STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 3, Randomized, Observer-blind, Controlled, Multicenter, Clinical Study to Evaluate Immunogenicity and Safety of an MF59-adjuvanted Quadrivalent Subunit Inactivated Influenza Vaccine in comparison with a Licensed Quadrivalent Influenza Vaccine, in Adults 50 to 64 years of Age
Study Number:	V118_23
Protocol Version and Date:	Final Version 3.0, 11JUL2022
Phase of Development:	Phase 3
Plan Prepared by:	[redacted], Principal Biostatistician II
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Approvers:	[redacted], Global Director Biostatistics [redacted], Global Principal Clinical Scientist

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TABLE OF CONTENTS

APPROVALS

[redacted], Principal Biostatistician II Date

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[redacted], Global Director Biostatistics, Seqirus Date

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Event of Special Interest
ANCOVA	Analysis of Covariance
aQIV	MF59-adjuvated Quadrivalent Influenza Vaccine
CI	Confidence Interval
CSR	Clinical Study Report
EOS	End of Study
FAS	Full Analysis Set
FWER	Family Wise Error Rate
GMT	Geometric Mean Titer
GMTr	Geometric Mean Titer Ratio
HI	Hemagglutination Inhibition
ICH	International Council for Harmonization
IRT	Interactive Response Technology
MCAR	Missing Completely At Random
MedDRA	Medical Dictionary for Regulatory Activities
MN	Microneutralization
NI	Non-inferiority
PD	Protocol Deviation
PPS	Per Protocol Set
PT	Preferred Term
QIV	Quadrivalent Influenza Vaccine
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion Rate
SD	Standard Deviation
SE	Standard Error
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction

1. BACKGROUND AND RATIONALE

Vaccination is considered the best approach to lower the burden of influenza disease. The efficacy in older individuals is significantly lower due to the aging of the immune system (i.e. immunosenescence) and underlying medical conditions that can both increase the risk of influenza complications as well as interfere with immune responses (Sasaki et al. 2011).

One way to increase the immunogenicity of influenza vaccines is by using adjuvants, such as the squalene and water emulsion adjuvant, MF59.

Although the current licensure of Fluad (trivalent and quadrivalent) is limited to persons 65 years of age and older, the results of clinical studies conducted in healthy individuals 50-64 years and subjects 18-60 years of age with chronic medical conditions, demonstrated similar benefits as has been seen in adults 65 years of age and older in terms of immunogenicity (O'Hagan et al. 2011; Noh et al. 2016; Baldo et al. 2007). Like the findings with older adults an acceptable safety and tolerability profile was seen in adults under 65 years of age.

Apart from the medical need for improved vaccines in people aged 50 to 65 years, determining persistence beyond a typical seasonal epidemic is important, considering that influenza persists in the temperate climate zone for more than 6 months. Persistence of vaccine-induced immunity over periods longer than a typical winter season has not been widely investigated.

The purpose of this study is to demonstrate that vaccination with MF59-adjuvated Quadrivalent Influenza Vaccine (aQIV) elicits an immune response that is non-inferior to Quadrivalent Influenza Vaccine (QIV) with respect to the Geometric Mean Titer ratio (GMTr) and Seroconversion Rate (SCR) difference for each vaccine strain contained within the vaccine. Furthermore, if the non-inferiority comparisons are successful, the study is designed to assess superior immune response with regards to GMTr of aQIV compared with a licensed non-adjuvanted quadrivalent influenza vaccine for two out of four vaccine strains, three weeks after vaccination in adults 50-64 years of age. In addition, antibody persistence, reactogenicity and safety will be assessed. Data from this study will be used to expand the licensure of the quadrivalent version of Fluad for the prevention of seasonal influenza in adults 50-64 years of age.

For further details of the background of the study, please refer to section 1.0 of the protocol.

This Statistical Analysis Plan (SAP) describes the data and variables to be summarized and analyzed, including specifics of the statistical analyses to be performed and is based on protocol version 3.0 dated 11JUL2022. It is compliant with International Conference on Harmonization (ICH) Harmonized Tripartite Guideline, 5 February 1998, Statistical Principles for Clinical Trials, E9; World Health Organization, WHO Technical Report, Series No. 924. 2004, Annex 1: Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations; and FDA Center for Biologics Evaluation and Research (CBER) Guidance for Industry, May 2007, Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines.

2. OBJECTIVES

2.1 Primary Objectives

2.1.1 Primary Immunogenicity Objective

1a. To demonstrate immunological non-inferiority of aQIV versus a non-adjuvanted quadrivalent influenza vaccine comparator (QIV) in subjects 50-64 years of age, as measured by hemagglutination inhibition (HI)[1] geometric mean titers (GMTs) and seroconversion rates (SCRs) for each vaccine strain, at 3 weeks after vaccination.

Success criteria:

Non-inferiority will be demonstrated if the UL of the two-sided 95% confidence interval (CI) for inter-group GMT ratio[2] (QIV/aQIV) is ≤1.5 for each vaccine strain, and the upper limit (UL) of the two-sided 95% CI around the difference in SCR[3] (QIV-aQIV) is ≤10% for each vaccine strain.

1b. To demonstrate that aQIV induces a superior immune response compared with QIV in subjects 50-64 years of age as measured by HI[1] GMTs at 3 weeks after vaccination for at least 2 of the 4 vaccine strains.

Success criteria (after objective 1a is reached):

Superior immune response will be demonstrated if the UL of the two-sided 95% CI for inter-group GMT ratio (QIV/aQIV) <1.0 for at least 2 of the 4 vaccine strains

2.2 Secondary Objectives

2.2.1 Secondary Immunogenicity Objective(s)

2a. To demonstrate that aQIV induces a superior immune response compared with QIV in subjects 50-64 years of age as measured by HI GMT for at least one vaccine strain at 3 weeks after vaccination.

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[1] In case of lack of agglutination or agglutination mediated through neuraminidase for a specific strain using HI assay, immunogenicity for that strain will be assessed as measured by microneutralization (MN) assay as an acceptable alternative.

[2] The GMT ratio is defined as the geometric mean of the post-vaccination HI titer for QIV over the geometric mean of postvaccination HI titer for aQIV.

[3] The SCR is 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 ≥4-fold increase in postvaccination HI titer. Difference in SCR is defined as $SCR_{QIV} - SCR_{aQIV}$.

Success criteria:

Superior immune response will be demonstrated if the UL of the two-sided 98.73% CI for inter-group GMT ratio (QIV/aQIV) < 0.67 for one or more vaccine strains.

2b. To demonstrate greater persistence of the immune response for at least one vaccine strain at 6 months after vaccination with aQIV compared with QIV as measured by HI in subjects 50-64 years of age.

Success criteria:

Greater persistence of the immune response will be demonstrated if the UL of the two-sided 98.73% CI for inter-group GMT ratio (QIV/aQIV) <1.0 for one or more vaccine strains.

2c To evaluate the immunogenicity of aQIV compared with QIV as measured by HI in subjects 50-64 years of age.

2.2.2 Secondary Safety Objective:

To assess the safety and reactogenicity of aQIV and QIV in adults 50-64 years.

2.3 Exploratory Objectives

To evaluate persistence of the immune response at 9 months after vaccination with aQIV compared with QIV as measured by HI in subjects 50-64 years of age.

To further evaluate the immunogenicity of aQIV compared with QIV in subjects 50-64 years of age, with alternative assays, if sera permit.

3. STUDY DESIGN

Experimental design: This is a Phase 3, randomized, comparator controlled, observer-blind, multi-center study in approximately 2,018 adults 50-64 years of age.

Duration of the study: The study duration is approximately 9 months for each subject.

Vaccination schedule: single vaccination (Day 1).

Study vaccine: aQIV vaccine (Fluad Tetra/Quadrivalent).

Comparator vaccines: non-adjuvanted QIV ([redacted]).

Treatment groups:

aQIV group: approximately 1,009 subjects receiving one dose of aQIV at Day 1.

QIV group: approximately 1,009 subjects receiving one dose of QIV at Day 1.

