section 4.4 FAS Immunogenicity.

Duplicate tables of primary and secondary immunogenicity analyses may also be produced based on the FAS Immunogenicity if there is >5% difference in the total number of subjects between the PPS and the FAS Immunogenicity.

Exclusions of Individual Values for Safety Analyses

Some local and systemic AEs will be directly measured by the subject and will not be subject to a reconciliation process, even if they are biologically implausible.

Therefore, these implausible measurements will be removed from the analysis but included in listings.

Implausible measurements are summarized in the table below:

Table 5.2-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 33°C or ≥ 42°C
Erythema Measurements	≥ 450 mm or < 0 mm
Induration Measurements	≥ 250 mm or < 0 mm
Ecchymosis Measurements	≥ 250 mm or < 0 mm

6. Statistical Methods

6.1 General Principles

All data processing, summarization and analyses will be performed using [redacted]'s SAS Environment / Version 9.3 (or later) of the SAS® statistical software package.

The FAS will be used for summaries of baseline characteristics and background data, analyses will be repeated on the All Enrolled Set and PPS; the Safety population will be used for all safety analyses; the PPS will be used for the primary and secondary analyses; and the FAS population will be used for supporting analysis of the primary endpoint, and all other immunogenicity endpoints.

Descriptive statistics will be used to present all safety and immunogenicity results: number of observations (n), mean, standard deviation (SD), median, minimum (min), maximum (max) for continuous data and frequency and percentage relative to the appropriate population for categorical data.

Binary data (ie, percentages of subjects with SCR) will be summarized for each group using unadjusted estimates and will be reported together with two-sided exact 95% Confidence Intervals (CIs) (Clopper-Pearson).

The difference between the proportions of treatment groups will be determined and the corresponding two-sided 95% CIs calculated by Miettinen-Nurminen method.

Geometric means and 95% CIs will be calculated by taking the anti-logs of the means and 95% CI of the log transformed immunogenicity parameters. Exact CIs based upon the binomial distribution will be calculated for percentages.

Statistics will be displayed for the following:

- Seqirus QIVc
- Comparator QIV
- Overall

In the summary tables, the strains will be displayed as follows:

- Cell antigen:

  - H1N1: A/Idaho/07/2018
  - H3N2: A/Indiana/08/2018
  - B-yam: B/Singapore/INFTT-16-0610/2016
  - B-vic: B/Iowa/06/2017
- Egg antigen:
  - H1N1: A/Brisbane/02/2018 (IVR-190)
  - H3N2: A/Kansas/14/2017 (X-327)
  - B-yam: B/Phuket/3073/2013 (BVR-1B)
  - Bvic: B/Maryland/15/2016

All data will be listed.

6.2 Subject Disposition and Data Sets Analyzed

The number of subjects screened, enrolled into the study, in each study population, who completed the study, and the reasons for any premature discontinuation from the study will be presented in summary tables by treatment group, by age strata (6 through 23 months, 24 through 47 months) and overall. The number in the full analysis set will be used as the denominator.

The number of subjects who are excluded from each of the FAS Immunogencity and Per-Protocol populations will be summarized by treatment group, by age strata (6 through 23 months, 24 through 47 months) and overall.

6.3 Protocol Deviations

Major protocol deviations will tabulated by vaccine group and will also be listed for subjects who have been entered into the study and assigned a subject number.

The following criteria will exclude a subject from the FAS population. These will be listed in the deviation listing:

- Not provided both pre- and post-vaccination blood samples
- Laboratory-confirmed influenza infection during the active period (Day 1 to Exit Visit)
- Received prohibited medication during the active study period which medically assessed to potentially impact immunogenicity results

Note: Other reasons for deviation may be added to this list, but will be done prior to unblinding of the study. The reasons highlighted will be used in defining the FAS population and per-protocol population.

6.4 Demographics and Other Baseline Characteristics

All baseline and demographic characteristics will be summarized by vaccine group, age strata, and overall on the FAS population. This will include age (months), gender, race, ethnicity, weight, height, BMI, pre-vaccination temperature, medical history, and previous vaccination status on the FAS population.

Derived variables:

Body Mass Index will be calculated according the following formula:

Body Mass Index (kg/m2): Weight (kg) / Height^2 (m^2)

6.4.1 Medical History

Medical history will be coded according to Medical Dictionary for Regulatory Activities (MedDRA) version 22.0 or higher and will be displayed by system organ class and preferred term, using the MedDRA internationally agreed order.

The numbers and percentages of subjects with medical history will be presented by MedDRA system organ class (SOC) and preferred term (PT) by vaccine group and overall. Medical history data will be tabulated for FAS.

For prior vaccination history, the number and percentage of subjects who have ever been vaccinated, who have been vaccinated in the current season, and have been assigned to receive 1 or 2 vaccinations during the study, will be summarised.

6.4.2 Prior and Concomitant Medications

Medications will be coded using the WHO Drug dictionary version B3 MARCH 2019 or higher.

A prior medication is a medication used only before the first study vaccination (i.e. medication end date < first study vaccination date). Concomitant medications are all medications taken during the study period, including those started before but ongoing at vaccination.

