STATISTICAL ANALYSIS PLAN

Study Title:	A Phase III/IV, Stratified, Randomized, Observer Blind, Multicenter Clinical Study to Evaluate the Efficacy, Safety and Immunogenicity of a Cell-Based Quadrivalent Subunit Influenza Virus Vaccine Compared to Non-Influenza Comparator Vaccine in Subjects ≥2 years to <18 Years of Age
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Sponsor:	Seqirus UK Ltd.
Plan Prepared by:	[redacted], Biostatistician ([redacted])
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LIST OF ABBREVIATIONS

aVE	Absolute Vaccine Efficacy
AE	Adverse Event
BLA	Biologics License Application
BMI	Body Mass Index
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence Interval
CRF	Case Report Form
CRO	Contract Research Organization
CSR	Clinical Study Report
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
ER	Event Rate
FAS	Full Analysis Set
FDA	Food and Drug Administration
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HI	Haemagglutination Inhibition
ID	Identification
ICF	Informed Consent Form
DMC	Data Monitoring Committee
ILI	Influenza-Like Illness
IRT	Interactive Response Technology
MedDRA	Medical Dictionary for Regulatory Activities
Men ACWY	Meningococcal (Groups A, C, Y, W-135) Conjugate Vaccine
MN	Microneutralization
NOCD	New Onset of Chronic Disease
NH	Northern Hemisphere
NP	Nasopharyngeal
PCR	Polymerase Chain Reaction
PH	Proportional Hazards
PPS	Per Protocol Set
QIVc	Cell-derived Quadrivalent Influenza Vaccine
QIV	Quadrivalent Influenza Vaccine
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
sBLA	Supplement Biologics License Application

SDA	Source Data Agreement
SH	Southern Hemisphere
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

This document presents the statistical analysis plan (SAP) for Seqirus, Protocol No. V130_12: A Phase III/IV, Stratified, Randomized, Observer Blind, Multicenter Clinical Study to Evaluate the Efficacy, Safety and Immunogenicity of a Cell-Based Quadrivalent Subunit Influenza Virus Vaccine Compared to Non-Influenza Comparator Vaccine in Subjects ≥2 years to <18 Years 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 compliant with ICH Harmonized Tripartite Guideline, 5 February, 1998, *Statistical Principles for Clinical Trials, E9*[2]*;* World Health Organization, WHO Technical Report, Series No. 924. 2004, Annex 1: *Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations*.[3]; and FDA CBER Guidance for Industry, May 2007, *Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines*[4].

The DMC analysis will be detailed in the Charter and follow definitions noted in this SAP.

The goal of the current randomized, observer-blind, controlled absolute efficacy study is to demonstrate that QIVc prevents influenza in pediatric subjects in subjects 2 to < 18 years of age, and obtained data will be used to support the licensure of QIVc for the prevention of seasonal influenza in pediatric subjects. Direct comparison with a non-influenza comparator vaccine, Menveo, (licensed for use in pediatric subjects) will enable an estimation of the absolute efficacy of QIVc in preventing influenza in pediatric subjects. A non-influenza vaccine comparator has recently been used to show efficacy of an egg-based quadrivalent influenza vaccine in the pediatric population (Jain et al. 2013). This approach is consistent with Center for Biologics Evaluation and Research (CBER) Guidance for the licensure of seasonal influenza vaccines.

For further details on the background and rationale of the study, please refer to protocol version 5.0, dated 13 December 2018, sections 1.1 (background) and 1.2 (rationale).

Any deviations from the current statistical plan and changes in the conduct or planned analysis will be described and justified in the final Clinical Study Report (CSR), whether written post interim or final analysis.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objectives and Endpoint

2.1.1 Primary Efficacy Objective

The primary efficacy objective of the study is to demonstrate absolute vaccine efficacy of QIVc versus a non-influenza comparator determined by first occurrence RT-PCR or culture confirmed influenza, due to any influenza Type A and B strain in *subjects ≥2 years to <18 years of age.*

In case of successful demonstration of the primary efficacy objective:

Co-Primary Efficacy Objective

The co-primary efficacy objective is to demonstrate absolute vaccine efficacy of QIVc versus a non-influenza comparator determined by first occurrence RT-PCR or culture confirmed influenza, due to any influenza Type A and B strain in *subjects ≥3 years to <18 years of age*.

2.1.2 Primary and Co-Primary Endpoint

The primary and co-primary efficacy endpoint is the time from the last study vaccination to the onset of the first occurrence confirmed influenza by either RT-PCR-confirmed or culture-confirmed, due to any influenza Type A or B strain regardless of antigenic match to the strains selected for the seasonal vaccine, occurring >14 days after the last vaccination and until the end of the influenza season.

2.2 Secondary Objectives and Endpoints

2.2.1 Secondary Efficacy Objectives

The following objectives will be evaluated in the age cohorts: ≥2 years to <9 years of age, ≥4 years to <18 years of age and ≥ 9 to < 18 years of age:

1. To demonstrate absolute vaccine efficacy of QIVc versus a non-influenza comparator determined by first occurrence RT-PCR or culture-confirmed influenza, due to any influenza Type A and B strain.

The following objectives will be evaluated in the age cohorts: ≥2 years to <18 years of age, ≥4 years to <18 years of age, ≥2 years to <9 years of age, and ≥ 9 years to < 18 years of age:

2. To demonstrate absolute vaccine efficacy of QIVc versus a non-influenza comparator determined by first occurrence RT-PCR influenza, due to any influenza Type A and B strain.

3. To demonstrate absolute vaccine efficacy of QIVc versus a non-influenza comparator determined by first occurrence culture-confirmed influenza, due to any influenza Type A and B strain.

4. To demonstrate absolute vaccine efficacy of QIVc versus a non-influenza comparator determined by first occurrence culture-confirmed influenza, caused by influenza strains antigenically matched to the strains selected for the seasonal vaccine.

2.2.2 Secondary Efficacy Endpoints

1. The efficacy endpoint for secondary objective 1 is the time from the last study vaccination to the onset of the first occurrence confirmed influenza by either RT-PCR-confirmed or culture-confirmed, due to any influenza Type A or B strain regardless of antigenic match to the strains selected for the seasonal vaccine, occurring >14 days after the last vaccination and until the end of the influenza season.

2. The efficacy endpoint for secondary objective 2 is the time from the last study vaccination to the onset of the first occurrence confirmed influenza by RT-PCR-confirmed, due to any influenza Type A or B strain regardless of antigenic match to the strains selected for the seasonal vaccine, occurring >14 days after the last vaccination and until the end of the influenza season.

3. The efficacy endpoint for secondary objective 3 is the time from the last study vaccination to the onset of the first occurrence confirmed influenza by culture-confirmed, due to any influenza Type A or B strain regardless of antigenic match to the strains selected for the seasonal vaccine, occurring >14 days after the last vaccination and until the end of the influenza season.

4. The efficacy endpoint for secondary objective 4 is the time from the last study vaccination to the onset of the first occurrence confirmed influenza by culture-confirmed, due to influenza Type A or B strain antigenic matched to the strains selected for the seasonal vaccine, occurring >14 days after the last vaccination and until the end of the influenza season.

2.2.3 Secondary Immunogenicity Objective

The following objective will be evaluated in a subset of subjects in the age cohort: ≥2 years to <9 years of age:

To characterize the immunogenicity of QIVc by haemagglutination inhibition (HI) assay 3 weeks after last vaccination.

2.2.4 Secondary Immunogenicity Endpoints

The immunogenicity of study vaccines will be assessed 21 days after the last vaccine administration by measuring the hemagglutination inhibition (HI) assay to the four viral strains included in the vaccines.

The measures for assessing immunogenicity as determined by HI are as follows:

1. HI Geometric mean titers (GMTs) on Day 1 (all subjects), Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Days 29 and 50 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

2. Percentage of subjects achieving seroconversion (defined as: either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1:40 or a pre-vaccination HI titer ≥ 1:10 and a ≥ 4-fold increase in post-vaccination HI titer) on Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Days 29 and 50 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

3. HI Geometric Mean Ratio (GMR)[1] of Day 22/Day 1 (all "previously vaccinated" subjects receiving a single vaccine dose) or Day 29/Day 1 and Day 50/Day 1 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

4. Percentage of subjects with HI titer ≥ 1:40 on Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Days 29 and 50 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

2.2.5 Secondary Safety Objective

To assess the safety and tolerability of QIVc.

2.2.6 Secondary Safety Endpoints

Safety will be assessed by calculating:

[1] Geometric mean ratios (GMRs) measure the ratio in immunogenicity titers *within* subjects.

1. The percentage of subjects with solicited local and systemic adverse events (AEs) for 7 days following vaccination at Day 1 ("previously vaccinated" subjects) or Day 1 and Day 29 ("not previously vaccinated" subjects) in the QIVc group and the non-influenza comparator vaccine group.

2. The percentage of subjects with all unsolicited AEs will be assessed from Day 1 to Day 22 for "previously vaccinated" subjects or Day 1 to Day 50 for "not previously vaccinated" subjects in the QIVc group and in non-influenza comparator vaccine group.

3. Percentage of subjects with SAEs, AEs leading to withdrawal from the study and NOCDs reported during the subject's entire participation in the study, i.e. from Day 1 to Day 181 (for "previously vaccinated" subjects) or to Day 209 (for "not previously vaccinated" subjects), or until the end of influenza season, whichever is longer, and all medications associated with these events.