Randomization: An Interactive Response Technology (IRT) system will be used in the study. Subjects will be enrolled and stratified equally into two age groups (50-59 years and 60-64 years) with approximately 50% of subjects per age group. Within each age group subjects will be randomized to the aQIV or QIV according to a 1:1 ratio. Stratification for history of any influenza vaccination within 3 previous seasons (yes/no) will be applied to all subjects.

Blinding: Observer-blind study.

Blood sample schedule: Four blood samples (approximately 10 mL each)[4] will be collected from all subjects on Days 1, 22, 181 and 271.

Data collection: electronic Diary (eDiary), electronic Case Reporting Form (eCRF).

Study clinic visits: Four clinic visits for each subject at Days 1, 22, 181 and 271.

Safety phone call: Two safety phone calls (Day 15 and Day 91) will be conducted: on Day 15 to collect any unsolicited adverse events (AEs), including associated medications and any vaccinations, on Day 91 to collect only serious AEs (SAEs), AEs leading to withdrawal, AEs

[4] Subjects may be asked to voluntarily provide extra blood samples of 50 mL at Day 1 and Day 22, that can be used for future research not directly related to this study, but with the purpose to improve the understanding of the influenza vaccines or disease.

of special interest (AESIs), and concomitant medications associated with these events and any vaccinations.

Solicited AEs occurring on the day of study vaccination through the following 6 days (Day 1 through Day 7, or longer if the events are not resolved), will be recorded daily using an eDiary as completed by the subject.

Safety data collection: During the treatment period (Days 1-22) all unsolicited AEs occurring within 21 days (Day 22) after study vaccination will be collected. During the follow-up period (Days 23-271) only SAEs, AEs leading to study withdrawal, and AESIs will be collected. These data will be captured by interviewing the subject during the clinic visits, during the safety phone calls scheduled to occur on Days 15 and 91 and by review of available medical records. Subjects will be instructed to call the site in the event of any AE which they perceive as being of concern during the entire study period.

Serological assays:

HI assay for homologous vaccine strains, using egg-derived target virus, will be performed for all subjects on serum samples collected on Days 1, 22, 181 and 271.

Written informed consent will be obtained before conducting any study-specific procedures.

Table 1 Time and Events Schedule

	Visit Type	Clinic Visit	Safety Phone Call	Clinic Visit*	Safety Phone Call	Clinic Visit*	Clinic Visit*
	Study Day	**1**	**15**	**22**	**91**	**181**	**271**
	Visit Window (Days)	**n/a**	**-3/+3**	**-3 to +7**	**-7 to +7**	**-14 to +14**	**-14 to +14**
	Visit Number	**1**	**2**	**3**	**4**	**5**	**6**
Study Event	**References****						
Study Treatment							
Vaccination	Section 5.2	X					
Screening and Safety							
Informed Consent[a]	Section 5.1.1	X					
Demographics and baseline characteristics	Section 5.1.2	X					
Medical History[b]	Section 5.1.2	X					
Physical Exam[c]	Section 5.1.2	X		X		X	X
Pregnancy Test[d]	Section 5.1.2	X					
Exclusion/Inclusion Criteria	Section 4	X					
Randomization	Section 5.1.4	X					

	Visit Type	Clinic Visit	Safety Phone Call	Clinic Visit*	Safety Phone Call	Clinic Visit*	Clinic Visit*
	Study Day	**1**	**15**	**22**	**91**	**181**	**271**
	Visit Window (Days)	**n/a**	**-3/+3**	**-3 to +7**	**-7 to +7**	**-14 to +14**	**-14 to +14**
	Visit Number	**1**	**2**	**3**	**4**	**5**	**6**
Study Event	**References****						
30 Minutes Post Injection Assessment of unsolicited AEs	Section 5.3	X					
Subject eDiary Dispensed with Training	Section 5.3	X					
Review of eDiary data and compliance	Section 3.6.2	Ongoing during eDiary use					
Assess all AEs	Section 7.1.2	X	X	X			
Assess SAEs	Section 7.1.4	X	X	X	X	X	X
Assess for AEs leading to withdrawal, and AESIs	Section 7.1.4.1	X	X	X	X	X	X
Assess relevant medications and vaccinations	Section 6.5	X	X	X	X	X	X
Immunogenicity							
Serology blood draw[e]	Section 5.1.5	X[f]		X		X	X
Study Completion Procedure							

	Visit Type	Clinic Visit	Safety Phone Call	Clinic Visit*	Safety Phone Call	Clinic Visit*	Clinic Visit*
	Study Day	**1**	**15**	**22**	**91**	**181**	**271**
	Visit Window (Days)	**n/a**	**-3/+3**	**-3 to +7**	**-7 to +7**	**-14 to +14**	**-14 to +14**
	Visit Number	**1**	**2**	**3**	**4**	**5**	**6**
Study Event	**References****						
Study Completion/early termination[g]	Section 5.6						X

Notes: * In the exceptional case that a clinic visit is not possible, and if in line with country and site regulations with appropriate sponsor approvals a home visit may be considered.

** All sections refer to sections of the protocol.

[a] Consent form should be signed prior to performing any study procedures. The informed consent process may be conducted earlier, but within 10 days prior to Day 1;

[b] Medical history includes existing comorbidities

[c] A physical examination will be based on a review of systems, i.e., a structured interview for complaints for each organ system;

[d] A pregnancy test should be done for females of childbearing potential;

[e] Subjects may be asked to voluntarily provide extra blood samples of 50 mL at Day 1 and Day 22 (see section 3.7 of the protocol, Collection of Clinical Specimens)

[f] Blood sample for serology to be taken after temperature measurement, but prior to vaccination;

[g] Subjects who terminate the study early will be requested to complete all safety-related Study Completion procedures.

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Enrolled subjects will be assigned a subject ID and randomized in the IRT system in a 1:1 ratio to receive either aQIV or QIV with age group (50 to 59, and 60 to 64 years of age), and history of any influenza vaccination within 3 previous influenza seasons (yes/no) as stratification factors. Approximately 50% of subjects will be enrolled into each age group. The Subject ID will be the subject's unique identification number for all eCRFs and associated study documentation that will be used for duration of the study. After randomization, the Screening Number ceases to be used and remains in the Screening and Enrolment Log only. The list of randomization assignments is produced by the IRT service provider and approved by Seqirus according to applicable Seqirus Standard Operating Procedure (SOP).

If for any reason, after signing the informed consent form (ICF), the subject who is eligible and enrolled fails to be randomized, this is called a randomization failure and the early termination study procedures must be applied. The reason for all randomization failures should be recorded in the Screening and Enrolment Log and in the source document. The information on subjects who are randomization failures should be kept distinct from subjects who are screen failures, as described in protocol section 5.1.2, Screening.

If for any reason, after randomization the subject fails to undergo treatment or the subject has discontinued, the reason should be recorded in source document and in the eCRF. The information on discontinued subjects should be kept distinct in the source documentation from randomization failures.

4.1.1 Definition of Randomization/Vaccination Errors

The list below provides categories for errors that may occur during randomization and/or vaccination.

Randomization errors:

- Administered wrong kit (Subject received vaccine from a kit different from the kit number that was assigned at randomization).

Vaccination errors:

- Administered only part of the study vaccine.
- Incorrect vaccine administration (e.g. incorrect location or route)
- Administered expired vaccine.
- Administered temperature deviated vaccine.

Stratification error:

- Subject randomized in the wrong stratification stratum.

Differentiate between incorrect stratification and randomization errors.

A randomization error is defined as a subject receiving a vaccine from another kit than the one assigned by randomization. If after unblinding, it appears that the vaccine from the incorrect kit is identical to the vaccine from the assigned kit, the error will be reported as a major protocol deviation (PD), but the data from the subject will not be excluded from any analyses sets. If the vaccine is different from the vaccine in the assigned kit, the randomization error is a Clinical Study Report (CSR) - reportable PD and the subject's data should be analyzed as randomized in Full Analysis Set (FAS), excluded from Per Protocol Set (PPS) and analyzed as received for Safety.