When start and/or end dates of a medication intake are missing, the medication is considered as concomitant with the study vaccination schedule.

If the study vaccination date is missing, then the medication is considered as concomitant with the study vaccination schedule, provided that the study vaccine was administered to the subject.

Use of concomitant medication will be tabulated by treatment, age strata, therapeutic area, and preferred drug name.

Prior medications will only be listed.

6.5 Primary and Secondary Analysis

Derived immunogenicity variables:

Values below the limit of quantification (recorded as "< LOQ") will be set to half that limit (LOQ/2).

The rate of seroconversion is defined as the percentage of subjects with either a prevaccination HAI (or MN) titer < 1:10 and a post vaccination HAI (or MN) titer ≥ 1: 40 or a prevaccination HAI (or MN) titer ≥ 1:10 and a ≥ 4-fold increase in post vaccination HAI (or MN) titer.

All statistical analyses for HAI (or MN) titers will be performed on the logarithmically transformed (base 10) values. Individual HAI titers below the detection limit (<10) will be set to half of that limit (5). Individual MN titers below the lower limit of quantification (LLOQ), will be set to half of that limit (1/2* LLOQ).

Unadjusted for GMT, GMRs and pertaining two-sided 95% CIs will be calculated assuming log-normal distribution of the titers and will be completed by providing minimum, maximum and median titers for each vaccine group.

Seroconversion is defined as binary variable for subjects with non-missing values prevaccination and post-vaccination as:

= 1, if seroconverted (defined as the percentage of subjects with either a pre-vaccination HAI (or MN) titer < 1:10 and a post-vaccination HAI (or MN) titer ≥ 1:40 or a pre-vaccination HAI (or MN) titer ≥ 1:10 and a minimum 4-fold rise in post-vaccination HAI (Or MN) antibody titer)

= 0, otherwise

Geometric Mean Titer (GMT)

Serum HAI antibody levels of all participants will be determined in triplicate (HAI1, HAI2 and HAI3) on serum separated from the whole blood. Pre- and post-vaccination samples will be titrated in duplicate, simultaneously. The titer assigned to each sample shall be the geometric mean of three independent determinations i.e. Assigned titer=exp[(log(HAI1) +log(HAI2) +log(HAI3))/3

GMT will be based on the following:

- HAI antibody titer for each strain: All analyses involving HAI antibody titer (namely group GMT within a vaccine group) will be performed on the log scale and the resultant summary statistic back-transformed to derived GMT

The GMT will be calculated using the following formula:

$$10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}(t_i)}{n}\right\}}$$

where n, t_1, t_2, K, t_n are n observed immunogenicity titers.

The 95% confidence intervals for GMT will be calculated as $10^{\{M-t_{0.975,n-1}SE)\}}, 10^{\{M+t_{0.975,n-1}SE)\}}$; where M and SE are the means and standard error of logarithm base 10 -transformed titers, respectively.

Geometric Mean Ratio (GMR)

GMRs measure the changes in immunogenicity titers *within* subjects.

The GMR will be calculated using the following formula:

$$10^{\left[\frac{\sum_{i=1}^{n}\log_{10}\left(t_{ij}/t_{ik}\right)}{n}\right]} = 10^{\left[\frac{\sum_{i=1}^{n}\left(\log_{10}(t_{ij})-\log_{10}(t_{ik})\right)}{n}\right]}$$

where, for n subjects, t_{ij} and t_{ik} are observed immunogenicity titers for subject i at time-points j and k, j ≠ k. The 95% confidence intervals for GMR will be calculated as $10^{\{M-t_{0.975,n-1}SE)\}}, 10^{\{M+t_{0.975,n-1}SE)\}}$; where M and SE are the means and standard error of log 10(t_{ij}) - log 10(t_{ik}) respectively.

6.5.1 Analysis of Primary Immunogenicity Objective(s)

The primary immunogenicity objective of this study is to demonstrate that vaccination with Seqirus QIVc elicits an immune response that is not inferior to that of an US-licensed QIV containing the recommended strains for the season, in subjects 6 months through 47 months of age, as measured by HAI and MN.

6.5.1.1 Statistical Hypothesis

The non-inferiority of Seqirus QIVc compared to the US-licensed comparator QIV will be assessed by the 8 co-primary endpoints of GMT and SCR for A/H1N1, A/H3N2 and the B Strains. Definitions for noninferiority based on endpoints measured by HAI are derived from the FDA Guidance on seasonal inactivated influenza vaccines (Guidance for Industry Clinical Data Needed to Support Licensure of Seasonal Inactivated Influenza Vaccines 2007). A modified definition will be applied to assessment of the endpoint based on the MN assay.

The study will be considered successful if all the 8 co-primary endpoints are achieved.

Specifically, Seqirus QIVc will be considered to be noninferior to the US-licensed QIV if, for each of the four strains, the following statistical criteria are met:

A/H1N1 and B strains:

- The upper bound of the two-sided 95% CI on the ratio of the HAI GMTs does not exceed 1.5. The HAI GMT ratio will be calculated as HAI GMT $_{\text{US-licensed QIV}}$ / HAI GMT $_{\text{Seqirus QIVc}}$

- The upper bound of the two-sided 95% CI on the difference between the HAI SCRs does not exceed 10%. The difference in HAI SCRs will be calculated as (HAI Seroconversion $_{\text{US-licensed comparator QIV}}$–HAI Seroconversion $_{\text{Seqirus QIVc}}$). The 95% CI will be computed based on the binomial distribution.