4. Percentage of subjects with medically-attended adverse events within 30 days after the first occurrence ILI.

2.3 Exploratory Objectives and Endpoints

2.3.1 Exploratory Efficacy Objectives

The following objectives will be evaluated in the age cohort: ≥2 years to <18 years of age:

1. To further characterize the efficacy of QIVc, with specific attention for all-cause mortality, all-cause pneumonia and all-cause otitis media.

2. To describe the absolute vaccine efficacy of QIVc versus a non-influenza comparator determined by occurrence of culture-confirmed illness caused by influenza H3N2 virus strains antigenically matched to the influenza H3N2 A / Singapore / GP2050 / 2015 (cell seed) strain.

2.3.2 Exploratory Efficacy Endpoints

1. The measures for the exploratory efficacy objective 1 are as follows:

   - Number of deaths as derived from SAE forms

   - Number of subjects with pneumonia as derived from AE forms

- Number of subjects with physician-confirmed otitis media as derived from AE forms

2. The efficacy endpoint for the exploratory efficacy objective 2 is the time from the last study vaccination to the onset of the first occurrence of culture-confirmed influenza, due to any influenza H3N2 virus strains antigenically matched to the influenza H3N2 A/Singapore/GP2050/2015 (cell seed) strain, occurring at >14 days after the last vaccination and until the end of the influenza season.

2.3.3 Exploratory Immunogenicity Objective

The following objectives will be evaluated in a subset of subjects in the age cohort: ≥2 years to <9 years of age:

To further characterize the immune response, additional immunogenicity analyses may be conducted using other assays such as MN.

2.3.4 Exploratory Immunogenicity Endpoints

In case of additional immunogenicity analyses, such as MN, the immune response will generally be characterized in a similar manner as described in Secondary Immunogenicity Endpoints:

1. MN Geometric mean titers (GMTs) on Day 1 (all subjects), Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Days 29 and 50 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

2. MN Geometric Mean Ratio (GMR) of Day 22/Day 1 (all "previously vaccinated" subjects receiving a single vaccine dose) or Day 29/Day 1 and Day50/Day 1 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

3. Percentage of subjects with at least a 4-fold rise in MN titer on Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Day 29 and Day 50 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

3. STUDY DESIGN

This is a phase III/IV, stratified, randomized, observer blind, multicenter clinical study to evaluate the efficacy, safety and immunogenicity of a cell-based quadrivalent subunit influenza virus vaccine compared to non-influenza comparator vaccine in subjects ≥2 years to <18 years of age.

In this study, a total of 7692 healthy male and female subjects aged ≥2 years to <18 years of age are planned to be enrolled. Approximate numbers of subjects planned for enrollment in the age groups are presented below.

Table 3-1: Approximate Number of Subjects Planned for Enrollment

Age cohort	**≥2 to <9 years of age**		**≥9 to <18 years of age**	**Total**
Vaccine	"not previously vaccinated" Subjects	"Previously Vaccinated" Subjects		
QIVc	1022 to 1430	614 to 1022	1802	3846
Men ACWY	1022 to 1430	614 to 1022	1802	3846
Total	2044 to 2860	1228 to 2044	3604	7692

A subset of subjects will be required to provide a blood sample and immunogenicity assessments will be conducted. The subset will comprise a total of maximum 444 subjects per season in the ≥2 to <9 years of age cohort for the second and for the third season, resulting in approximately 400 evaluable subjects per season: approximately 200 evaluable subjects in the QIVc group (100 in "not previously vaccinated" subjects and 100 "previously vaccinated" subjects) and approximately 200 evaluable subjects in the non-influenza comparator vaccine group (100 "not previously vaccinated" subjects and 100 "previously vaccinated" subjects). For the subsequent seasons, the number of subjects enrolled into the immunogenicity subset is maximized at 200 evaluable subjects per season (100 from the active arm and 100 from the non-influenza comparator arm). Assuming a 10% drop out rate approximately 222 subjects will be enrolled per any subsequent season after season three (fourth and fifth season, etc) depending on the duration of the study.

Subjects ≥2 to <9 years of age who are "not previously vaccinated" will receive two (2) vaccinations separated by approximately 28 days. Subjects ≥2 to <9 years of age who are "previously vaccinated" and subjects ≥9 to <18 years of age will receive one (1) vaccination. A subset of subjects ≥2 to <9 years of age will be selected to participate in an assessment of immunogenicity, balanced by vaccination status and assigned vaccination allocation. Solicited adverse events will be collected for all subjects.

After randomization, all subjects will receive a dose of 0.5 mL of study vaccine to which they were assigned (QIVc or non-influenza comparator vaccine) on Day 1, administered intramuscularly in the deltoid muscle, preferably of the non-dominant arm. For those subjects who are "not previously vaccinated" a second administration will follow on Day 29, as presented below:

Table 3-2: Vaccination Schedule

Subjects	QIVc Group	Comparator Group
"previously vaccinated" ≥2 years to <9 years of age, and ≥9 years to <18 years of age	Day 1: QIVc	Day 1: Men ACWY
"not previously vaccinated" ≥2 years to <9 years of age	Day1: QIVc Day 29: QIVc	Day 1: Men ACWY Day 29: Saline

To maintain the observer-blind design of the study, the roles and responsibilities of "blinded" and "unblinded" team members will be defined. After vaccination, safety assessments and study related procedures and monitoring thereof must be performed by "blinded" team members.

This study is planned for at least three influenza seasons. Laboratory confirmed influenza cases will be reviewed on a regular basis (blinded review). If decided by the Sponsor, and after observing at least 50% of planned events meeting the co-primary endpoint, interim analyses for efficacy may be performed for evaluation by a DMC.

Study Definition of Influenza-Like-Illness and Influenza Case

An Influenza Like Illness (ILI) Case is defined as follows: The Centers for Disease Control and Prevention (CDC) criteria ILI is modified for young children for the purposes of this study to include additional symptoms): fever of ≥100.0°F / ≥37.8°C along with any of the following: cough, sore throat, nasal congestion, or rhinorrhea.

The ILI onset day is defined as the first day that the subject meets the primary protocol defined ILI.

The ILI end date is defined as the date the last symptom resolves.

A new ILI episode will only be taken into account after resolution of the previous one, as judged by the investigator (suggested interval between two ILI episodes is 14 symptom free days).

A detailed schedule and listing of procedures are shown in the Time and Events Tables 3-7 (see protocol synopsis).

4. RANDOMIZATION AND BLINDING

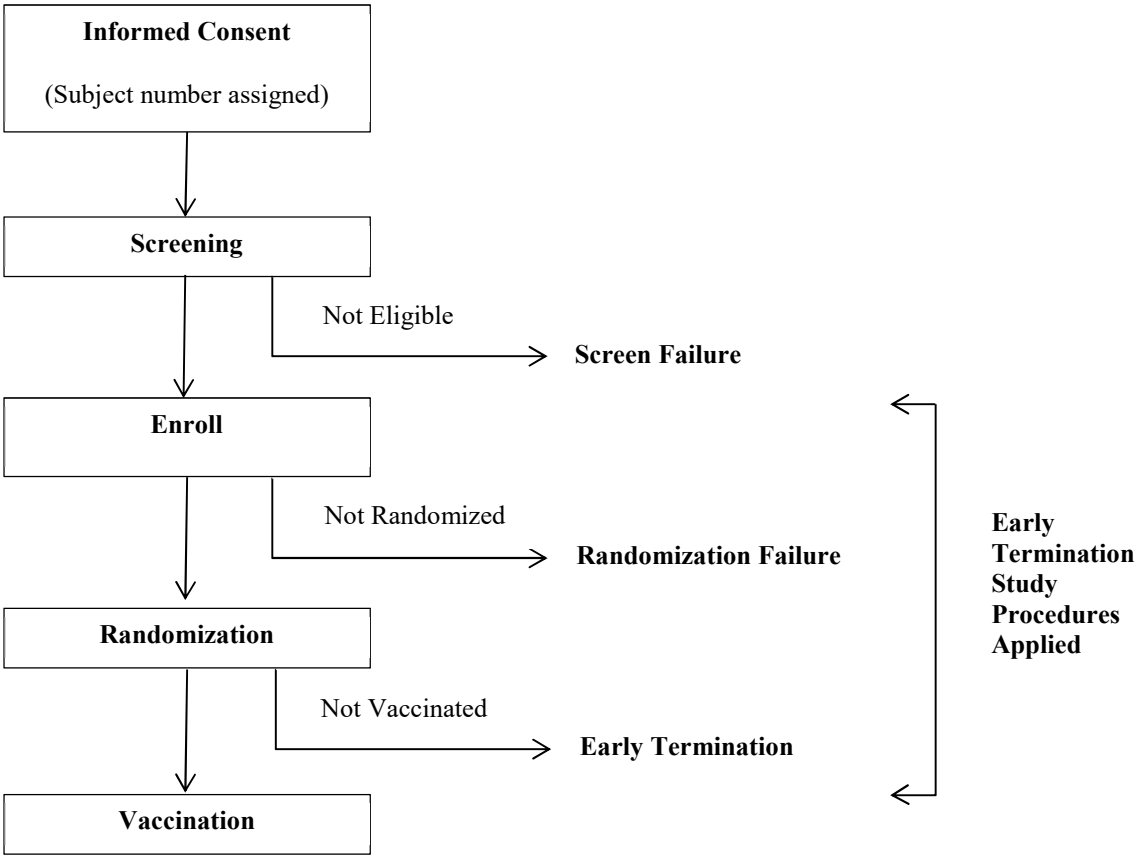
4.1 Method of Group Assignment and Randomization

Enrolled subjects will be randomized in the IRT system by a 1:1 ratio to receive either QIVc or the non-influenza comparator vaccine (MenACWY), and will be automatically assigned a unique Subject ID. The Subject ID will be the subject's unique identification number for all CRFs, ILI booklet, Subject diary card and any associated, relevant study source documentation that will be used for duration of the study. The Subject ID consists of an 11-digit number resulting from the combination of the site number, and the subject's order of randomization at the site. 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 IRT service provider and approved by Seqirus or delegate.

