STATISTICAL ANALYSIS PLAN

Study Title:	A Phase III, Randomized, Observer-Blind, Controlled, Multicenter Clinical Study to Evaluate the Efficacy, Safety and Immunogenicity of an MF59-Adjuvanted Quadrivalent Influenza Vaccine Compared to Non-influenza Vaccine Comparator in Adults ≥ 65 Years of Age
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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
APAC	Asia Pacific
aQIV	Adjuvanted Quadrivalent Influenza Vaccine
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CPSL	Clinical Program Scientific Lead
CPML	Clinical Program Medical Lead
CRF	Case Report Form
CSR	Clinical Study Report
DMC	Data Monitoring Committee
eTMF	electronic Trial Master File
FAS	Full Analysis Set
FWER	Familywise Error Rate
GCOL	Global Clinical Operations Lead
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
GMTr	Ratio of Geometric Mean Titer
HI	Hemagglutination Inhibition
HR	Hazard Ratio
ICH	International Conference on Harmonization
ID	Identification
ILI	Influenza-Like Illness
IRT	Interactive Response Technology

LQ	Limit of Quantification
MCAR	Missing Completely at Random
MedDRA	Medical Dictionary for Regulatory Activities
ML	Maximum Likelihood
NH	Northern Hemisphere
NOCD	New Onset of Chronic Diseases
PH	Proportional Hazard
PD	Protocol Deviation
PPS	Per Protocol Set
RT-PCR	Reverse Transcription Polymerase Chain Reaction
rVE	Relative Vaccine Efficacy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SH	Southern Hemisphere
SUSAR	Suspected Unexpected Serious Adverse Reactions
TFL	Tables, Figures and Listings
TIV	Trivalent Influenza Vaccine
TOC	Table of Content
VE	Vaccine Efficacy
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

The purpose of this study is to demonstrate the efficacy, safety and immunogenicity of an MF59-adjuvanted inactivated egg-derived quadrivalent influenza vaccine (aQIV) in preventing seasonal influenza in elderly adults. Seasonal influenza is a significant cause of morbidity and mortality, particularly in children and elderly (Neuzil, Mellen et al. 2000); (Mullooly, Bridges et al. 2007); (MMWR,2010). The efficacy of vaccination in preventing influenza in the elderly appears to be lower compared with younger adults (Osterholm, Kelley et al. 2012; Beyer, McElhaney et al. 2013). One potential explanation for the absolute decrease in vaccine efficacy compared with younger adults may be a less robust immune response after influenza vaccination (Goodwin, Viboud et al. 2006; Sasaki, Sullivan et al. 2011).

Therefore, novel approaches are sought to improve the immunogenicity of influenza vaccines in the elderly population. One way to increase immunogenicity of influenza vaccines is through the use of adjuvants, such as the squalene and water emulsion, MF59. FLUAD®, Seqirus' trivalent influenza vaccine combined with MF59, has been licensed for use in Europe since 1997. The administration of FLUAD® to elderly patients results in significantly higher geometric mean Haemagglutination Inhibition (HI) titers and rates of seroconversion in comparison to non-adjuvanted trivalent influenza vaccine (TIV).

This document presents the statistical analysis plan (SAP) for Seqirus UK Ltd., Protocol No. V118_18 V5: A Phase III, Randomized, Observer-Blind, Controlled, Multicenter Clinical Study to Evaluate the Efficacy, Safety and Immunogenicity of an MF59-Adjuvanted Quadrivalent Influenza Vaccine Compared to Non-influenza Vaccine Comparator in Adults ≥ 65 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 based on protocol amendment 1 final version 4.0 dated 24 March 2016 and 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].

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

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 PRIMARY OBJECTIVE AND ENDPOINT

2.1.1 Primary Efficacy Objective

The primary efficacy objective of the study is:

1. To demonstrate absolute vaccine efficacy of aQIV versus non-influenza comparator (Boostrix®) when administered as a single dose to prevent first occurrence of reverse transcription polymerase change reaction (RT-PCR)-confirmed influenza, due to any strain of influenza regardless of antigenic match to the strains selected for the seasonal vaccine, in subjects ≥ 65 years of age.

2.1.2 Primary Efficacy Endpoints

The primary efficacy endpoint is the time of first-occurrence of RT-PCR-confirmed influenza due to A (H1N1 and H3N2) or either B lineage due to any strain of influenza regardless of antigenic match to the strains selected for the seasonal vaccine from 21 through 180 days after vaccination or end of the influenza season, whichever is longer.

2.1.3 Primary Safety Objective

1. To evaluate the safety of aQIV through assessment for local and systemic solicited adverse events through Day 7 in a subset of subjects.
2. To evaluate the rates in each vaccine group of medically-attended adverse events within 30 days after the first occurrence RT-PCR confirmed influenza-like illness (ILI).
3. To evaluate the rates in each vaccine group of unsolicited adverse events for 21 days after vaccination and adverse events leading to withdrawal, serious adverse events (SAEs), adverse events of special interest (AESI), and new onset of chronic diseases (NOCD) for 365 days after vaccination.