A/H3N2 strain only:

- The upper bound of the two-sided 95% CI on the ratio of the MN GMTs does not exceed 1.5. The MN GMT ratio will be calculated as MN GMT $_{\text{US-licensed QIV}}$ / MN GMT $_{\text{Seqirus QIVc}}$

- The upper bound of the two-sided 95% CI on the difference between the MN SCRs does not exceed 10%. The difference in MN SCRs will be calculated as (MN Seroconversion $_{\text{US-licensed comparator QIV}}$–MN Seroconversion $_{\text{Seqirus QIVc}}$). The 95% CI will be computed based on the binomial distribution.

In mathematical notation the statistical hypotheses to be tested for the primary noninferiority immunogenicity analysis corresponds to:

- H0: Ri > 1.5, for any strain
- Ha: Ri ≤ 1.5, for all strain and
- H0: Di > 10, for any strain
- Ha: Di ≤ 10, for all strain

where Ri is any of the 4 strain-specific post immunogenicity dose GMT ratios:

- (US-licensed comparator QIV) / (QIVc) for B/Yamagata strain
- (US-licensed comparator QIV) / (QIVc) for B/Victoria strain
- (US-licensed comparator QIV) / (QIVc) for A/H1N1 strain
- (US-licensed comparator QIV) / (QIVc) for A/H3N2 strain

and Di is the 4 strain-specific post-dose SCR difference, namely

- (US-licensed comparator QIV) - (QIVc) for B/Yamagata strain
- (US-licensed comparator QIV) - (QIVc) for B/Victoria strain
- (US-licensed comparator QIV) - (QIVc) for A/H1N1 strain
- (US-licensed comparator QIV) - (QIVc) for A/H3N2 strain

No adjustment of Type I error will be made for multiple comparisons.

6.5.1.2 Analysis Sets

The Per Protocol Set will be used for the primary immunogenicity analysis and a supporting analysis will be performed using the FAS – Immunogenicity Analysis. Duplicate tables of primary and secondary immunogenicity analyses may also be produced based on the FAS Immunogencity population if there is >5% difference in the total number of subjects between the Per-Protocol Population and the FAS Immunogenicity Population.

6.5.1.3 Statistical Methods

All statistical analyses for HAI (or MN) titers will be performed on the logarithmically transformed (base 10) values. Individual HAI titers below the detection limit (<10) will be set to half of that limit (5). Individual MN titers below the LLOQ, will be set to half of that limit (1/2*LLOQ).

Co-primary immunogenicity endpoints of GMT and SCR for each virus strain contained in the vaccine will be assessed in subjects 6 Months through 47 Months of Age overall.

Primary analysis will be performed in subjects 6 Months through 47 Months of Age for the Per-Protocol Set. The difference in SCRs will be presented with exact 95% CIs. Each of the four strains will be analyzed separately.

For immunogenicity data, the immunogenicity analysis will comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

To determine the GMT ratio (adjusted analysis) a general linear model (GLM) will be fitted on log transformed (base ten) post-vaccination HAI (or MN) titer as the outcome variable and terms for covariates: vaccine treatment, pre-vaccination HAI (or MN) titer, age stratum, gender, vaccination history, age-by-vaccine interaction and study site. Potential covariate interaction effects will also be examined in the fit of the GLM. From the model, an adjusted difference in least square means (on the log scale) will be produced with 95% confidence limits. The estimated difference and the confidence limits will be back transformed to obtain an *adjusted GMT ratio* with 95% confidence limits. Each of the four strains will be analyzed separately. The adjusted GMT ratio will be the result for which the non-inferiority assessment of the HAI (or MN) GMT co-primary endpoint will be based on.

The statistical models might be reduced in case they fail to converge.

The complete set of covariates that will be used in the model to calculate the adjusted GMT ratio will include treatment group (2 treatments), pre-vaccination GMT titer (value), age strata (2 categories, 6 through 23 months or 24 through 47 months), gender (male or female), influenza vaccination received prior year (Yes or No), and investigator site (site identifier).

The GLM specification is:

Adjusted Analysis GMT Model: Log-transformed Post-vaccination HAI (or MN) Titer = Vaccine + Age Strata + Gender + Vaccination History [y/n] + Log-transformed Pre-vaccination HAI (or MN) Titer + Site + Age Strata*Vaccine.

For any strain, the interaction term Age Strata*Vaccine will be removed from the fit of the model if it is assessed to be not significant. For all immunogenicity subgroup analysis, the interaction term will be removed in the fit of the model.

The measure of the unadjusted GMT ratio based on post-vaccination GMTs only will also be presented.

Binary data (i.e., percentages of subjects with seroconversion and with titer ≥ 1:40) will be summarized for each group using unadjusted estimates and will be reported together with two-sided exact 95% CIs. No multiplicity adjustment to the CI levels will be implemented.