If for any reason, after randomization the subject fails to undergo treatment, this is an early termination and the early termination study procedures must be applied.

The procedure described above is displayed in the Figure 4.1-1.

Figure 4.1-1**: Description of Study Procedures**



4.1.1 Definition of Vaccination/Randomization Errors

The list below provides some examples of potential errors that may occur during vaccination:

- Subjects vaccinated with a vaccine different from the one assigned at randomization.
- Subjects vaccinated with the correct vaccine but containing a lower volume.
- Subjects randomized into the wrong stratum.

Guidance on how vaccination/randomization errors are handled in analysis is described in Section 6.

4.2 Blinding and Unblinding

4.2.1 Randomized Studies

4.2.1.1 Controlling Access to the Study Randomization

Unblinded teams will be used for preparation of the reports for the DMC and the interim analysis, see Sections 14 and 15. For other details please refer to section 3.3 of the current protocol (Version 5.0, dated 13 DEC 2018).

If a subject is unblinded during the study, it is to be reported as major protocol deviation, except for subjects unblinded by Pharmacovigilance due to suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately. The first-line analysis excludes unblinded subject(s) in immunogenicity statistical analyses based on the per-protocol set (PPS). The unblinded subjects will be included in the full analysis set (FAS) and safety sets.

4.2.1.2 Blinding of Study Vaccine

Study vaccine will be administered in observer blinded fashion (described in Section 4.2.1.1).

For this study using IRT, vaccine will be packed using the ‘scrambled pack numbering’ methodology. All vaccine outer packs will look identical and are blinded at the kit level. Each pack will be given a unique pack number. IRT will know the content of each pack. Once the unblinded site staff performing the vaccination has opened the outer pack, this person is unblinded for that subject.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

This study is planned using a group sequential design, with one or more interim analyses for efficacy using O'Brien-Fleming efficacy bounds. The statistical test performed will depend only on the number of confirmed ILI cases (events), so the sample size estimate is only for operational reasons (an estimate of number of subjects needed to assess the endpoint).

Primary Efficacy Objective *≥2 years to <18 years of age*

Estimated sample size to arrive at 298 events, is 4,814 evaluable subjects (or 2,407 evaluable subjects per treatment group), assuming attack rate in non-influenza comparator vaccine subjects of 8%, vaccine efficacy of 45%, and the risk of infection contained entirely within period covered by follow-up. Accounting for early dropout and uncertainty about the assumed parameters, 5,349 subjects are planned to be enrolled to demonstrate that the lower limit of the two-sided 95% CI for the VE is greater than 20% for the primary endpoint assessment, with approximately 90% power.

Co-primary Efficacy Objective *≥3 years to <18 years of age*

Assuming a true vaccine efficacy of 50% it was calculated that approximately 381 observed confirmed ILI cases would be needed to demonstrate that the lower limit of the two-sided 95% CI for the VE is greater than 30% with approximately 90% power.

The statistical test performed will depend only on number of confirmed ILI cases (events), so sample size estimate is only for operational reasons. Estimated sample size to arrive at 381 events, is 6,350 evaluable subjects (or 3,175 evaluable subjects per treatment group), assuming attack rate in non-influenza comparator vaccine subjects of 8%, assumed vaccine efficacy of 50%, VE is greater than 30%, and the risk of infection contained entirely within period covered by follow-up. Accounting for early dropout and uncertainty about the assumed parameters, 7,056 subjects are planned to be enrolled to demonstrate that the lower limit of the two-sided 95% CI for the VE is greater than 30%**.**

Table 5-1 summarizes the power calculations assumptions and the number of events required to meet primary and co-primary endpoint.

Table 5-1 Power calculation for Primary and Co-Primary Endpoints

Age group	VE Success Criteria	Assumed Vaccine Efficacy	Influenza attack rate in comparator group	Power	Minimal total evaluable subjects per Treatment Group	Minimal enrolled subjects needed per Treatment group*	Minimal total Number Enrolled*	Total Number of ILIs to demonstrate LL 95% CI for VE is > 30% or 20%
≥2 years to <18 years of age	20%	45%	8%	>90%	2,407	2,674	5,349	298
≥3 years to <18 years of age	30%	50%	8%	>90%	3,175	3,528	7,056	381

*accounted for early dropout and uncertainty

A provision for triggering additional enrollment of subjects is also included, either due to inadequate geographical or temporal distribution of cases or insufficient total number laboratory confirmed influenza cases, or paired with the outcome of an interim analysis (noted below).

Nasopharyngeal swab samples will be analyzed in batches and the number of laboratory-confirmed influenza cases will be reviewed on a regular basis (blinded review). Following the end of the second influenza season, and after observing at least 50% of planned events meeting the co-primary endpoint, an interim analysis for efficacy may be performed and evaluated by a Data Monitoring Committee (DMC). Stopping rules for efficacy and any additional details regarding the interim analysis is specified in Section 14.

For this analysis a restricted unblinding will be done, i.e. only external DMC members and Contract Research Organization (CRO) employees executing it will receive access to the randomization codes and unblinded data for the purpose of preparing the interim analyses.

Strata such as age group and history of previous vaccination will be accounted for in the final analysis as covariate “strata” in proportional hazard’s model. The use of strata in such context does not affect sample size calculation.

To account for the sample size requirements to demonstrate that the lower limit of the two-sided 95% CI for the VE is greater than 30% in ≥3 to <18 years-old (co-primary objective) and the contributable 2 year plus enrolment for the co-primary objective, the total sample size is estimated to be minimally 7,692 subjects. While minimum enrolment for the 2+ age cohort will not be pre-defined a maximum of 635 subjects (=7,692 – 7,056) in this age group can be enrolled to maintain a power >90% for the co-primary endpoint. Thus, in total minimally 7,692 subjects are planned be enrolled over the entire age distribution of ≥2 to <18 years of age.

Sample Size for Secondary Immunogenicity Objectives (Immunogenicity Subset):

The immunogenicity study will be performed in the second season and subsequent seasons.

The immunogenicity endpoint is descriptive and the number of subjects aged ≥ 2 to < 9 years of age is maximized at 444 subjects with approximately 400 evaluable subjects, per season for the second season and for the third season. With a 1:1 allocation, approximately 200 evaluable subjects will be enrolled from the active arm and 200 evaluable subjects would be from the non-influenza active comparator arm. Assuming a 10% drop out rate approximately 444 subjects will be enrolled per season. For the subsequent seasons, the number of subjects enrolled into the immunogenicity subset is maximized at 200 evaluable subjects per season (100 from the active arm and 100 from the non-influenza comparator arm). Assuming a 10% drop out rate approximately 222 subjects will be enrolled per every subsequent season after season three (fourth season, fifth season, etc) depending on the duration of the study.

In the case of early study termination, in the event that the number of ILI cases has been reached, the total number of subjects evaluated may be lower than the number of evaluable subjects described per season.

Sample Size for Safety:

With a Safety Population of 2,674 evaluable subjects in the safety set of QIVc, AEs with population rates of 1 in 1,000 have a 93.1% probability of being detected.

Events with population rates of 1 in 893 have a 95% chance of being observed with n=2,674. Events with population rates of 1 in 500 have a 99.7% chance of being observed with n=2,674.

Sample size calculations were performed using PASS v12.0.2.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

- Deviations from the protocol will be assessed as 'minor' or 'major'. Major (reportable) protocol deviations (PDs) are those deviations from the protocol that are likely to have an impact on the subjects' rights, safety, well-being, and/or the validity of the data for analysis. This includes all important PDs to be reported in the CSR. Major PDs may lead to the exclusion of the subject from the relevant analysis set or exclusion of subject data from one or more analysis sets. 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 and prescribed action to be taken with regard to excluding of subjects or affected data from specific analyses are defined in the PD specification document. The final evaluation and classification of PDs as major, as well as the specification of exclusion actions taken will be performed and documented prior to unblinding the database, in accordance with the relevant SOP. A list of major PDs include but are not limited to:Subject randomized and did not satisfy entry criteria

- Subject developed withdrawal criteria during the study, but was not withdrawn

- Subject received the wrong treatment or incorrect dose

- Subject took an excluded concomitant medication

- Key study procedures missed or performed out of window

The following PD summary will be provided:

- Number and percentage of subjects with a major protocol deviation by type of deviation and vaccine group

A by-patient listing of protocol deviations will be provided.

6.2 Determination of Protocol Deviations

On an ongoing basis and prior to unblinding, a combined PD report, including programmable and observable PDs will be reviewed by the Clinical Research Organization (CRO). 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' or 'Minor' by the Biostatistics Lead (BL). In addition, Clinical Operations Lead (COL) will review PDs to identify trends that might be evident across sites or countries.