Incorrect stratification is defined as enrollment and randomization of a subject based on incorrect stratification information at baseline. Incorrect stratifications should be split for major and minor errors.

- Major stratification errors are those resulting in administration of incorrect dosage or schedule~~,~~ not corrected on time, i.e. not corrected before administration of the vaccine. These will be handled as CSR-reportable PD.
- Minor stratification errors are those not having impact on dose/schedule administered. These will not be considered as CSR reportable PD.

Since the vaccine dosage and regimen in this study are independent from the stratification factors, major stratification errors are not applicable. In the analysis of covariance (ANCOVA) and in the subgroup analyses, subjects will be analyzed according to their actual age and/or influenza vaccination history.

Stratification error	**FAS**	**PPS**	**Safety Sets All-Enrolled Set All-Exposed Set**
Minor	Analyze as corrected	Analyze as corrected	Analyze as corrected
Major*	Analyze as originally stratified	Exclude from analysis	Analyze as corrected

* Since the vaccine dosage and regimen in study V118_23 are independent from the stratification factors, major stratification errors are not applicable.

4.1.2 Forced Randomization

Forced randomization will not be utilized in this trial.

4.2 Blinding and Unblinding

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

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

All personnel involved in the conduct of the study or in the analysis of the final study results, or who have contact with study centers, will remain blinded to the treatment codes until the clinical database has been locked, protocol deviations (except for Day 271 serum sample analysis PDs) have been assessed, and the data have been released for statistical analysis. The analysis on the primary and secondary objectives for the final CSR will be conducted on this data.

All personnel involved in processing samples and performing laboratory assays will remain blinded to the treatment codes until all Day 271 serum samples have been tested and results have been transferred. The exploratory analysis on the 9 month persistence objective will be conducted on this data and reported in a CSR addendum.

If a subject is unblinded during the study, it is to be documented as a CSR-reportable PD. The unblinding will be documented appropriately. The unblinded subject(s) may be excluded from the PPS. Unblinded subjects will be included in the FAS and safety sets. Data from subjects who are unblinded by Pharmacovigilance due to suspected unexpected serious adverse reactions (SUSAR) do not need to be excluded from any analysis set or reported as CSR reportable PD, as long as the clinical study team involved in the data review and analysis remains blinded.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

aQIV will be tested against QIV. The randomization ratio is 1:1 (aQIV:QIV). The study is designed to have at least 90% power to achieve the primary objectives: to demonstrate the noninferiority of GMT and SCR of aQIV vs QIV for all four vaccine strains, and the superiority assessment (superiority margin of 1) of GMT of aQIV vs QIV for at least two out of the four vaccine strains with one-sided significance level α=0.025.

The results of a previous clinical study with aTIV and TIV vaccine in subjects 50-64 years of age (V7P38) was used to generate assumptions for the vaccine effects and sample size.

It is assumed that the differences (aQIV minus QIV) of $\log_{10}$(GMT) of A/H1N1 is 0.07, with standard deviation of 0.46 for both treatment arms, 1,816 evaluable subjects will provide at least 90% of power to demonstrate a superiority (GMTr margin of 1) of aQIV vs QIV for the A/H1N1 strain. The table below shows the power of each comparison based on GMT ratio and SCR data from study V7P38:

Table 2 Sample size and power estimates based on the GMT ratio endpoint

Strain	Log10(GMT) Differences (SD)	Sample Size* (Evaluable) for 90% Power	Power with Sample size of 2,018 for superiority (GMTr margin of 1) (1816 evaluable)	Power with Sample size of 2,018 for NI (GMTr Margin of 0.67)
A/H1N1	0.07 (0.46)	2,018 (1,816 evaluable)	90%	~100%
A/H3N2	0.26 (0.61)	260 (234 evaluable)	~100%	~100%
B (Victoria and Yamagata lineage)	0.14 (0.48)	552 (496 evaluable)	~100%	~100%

*Based on 10% drop-out rate

Table 3 Sample size and power estimates based on the SCR endpoint

Strain	SCR in aQIV group	SCR in QIV group	Power with Sample size of 2,018 (1,816 evaluable) for NI (SCR margin -10%)
A/H1N1	86%	76%	~100%
A/H3N2	87%	77%	~100%
B (Victoria and Yamagata lineage)	82%	80%	~100%

Thus with 1:1 randomization, assuming that B/Victoria and B/Yamagata are similar, 1,816 evaluable subjects will provide an overall power of 90% to demonstrate the primary objectives of noninferiority and superiority of aQIV vs QIV with one-sided alpha=0.025. Assuming a 10% drop out rate, the total sample size for the study needed is 2,018.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

The CSR-reportable PDs are defined in accordance with ICH E3 as important PDs related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment resulting in the potential to jeopardize the safety or rights of the trial subjects or the scientific value of the trial. Protocol deviations will be classified as CSR-reportable and non-CSR-reportable.

The Protocol Deviation Specification Document for this study lists all the pre-specified observable and programmable PDs, including their classification, categories, subcategories and impact on the analysis.

CSR-reportable PDs may lead to exclusion of the subject or part of the subject's data from at least the PPS.

The number of subjects with any major PD and by PD category and subcategory will be summarized by study treatment and overall. Individual subject listings of major PD will be provided sorted by study treatment, subject, visit and date.

6.2 Determination of Protocol Deviations

The source/method of identification can be either observable or programmable. Programmable PDs are those which can be programmed from the data recorded in the clinical database. Depending on the type of PD, this can be done directly in the database using edit checks. Or separate offline PD listings are created, which are reviewed manually and will lead to triggering PDs in the clinical database. Observable PDs are those that can only be identified by Clinical Research Associates during monitoring or by other team members and are recorded in Impact Harmony.

All PDs will be collected according to the Protocol Deviation Specification document in a web-based protocol deviation tool. The protocol deviation tool and process are overseen by Data Management. Extracts from the tool will be provided regularly for review. Categorization of PDs as major / minor and potential exclusion of the subject from an analysis set will be captured as agreed during the review in the PD tool.

Prior to database lock and unblinding, all PDs will have been reviewed and categorized. A Blinded Data Review Meeting will take place to discuss and confirm assignment of subjects to analysis sets based on the final protocol deviations. Blinded Data Review Meeting minutes and analysis sets will be signed off by at least the Biostatistician and the Clinical Scientist prior to unblinding.

Classification of protocol deviations (and associated actions for analysis sets) that can only be assessed after unblinding, e.g. administration of study drug from the wrong treatment arm, will be

discussed as much as possible before unblinding considering all potential scenarios. However, the final assessment can only be confirmed based on the unblinded locked database. Therefore, final classification of such PDs will be carried out after unblinding. Documentation will be signed off again by at least the Biostatistician and the Clinical Scientist.

6.3 Exclusions of Individual Values for Safety Analysis

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

Therefore, these implausible measurements will be removed and counted as missing in the analysis but included in listings. Implausible measurements are summarized in the table below.

Table 4 Implausible Solicited Adverse Events

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

7. ANALYSIS SET

7.1 All-Enrolled Set

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

Demography and baseline characteristics tables as well as subject listings will be produced on the All-Enrolled Set. Subjects will be analyzed according to the randomized treatment.

7.2 All-Exposed Set

All subjects in the All-Enrolled Set who received a study vaccination. Subjects will be analyzed according to the actual treatment received.

7.3 Full Analysis Set (FAS), Immunogenicity

All subjects in the All-Enrolled Set who are randomized, received study vaccination and provide immunogenicity data at any time point.

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

See section 4.1.1 for details on how to handle subjects randomized in the wrong stratum.

The FAS Immunogenicity will be used for immunogenicity superiority comparisons on Day 22 and for evaluation of immune response persistence. FAS Immunogenicity will also be used for sensitivity analysis for PPS Immunogenicity based primary non-inferiority analyses.

7.4 Per Protocol Set (PPS), Immunogenicity

All subjects in the FAS Immunogenicity who:

- Have both Day 1 and Day 22 immunogenicity assessment.
- Correctly receive the vaccine (i.e., receive the vaccine to which the subjects are randomized and at the scheduled time points).
- Have no protocol deviations leading to exclusion (see section 6, Determination of Protocol Deviations) as defined prior to unblinding / analysis.