If all 8 co-primary endpoints result in a conclusion of non-inferiority, then overall non-inferiority of Seqirus QIVc compared to the US-licensed comparator QIV will be concluded.

Handling of Missing Values for Immunogenicity Data

For immunogenicity data, it may be reasonable to consider missing immunogenicity values as missing completely at random (MCAR), i.e., not informative. Therefore, the immunogenicity and key secondary analyses will comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

Sensitivity Analyses for Handling of Missing Immunogenicity Data

To verify the robustness of the primary analysis results, an exploratory analysis may be performed, in which missing titers were imputed using *multiple imputation.* The evaluation of the robustness of the primary analysis results will be based on a pattern mixture model and a 'tipping analyses' approach using multiple imputation methods.

This sensitivity analysis, if conducted, will be based on the per-protocol analysis set and will serve to evaluate the robustness of the non-inferiority conclusions. Appendix C specifies the details of the exploratory sensitivity analysis.

6.5.2 Analysis of Secondary Safety Objective(s)

The analysis of safety assessments in this study will include summaries of the following categories of safety data collected for each subject:

- Vaccine exposure
- Solicited local and systemic AEs.
- Unsolicited AEs.
- SAEs, AE leading to withdrawal, NOCD

6.5.2.1 Analysis of Extent of Exposure

The number and percentage of subjects with vaccinations will be summarized overall and by vaccine group.

6.5.2.2 Analysis of Solicited Local, Systemic and Other Adverse Events Definitions

For details refer to section 7.1.1 of Study Protocol.

Analysis

Solicited Adverse Events

All solicited adverse events will be summarized according to defined severity grading scales (Table 6.5.2.2-1).

Frequencies and percentages of subjects experiencing each adverse event will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic adverse event overall and at each time point will also be presented.

Post-vaccination solicited adverse events reported for 7 days after each vaccination and will be summarized for the intervals day <30 min and day 1 to 7 by maximal

severity and by vaccine group, the latter excluding the 30-minute measurement. Injection-site erythema, ecchymosis, and induration will be summarized according to categories based on linear measurements: Type I: none (0 mm), any (1 to <10 mm, 10 to 25 mm, 26 to 50 mm, >50 mm) and Severe (>50mm).

Injection site tenderness and systemic adverse events (except fever) occurring up to 7 days after each vaccination will be summarized according to "mild, moderate" or "severe" categorization. Severity grades are defined in Tables 6.5.2.2-1 below.

Table 6.5.2.2-1 Solicited AE Grading

Symptom	**Grading**			
	0 (None)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**
Solicited Local AEs*				
Tenderness		For subjects less than 24 months of age at the time of the first dose of study vaccine:		
Tenderness		Minor reaction on touch	Cried/protested on touch	Cried when limb was moved/ spontaneously painful
Tenderness		For subjects 24 months of age and older at the time of the first dose of study vaccine:		
Tenderness		Does not interfere with daily activities	Interferes with daily activities	Prevents daily activity
Solicited Systemic AEs**				
Change of eating habits	None	Eating less than normal for 1 - 2 feeds / meals	Missed 1 or 2 feeds / meals	Missed more than 2 feeds / meals
Sleepiness	None	shows an increased drowsiness	sleeps through feeds / meals	sleeps most of the time and it is hard to arouse him/ her

Vomiting (throwing up)	None	1 - 2 times in 24 hours	3 - 5 times in 24 hours	6 or more times in 24 hours or requires intravenous hydration
Diarrhoea (loose stools)	Fewer than 2 loose stools in 24 hours	2-3 loose stools in 24 hours	4-5 loose stools in 24 hours	6 or more loose stools in 24 hours or requires intravenous hydration
Irritability	None	Requires more cuddling and is less playful than usual	More difficult to settle	Unable to console
Shivering	None	Present but does not interfere with daily activity	Interferes with daily activity	Prevents daily activity
Temperature***	<38°C	≥38°C to <39°C	≥39°C to <40°C	≥40°C

* Injection-site erythema, ecchymosis, and induration will be summarized according to categories based on linear measurements: Type I: none (0 mm), any (1 to <10 mm, 10 to 25 mm, 26 to 50 mm, >50 mm); Type II: Grade 0 (<10 mm), any (10 to 25 mm, 26 to 50 mm, >50 mm

** Use of antipyretics and analgesics will be summarized by frequency, by type of use (prophylactic versus treatment) and percentage of subjects reporting use.

*** Body temperature will be summarized by 0.5° C and 1.0° C increments from 36.0° C to ≥40° C and will be broken down by route of measurement and by age cohort.

Each solicited local and systemic adverse event will also be further summarized as "none" versus "any." "Any" will include measured reactions (erythema, ecchymosis, and induration) with a diameter of at least 1 mm.

Time to onset and duration of solicited AEs will be analysed using descriptive statistics.

Implausible measurements will not be taken into consideration in the analysis.

Use of antipyretics and analgesics will be summarized by frequency, by type of use (prophylactic versus treatment) and percentage of subjects reporting use.

Body temperature will be summarized by 0.5°C and 1.0°C increments from 36.0°C to ≥40°C and will be broken down by route of measurement and by age cohort.