2.1.4 Primary Safety Endpoint(s)

Safety objective 2 will be assessed by calculating the percentage of subjects in the solicited safety subset with solicited local and systemic adverse events from Day 1 through Day 7. Solicited local and systemic adverse event data from Day1-7 will be obtained from data collected on the subject diaries. Only subjects randomly selected for participation in the solicited safety subset will be asked to fill out a Subject Diary from Day 1-7.

Safety objectives 3 and 4 will be assessed by comparing the following between cohorts that received aQIV or non-influenza comparator:

- Percentage of subjects with medically-attended adverse events within 30 days after the first occurrence ILI.
- Percentage of subjects with any unsolicited AEs and concomitant medications reported from Day 1 through Day 22.
- Percentage of subjects reporting SAEs, AEs leading to withdrawal from the study, NOCD, AESI and all concomitant medications associated with these events from Day 1 to Day 366.

2.2 SECONDARY OBJECTIVES AND ENDPOINTS

2.2.1 Key Secondary Efficacy Objective

1. To demonstrate absolute vaccine efficacy of aQIV versus non-influenza comparator when administered as a single dose to prevent first occurrence culture-confirmed influenza, due to strains antigenically matched to the strains selected for the seasonal vaccine.

Key Secondary Efficacy Endpoints

Time to first occurrence of culture-confirmed influenza due to any strain of influenza antigenically matched from 21 days to 180 days after vaccination or at the end of influenza season whichever is longer.

2.2.2 Secondary Efficacy Objectives

1. To evaluate absolute vaccine efficacy of aQIV versus non-influenza comparator when administered as a single dose to prevent first occurrence culture-confirmed influenza, due to any strain of influenza regardless of antigenic match to the strains selected for the seasonal vaccine.
2. To evaluate absolute vaccine efficacy of aQIV versus non-influenza comparator when administered as a single dose to prevent first occurrence culture-confirmed influenza, due to strains antigenically unmatched to the strains selected for the seasonal influenza vaccine.
3. To evaluate the absolute efficacy of aQIV versus non-influenza comparator when administered as a single dose to prevent first occurrence RT-PCR-confirmed influenza due to any strain of influenza regardless of antigenic match from 7 days to

180 days after vaccination or at the end of influenza season, whichever is longer (early efficacy).

2.2.3 Secondary Efficacy Endpoints

In addition to the primary efficacy endpoint, additional endpoints for the secondary efficacy objectives are sought, based on antigenic match of culture isolated influenza to the strains of virus contained in the seasonal vaccine.

The secondary efficacy endpoints are the time to first occurrence of culture-confirmed influenza, due to

- Influenza strains regardless of antigenic match (Secondary Efficacy Objectives 2)
- Antigenically unmatched strains of influenza virus (Secondary Efficacy Objective 3).

selected for the seasonal vaccine from Day 21 to Day 180 after vaccination or the end of the influenza season, whichever is longer.

and

- Time to first occurrence of RT-PCR-confirmed influenza due to any strain of influenza regardless of antigenic match from 7 days to 180 days after vaccination or at the end of influenza season, whichever is longer (Secondary Efficacy Objective 4).

2.2.4 Secondary Immunogenicity Objective

1 To evaluate the immunogenicity of aQIV measured by hemagglutination inhibition (HI) titer 21 days after vaccination, against influenza strains homologous to the seasonal vaccine.

2.2.5 Secondary Immunogenicity Endpoints

Immunogenicity Endpoint(s):
The measures of immunogenicity used for Objective 5 as determined by the HI assay against homologous strains at Days 1 and 22 (unless indicated otherwise), include the following:

- Geometric mean HI titer (GMT).
- Geometric mean ratio (GMR) calculated at Day 22/Day 1,
- Percentage of subjects with an HI titer ≥1:40.

- Percentage of subjects achieving seroconversion (defined as: HI titer ≥1:40 for subjects sero-negative at baseline [HI titer <1:10]; or a minimum 4-fold increase in HI titer for subjects sero-positive at baseline [HI titer ≥1:10]) on Day 22.
- Reverse cumulative distributions of HI titers at Day 22.

2.3 EXPLORATORY OBJECTIVES AND ENDPOINTS

2.3.1 Exploratory Immunogenicity Objectives

1 To characterize the immunogenicity of aQIV using other immunological assays (such as microneutralization assay).

2 To explore potential immune correlates of protection based on HI and/or other immunological assays (such as microneutralization assay)

2.3.2 Exploratory Endpoints

The exploratory endpoints determined by the MN assay against homologous strains at Days 1 and 22 (unless indicated otherwise), include the following:

- Geometric mean titer (GMT).
- Geometric mean ratio (GMR) calculated at Day 22/Day 1.
- Percentage of subjects with a four-fold rise in MN antibody titer at Day 22
- Reverse cumulative distributions of MN titers at Day 22.