In case of randomization error, if a subject receives a vaccine kit, labelled for another subject but containing the same vaccine as the one the subject was randomized to, the subject will not be removed from the PPS.

In case of vaccination error, the subject is excluded from the PPS.

See section 4.1.1 for details on how to handle subjects randomized in the wrong stratum.

If a subject is unblinded during the study (except for possible SUSARs), he/she will be excluded from the PPS (see also section 4.2).

The PPS Immunogenicity will be used for noninferiority comparisons and exploratory analyses. PPS Immunogenicity will also be used for sensitivity analysis for FAS immunogenicity analysis where is applicable. Immunogenicity blood samples on Day 181 or 271 qualified to be excluded by protocol deviation review will not be included in Day 181 or Day 271 immunogenicity analyses.

7.5 Safety Set

Solicited Safety Set

All subjects in the All-Exposed Set with any solicited AE data including temperature measurements or use of analgesics/antipyretics.

All solicited safety analyses will be performed based on the Solicited Safety Set.

Unsolicited Safety Set (unsolicited adverse events)

All subjects in the All-Exposed Set who provided unsolicited AE data. Subjects are excluded when they have a confirmed PD of the following category "Subject did not provide or was not available for unsolicited post-vaccination safety assessment at all".

All unsolicited safety analyses will be performed based on the Unsolicited Safety Set.

Overall Safety Set

All subjects who are in the Solicited Safety Set or Unsolicited Safety Set.

In case of randomization 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) in all safety sets.

In case a subject is randomized in the wrong stratum:

- Subject will be analyzed in their actual subgroup for all safety analyses.

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

7.6 Other Analysis Set

Not applicable

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

All EDC data up to Visit 6 (Day 271) at the time of database lock, immunogenicity serum lab samples supporting primary and secondary objectives collected up to Visit 5 (Day 181) and protocol deviations (except for Day 271 serum sample analysis PDs) will be used to support final CSR analysis.

For the exploratory objective of persistence of the immune response at 9 months after vaccination, HI antibody responses for all strains included in the study vaccines will be evaluated using an egg-derived target virus at a later stage. For this analysis, Day 1 serum samples obtained for the primary and secondary study objectives (noninferiority, superiority assessments at Day 22 and persistence at Day 181) will be retested together with the Day 271 sample. Day 1 data from the exploratory assessment of persistence at 9 months (retested result) will not replace data obtained for the primary and secondary endpoint analyses. Similarly, if there is insufficient Day 1 serum sample for retesting, the original Day 1 result will not be imputed in the exploratory analysis of persistence at Day 271 in the CSR addendum. and results have been transferred and will be reported in a CSR addendum.

8.1 Adjustment for Covariates

The main statistical analysis includes descriptive statistics for the overall population. Subgroup analyses will be done by age cohort (50-59 and 60-64 year of age), previous vaccination history, sex, race, ethnicity, and comorbidity risk scores (Hak score, <50 and ≥50). Summary tables will show unadjusted GMTs for each vaccine group by time point.

Adjusted GMTs will be calculated based on the $\log_{10}$-transformed antibody titers at Day 22/181/271 using an ANCOVA model which includes the vaccine group (aQIV and QIV), $\log_{10}$-transformed pre-vaccination antibody titer, age cohort (50-59 or 60-64 year of age), sex, and history of any influenza vaccination within the 3 previous seasons (yes/no).The main analysis of binary immunogenicity endpoints (i.e., percentages of subjects with seroconversion) will not be adjusted for any of the covariates. Binary data will be summarized for each group using unadjusted estimates and will be reported together with two-sided 95% CIs calculated according to the Clopper-Pearson method. Sensitivity analysis may be done to include the vaccine group (aQIV and QIV), stratification factors age and previous vaccination history, and sex in a generalized linear model

8.2 Handling of Dropouts, Missing Data

The distribution of subjects excluded from FAS/PPS will be described by vaccine group.

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

sensitivity analysis will be considered if the percentage of subjects with missing data is more than 10%.

Solicited AEs are collected using eDiary from Day 1 to Day 7 post-vaccination. If a solicited local or systemic AE continues beyond day 7 after vaccination, recording in the Subject eDiary will be continued until the event(s) resolved or for a maximum of 14 days after vaccination. If the data have not been recorded for all 7 days, the assessment is considered missing and excluded from analysis. In case at least one day is filled in but other days are missing the presence or absence of the event will be based on the available data

8.3 Multicenter Studies

There will be no adjustment for multiple centers.

8.4 Multiple Comparisons and Multiplicity

Adjustment for multiple comparison and multiplicity is reflected in the CI of the success criteria, which kept the type I error under 5%. For secondary endpoint analyses, sequential testing and significance level adjustment will be applied to keep type I error under 5%.

For four out of four strain successes, with α=0.05 for each strain, the overall type I error is α^4=0.00000625.

For two out of four strain successes, with α=0.05 for each strain, the overall type I error is

$0.05^4+4 \times 0.05^3 \times 0.95+6 \times 0.05^2 \times 0.95^2=0.014019$.

For 1 out of four strain successes, with α=0.05 for each strain, the overall type I error is

$1-0.95^4=0.1855$.

Thus, for objective 1b): two out of four strain success, there is no need to adjust for α=0.05 to keep the overall type I error under 0.05. But for objectives 2a) and 2b): 1 out of four strain success, α needs to be adjusted to 0.01274 so that the overall type I error $=1-(1-0.01274)^4=0.0499944$ which is less than 0.05. Therefore, the CI for the secondary objectives 2a and 2b have been adjusted to 98.73% to keep overall family wise error rate (FWER) under 0.05.

Confirmatory flow of tests and objectives, using a hierarchical testing approach

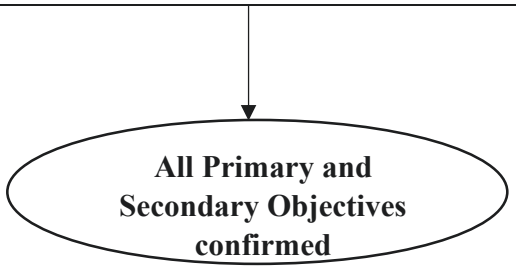
As soon as any success criterion is not met, confirmatory testing will stop.

PRIMARY OBJECTIVE 1a: Non-inferiority	
Tests using Antibody titer at Day 22: UL of 95% CI for GMT ratio (QIV/aQIV) for strain i ≤1.5 and UL of 95% CI for SCR difference (QIV-aQIV) for strain i ≤10%, for all i, i = 1 to 4	**Multiplicity consideration:** not required, overall type I error is < α, where α =0.05
	Analysis Set: PPS
Criterion: UL ≤ threshold for all 8 CIs => Non-inferiority confirmed and continue testing	

PRIMARY OBJECTIVE 1b: Superiority for at least 2 strains - basic threshold	
Tests using Antibody titer at Day 22: UL of 95% CI for GMT ratio (QIV/aQIV) for strain i <1.0, for at least 2 out of 4 strains	**Multiplicity consideration:** not required, overall type I error is < α
	Analysis Set: FAS
Criterion: CI UL < threshold for at least 2 out of 4 strains => Superiority (basic threshold) confirmed and continue testing	

SECONDARY OBJECTIVE 2a: Superiority for at least 1 strain - higher threshold	
Tests using Antibody titer at Day 22: UL of 98.73% CI for GMT ratio (QIV/aQIV) for strain i <0.67, for at least 1 out of 4 strains	**Multiplicity consideration:** α-adjusted CI
	Analysis Set: FAS
Criterion: CI UL < threshold for at least 1 strain => Superiority (higher threshold) confirmed and continue testing	

SECONDARY OBJECTIVE 2b: Persistence for at least 1 strain - Day 181	
Tests using Antibody titer at Day 181: UL of 98.73% CI for GMT ratio (QIV/aQIV) for strain i <1.0, for at least 1 out of 4 strains	**Multiplicity consideration:** α-adjusted CI
	Analysis Set: FAS
Criterion: CI UL < threshold for at least 1 strain => Persistence at Day 181 confirmed and continue testing	



8.5 Subgroups

Adjusted (same model as the main analysis by subgroup with subgroup factor removed if it is in the main model) and unadjusted immunogenicity analysis of the GMTs and SCRs will be performed by stratifying for the following subgroups:

- Age cohort (50-59 and 60-64 years of age)
- Previous vaccination history (Yes and No)
- Sex
- Race
- Ethnicity
- Comorbidity risk score (<50 and ≥50)

Safety analysis will be performed by stratifying for the following subgroups:

- Age cohort (50-59 and 60-64 years of age)
- Previous vaccination history (Yes and No)
- Sex
- Race
- Ethnicity
- Comorbidity risk score (<50 and ≥50)

8.6 Data Transformation

Distributions of antibodies are generally skewed to the right and approximately log-normally distributed. Therefore, prior to any statistical analysis that assumes normally distributed observations, antibody titers will be $\log_{10}$-transformed. GMTs and their 95% CIs will be then computed by exponentiating (base 10) the means and 95% CIs of the $\log_{10}$ transformed titers.