6.5.2.3 Analysis of Unsolicited Adverse Events

Definitions

All AEs will be characterized according to the date of occurrence related to the vaccination phase as follows:

- **Non-Treatment Emergent:** start date before the date of injection of study vaccine.
- **Treatment Emergent**: start date on or after the date of injection of study vaccine or, AE increase in severity, including to "serious" AE.

If start date is equal to the first date of injection, then "timing" variable ("Did event start before or after vaccination?") will be used to define whether the AE occur before or after the injection.

If an AE happened on the same day of injection and the time stamp is missing, then the AE is assumed to be treatment emergent.

If an AE start date is missing or unknown, the AE will be considered as emergent.

When start and/or end dates of an AE are only partially known, AEs will be categorized as emergent before, during, or after vaccination phase using the following rules:

- If the partial end date is before (<) the vaccination (i.e., year or year & month is/are before the study vaccination year or year & month) then the AE is not treatment-emergent before vaccination phase.
- If the partial start date is equal or after (≥) the first study vaccination (i.e., year or year & month is/are after or the same as the first study injection year or year & month) then the AE is considered treatment-emergent.

The **maximum event severity** is the greatest severity associated with a PT for a reported AE according to the following order: Mild < Moderate < Severe. Unknown/ Missing severity is considered as severe.

Multiple AEs with the same PT for the same subject are counted only once.

Vaccination-related AEs are those for which the cause has been evaluated by the investigator and recorded as related.

Analysis

This analysis applies to all Treatment-Emergent adverse events occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in AE CRF, with a start date on or after the date of first vaccination. AE starting prior to the first vaccination will only be listed.

The original verbatim terms used by investigators to identify AEs in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The AEs will then be

grouped by MedDRA preferred terms into frequency tables according to SOC. All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccination group and by interval of study observation (Day 1 to Day 29, Day 29 to Day 181 in "previously vaccinated" subjects and Day 1 to Day 57, Day 57 to Day 209 in "not previously vaccinated" subjects). When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Separate summaries will be produced for the following categories:

- SAE
- NOCDs
- Adverse events that are possibly or probably related to vaccine
- SAEs that are possibly or probably related to vaccine
- Adverse event leading to withdrawal or early termination
- Adverse event resulting in death
- Adverse events leading to a medically-attended visit during the treatment period

Data listings of all adverse events will be provided by subject. In addition, adverse events in the categories above will be provided as listed data.

6.5.2.4 Combined Solicited and Unsolicited Adverse Events

A summary of the number of subjects with all combined solicited (regardless of their duration) and unsolicited AEs will be provided, regardless of their duration and recurrence. A further differentiation of combined AEs according to seriousness, severity, or relationship will not be performed. For clintrial.gov and EudraCT posting purposes, a summary of combined solicited and unsolicited non-serious AEs will be produced by System Organ Class and according to occurrence of each event.

6.5.2.5 Body weight and temperature

Body weight and temperature, recorded prior to vaccination, will be provided as a by–subject listing.

6.5.2.6 Physical Examination

Physical Examination Findings will be provided as a by-subject listing.

6.5.3 Analysis of Secondary Immunogenicity Objective(s)

The measures for immunogenicity will be determined by HAI and MN assay for Day 1 and Day 29/57.

Humoral immune response in terms of HAI antibodies against A/H1N1, B/Victoria and B/Yamagata strains, using cell- and egg- derived target virus for both Seqirus QIVc and the QIV comparator.

- GMTs: Geometric mean of HAI titers pre-vaccination (Day 1) and post-vaccination (Day 29/57);
- Geometric mean ratio (GMR), defined as the fold increase in serum HAI GMTs post vaccination (Day 29/57) compared to prevaccination (Day 1). Geometric Mean Ratio from Day 1 to Day 29 or Day 57.
- Seropositivity rates (percentages of subjects with HAI titer $\geq$ 1: 10) at Days 1 and 29/57.
- Percentage of subjects with HAI titer ≥ 1:40 at Days 1 and 29/57.
- SCR by HAI assay.

Neutralizing antibody titers against A/H3N2 vaccine strains, using cell- and egg-derived target virus:

- GMT by MN assay at Days 1 and 29/57
- GMR, defined as the fold increase in serum MN GMT post vaccination (Day 29/57) compared to prevaccination (Day 1)
- Seropositivity rates (percentages of subjects with MN titer $\geq$ 1:10(LLOQ)) at Days 1 and 29/57
- SCR by MN assay

Neutralizing antibody titers against A/H1N1, B/Victoria and B/Yamagata vaccine strains, in a subset of subjects:

- GMT by MN assay at Days 1 and 29/57
- GMR, defined as the fold increase in serum MN GMT post vaccination (Day 29/57) compared to prevaccination (Day 1)
- Seropositivity rates (percentages of subjects with MN titer $\geq$ 1:10(LLOQ)) at Days 1 and 29/57
- SCR by MN assay

6.5.3.1 Statistical Hypothesis

No statistical testing will be performed for the secondary immunogenicity objectives.