After primary review performed by the CRO Biostatistician and COLs, the PD combined report will be provided to the Sponsor for the oversight review and final agreement.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events (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 6.3-1 below:

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 33°C or ≥ 42°C
Erythema	Measurements ≥ 900 mm Measurements < 0 mm
Induration	Measurements ≥ 500 mm Measurements < 0 mm
Ecchymosis	Measurements ≥ 500 mm Measurements < 0 mm

7. ANALYSIS SETS

7.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or other baseline screening measurements, regardless of the subject's randomization and vaccination status in the study, and received a subject ID.

7.2 Exposed Set

All subjects in the All Enrolled Set who receive a study vaccination.

7.3 Full Analysis Set (FAS), Efficacy/Immunogenicity Set

FAS, Efficacy: All subjects in the All Enrolled Set who received at least one dose of study vaccine and are evaluated for efficacy from 14 days after the last vaccination.

FAS, Immunogenicity: All subjects in the All Enrolled Set who received at least one dose of study vaccine and provide evaluable serum samples at both baseline and after the last vaccination.

Last vaccination is at Visit 2 for "previously vaccinated" subjects and at Visit 3 for "not previously vaccinated" subjects.

FAS will also be defined by time point and objective:

- *FAS-Eff1* will include all subjects ≥2 years to <18 years of age who received at least one dose of study vaccine and are evaluated for efficacy from 14 days after the last vaccination (primary objective).

- *FAS-Eff2* will include all subjects ≥3 years to <18 years of age who received at least one dose of study vaccine and are evaluated for efficacy from 14 days after the last vaccination (Co-primary objective).

- *FAS-HI1* will include all subjects ≥2 years to <9 years of age who received at least one dose of study vaccine and provide evaluable serum samples for HI analysis at both baseline and after the last vaccination (secondary objective).

- *FAS-MN1* will include all subjects ≥2 years to <9 years of age who received at least one dose of study vaccine and provide evaluable serum samples for MN analysis at both baseline and after the last vaccination (exploratory objective).

In case of vaccination error, 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).

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

7.4 Per Protocol Set (PPS), Efficacy/Immunogenicity Set

All subjects in the FAS Efficacy/Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subjects is randomized and at the scheduled time points).
- Have no major protocol deviations leading to exclusion (see Section 6.1) as defined prior to unblinding / analysis.
- Are not excluded due to other reasons (e.g. subjects who withdrew informed consent) defined prior to unblinding or analysis (see Section 6.2)

PPS are subsets of FAS and should be always defined even if the objectives do not require it.

In case of vaccination error, subjects in the PPS will be analyzed "as randomized" and the subject who received the wrong vaccination will be excluded from the PPS. If a subject receives a vaccine from the wrong kit number, but the same as the one the subject was randomized to, the subject will not be removed from the PPS.

Subjects randomized in the wrong stratum will be excluded from the PPS.

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

Similarly to FAS, PPS will also be defined per objective and time point.

- *PPS-Eff1* will include all subjects ≥2 years to <18 years of age who received at least one dose of study vaccine and are evaluated for efficacy from 14 days after the last vaccination (primary objective).
- *PPS-Eff2* will include all subjects ≥3 years to <18 years of age who received at least one dose of study vaccine and are evaluated for efficacy from 14 days after the last vaccination (Co-primary objective).

- *PPS-HI1* will include all subjects ≥2 years to <9 years of age who received at least one dose of study vaccine and provide evaluable serum samples for HI analysis at both baseline and after the last vaccination (secondary objective).

- *PPS-MN1* will include all subjects ≥2 years to <9 years of age who received at least one dose of study vaccine and provide evaluable serum samples for MN analysis at both baseline and after the last vaccination (exploratory objective).

Exclusions will be considered by objective/time point, i.e., sometimes not all data of a subject but only part of the subject's data will be removed from the PPS analysis.

7.5 Safety Set

Solicited Safety Set (solicited local and systemic adverse events and other solicited adverse events)

All subjects in the Exposed Set who have gone through any assessment of local and systemic site reaction and/or assessment of any use of analgesics/antipyretics.

Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set who have gone through any adverse event assessments i.e., a subject does not have to have any adverse events to be included in this population.

Overall Safety Set

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

Subjects providing only 30 minutes post-vaccination safety data will also be reported separately in a 30-minute post-vaccination safety analysis.

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).

Subjects randomized in the wrong stratum will be reassigned to the correct stratum and will be analyzed using corrected stratum for all safety sets (i.e., solicited safety set, unsolicited safety set and overall safety set). If a subject is unblinded during the study, he/she will be included in all the safety sets.

7.5.1 Restricted Safety Set

Not applicable.

7.6 Other Analysis Set

Not applicable.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The estimate of the hazard ratio and the respective estimate for absolute vaccine efficacy (aVE) and pertaining two-sided 95% confidence intervals will be calculated based on the Cox proportional hazards model with vaccine group as the main effect. Randomization stratifying factors (i.e by age stratifying factors, season) as well as previous influenza vaccination will be modelled as random effects as suggested by Kahan and Morris (2012). In case of computational difficulties due to small number of events, an unadjusted hazard ratio will be estimated and reported (for example, in a subgroup analysis).

The log10-transformed antibodies at Day 1, Day 22, and Day 50 for not previously vaccinated subjects and Day 1 and Day 22 for not previously vaccinated subjects will be modeled using an Analysis of Covariance (ANCOVA) model with a factor for vaccination group, age-group, baseline-titer, country and previous influenza vaccination. Summary tables will show both adjusted and unadjusted GMTs and adjusted and unadjusted ratios of GMTs for each vaccine group, except for subgroup analyzes which will present unadjusted estimates only.

Binary data tables will show unadjusted percentages.

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

8.2 Handling of Dropouts, Missing Data

The analyses will be done without adjustments for missing values.

8.2.1 Safety Data

For unsolicited adverse events, the entire study period will be divided into the following intervals: Day 1 to Day 22, Day 23 to Day 181 in “previously vaccinated” subjects and Day 1 to Day 50, Day 51 to Day 209 in “not previously vaccinated” subjects.

For solicited adverse events, the solicited study period 30 min - Day 7 after each vaccination will be divided into: 30 min and Day 1-7 (without the 30-minute interval).

No imputation of missing solicited or unsolicited AEs will be used. The percentage of subjects with missing solicited AE assessments and missing safety phone calls or safety assessment, including subjects who discontinued the study, will be reported for each time.

8.2.2 Immunogenicity Data

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 secondary immunogenicity objectives will be analyzed using the FAS immunogenicity. The exploratory immunogenicity objectives will be analyzed in the similar manner as the secondary immunogenicity objectives.

If the percentage of vaccinated subject excluded from the FAS (immunogenicity) is greater than 5%, a secondary analysis based on the PPS will be performed to complement the FAS. The exploratory immunogenicity objectives will be analyzed on the FAS only.

8.2.3 Efficacy Data

Primary efficacy data will be analyzed using FAS as primary analysis set but the analysis will be repeated on PPS for robustness, irrespective of the difference in size between the two analysis sets.

The following algorithm will be applied:

1. If less than 20% of subjects are without efficacy data, then the analyses (e.g., ILI swabs collected without any RT-PCR assessment record) will be run on FAS and PPS and no further statistical evaluation will be performed.
2. If observations are missing for 20% or more of subjects, the missing mechanism will be analyzed by vaccine group using a newly created variable indicating whether a subject's efficacy-value is missing or not (1=efficacy record present; 0=efficacy record not present). It will be tested, by chi-square test, if the proportion of missing observations/subjects varies significantly between vaccine groups. If the difference is significant with $P<0.05$ then a sensitivity analysis will be performed of the primary efficacy analysis imputing randomly x% (from 0% to 100% in 10% increments) of missing data as PCR-confirmed cases.

8.3 Multiple Comparisons and Multiplicity

The K-stage group-sequential design for efficacy introduces a multiple test problem. Therefore, the familywise error rate (FWER) will be adjusted via an error-spending function (Jennison and Turnbull 1999) for each stage of the interim analyses that maintains the alpha for each interim analysis.

The cumulative error-spending-function to be used is defined as:

$$E(t;\rho) = \begin{cases} 1, if\ t \geq 1 \\ t^{\rho}, if\ 0 < t < 1, \\ 0, otherwise \end{cases}$$

where ρ is the power parameter (for this trial chosen as ρ=2) and where t is the information fraction. That is, with a specified error of α, the cumulative error spending at each stage k is $\alpha E(\Pi_k; \rho)$, with Π_k as information fraction at each stage k. For example for k=3 and equally spaced interim analyses this would lead to cumulative errors of $(1/3)^2$, $(2/3)^2$ and 1, multiplied by α.

8.4 Immunogenicity Subsets

For the overall study – a total of 7,692 healthy male and female subjects aged ≥2 years to <18 years of age will be enrolled.

Within this randomization, a subset of 444 subjects per season for the third season will be allocated for immunogenicity testing in a 1:1 ratio from either QIVc or non-influenza comparator, as described in Section 3. For the subsequent seasons, the number of subjects enrolled into the immunogenicity subset is maximized at 222 subjects per season.

8.5 Subgroups

The efficacy analyses will be performed stratified for the following subgroups:

- Subjects ≥2 to <4 years of age, ≥2 to <9 years of age, ≥2 to <18 years of age, ≥3 to <18 years of age, ≥4 to <18 years of age, and ≥9 to <18 years of age
- Subjects "previously vaccinated" and “not previously vaccinated”
- Subjects by race
- Subjects by sex
- Subjects by country or region
- Subjects by season/year treated

If, in any subgroup, the number of confirmed ILI cases is <10 then the statistical analysis in that subgroup will not be performed.