8.7 Derived and Computed Variables

Demographics

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

Body weight (kg) / Height^2 (m^2).

Immunogenicity

Values below the lower limit of quantification (LLOQ) will be set to half that limit. Values above the upper limit of quantification (ULOQ) will be set to the value of this upper limit.

Seroconversion based on **HI** (or microneutralization [MN]) antibodies titer is defined as binary variable for subjects with non-missing values pre-vaccination- and post-vaccination as:

= 1, if seroconverted (defined as a ≥ 4-fold increase in titer post-vaccination in those with pre-vaccination titer above or equal the LLOQ (1:10), or a post-vaccination titer ≥ 1:40 for subjects with pre-vaccination titer below the LLOQ (1:10))

= 0, otherwise

Fold increase is defined as the post-vaccination titer divided by the pre-vaccination titer.

Geometric Mean Titer

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

Comorbidity Score (Hak score)

For each subject, the Comorbidity Score (Hak score) is derived through the summation of the scores of the applicable characteristics. Missing values will not be replaced.

Characteristic	Score[a]
Age, years	
<70	0
70-74	14
75-79	28
80-89	42
≥90	56
Sex	
Female	0
Male	9
Outpatient visits during the previous year	
0	0
1-6	11
7-12	22
>13	33
Previous hospitalization due to pneumonia or influenza	
No	0
Yes	63
Comorbidity[b]	
Pulmonary disease	18
Heart disease	6
Renal disease or renal transplant	12
Dementia or stroke	22
Non-hematological and hematological cancer	48
Subject total score	

Notes:

a. The prognostic score for a given subject can be obtained by adding the scores for each applicable characteristic.

b. Pre-existing medical conditions of eligible subjects will be scored following a judgment by the investigator.

Solicited Adverse Events

For details see section 13.2.

Unsolicited Adverse Events

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

- **Pre-vaccination**: start date before the date of injection of study vaccine or indicated as on injection day but before injection are collected as medical history.
- **Emergence during vaccination phase**: all other cases.

Note: If an AE start date is missing or unknown and no indication is provided on the timing, the AE will be considered as emergent.

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

- If the partial end date is before (<) the vaccination (i.e., year or year & month is/are before the study vaccination year or year & month) then the AE is pre-vaccination.
- If the partial start date is equal or after (≥) the study vaccination (i.e., year or year & month is/are after or the same as the study injection year or year & month) then the AE is emergent during vaccination phase.

All AEs emergent during vaccination phase will be categorized as occurring during the period of 21 days following the study vaccination based on the start date, i.e. Day 1 to Day 22 included. If start date is missing or incomplete, events will be counted as yes during the period of 21 days following the date of study vaccination.

Adverse events that meet none of the following criteria SAE, AESI, or AE leading to withdrawal, and had a start date more than 21 days after the last vaccination are not to be recorded. However, if recorded, these AEs should be flagged (i.e. exclusion flag), excluded from analysis and listed separately. The same will be done with AEs starting before study vaccination injection.

The **maximum event severity** is the greatest severity associated with a preferred term (PT) for a reported AE according to the following order: Mild < Moderate < Severe.

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

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

Prior and Concomitant Medications and Vaccines

All medications and vaccines will be characterized according to the start and end date of occurrence related to the vaccination as follows:

- **Prior Medication and Vaccines**: start date before the date of injection of study vaccine.
- **Concomitant medications and vaccinations:** any medication and vaccination on or after the date of injection study vaccination.

Concomitant medication with partial date is treated in a similar way as treatment emergent AE with missing date. Concomitant medication and vaccines with start date after End of Study (EOS) date will be excluded from analysis. Concomitant medication that results from adverse events that meet none of the following criteria SAE, AESI, or AE leading to withdrawal, and had a start date more than 21 days after the study vaccination will be flagged (i.e. exclusion flag), and excluded from analysis.

8.8 Analysis Software

All analyses will be performed using SAS Software version 9.4 or higher.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All randomized subjects will be accounted for in this study. If a subject is enrolled but not randomized, the subject will not be included in other analysis tables (apart from the All-Enrolled Set). The numbers and percentages of subjects in each analysis set, study withdrawals, subgroups, and major protocol deviations will be presented by vaccine and overall. Number of subjects per site will be presented by vaccine group and overall for All-Enrolled Set.

The time in days (i.e. date of last assessment minus date of vaccination plus 1) the subjects are under observation for safety will be summarized by vaccine group and overall for Solicited and Unsolicited Safety Sets.

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

In general, all tables related to baseline characteristics should include a Total column across vaccine groups.

10.1 Demographics

Age, height, weight, and body mass index at screening will be summarized by reporting the mean, standard deviation, median, minimum and maximum, and will be calculated by vaccine group and overall.

In addition, the frequency of age categories will be reported as 50-59, and 60-64 years (age cohort). The number and percentages of subjects by sex, country, ethnic origin, race, and previous influenza vaccine history (within the past 3 years) will be presented by vaccine group and overall.

Demographic data will be tabulated for the All-Enrolled Set, FAS Immunogenicity, PPS Immunogenicity, and Overall Safety Set.

10.2 Medical History

The numbers and percentages of subjects with medical history will be presented by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and PT by vaccine group and overall. Medical history data will be tabulated for the All-Enrolled Set.

11. IMMUNOGENICITY ANALYSIS

11.1 Blood Samples

For each visit, the number and percentages of subjects with and without blood draws will be summarized overall and by vaccine group. Data will be tabulated for the All-Enrolled Set.

11.2 Primary Objectives Analysis

The primary immunogenicity objectives are:

1a. To demonstrate immunological non-inferiority of aQIV versus QIV in subjects 50-64 years of age, as measured by hemagglutination inhibition (HI) geometric mean titers (GMTs) and seroconversion rates (SCR) for each vaccine strain, at 3 weeks after vaccination.

1b. To demonstrate that aQIV induces a superior immune response compared to QIV in subjects 50-64 years of age as measured by HI GMTs at 3 weeks after vaccination for at least 2 of the 4 vaccine strains.

Primary Endpoints

Humoral immune responses in terms of HI antibody response against homologous egg-derived vaccine strains (A/H1N1, A/H2N3, B/Yamagata and B/Victoria):

- Geometric Mean Titer (GMT) of HI antibodies at Day 22;
- Seroconversion rate (SCR) defined as the percentage of subjects with either a prevaccination HI (or MN) titer <1:10 and a postvaccination (Day 22) HI (or MN) titer ≥1:40, or with a prevaccination HI (or MN) titer ≥1:10 and a ≥ 4 fold increase in post vaccination HI (or MN) titer.

The derived variables are:

- GMT ratios (QIV/aQIV) at Day 22 for each strain.
- The inter-group differences in the SCRs (QIV - aQIV) at Day 22 for each strain.