6.5.3.2 Statistical Methods

All statistical analyses for HAI (or MN) titers will be performed on the logarithmically transformed (base 10) values. Individual HAI titers below the detection limit (<10) will be set to half of that limit (5). Individual MN titers below the lower limit of quantification (LLOQ), will be set to half of that limit.

Unadjusted for GMT, GMRs and pertaining two-sided 95% CIs will be calculated assuming log-normal distribution of the titers and will be completed by providing minimum, maximum and median titers for each vaccine group.

Binary data (i.e. percentages of subjects with seroconversion and with titer ≥ 1:40) will be summarized for each group using crude estimates and will be reported together with 2- sided exact 95% CIs. No multiplicity adjustment to the CI levels will be implemented.

For immunogenicity data, it may be reasonable to consider missing immunogenicity values MCAR, i.e. not informative. Therefore, the key secondary analysis will

comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

6.5.4 Subgroup Analysis

The immunogenicity analyses will be performed by stratifying for the following subgroups:

- Subjects with pre-vaccination HAI titer <1:10 and pre-vaccination HAI titer ≥ 1:10.
- Subjects with pre-vaccination MN titer <LLOQ and pre-vaccination MN titer ≥ LLOQ.
- Subjects with and without recent seasonal influenza vaccine (defined as influenza vaccine within the past 12 months).
- Subjects "previously influenza vaccinated" and "not-previously influenza vaccinated".
- Subjects aged "6 through 23 months" and "24 through 47 months".
- Subjects by gender.
- Subjects by race.
- Subjects by ethnicity.

The safety analyses will be performed by stratifying for the following subgroups:

- Subjects "previously influenza vaccinated" and "not-previously influenza vaccinated".
- Subjects with and without recent seasonal influenza vaccine (defined as influenza vaccine within the past 12 months).
- Subjects aged "6 through 23 months" and "24 through 47 months".
- Subjects by gender.
- Subjects by race.
- Subjects by time interval as below:
    - Day 1 to Day 29, Day 29 to Day 181 in "previously vaccinated" subjects
    - Day 1 to Day 57, Day 57 to Day 209 in "not previously vaccinated" subjects.

6.5.5 Analysis of Exploratory Immunogenicity Objectives

Exploratory immunogenicity endpoints of homologous CMI responses will be summarized using descriptive statistics by treatment group at baseline (Day 1), and Day 29/57 in a subset of 84 subjects.

For each vaccine antigen analyzed and each pattern including single response variables, separated by vaccine group and visit, the mean percentage, the 2-sided 95% confidence interval, standard deviation, median, minimum, and maximum were calculated to characterize the vaccination groups. Between-group differences incl. 2-sided 95% CI and within-group differences (relative to baseline) incl. 2-sided 95% CI were calculated using ANCOVA with vaccine group as qualitative factor adjusted for baseline values. All available data were included in the analysis irrespective of any protocol deviation

In case of additional exploratory immunogenicity analyses, the immune response will be further characterized similar to the analysis of secondary immunogenicity endpoints.

6.6 Interim Analysis

No interim analysis will be performed for this study.

The final analysis of the primary and secondary immunogenicity endpoints will be conducted on cleaned and locked data once all subjects have completed all immunogenicity assessments (end of treatment period, i.e. up to 28 days following the last vaccination dose). At this time, the analysis of all solicited adverse events and of unsolicited adverse events reported during the treatment period will also be conducted. These results will be used to prepare a Clinical Study Report (CSR) with treatment period data. No individual unblinded listings will be generated at this stage.

This analysis constitutes the final analyses of the primary and secondary immunogenicity endpoints and therefore preserves the integrity of the clinical study results.

7. Changes in Planned Analysis

"Evaluable Set – Immunogenicity" population as been renamed as "Full Analysis Set – Immunogenicity".

Any deviations from the original statistical plan will be described and justified in the final CSR.

8. Data Issues

Not applicable.

9. References

1 Clinical Study Protocol V130_10 Protocol_v4_09DEC2019.pdf

2 ICH. *Statistical Principles for Clinical Trials*, Guideline E9, 1998. Available at http://www.emea.eu.int/pdfs/human/ich/036396en.pdf

3 World Health Organization, WHO Technical Report, Series No. 924. 2004, Annex 1: *Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations*

4 FDA CBER Guidance for Industry, May 2007, *Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines*

5 ICH guideline (Guidance for Industry, Clinical Safety Data Management: Definitions and Standards for Expedited Reporting – ICH-E2A), 27 October, 1994

Appendix A: Schedule of Assessments

The schedule chart is the master representation of the clinical trial. In case of (apparent) inconsistencies in the clinical trial protocol the information provided here is the binding one.