The immunogenicity analyses will be performed stratified for the following subgroups:

- Subjects ≥2 years to <9 years of age
- Subjects with pre-vaccination HI titer <1:10 and pre-vaccination HI titer ≥ 1:10
- Subjects "previously vaccinated" and “not previously vaccinated”
- Subjects by race
- Subjects by sex
- Subjects by season/year treated

The safety analyses will be performed stratified for the following subgroups:

- Subjects ≥2 to <6 years of age, ≥2 to <9 years of age, ≥2 to <18 years of age, ≥3 to <18 years of age, ≥4 to <18 years of age, ≥6 to <9 years of age, and ≥9 to <18 years of age
- Subjects "previously vaccinated" and “not previously vaccinated”
- Subjects by race
- Subjects by sex
- Subjects by country or region

For by-season analysis, the efficacy/immunogenicity analyses will be presented by Season 1 in which egg-derived seed virus was used, compared to the vaccine efficacy in Seasons 2 and 3, in which cell-derived seed viruses were used. Safety and immunogenicity analyses will also be performed by time periods.

For details regarding the analyses by center please see Section 8.3 above. Analyses by center will be presented in Appendix 16.1.9 of the CSR.

8.6 Derived and Computed Variables

Demographics

Age, if not already provided, will be calculated in years using the following formula:

Age (years) = (Date of Visit 1 – Date of Birth + 1) / 365.25, round to smallest integer

Body Mass Index (BMI, kg/m^2) will be calculated using the following formula:

BMI = Weight (kg) / Height^2 (m^2)

Immunogenicity

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

Titer greater or equal to a given threshold is defined as binary variable for non-missing values as:

= 1, if the titer is superior or equal to the given threshold

= 0, otherwise

HI antibody titer ≥1:40 is defined as binary variable for subjects with non-missing values as:

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

= 0, otherwise

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

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

= 0, otherwise

Geometric Mean Titer/Concentration

The GMT will be calculated using the following formula:

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

where $t_1, t_2, \ldots, t_n$ are n observed immunogenicity titers/concentrations.

Geometric Mean Ratio

Geometric mean ratios (GMRs) measure the changes in immunogenicity titers/concentrations *within* subjects.

The GMR will be calculated using the following formula:

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

where, for n subjects, v_{ij} and v_{ik} are observed immunogenicity titers/concentrations for subject i at time-points j and k, $j \neq k$.

Duration in the Study

Duration of study per subject based on last phone call defined as Day 181 (for "previously vaccinated" subjects) or Day 209 (for "not previously vaccinated" subjects), or until the end of influenza season, whichever is longer.

Duration in the study is defined in days as:

Last visit date (last visit)[a] – Enrollment date (visit 1) + 1

[a]or premature discontinuation date (in case of withdrawal from the study)

The duration is missing if one of the dates is missing or incomplete.

Solicited Adverse Events

For details, see Section 13.2.

Unsolicited Adverse Events

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

- **Emergence before vaccination phase**: start date before the first date of injection of study vaccine.
- **Emergence during vaccination phase**: start date on or after the first date of injection of study vaccine or, adverse event increase in severity including to "serious" adverse event.

If the start date is equal to the first date of injection then a "timing" variable ("On injection day, before injection" / "On injection day, after injection") will be used to define whether the adverse event occurred before or after the injection.

If there are several vaccinations, the adverse event will be associated with the most recent vaccination.

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

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

- If the partial end date is before (<) the first study vaccination (i.e., year or year & month is/are before the first study vaccination year or year & month) then the adverse event is 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 adverse event is emergent during vaccination phase.

The **maximum event severity** is the greatest severity associated with a preferred term for a reported adverse event according to the following order: Mild < Moderate < Severe. Unknown/ Missing severity is considered as severe (except for the definition of emergence).

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

Vaccination-related Adverse Events are those for which the cause has been evaluated by the investigator, and recorded either as possibly related, probably related or unknown/ missing.

Safety Laboratory Data

Not applicable.

Pre-study, Concomitant and Post-Study Medications

A **pre-study medication** is a medication used only before the first study vaccination (i.e. medication end date < first study vaccination date).

A **post-study medication** is a medication used only after study termination (i.e. medication start date > study termination date). This will not be collected in the clinical database and will not be reported in the CSR.

All other medications are **concomitant**.

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 first 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.

8.7 Analysis Software

All analyses will be performed using SAS® Software version 9.2 or higher.

8.8 Data Transformation

Distributions of antibodies are generally skewed to the right (Nauta, 2010). Therefore, prior to any statistical analysis that assumes normally distributed observations, antibody titers or concentrations will be log10-transformed. GMTs and their 95% CIs will be computed by exponentiating (base 10) the least squares means and associated 95% CIs of the log10 titers.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All enrolled subjects will be accounted for in this study and displayed along with the frequencies and percentages of subjects enrolled, vaccinated, completed and withdrawn (including reason for withdrawal) for the Enrolled Set. The frequencies and percentages of subjects with CSR reportable PDs will be presented for the FAS (Efficacy and Immunogenicity).

The time the subjects are under observation will be summarized by vaccine and overall using summary statistics (mean, standard deviation, minimum, median, maximum) and compared between treatment arms.

The numbers and percentages of subjects in each analysis set, study withdrawals, subgroups, and major protocol deviations will also be presented.

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

10.1 Demographics

Age, height, weight, body mass index will be summarized by reporting the mean, standard deviation, median, minimum and maximum, and will be calculated by vaccine group and overall. They will be also reported by age groups.

The frequencies and percentages of subjects by country, sex, ethnic origin, race and age, meeting or not-meeting of protocol entrance criteria and "previously vaccination" status will be presented by vaccine group and overall, stratified by age categories and Season and then by Season and country. Demographic data will be tabulated for the Enrolled, FAS (Efficacy, Immunogenicity HI), PPS (Efficacy) and Safety sets.

10.2 Medical History

The frequencies and percentages of subjects with medical history and by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT) will be presented overall and by vaccine group and for the different age categories, by Season, and for "previously vaccination" status. Medical history data will be tabulated for the Enrolled Set, FAS (Efficacy) and Safety Sets.

11. EFFICACY ANALYSIS

11.1 Primary Efficacy Objectives Analysis

Efficacy will be evaluated in all subjects in relation to cases occurring >14 days after last vaccination and until the end of study or the end of the influenza season, whichever is longer, using the following null (H_0) and alternative (H_1) hypotheses:

$$H_0: 1 - HR = VE \leq 0.2 \qquad \text{versus} \qquad H_1: 1 - HR = VE > 0.2,$$

where HR is a hazard ratio of QIVc versus non-influenza comparator and VE is vaccine efficacy. The primary objective will be achieved if the lower limit of the two-sided confidence interval of VE estimate, with at least 95% coverage in a multiple sequential hypothesis testing, exceeds 0.2 in subjects ≥2 years to <18 years of age.

The co-primary objective will be achieved if the lower limit of the two-sided confidence interval of VE estimate, with at least 95% coverage in a multiple sequential hypothesis testing, exceeds 0.3 in subjects ≥3 years to <18 years of age.

The co-primary objective will only be demonstrated in case of successful demonstration of the primary efficacy objective (i.e. in hierarchical testing procedure, only after demonstration of absolute vaccine efficacy of QIVc in subjects ≥2 years to <18 years of age).

In case an interim analysis will be performed the 95% CI will be adjusted accordingly. If an interim analysis is performed and the trial doesn't stop, then all the subsequent analysis will be tested at a reduced alpha level, i.e. what's left from the interim analysis. The confidence interval will be higher than 95% instead of 95% for the final analysis.

(Co-)Primary vaccine efficacy analyses will be based on the Efficacy FAS, and repeated on the Efficacy PPS.

For each of the age groups (≥2 to <18 and ≥3 to <18 years) , the HR[2]and the related 95% CI of HR, for onset of first RT-PCR or culture confirmed influenza will be estimated by a proportional hazards regression model with treatment effect as a fixed effect and stratifying covariates as random effect:

$h_i(t|X) = h_0(t) \exp(\beta^T X + b^T Z)$, with t denoting time to the influenza, β is the effect of treatment group indicated by X, b is random effect (assumed as a multivariable random gaussian variable with zero mean and diagonal covariance matrix), Z is random effect covariate (reflecting randomization strata, see Section 8.1 for further discussion of the covariates utilized).

[2] Formula: VE=1-HR

Subjects that did not experience ILI during observation period and subjects that dropped out from the study during observational period will be censored (right-censoring). The estimate of the hazard ratio, the respective estimate for absolute VE and the pertaining two-sided CIs will be calculated based on this model. If the study continues over several seasons, estimates will be also adjusted for the factor season (s). In case of one or two (interim) analyses, confidence levels at each stage will be adjusted, as discussed in section 14.1, to provide 95% overall coverage.

For each of the age groups (≥2 to <18 and ≥3 to <18 years), estimates for hazard ratio in Cox Proportional hazard (PH) model will be calculated using Maximum Likelihood (ML) method. In case of problems with convergence (algorithm does not converge or converges to infinite estimates) penalized ML approach will be used (Heinze and Schemper).

Vaccine efficacy VE = 1 – HR, that is, 1- $\exp(\widehat{\beta})$

with $\hat{\beta}$ with 100(1- α) percent confidence interval as:

[1- exp (+ Z(s.e.$(\widehat{\beta})$*; 1- exp (*$\hat{\beta}$ - Z(s.e.$(\widehat{\beta})$)]. Z is the 100(1- α) percent point of the standard normal distribution,and s.e. denotes the standard error of β

Subjects that did not experience ILI during observation period and subjects that dropped out from the study during observational period will be censored (right-censoring).