To evaluate the primary immunogenicity objectives 1a and 1b, the following derived variables of GMT ratios and SCRs differences will be assessed at Day 22:

1a. non-inferiority of aQIV compared to QIV will be assessed for the eight primary endpoints of HI (or MN) geometric mean titer (GMT) and seroconversion rate (SCR) for each virus strain included in the vaccines as follows:

- The GMT ratio (QIV/aQIV) for the A/H1N1 strain
- The GMT ratio (QIV/aQIV) for the A/H3N2 strain

- The GMT ratio (QIV/aQIV) for the B strain (Yamagata lineage)
- The GMT ratio (QIV/aQIV) for the B strain (Victoria lineage)
- The difference between the SCR (QIV-aQIV) for the A/H1N1 strain
- The difference between the SCR (QIV-aQIV) for the A/H3N2 strain
- The difference between the SCR (QIV-aQIV) for the B strain (Yamagata lineage)
- The difference between the SCR (QIV-aQIV) for the B strain (Victoria lineage).

1b. A superior immune response of aQIV compared to QIV will be assessed for the endpoints of HI (or MN) GMT for 2 of the 4 strains included in the vaccines as follows:

- The GMT ratio (QIV/aQIV) for the A/H1N1 strain
- The GMT ratio (QIV/aQIV) for the A/H3N2 strain
- The GMT ratio (QIV/aQIV) for the B strain (Yamagata lineage)
- The GMT ratio (QIV/aQIV) for the B strain (Victoria lineage)

Statistical Hypotheses

Noninferiority of aQIV to QIV (Objective 1a)

To demonstrate that vaccination with aQIV elicits an immune response that is not inferior to that of QIV containing the same virus strains among adults 50-64 years of age, aQIV will be considered to be noninferior to QIV if, for each of the four strains, the following statistical criteria are met:

- The UL of the two-sided 95% CI for the ratio of the Day 22 GMTs (GMTr) does not exceed 1.5 The GMTr will be calculated by GMT_{QIV}/GMT_{aQIV}
- The UL of the two-sided 95% CI for the difference between the SCRs does not exceed 10%. The difference in SCR will be calculated by $SCR_{QIV} - SCR_{aQIV}$

The statistical hypotheses to be tested for the primary immunogenicity objective 1a correspond to:

H_{0k}: $GMTr_k > 1.5$, for any strain k (k=1,2,3,4) at Day 22

H_{ak}: $GMTr_k \leq 1.5$, for all strains at Day 22

and

$H_{0(k+4)}$: $D_k > 10\%$, for any strain k (k=1,2,3,4) at Day 22

$H_{a(k+4)}$: $D_k \leq 10\%$, for all strains at Day 22

where D_k (k=1,2,3,4) is the 4 strain-specific Day 22 SCRs differences ($\boldsymbol{\pi}_{QIV,k}$-$\boldsymbol{\pi}_{aQIV,k}$), namely,

- D_1=$\boldsymbol{\pi}_{QIV,1}$ – $\boldsymbol{\pi}_{aQIV,1}$ for A/H1N1 strain at Day 22
- D_2=$\boldsymbol{\pi}_{QIV,2}$ – $\boldsymbol{\pi}_{aQIV,2}$ for A/H3N2 strain at Day 22
- D_3= $\boldsymbol{\pi}_{QIV,3}$ – $\boldsymbol{\pi}_{aQIV,3}$ for B/Victoria strain at Day 22
- D_4= $\boldsymbol{\pi}_{QIV,4}$ – $\boldsymbol{\pi}_{aQIV,4}$ for B/Yamagata strain at Day 22

where $\boldsymbol{\pi}_{QIV,k}$, $\boldsymbol{\pi}_{aQIV,k}$ (k=1,2,3,4) denotes the seroconversion rates for the four strains in QIV and aQIV respectively.

and $GMTr_k$ (k=1,2,3,4) is any of the 4 strain-specific Day 22 GMT ratios, namely,

- $GMTr_1$=GMT_{QIV}/GMT_{aQIV} for A/H1N1 strain
- $GMTr_2$=GMT_{QIV}/GMT_{aQIV} for A/H3N2 strain
- $GMTr_3$=GMT_{QIV}/GMT_{aQIV} for B/Victoria strain
- $GMTr_4$=GMT_{QIV}/GMT_{aQIV} for B/Yamagata strain.

Translating into $\log_{10}$ transformed GMT titer values, the hypothesis for GMT ratios comparisons become

H_{0k}: $\mu_{QIV,k} - \mu_{aQIV,k} > \log_{10}(1.5)$ for any strain k (k=1,2,3,4) at Day 22

H_{ak}: $\mu_{QIV,k} - \mu_{aQIV,k} \leq \log_{10}(1.5)$, for all strains at Day 22.

Where $\mu_{QIV,k}$ and $\mu_{aQIV,k}$ denote the mean of the $\log_{10}$ transformed QIV and aQIV HI titer of the kth strain.

According to the stepwise procedure the following superiority test(s) are only conducted if all above non-inferiority hypotheses can be rejected.

Superiority of aQIV to QIV (Objective 1b)

To demonstrate that aQIV induces a superior immune response compared to QIV in subjects 50-64 years of age as measured by HI GMTs at 3 weeks after vaccination for at least 2 of the 4 vaccine strains. aQIV will be considered to be superior to QIV if the following statistical criteria are met:

- The UL of the two-sided 95% CI for inter-group GMT ratio (QIV/aQIV) <1.0 for at least 2 of the 4 vaccine strains.

The statistical hypotheses to be tested for the primary immunogenicity objective 1b correspond to:

H_{0k}: $GMTr_k \geq 1$, for at least 3 of the 4 vaccine strains at Day 22

H_{ak}: $GMTr_k < 1$, for at least 2 of the 4 vaccine strains at Day 22

where $GMTr_k$ (k=1,2,3,4) are defined as above.

Translating into $\log_{10}$ transformed GMT titer values, the hypothesis for GMT ratios comparisons become

H_{0k}: $\mu_{QIV,k} - \mu_{aQIV,k} \geq 0$, for at least 3 out of 4 vaccine strains (k=1,2,3,4) at Day 22

H_{ak}: $\mu_{QIV,k} - \mu_{aQIV,k} < 0$, for at least 2 of the 4 vaccine strains at Day 22.

Statistical Models:

Let $\log_{10}(Y_{ijk})$ denote the $\log_{10}$-transformed HI titer Y_{ijk}. $\text{Log}_{10}(Y_{ijk})$ are identical, independent normal distributed random variables, where i denotes the vaccine group aQIV or QIV; j=1,..,n denotes subject j, k=1, 2, 3,4 denotes the four vaccine strains.

$\log_{10}(Y_{ijk}) \sim N(\mu_{ik}, \sigma_{ik}^2)$ with μ_{ik}, and σ_{ik}^2 unknown. μ_{ik} represents mean of the $\log_{10}$-transformed HI titer level in QIV, aQIV of strain k.

X_{ijk}, i= aQIV, QIV, j=1,..,n, k=1, 2, 3,4 independent Bernoulli distributed random variables: $X_{ijk} \sim B(1, \pi_{ik})$, where π_{ik} represents the probability for seroconversion in aQIV, QIV for strain k.

Statistical tests for GMT ratios

HI antibody titers will be $\log_{10}$ transformed and modeled using analysis of covariance with a qualitative factor for vaccine group (η_{ik}, i = aQIV, QIV), age stratum (α_{jk},j=1,2), history of influenza vaccination within the 3 previous seasons (yes/no) (γ_{lk}, l=1,2), sex (ξ_{mk}), and a common slope (β) representing the impact of the $\log_{10}$- prevaccination antibody titer τ_{0ik}, for subject n,

$$\mu_{ijlmkn} = \lambda_k + \eta_{ik} + \alpha_{jk} + \gamma_{lk} + \xi_{mk} + \beta\tau_{0ik} + \varepsilon_{ijlmkn} \qquad \text{(k=1, 2, 3, 4 strains)}$$

where $\varepsilon_{ijlmkn} \sim$i.i.d $N(0, \sigma_k^2)$. The statistical tests for noninferiority (1a) (H_0: $\mu_{QIV,k} - \mu_{aQIV,k} > \log_{10}(1.5)$, and for superiority(1b) (H_0: $\mu_{QIV,k} - \mu_{aQIV,k} \geq 0$), and translating into adjusted ratios of geometric means and pertaining 2-sided CIs will be calculated based on these models.