Time and Events Table – Previously Vaccinated Subjects

	Treatment Period			Follow-up Period	
	Clinic Visit	Reminder Phone Call	Clinic Visit	Safety Phone Call	Safety Phone Call
Visit Number	V1		V2	V3	V4
Study Day		V1 + 2	V1 + 28	V1 + 90	V1 + 180
Study Day Window	n/a	-1/+1	0 to +7	0 to + 7	0 to + 14
Informed Consent [a]	X [b]				
Medical History	X [b]				
Physical Examination and Clinical signs	X [b, c]		X		
Exclusion/Inclusion Criteria	X [b]				
Randomization	X [b]				
Vaccination	X				
30 Minutes Post Vaccination Assessment	X				
Subject Diary Card Dispensed with Training	X				
Subject Diary Card Reminder Call		X			
Subject Diary Card Reviewed and Collected			X		
Assess all AEs	X		X		
Assess SAEs, NOCDs, AEs leading to withdrawal	X		X	X	X
Assess Relevant Medications/Vaccinations	X		X	X	X
Blood Draw	X [b, d]		X [d]		

Abbreviations: AE = adverse event; n/a = not applicable; NOCD = new onset of chronic disease; SAE = serious adverse event; V = visit.
[a] Confirm consent form(s) signed prior to any procedures. The informed consent process may be conducted earlier, but within 10 days prior to Day 1.
[b] Procedures to be performed prior to vaccination.
[c] Includes measurement of height and weight..
[d] A blood sample will be drawn prevaccination (Day 1) and postvaccination (Day 29) for immunogenicity evaluation or for assessing cell-mediated immunity response.

Time and Events Table – Not Previously Vaccinated Subjects

	Treatment Period					Follow-up Period	
	Clinic Visit	Reminder Phone Call	Clinic Visit	Reminder Phone Call	Clinic Visit	Safety Phone Call	Safety Phone Call
Visit Number	V1		V2		V3	V4	V5
Study Day		V1 + 2	V1 + 28	V2 + 2	V2 + 28	V2 + 90	V2 + 180
Study Day Window	n/a	-1/+1	0 to+7	-1/+1	0 to +7	0 to +7	0 to +14
Informed Consent [a]	X [b]						
Medical History	X [b]						
Physical Examination and Clinical signs	X [b, c]		X		X		
Exclusion/Inclusion Criteria	X [b]		X [b, d]				
Randomization	X [b]						
Vaccination	X		X				
30 Minutes Post Vaccination Assessment	X		X				
Subject Diary Card Dispensed with Training	X		X				
Subject Diary Card Reminder Call		X		X			
Subject Diary Card Reviewed and Collected			X		X		
Assess all AEs	X		X		X		
Assess SAEs, NOCDs, AEs leading to withdrawal	X		X		X	X	X
Assess Relevant Medications/Vaccinations	X		X		X	X	X
Blood Draw	X [b, e]				X [e]		

Abbreviations: AE = adverse event; n/a = not applicable; NOCD = new onset of chronic disease; SAE = serious adverse event; V = visit.

[a] Confirm consent form(s) signed prior to any procedures. The informed consent process may be conducted earlier, but within 10 days prior to Day 1.

[b] Procedures to be performed prior to vaccination.

[c] Includes measurement of height and weight.

[d] Eligibility for subsequent vaccination (see 5.2.2).

[e] A blood sample will be drawn prevaccination (Day 1) and postvaccination (Day 57) for immunogenicity evaluation or for assessing cell-mediated immunity response.

Appendix B: Sample SAS® code for analyses

- **Tables that need descriptive statistics – continuous variables:**

[redacted]

- **Tables that need frequency counts:**

[redacted]

- **Tables that need exact (Clopper-Pearson)95% CIs between groups for proportions:**

[redacted]

Notes: 1 Estimates are computed for 2x2 tables only
2 This code also gives exact 95% CIs within group for binomial proportions

- **Tables that need Miettinen-Nurminen 95% CIs for proportion differences:**

[redacted]

- **Tables that need 95% CIs within group for binomial proportions:**

[redacted]

- **Code to create 95% CIs within group for continuous variables:**

[redacted]

- **General linear model (adjusted analysis for GMT ratio):**

[redacted]

[redacted]

- **The geometric mean is the antilog of the arithmetic mean of the logs:**

[redacted]

Appendix C: Sensitivity Analyses for Handling of Missing Immunogenicity Data

To verify the robustness of the primary analysis results, an exploratory analysis will be performed, in which missing titers were imputed using multiple imputation. The evaluation of the robustness of the primary analysis results will be based on a pattern mixture model and a 'tipping analyses' approach using multiple imputation methods.

This sensitivity analysis will be based on the per-protocol analysis set and will serve to evaluate the robustness of the non-inferiority conclusions.

An overview of the methods of imputation for each of the estimands GMT and binary endpoint SCR are specified below. The results based on the imputed data and the results of the same analyses performed on the original, non-imputed data using multiple imputation procedures will be compared.

GMT Missing Data Evaluation

The ANCOVA are valid when the missing data are Missing at Random (MAR). There is no reason to question MAR assumption for baseline data, but MAR cannot be established for follow-up observations.

The approach to evaluating the impact of missing serology data is based on finding the existence of evidence against the assertion that the observed results are not explained by the existence of missing data. The framework to be used for finding such evidence is the based on the Pattern Mixture Multiple Imputation (PMMI) (Little 2013). PMMI will provide assessments of missing data sensitivity.