The hazard ratio is the predicted ratio of cases of Influenza A and or B disease in subjects receiving QIVc and non-influenza vaccine comparator within each of the strata of interest. The term β_g is the estimate of treatment effect (or regression coefficient) between QIVc and non-influenza vaccine comparator within each of the stratum.

The estimate of the hazard ratio, the respective estimate for absolute VE and pertaining two-sided 95% CIs will be calculated based on this model. If the study continues over several seasons, estimates will be also adjusted for the factor season (s). Factor country might be added to the model if appropriate. In case of more than one interim analysis confidence level for the estimates at the final stage will be adjusted.

Primary analysis will take into account only first ILI episode for a subject.

In addition VE estimate estimated from Cox proportional model (for each of the age strata (≥2 to <18 and ≥3 to <18 years) a simple unadjusted estimate of VE will be also presented as 1-π_{QIVc}/ π_{Comp} along with 95% exact unconditional confidence interval; where π_{QIVc} and π_{Comp} will be attack rates for each group estimated as a binomial variable.

11.2 Secondary Objectives Analysis

Secondary efficacy objectives are not associated with any hypothesis testing.

All secondary efficacy objectives will be evaluated based on the Efficacy FAS, but analysis for the key secondary objective will be also repeated based on Efficacy PPS.

Similar to the primary efficacy objectives, a Cox PH model will be used to estimate absolute vaccine efficacy VE for each secondary objective.

11.3 Exploratory Objectives Analysis

All exploratory efficacy objectives will be evaluated based on the Efficacy FAS.

The measures for exploring efficacy are as follows:

- Number of deaths as derived from SAE forms
- Number of subjects with pneumonia as derived from AE forms
- Number of subjects with physician-confirmed otitis media as derived from AE forms

These will be evaluated by frequency tables both for QIVc and the non-influenza comparator.

The model used to estimate the exploratory efficacy objectives is similar to the model used for primary efficacy objectives, being related to the onset of the first occurrence of culture-confirmed influenza, due to any influenza H3N2 virus strains antigenically matched to the influenza H3N2 A / Singapore / GP2050 / 2015 (cell seed) strain.

12. IMMUNOGENICITY ANALYSIS

12.1 Blood Samples

The frequencies and percentages of subjects with blood draws will be summarized overall and by vaccine group, age group and "previously vaccination" status. Data will be tabulated for the FAS and PPS.

12.2 Primary Objectives Analysis

Not Applicable.

12.3 Secondary Objectives Analysis

Both Adjusted (see section 8.1) and Unadjusted estimates for GMTs, 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.

For the immunogenicity analysis, the measures for immunogenicity as determined by serum HI antibody titers for Day 1 (all subjects in immunogenicity subset), Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Days 29 and 50 (all "not previously vaccinated" subjects receiving 2 doses) for all four influenza strains are as follows:

- Geometric mean HI titer (GMT) on Day 1, Day 22 and Day 50 achieved by "previously vaccination" status of subjects.

- Percentage of subjects achieving seroconversion (defined as HI $\geq$ 1:40 for subjects negative at baseline [HI <1:10]; or a minimum 4-fold increase in HI titer for subjects positive at baseline [HI $\geq$ 1:10]) after last vaccination (i.e. on Day 50 for subjects "not previously vaccinated" and on Day 22 for subjects "previously vaccinated").

- Ratio of Geometric Mean HI titer (GMR) in subjects "not previously vaccinated" after Day 29 and Day 50 respectively relative to pre-vaccination.

- Ratio of Geometric Mean HI titer (GMR) in subjects "previously vaccinated" after Day 22 relative to pre-vaccination.

- Percentage of subjects "not previously vaccinated" with HI titer $\geq$ 1:40 on Day 29 and Day 50

- Percentage of subjects "previously vaccinated" with HI titer ≥ 1:40 on Day 22

Log-normal distributed data

All statistical analyses for HI will be performed on the logarithmically (base 10) transformed values. Results will be transformed back afterwards including the two-sided 95% confidence interval.

Individual HI titers below detection limit will be set to half that limit.

For each strain and each vaccine group, both unadjusted and adjusted estimates for GMTs, 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.

Log-transformed antibodies at Day 1, Day 22, and Day 50 for not previously vaccinated subjects and Day 1 and Day 22 for not previously vaccinated subjects will be modeled using ANCOVA with a factor for vaccination group, baseline-titer, country, and previous influenza vaccination. Adjusted Geometric means and adjusted geometric mean ratios and pertaining two-sided 95% confidence intervals will be calculated based on these models.

Analysis of binary data

Proportions of subjects with HI titers ≥1:40 will be summarized at all time-points.

Percentages of Subjects with Seroconversion: The number and proportion of subjects achieving seroconversion in HI titers from pre-immunization at all-time points at which blood samples were drawn will be summarized.

All secondary endpoints for binary data will be tabulated for each of the influenza vaccine strains and for each of the vaccine groups, including the associated two-sided 95% confidence intervals according to Clopper-Pearson (Clopper and Pearson 1934).

Secondary immunogenicity objectives will be evaluated based on the FAS (immunogenicity). If the percentage of vaccinated subject excluded from the FAS (immunogenicity) is greater than 5%, a secondary analysis based on the PPS will be performed to complement the FAS.

12.4 Exploratory Objectives Analysis

In case of additional immunogenicity analyses, such as MN, the immune response will generally be characterized in a similar manner as described in Secondary Immunogenicity Endpoints:

- MN Geometric mean titers (GMTs) on Day 1 (all subjects), Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Days 29 and 50 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

- MN Geometric Mean Ratio (GMR) of Day 22/Day 1 (all "previously vaccinated" subjects receiving a single vaccine dose) or Day 29/Day 1 and Day50/Day 1 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

- Percentage of subjects with at least a 4-fold rise in MN titer on Day 22 (all "previously vaccinated" subjects receiving a single vaccine dose) or Day 29 and Day 50 (all "not previously vaccinated" subjects receiving 2 doses) for all 4 influenza strains.

13. SAFETY ANALYSIS

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

- Vaccine exposure
- Solicited local and systemic adverse events
- Unsolicited adverse events
- Height and weight assessments

13.1 Analysis of Extent of Exposure

The overall time the subjects are under observation will be summarized by vaccine and overall and for naïve and non-naïve subjects and by age using summary statistics (mean, standard deviation, minimum, median, maximum) and compared between treatment arms.

13.1.1 Safety Completeness Analysis

Solicited adverse events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed subject diary cards, irrespective of severity. The analysis will show the number of subjects with valid data by solicited adverse event and time point. Valid data in the context of the safety completeness analysis are all data entered in the subject diary card (including implausible values) except “Not done/unknown”.

Three summaries will be produced:

1. The frequencies of subjects who provide subject diary cards by vaccine group and collection method.

2. For each type of solicited adverse event (local, systemic) [and indicators of solicited adverse events, such as use of analgesic use] the frequencies of subjects *with valid data* by vaccine group, aggregated over time points and intervals: 30 min and 6 h – Day 7.

3. For each solicited adverse event, the frequencies of subjects *with valid data* by vaccine group aggregated over time points and intervals: 30 min and 6 h – Day 7.

For the corresponding percentages, the denominator will be the respective numbers of exposed subjects, i.e., subjects who received a vaccination, irrespective of whether a

subject diary card was present or not. All analyses will be based on the Safety Set (i.e. 'as treated').

13.2 Solicited Local and Systemic Adverse Events

The following adverse events are included in the subject diary card check list. Each adverse event will be assessed using a grading system.

Subjects < 6 years of age

Solicited local adverse events:

- Injection site induration
- Injection site erythema
- Injection site ecchymosis
- Injection site tenderness

Solicited systemic adverse events:

- Change of eating habits
- Sleepiness
- Vomiting
- Diarrhea
- Irritability
- Shivering
- Fever is also solicited but this is based on actual recorded body temperatures rather than subjective interpretation of fever by the subject

Subjects ≥6 years of age

- **Solicited local adverse events:**
- Injection site induration
- Injection site erythema
- Injection site ecchymosis
- Injection site pain

Solicited systemic adverse events:

- Shivering
- Nausea
- Generalized myalgia

- Generalized arthralgia
- Headache
- Fatigue
- Vomiting
- Diarrhea
- Loss of appetite
- Fever is also solicited but this is based on actual recorded body temperatures rather than subjective interpretation of fever by the subject

Other solicited adverse events:

- Use of analgesics / antipyretics for prophylaxis
  Use of analgesics / antipyretics for treatment
- Body temperature described in degrees Celsius or degrees Fahrenheit and summarized by route of body measurement

For erythema, ecchymosis, and induration recorded originally as diameters (mm), the following categorization(s) will be used to summarize the data for subjects < 6 years of age:

- 1-9 mm
- 10-25 mm
- 26-50 mm
- >50 mm
- Any: ≥1 mm

and for subjects ≥ 6 years:

- 1-24 mm
- 25-50 mm
- 51-100 mm
- >100 mm
- Any: ≥1 mm

Grading for pre-selected local and systemic adverse events are presented in Table 13.2-1.