Summary statistics will be completed by providing minimum, maximum and median titers for each vaccine group.

Analysis of binary endpoints

Statistical tests for seroconversions

The number and proportion of subjects achieving the binary endpoints (percentage of subjects with seroconversion) will be summarized by assessment (Day 22) and vaccine group, overall.

These summaries will be reported together with the associated two-sided 95% CIs for the proportion according to Clopper-Pearson.

The binary endpoints (percentage of subjects with seroconversion) will be compared between vaccine groups by the differences of proportions with two-sided 95% CI using the Miettinen and Nurminen method (Miettinen and Nurminen 1985).

Additional supportive analyses may be done using generalized linear models with a qualitative factor for vaccine group, age stratum, history of any influenza vaccination within the 3 previous seasons (yes/no), and sex. Adjusted differences and pertaining 2-sided 95% CI between vaccine groups will be calculated based on the model.

11.3 Secondary Objectives Analysis

Superiority of aQIV vs QIV (higher threshold) (Objective 2a)

To demonstrate that aQIV induces a superior immune response *(higher threshold)* compared to QIV in subjects 50-64 years of age as measured by HI GMT for at least one of 4 vaccine strains at 3 weeks after vaccination. aQIV will be considered to be superior to QIV if, for at least one vaccine strain at Day 22, the following statistical criteria are met:

- *Superior immune response will be demonstrated if the UL of the two-sided 98.73% CI for inter-group GMT ratio (QIV/aQIV) <0.67 for one or more vaccine strains.*

The statistical hypotheses to be tested for the secondary immunogenicity 2a correspond to:

- H_0: $GMTr_i \geq 0.67$, for all four vaccine strains at Day 22
- H_a: $GMTr_i < 0.67$, for one or more vaccine strains at Day 22

where $GMTr_i$ is the Day 22 GMT ratio of GMT_{QIV}/GMT_{aQIV} for one of the four vaccine strains.

Persistence of immune response of aQIV compared to QIV (Objective 2b)

To demonstrate greater persistence of the immune response for at least one vaccine strain at 6 months after vaccination with aQIV compared with QIV as measured by HI assay in subjects 50-64 years of age. aQIV will be considered to be superior to QIV if, for at least one vaccine strain at Day181, the following statistical criteria are met:

- *Superior immune response will be demonstrated if the UL of the two-sided 98.73% CI for inter-group GMT ratio (QIV/aQIV) <1 for one or more vaccine strains.*

The statistical hypotheses to be tested for the secondary immunogenicity 2b correspond to:

H_0: $GMTr_i \geq 1$, for all four vaccine strains at Day 181

H_a: $GMTr_i < 1$, for one or more vaccine strains at Day 181

where $GMTr_i$ is 6-month GMT ratio of GMT_{QIV}/GMT_{aQIV} for any of the four vaccine strains.

Secondary objective analysis 2a, 2b will be carried out using the same model for GMT ratio analysis as specified for the primary objective analysis at Day 22. Endpoints at Day 22, and Day 181 for objective 2a and 2b will be tested sequentially, each at 0.0127% two-sided significance level.

To evaluate the immunogenicity of aQIV compared with QIV as measured by HI in subjects 50-64 years of age (Objective 2c)

Secondary immunogenicity endpoints 2c include the measures of immunogenicity of aQIV and QIV as determined by the HI assay against homologous strains at Day 1, Day 22, and Day 181 (unless indicated otherwise), include the following:

- GMT of HI antibodies on Day 1, Day 22, and Day 181
- Geometric mean fold increase (GMFI): The geometric mean of the fold increase of post- vaccination HI titer over the pre-vaccination HI titer (Day 22/Day 1, Day 181/Day 1)
- The percentage of subjects with a HI titer ≥1:40 at Day 1, Day 22, and Day 181
- SCR: the percentage of subjects with either a pre-vaccination HI titer < 1:10 and a postvaccination HI titer ≥ 1:40 or a pre-vaccination titer ≥ 1:10 and a ≥ 4-fold increase in postvaccination titer on Day 22, and Day 181

Unadjusted Estimates for GMTs, GMFIs, and pertaining 2-sided 95% CIs will be calculated assuming a log-normal distribution of the titers and will be completed by providing minimum, maximum, and median titers for each vaccine group. Binary data (i.e., percentages of subjects with seroconversion and percentage of subjects with a HI titer ≥1:40) will be summarized for each group using crude estimates and will be reported together with 2-sided 95% CIs calculated according to Clopper's and Pearson's method (Clopper and Pearson 1934).

The immune response profiles and subgroup analyses by age cohort, previous vaccination history for the A/H1N1, A/H3N2, B/Yamagata, and B/Victoria strains in the aQIV and QIV groups will be graphically displayed by visit using reverse cumulative distribution (RCD) curves. The RCD curves display titer levels (x-axis) by the percentage of subjects (y-axis) having a titer value greater than or equal to the value on the x-axis.

11.4 Sequential Testing and Significance Levels

As described in section 8.4, the following test procedure will be used to keep the FWER at 2-sided 5% for primary and secondary hypotheses:

- Test hypothesis 1a) (noninferiority) and hypothesis 1b) (superiority) sequentially at 2-sided level of 5%.
- Only after the goal of hypothesis 1a) is achieved hypotheses 1b) will be tested at α=0.05 level.

- Only after the primary goals 1a) and 1b) are achieved, the secondary hypotheses will be tested sequentially at α=0.05 level.
- For secondary objectives, if both primary objectives are achieved, then 2a will be tested at alpha-0.01274 level (two-sided) for each strain.
- If hypothesis 2a is successful, then hypothesis 2b will be tested at alpha=0.01274 for each strain at Day 181.

The study is considered successful if the primary objective 1a (non-inferiority) is achieved.

11.5 Exploratory Objectives Analysis

Exploratory objective analysis - to evaluate persistence of the immune response at 9 months after vaccination with aQIV compared with QIV as measured by HI in subjects 50-64 years of age - will be carried out in a similar fashion as for secondary endpoint 2b and 2c after receiving Day 1 retest and Day 271 sera sample testing results. All exploratory analysis results will be presented with 95% CI when applicable. The analysis and result will be included in the CSR addendum. If there is insufficient Day 1 serum sample for retesting, the Day 1 retest data will be considered missing: the original Day 1 result will not be imputed in the exploratory analysis of persistence at Day 271.

Exploratory objective analysis - to further evaluate the immunogenicity of aQIV compared with QIV in subjects 50-64 years of age, with alternative assays, if sera permit - will be carried out in a similar fashion as secondary endpoint 2c.

Exploratory immunogenicity endpoints that may be assessed in the study include the measures of immunogenicity of aQIV and QIV as determined by the HI, the MN or any alternative assay against homologous or heterologous strains at Day 1, Day 22, Day 181, and Day 271 (depending on availability of adequate sera and on assay availability).

Exploratory analysis objective might be specified in an SAP addendum if performed.

12. EFFICACY ANALYSIS

Not applicable

13. SAFETY ANALYSIS

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

- Vaccine exposure
- Solicited local and systemic AEs and other indicators of reactogenicity
- Unsolicited AEs
- Serious AEs, AEs leading to withdrawal, and AESIs

13.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects vaccinated will be summarized overall, by vaccine group and by age group. Data will be tabulated for the All-Exposed Set.

13.2 Solicited Local and Systemic Adverse Events

Solicited AEs are reported daily from Day 1 up to and including Day 7 post-vaccination. If a solicited local or systemic AE continues beyond day 7 after vaccination, recording in the Subject eDiary will be continued until the event(s) resolved or for a maximum of 14 days after vaccination. If the reaction continues to be present on Day 14, the event and follow-up is to be captured as an unsolicited AE in the eCRF. A solicited AE will be defined as “present” or at least mild on any of the recorded days to be counted. This will be summarized as total and for local, systemic and the other category (use of analgesics/antipyretics) separately. The same tables will be created at each time point.