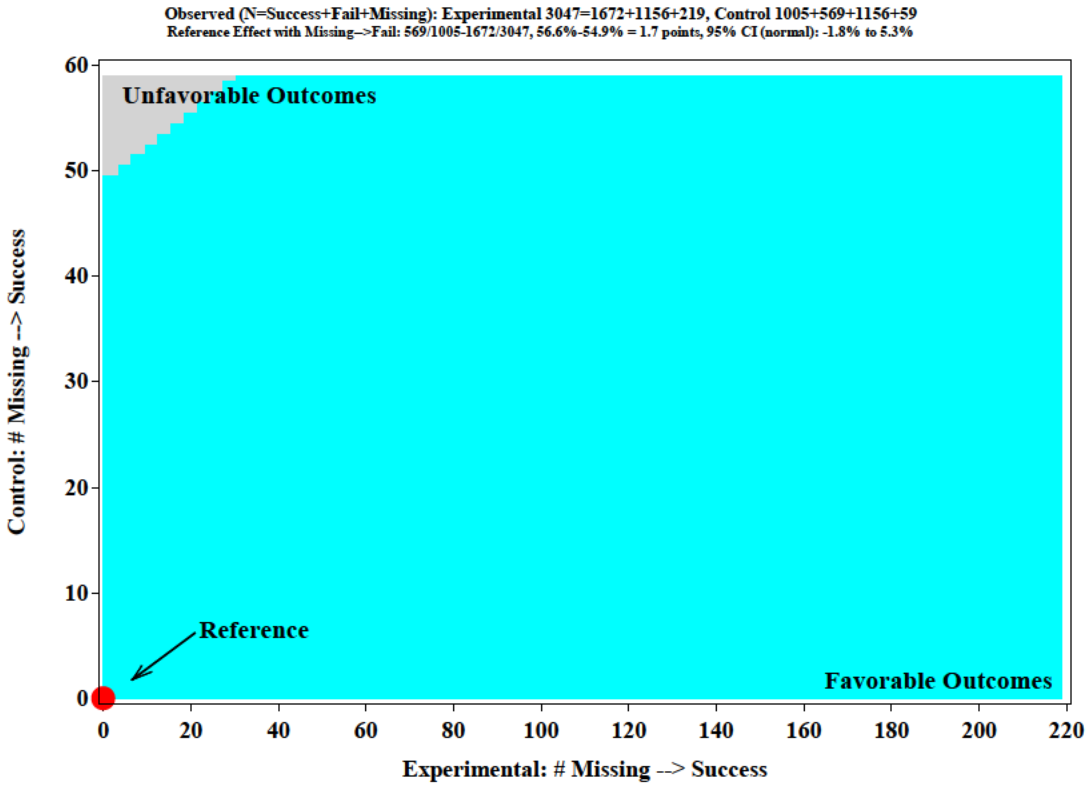
For this exploratory analysis imputation sampling will be done using the non-missing data in pattern subsets because the expected data do not follow a defined statistical distribution. Usually PMMI implementations use Markov Chain Monte Carlo (MCMC) imputation sampling based on known statistical distributions, for example a multivariate normal. The disadvantage of sampling from non-missing data is the loss of a specified correlation structure possible when using a defined statistical distribution. The use of smaller pattern groups, however, do provide some preservation of observed within patient correlation.

A SAS macro has been created for doing PMMI as described. This macro allows sampling from subsets (patterns) defined for a list of categorical variables. It computes the mean results over multiple imputations using the previously discussed models. The missing data in either arm can be sampled from the non-missing data from either arm to impute data in each arm. The missing baseline data can also be imputed according to the patterns specified. The primary focus will be to estimate the results when missing data in both the control and experimental arms are imputed from sampling from the non-missing data in the control arm, and when missing data in both the control and experimental arms are imputed from sampling from the non-missing data in the experimental arm, The latter analysis provides an additional

sensitivity assessment appropriate for non-inferiority evaluations. The macro allows for covariates other than baseline (as used in the ANCOVA model specification for the primary analysis).

Seroconversion Binary Outcome Analyses

Analysis of the seroconversion binary outcome, SCR, is also expected to have missing data. An excellent method of assessing the impact of missing outcomes for a binary outcome is to make a tipping point graph (Yan 2009). A tipping point graph has as its two axes corresponding to the two arms. Each axis ranges from zero to the number of missing values in the corresponding arm. For each possible combination of possible assignments of missing from non-success to success the statistical test is recomputed. In this evaluation we apply sensitivity analysis based upon multiple imputations under the by searching for a tipping point by using "shift" approaches until the inferences change from significance to non-significance, or vice versa. Those analyses meeting statistical criterion are graphed as a point with a colored symbol showing success and the analyses failing statistical criterion are graphed as a point using a symbol of a contrasting color. In general, this graph will then show two regions defined by the colors assigned. The location of the analysis using the default assignment for missing outcome (usually missing is regarded as a non-success) will then be in one of these regions, and the distance this point is from the other region can be assessed as a sensitivity analysis. The statistical test can be based on the usual two group assessment (asymptotic or exact) or with covariates using logistic regression. The following is an example of a tipping point graph.



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Yan 2009] Yan X, Lee S, Li N. Missing data handling methods in medical device clinical trials, J Biopharm Stat. 2009 19(6):1085-98.