Table 13.2-1: Grading of Solicited Local Adverse Events for All Subjects

	Reaction	Grade 0/None	Grade 1/Mild	Grade 2/Moderate	Grade 3/Severe
Local	Injection Site Tenderness/Pain	None	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Systemic	Temperature	< 38.0°C	≥ 38.0 - 38.9°C	≥ 39.0 - 39.9°C	≥ 40.0°C
	Change of Eating Habits/ Loss of Appetite	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	None	1-2 times in 24 hours	3 – 5 times in 24 hours	6 or more times in 24 hours or requires intravenous hydration
	Diarrhea	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	Requires more cuddling and is less playful than usual	More difficult to settle	Unable to console
	Nausea	None	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Generalized Myalgia	None	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Generalized Arthralgia	None	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Headache	None	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Fatigue	None	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Fever	Captured as No (<38ºC) or Yes (≥38ºC)			

Other	Use of analgesics / antipyretics for treatment.	Captured as No or Yes
	Use of analgesics / antipyretics for prophylaxis.	Captured as No or Yes

Solicited adverse events will be reported at 30 minutes, at 6 hours on Day 1 and then daily until Day 7 using structured diaries. The analyses of solicited adverse events will be done based on the intervals: 30min and 6h – Day 7. In addition, solicited adverse events ongoing after Day 7 will be presented as unsolicited AE.

Body temperature will be summarized separately according to the 3 schemes described below and will be broken down by route of measurement according to the recommendations of the Brighton collaboration.

- by 0.5 °C increments from 36.0°C up to ≥40°C: <36.0, ≥36.0–<36.5, ≥36.5–<37.0, ≥37.0–<37.5, ≥37.5–<38.0, ≥38.0–<38.5, ≥38.5–<39.0, ≥39.0–<39.5, ≥39.5–<40.0, ≥40.0°C.
- by 1.0 °C increments from 36.0°C up to ≥40°C: <36.0, ≥36.0–<37.0, ≥37.0–<38.0, ≥ 38.0–<39.0, ≥39.0–<40, ≥40°C.
- <38.0, ≥38.0 °C.

Fever (defined as a body temperature of ≥38°C irrespective of route of measurement) will be integrated to the summaries as an indicator of a systemic adverse event.

Injection site pain and systemic AEs (except fever) occurring for the 7 days including each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic AE will also be further summarized as “none” versus “any”.

The analyses will encompass summaries of the data on five levels:

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events (excluding 30 min measurement).
3. Solicited adverse events, maximum event severity by event on Day 1-7 without 30 min.
4. Duration of solicited adverse events.

5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval Day 1-7 without 30 min.

For each of the time points or time intervals presented in the summaries, only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse events in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero).

Level 1: Daily reports of solicited adverse event

For each of the time points only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse event in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator in order to prevent a downward bias (towards zero). Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 2: Time of first onset of solicited adverse events

The time of first onset is defined, for each subject, for each solicited adverse event, as the time point on which the respective solicited adverse event first occurred. For erythema, ecchymosis and induration two thresholds will be used: ≥ 1 mm and ≥ 25 mm for children older than 6 years or ≥ 1 mm and ≥ 9 mm for children aged 6 and below.

Table 13.2-2: Example for Time to First Onset of Solicited Adverse Events

Vaccination	Subject Number	Day 1[a]	Day 2	Day 3	Day 4	…	Day 7
1	001	None	Severe	Moderate	None	…	None
	002	Mild	None	None	Moderate	…	Missing
	003	Moderate	Mild	None	Severe	…	Mild
	004	Mild	Mild	None	None	…	None
2	001	None	None	None	None	…	Not done
	002	None	Mild	Mild	Missing	…	Missing
	003	Severe	None	Mild	Missing	…	None
	004	Missing	Missing	Missing	Severe	…	Mild

[a] Exclude 30 minutes after vaccination.

For each vaccination the first onset of the adverse event will be used for each subject. For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, 'not done' is treated identical to 'missing'. A mock-up table is shown in Table 13.2-3 below.

Table 13.2-3: Time to First Onset of Solicited Adverse Events

Vaccine group A

Vaccination	Adverse event		Number (%) of Subjects					
			DAY 1[a] (N=4)	DAY 2 (N=4)	DAY 3 (N=4)	DAY 4 (N=4)	…	DAY 7 (N=4)
1	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	1 (25.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	0 (0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
2	XY	n	3	3	3	2	…	2
		ANY	1 (33.3%)	1 (33.3%)	0 (0%)	1 (50.0%)	…	0 (0%)
		Mild	0 (0%)	1 (33.3%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (33.3%)	0 (0%)	0 (0%)	1 (50.0%)	…	0 (0%)
ANY	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (25.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)

N: no. of subjects with data at a time point across all vaccinations.
n: no. of subjects with data at a time point for that specific vaccination.
[a] Exclude 30 minutes after vaccination.

Level 3: Solicited adverse events, maximum event severity by event and interval

The **maximum event severity** will be defined if there is at least one plausible non-missing observation (excluding "Not done/unknown" and implausible values) within this time interval, Each subject's data will be aggregated across the time points of the interval and summarized according to the maximal severity observed for each adverse event, followed by a summary across subjects for each vaccine. Subjects without any solicited

adverse events in the interval, i.e., missing values at each of the requested time points, will be removed from the denominator.

Level 4: Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. This means at least 'mild' solicited adverse event that are assessed qualitatively. For erythema, ecchymosis and induration two thresholds will be used: ≥ 1 mm and ≥ 25 mm for children older than 6 years or ≥ 1 mm and ≥ 9 mm for children aged 6 years and below. If a solicited adverse event continues beyond Day 7, the period after Day 7 is added as an unsolicited adverse event.

The frequency distribution of the number of days will be provided in a summary table by vaccine and by adverse event.

Level 5: Solicited adverse events, occurrence of at least one event by category (local, systemic) and interval.

The **occurrence of at least one solicited adverse event** is defined as "any" for a subject if he/she reports greater than "none" for the respective event and "none" otherwise. For erythema, ecchymosis and induration two thresholds will be used for an event: ≥ 1 mm and ≥ 25 mm for children older than 6 years or ≥ 1 mm and ≥ 9 mm for children aged 6 years and below. The occurrence of at least one solicited adverse event (i.e., none versus any) will be summarized by category (i.e., local, systemic, any), by vaccine group, by vaccination (after each vaccination and after any vaccination) and by time interval.

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval 30 min, Day 1 – 7 (excluding 30 minutes after vaccination).

For details please refer to section 7.1.1 of the protocol.

13.3 Unsolicited Adverse Events

The first-line analysis will use unsolicited adverse event data from all reporting sources combined. A second-line analysis will encompass the analysis of unsolicited adverse events by source.

All the unsolicited adverse events occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded. The original verbatim terms used by investigators to identify adverse events in the case report forms (CRFs) will be mapped to preferred terms using the MedDRA dictionary. The unsolicited adverse events will then be grouped by MedDRA preferred

terms into frequency tables according to system organ class. Adverse events judged by the investigator as at least possibly related to study vaccine will be summarized by vaccine group, according to system organ class and preferred term within system organ class. When an unsolicited adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Only vaccine-emergent adverse events (see protocol section 8.7 for definition) will be analyzed, i.e., excluding those after a subject has given informed consent but before vaccination. The selection of unsolicited adverse events and the assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

The summaries will be presented by period of onset and will include frequency distributions of the different adverse events:

Previously vaccinated subjects:

- Onset between Day 1 and Day 22
- Onset between Day 23 and Day 181

Not previously vaccinated subjects:

- Onset between Day 1 and Day 50
- Onset between Day 51 and Day 209

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited adverse event
- Possibly or probably related unsolicited adverse events
- Unsolicited adverse events leading to death
- Serious adverse events
- Possibly or probably related serious adverse event
- Unsolicited adverse events leading to premature withdrawal from study
- Unsolicited adverse events leading to dose reduction, interruption or delay in study vaccination
- Medically attended adverse events

- Unsolicited adverse events leading to new onset of chronic disease (NOCD)

13.4 Combined Solicited and Unsolicited Adverse Events

Solicited adverse events continuing beyond Day 7 will be reported as an unsolicited AE by the PI and subsequently coded by MedDRA and combined with the unsolicited adverse events as routinely collected beyond Day 7.

A summary of subjects with all combined solicited and unsolicited adverse events will be provided using preferred MedDRA terms for solicited adverse events.

Out of window solicited and unsolicited adverse events and reason for exclusion of these AEs from the analysis of the safety data will be presented as separate listings.

13.5 Clinical Safety Laboratory Investigations

Not applicable.

13.6 Concomitant Medication

The frequencies and percentages of concomitant medications will be tabulated overall and by vaccine group. Medications (generic drug name) will be coded using the WHODRUG dictionary.

14. INTERIM ANALYSIS

14.1 Interim Analysis

As the circulation of influenza viruses is seasonal and the event rates of influenza are difficult to predict, this study is group sequentially, with a case count-driven interim analysis if decided by the Sponsor. The goal of the Interim Analyses is, first, to minimize the risk of not being able to take a significant test decision after the end of the study, and second, to be able to stop the study for early evidence of efficacy.

The number of laboratory-confirmed influenza cases will be reviewed on a regular basis. If decided by the Sponsor and after observing at least 50% of planned events meeting the co-primary endpoint, an interim analysis for efficacy may be performed for evaluation by a DMC.

- If the number of RT-PCR confirmed influenza cases is less or equal to 190 overall, no interim analysis for efficacy may be done and the study will be extended because the probability to make a conclusion for efficacy is too low.