Frequencies and percentages of subjects reporting an AE will be presented overall and for each maximum symptom severity and by vaccine group. Post-vaccination solicited AEs reported from Day 1 to Day 7 will also be summarized for the intervals Day 1-3 and Day 4-7 overall and for each maximal severity and by vaccine group.

Each solicited AE is to be assessed for 7 days following the vaccination according to a defined severity grading scale; see specifics of the solicited event and grading system below in Table 5.

Table 5 Severity Grading for Solicited Local and Systemic Adverse Events

Solicited Local Adverse Event	**Grade 1/Mild**	**Grade 2/Moderate**	**Grade 3/Severe**
Injection site pain	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Erythema	25-50 mm	51-100 mm	>100 mm
Induration	25-50 mm	51-100 mm	>100 mm
Ecchymosis	25-50 mm	51-100 mm	>100 mm
Solicited Systemic Adverse Event	**Grade 1/Mild**	**Grade 2/Moderate**	**Grade 3/Severe**
Loss of appetite	Eating less than usual with no effect on normal activity	Eating less than usual /interfered with normal activity	Not eating at all
Nausea	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Fatigue	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Myalgia	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Arthralgia	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Headache	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Chills	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Vomiting	1-2 times per 24 hours	3-5 times per 24 hours	6 or more times per 24 hours or requires intravenous hydration
Diarrhea	2-3 loose stools per 24 hours	4-5 loose stools per 24 hours	6 or more loose stools per 24 hours or requires intravenous hydration
Fever	38.0-38.4°C	38.5-38.9°C	≥39.0°C

Note: presence of an event on a day is defined as mild, moderate, or severe; absence is defined as none. For Ecchymosis, Erythema and Induration: grading will be derived from the actual measurements in mm; Fever will be derived from the actual measured body temperature.

The use of analgesics/antipyretics will be captured as “absent” or “present” separately by reason “for treatment” or “for prevention”.

The analyses will encompass various summaries of the data by vaccination:

1. Overall summary of subjects with solicited AEs
2. Solicited local AEs, maximum event severity by event and interval
3. Solicited systemic AEs, maximum event severity by event and interval

4. Number of days of solicited AEs, including ongoing AE after Day 7
5. Daily reports of subjects with solicited AEs
6. Day of first onset of solicited AEs
7. Solicited AEs ongoing after Day 7
8. Distribution of maximum body temperature
9. Other use of analgesics/antipyretics

All tables are run by vaccine group.

1. Overall summary of subjects with solicited adverse events.

Any solicited AE presence is defined as at least one day recorded a presence of a local or a systemic AE. No solicited AE is defined as for all days 'No' for all pre-defined solicited AEs. Same convention is used considering local and systemic events separate.

The use of analgesics/antipyretics will be considered in this summary as a separate category under "other", however will be considered for summaries showing "any solicited AE".

2. Solicited local adverse events, maximum event severity by event and time interval

The maximum event severity will be defined if there is at least one plausible non- missing observation (excluding 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 local AE, followed by a summary across subjects.

The time intervals will be Day 1 to Day 7, Day 1 to Day 3 and Day 4 to Day 7. A summary tables will be created with the frequency for all grade of AEs.

3. Solicited systemic adverse events, maximum event severity by event and interval

The analysis on the maximum severity of the systemic AEs will be done along the same methods as for the local solicited AEs.

4. Number of days with solicited adverse events

The number of days with the AE is defined irrespective of severity. If a solicited AE symptom occurs more than 7 days, it will be counted into the ">7 days" category.

The frequency distribution of the number of days will be provided in a summary table by AE.

5. Daily reports of solicited adverse events

For each day, only subjects with at least one plausible observation (i.e., any non-missing values but excluding implausible values) for the solicited AE will be considered. Data collected will be

summarized (frequencies and percentages of subjects) by vaccine group, solicited AE and time point.

6. Day of first onset of solicited adverse events

The day of first onset is defined, for each subject, for each solicited AE, as the time point at which the respective solicited AE first occurred. The summary will provide the frequencies and percentages of subjects with first onset of each solicited AE by vaccine group and by each time point (i.e., by day).

7. Solicited adverse events ongoing after Day 7

For each of the solicited AEs, the number of subjects that reported the event ongoing after Day 7 (AE present prior or on Day 7 and beyond) will be summarized. Severity grading of solicited AEs that are reported from Day 8 up to Day 14 will be reported in listings only.

8. Distribution of maximum body temperature

Body temperature will be summarized by 0.5 °C increments from 36.0 °C up to ≥40 °C by frequency tables.

The following conversion rule is used for the conversion of temperature to °C, and the result is rounded to 1 decimal:

Temperature in °Celsius = (Temperature in °Fahrenheit - 32) *5/9.

9. Use of analgesics/antipyretics

The use of antipyretics and analgesics will be summarized by type of use (prophylactic versus treatment) as the number and percentage of subjects reporting at least one day of use during Day 1-7.

13.2.1 Safety Completeness

Analysis Solicited Adverse Events

The safety completeness analysis on solicited AEs aims to identify subjects who completed the electronic diaries, irrespective of severity. The analysis will show the number of subjects with results by solicited AE and day.

Three summaries will be produced:

1. The frequencies of subjects who provided data on the electronic diary cards by vaccine group.
2. For each solicited AE – including analgesic use, the frequencies of subjects with data will be presented by vaccine group and day.

3. For each solicited AE – including analgesic use, frequency of the number of days with data on the eDiary by vaccine group.

For the corresponding percentages, the denominator will be the respective number of subjects vaccinated (All-Exposed Set – analyzed as treated).

13.3 Unsolicited Adverse Events

This analysis applies to all unsolicited AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in the AE eCRF, with a start date on (but with onset after vaccination) or after the date of vaccination.

The original verbatim terms used by investigators to identify AEs in the eCRFs will be mapped to PTs using the MedDRA dictionary.

The AEs will then be grouped by MedDRA PTs into frequency tables according to SOC. All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine, will be summarized according to SOC and the PT within SOC. These summaries will be presented by the vaccine group and by interval of study observation (Day 1-Day 22, Day 23-EOS, Day 1-EOS). When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine will be counted.

The assignment to time intervals will be done by day of onset.

The AE summaries will be presented by SOC and PT and different periods of onset depending on the category of events.

- Unsolicited AEs during the 30 minutes post vaccination
- SAEs
- Unsolicited AEs that are possibly or probably related to vaccine
- AESIs
- AEs leading to withdrawal
- Deaths

All tables will be produced by treatment.

An overview summary of AEs, including frequencies and percentages of any AEs (by mild/moderate/severe grade), related AEs, AEs leading to withdrawal, any SAEs, related SAEs, AESIs and deaths will also be presented.

In addition, summary of subjects with unsolicited non-serious adverse event reported by >5% of subjects and summary of subjects with unsolicited non-serious or solicited adverse event reported

by >5% of subjects in any vaccine group sorted by SOC and PT will be provided for clinicaltrials.gov and EudraCT.eu posting purposes.

Separate data listings of all AEs, SAEs, AESIs, AE leading to withdrawal and AE leading to death will be provided by study treatment, subject and onset date.

13.4 Clinical Safety Laboratory Investigations

Not applicable

13.5 Concomitant Medication

Concomitant medications include all medications (including vaccines) taken by/administered to the subject at and after enrolment and must be documented on the Concomitant Medications eCRF (See protocol section 6.5 for definitions).

Medications (generic drug name) will be coded using the WHODRUG dictionary.

The frequencies and percentages of subjects reporting prior or concomitant medications will be tabulated by vaccine group and overall for All-Enrolled Set.

Summaries for concomitant medications will be presented by ATC level 2 code and preferred medication name, based on the All-Enrolled Set.

14. INTERIM ANALYSIS

There are no planned interim analyses for this study.

15. DATA MONITORING COMMITTEES

Not applicable

16. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

This list of tables, listings and figures are defined and combined with the table shells.

17. REFERENCES

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