- If the number of RT-PCR confirmed influenza cases is greater or equal to 191 but less than 381 unblinded interim analyses for efficacy may be performed for evaluation by the DMC (see below for description and DMC charter). To maintain the overall alpha, α = 2.5% (1-sided), for the hypotheses testing for the primary objectives, an error-spending-function will be used. The benefit of using an error-spending-function is that no maximum number of analysis stages and the timing of the analyses need to be pre-specified, what in practice means that the duration of the study in terms of number of seasons can be left open. In this case α-boundaries, forming the adjusted probabilities for the type I error, are calculated using error-spending function and if the p-values for both primary objectives are lower than the respective α-boundary the trial stops early (i.e., without reaching the targeted number of cases of 381) for efficacy. Otherwise, the trial continues enrollment. Decisions to stop or continue the trial will be made on the basis of discussions between the DMC and Senior Management.

- If the number of RT-PCR confirmed influenza cases is greater or equal to 381 (targeted number of cases to be able to evaluate the co-primary objective) and subjects have been enrolled over at least three seasons the study will be unblinded and the final analysis will be performed by the sponsor. If the trial proceeds to the final analysis (upon reaching 381 cases) the boundaries for acceptance or rejection are identical to the assumed type 1 and type 2 errors for the overall design, and the trial stops to either reject or not reject the null hypothesis of the primary objective.

That is, the primary objective, i.e. VE against RT-PCR or culture confirmed influenza A and or B will be assess the one-sided 0.025 alpha level.

In case the DMC states that the observed data provides already the full information level needed for the final test decision, then the final analysis can be done on full alpha level of (2.5 % 1-sided) and no further enrollment is needed. If, however, the decision of the group-sequential test is to continue the study then it is on the DMC to determine the number of subjects needed to be enrolled. The DMC should not be influenced by the individual results of the vaccine groups observed at an interim analysis stage when planning further subjects' accrual or the times of future analysis. Only the overall number of cases is allowed to be used for further planning. The following formula for determination of sample sizes for further enrollment may be used:

$$N_{total} = (C_{planned} - C_{observed}) / [(ER_{QIVc} + ER_{comp})/2],$$

where N_{total} denotes the total number of subjects needed for further enrollment, $C_{planned}$ is the overall number of cases needed for the test, i.e. 381 cases, $C_{observed}$ are the at that stage observed number of cases overall, and ER are the respective event rates assumed for each group, i.e., 8.0% for non-influenza comparator group and 4.0%. for QIVc.

For the analysis of for efficacy, an error-spending function will be applied to provide statistical stopping rules for efficacy (α-boundaries) for the first interim analysis and second interim analysis, if necessary, based on the information accumulated until that specific interim stage, i.e. based on the accumulated variance of the parameter of interest.

These boundaries will be calculated on a p-value see:

https://support.sas.com/documentation/onlinedoc/stat/131/seqdesign.pdf

At each interim stage, α-boundaries, forming the adjusted probabilities for the type 1 error, are calculated using error-spending function and if the p-value for the test of primary objective is lower than the respective α-boundary the trial stops for efficacy at this stage.

In other words the trial stops early for efficacy if data collected at this stage allows to demonstrate both primary and co-primary efficacy objectives (reject null-hypothesis). Further guided by efficacy estimates of QIVc described in secondary efficacy objective 4 (prevention of PCR confirmed influenza antigenic matched to the strains selected for the seasonal vaccine). Otherwise, the trial continues to the next stage and more subjects will be enrolled.

The cumulative O'Brien-Fleming type error-spending-function (Lan and DeMets, 1983, option ERRFUNCOBF in SAS® PROC SEQDESIGN) will be used for both α- and β-boundaries; the error-spending function is defined as:

$$E(t;a) = \begin{cases} 1 & \text{if } t \geq 1 \\ \frac{1}{a}\,2\,(1-\Phi(\frac{z_{(1-a/2)}}{\sqrt{t}})) & \text{if } 0 < t < 1 \\ 0 & \text{otherwise} \end{cases}$$

where a equals α for α spending function and β for beta spending function, and where t is the information fraction.

If the trial proceeds to the final analysis, the boundary for rejection of null hypothesis is identical to the type 1 error (that is as the 0.025 one-sided alpha level) with power specified for the overall design, and the trial stops to either reject or not reject the null hypothesis of the primary objective.

Additional interim analyses may be requested at a future date (by the DMC), particularly if the first interim analysis is conducted soon after 191 cases of influenza have been reported. In this case, the DMC may request that a second interim analysis be conducted to allow for greater accuracy to determine if the trial should stop or continue.

Any modifications of a trial design (i.e. introducing additional interim analysis stage) and calculations of number of subjects to be additional enrolled will be based on the assumptions as defined in the protocol Section 8.6, and will not be influenced by the results of an interim analysis.

Table 14.1-1 shows the needed total number of cases at each stage of the two-stage design together with boundaries for early stopping efficacy, calculated on p-value scale using the cumulative error spending function. With ½ information collected at the stage of interim analysis (that is, with exactly 191 confirmed ILIs) the trial would stop early for efficacy if the p-value is lower than 0.00282. Otherwise the trial will be continued and more subjects will be enrolled. The actual boundaries used for decision making would depend on number of confirmed ILIs occurring and reported for Interim Analysis.

Table 14.1-1: Example of a Two Stages Group-Sequential Design

	Stage 1 First Interim	**Stage 2 Final**
Number of Events	191	382
Alpha Boundary on p-value scale (equivalent to Type 1 error, 1-sided), early stopping for efficacy	0.00282	0.02518

The stopping rules are statistically determined and should be complemented by clinical and strategic stopping rules that allow the DMC to make a decision on a broader picture of the data which includes safety endpoints and the other endpoints of the study being for example secondary efficacy objective 4. Another interim look at the data with appropriate adjustment of type 1 error might be recommended before reaching the targeted 381 cases.

For the interim analyses, if needed a restricted unblinding will be done, i.e. only independent DMC members and unblinded individuals responsible for the analyses will receive access to the randomization codes and unblinded data for the purpose of preparing the interim analyses (further information on handling of the blinding for the interim analyses can be found in the DMC charter and protocol section 3.3). The results of the interim analyses will be used only for DMC purposes and will not be reported in a CSR.

The comparison of the test statistic with its boundary values will be performed by using the SAS® SEQTEST procedure. The boundary information tables calculated by PROC SEQDESIGN at an analysis stage are structured for input to the SEQTEST procedure. At each subsequent stage, the boundary values are derived by using the test information tables created by the SEQTEST procedure at the previous stage. These test information tables are also structured for input to the SEQTEST procedure. PROC SEQTEST can also be used to compute parameter estimates, confidence limits, and p-values after the trial stops.

15. DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be constituted for this trial. The members of the DMC shall have no involvement in the design or conduct of the trial and no financial interest in the outcome of the trial. The DMC will comprise solely of non-Seqirus employees, and include medical experts and a biostatistician. The main purpose of the DMC will be to ensure the safety of subjects and scientific integrity of the study on an ongoing basis during the trial.

If decided by the Sponsor, an unblinded interim analysis for efficacy may be performed for evaluation by the DMC after observing at least 50% of planned events meeting the co-primary endpoint. In addition, the DMC will also review periodically general safety of subjects (especially SAEs and NOCDs) to advise the sponsor as appropriate.

A DMC Charter will be written documenting that procedures were pre-specified and well defined, and thereby reduce concerns that operations inappropriately influenced by interim data could bias the study results and interpretation. The independent DMC is comprised solely of non-Seqirus employees, and consists of medical experts and a biostatistician. The actual data handling and programming will be done by a CRO who will provide blinded and unblinded results to the DMC.

After the efficacy interim analysis, the CRO will produce a report containing an aggregate of summary tables, figures, and/or listings of study conduct or outcome data that are prepared for the DMC to review. The DMC reports will have two parts: an “open” section which includes only aggregate data and focuses on study conduct issues such as accrual, eligibility rates, dropout rates, and timeliness of data; and a “closed” section in which comparative efficacy data are presented. The “open” section of the report may be shared with Seqirus if necessary and appropriate. The “open” section will not contain any unblinded data. Seqirus personnel who are involved in the treatment or clinical evaluation of the subjects during the trial will remain blinded. For the interim analyses a restricted unblinding will be done, i.e. only external DMC members and CRO employees executing the analyses will receive access to the randomization codes and unblinded data for the purpose of preparing the interim analyses.

If safety signals of concern are observed by the DMC, the DMC may recommend that study vaccination be halted until the DMC determines it is appropriate to proceed with vaccination. Further details on the working of the DMC will be described in the charter.

16. PEER REVIEW

The type of peer review required for each output is to be identified by the study Biostatistician and Statistical Programmer (SP). Analyses/Outputs requiring statistical peer review correspond usually to the analyses of the primary and secondary objectives, and data conversion programs. Peer review of these analyses should be performed in accordance with the applicable procedures for validation of SAS programs used in clinical data analysis.

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

For the complete list of tables, listings and figures (TLFs), please refer to the post-text TLF Table of Contents (TOC).

18. LAYOUT SPECIFICATIONS FOR TABLES, LISTINGS AND FIGURES

All TLFs will include a header with the following components, e.g.:

Seqirus Inc.	Vaccine: QIVc
Final Report: Study 130_12	Phase III/IV, Subjects ≥2 years to <18 Years

In all tables, listings and figures, vaccine groups will be labeled as “QIVc”, “Vaccine Comparator”.

The mock-up catalogue will be used during programming.

Since all TLFs will be produced using SAS®, the output actually generated may slightly differ from the mock-ups presented in the study specific mock-up catalogue.

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