Additionally, an estimate of the serologic correlate of protection on the basis of HI or other immunological responses including MN, measured three weeks after vaccination against the respective strain.

2.4 Definitions

Two definitions of influenza-like-illness (ILI) will be used in this study. Primary protocol-defined ILI requires at least one of the following respiratory symptoms: sore throat, cough, sputum production, wheezing, or difficulty breathing; concurrently with at least one of the following systemic symptoms: temperature of > 37.2°C/ 99°F, chills, tiredness, headache, or myalgia and is based on that used to demonstrate the efficacy of Fluzone High Dose (DiazGranados, 2014).

The end of the influenza season will be defined as the end of June for Northern Hemisphere (NH) influenza season and end of December for Southern Hemisphere (SH) influenza season. For tropical countries, with no typical NH or SH influenza season, the season is defined by the use of the strains in the vaccine formulation (i.e. strains as recommended for the NH or the SH influenza season) and the timing of vaccination. The investigators will be advised of the date when enrollment should stop in their country or hemisphere.

The second (modified Centers for Disease Control and Prevention [CDC]) definition of ILI will be fever (temperature of > 37.2°C/ 99°F) with cough or sore throat. The respiratory symptoms in both definitions are considered a new onset or exacerbation of a pre-existing condition.

The primary and key secondary efficacy endpoints will be assessed using the primary protocol-defined ILI definition to determine success in the study.

Primary and secondary efficacy objective 1 and secondary objectives 2, 3, and 4 will also be analyzed using both definitions of the first occurrence of ILI.

CSR-reportable Protocol Deviations are defined as important Protocol Deviations related to study inclusion or exclusion criteria, conduct of the study, subject management or subject assessment which can potentially jeopardize the safety or rights of the subjects, or the scientific value of the study. Specifically, CSR-reportable Protocol Deviations are those Protocol Deviations which directly or indirectly have a significant impact on one or more of the following: A) subject's rights, safety, or well-being B) data integrity, i.e., completeness, accuracy, and reliability of safety, efficacy, and immunogenicity outcomes of the clinical study C) regulatory compliance. CSR-reportable PDs will lead to exclusion of the subject from at least one analysis set and are described in Section 10.2 of the CSR.

CSR-non reportable Protocol Deviations are defined as changes or alterations in the conduct of the study which are unlikely to have a significant impact on one or more of the

following: A) subject's rights, safety, or well-being, B) data integrity, i.e., completeness, accuracy, and reliability of safety, efficacy, and immunogenicity outcomes of the clinical study, and C) regulatory compliance. CSR-non-reportable Protocol Deviations do not lead to the exclusion from any analysis set. Additionally, they should not be reported in the CSR; they are reported and followed up in monitoring visit reports.

3. STUDY DESIGN

The study is a phase III, stratified, randomized, observer-blind, non-influenza comparator-controlled, multicenter study to evaluate the efficacy, safety and immunogenicity of an MF59-adjuvanted quadrivalent subunit influenza vaccine compared with a non-influenza comparator vaccine in subjects ≥ 65 years of age.

The study vaccine, aQIV, contains 15 µg of each of the four seasonal influenza strains (A/H1N1, A/H3N2, B [Yamagata and Victoria lineages]) and MF59 adjuvant. The non-influenza comparator (Boostrix®) vaccine will be used to provide a comparative assessment for efficacy, immunogenicity and safety.

Approximately 10,692 subjects ≥ 65 years, 5,346 per vaccine group will be randomized to receive either aQIV or non-influenza comparator in a 1:1 allocation ratio, stratifying according to age (≥ 65 to 74 and ≥ 75 years), study site and comorbidity status (at risk/not at risk). Each subject will have two periods of study participation: Treatment Period (Day 1 to Day 22) and Follow-up Period (Day 23 to Day 366). The final study size is based on the number of laboratory confirmed cases of influenza, so that the ultimate number of subjects enrolled will be determined by the actual attack rates of influenza during the conduct of the study. If the number of influenza cases range between 119 and 238, unblinded interim analyses for efficacy and futility will be performed by an independent Data Monitoring Committee (DMC). Once the number of RT-PCR confirmed influenza cases is ≥ 238 the trial will be stopped and unblinded, and the final analysis will be performed. Stopping rules for futility and efficacy and any additional details regarding the DMC can be found in the DMC Charter.

The study procedures consist of four (4) clinic visits (on Days 1, 22, 181 and 366), and three (3) safety phone calls on Days 15, 91, and 271. Subjects participating in the solicited safety subset will receive an additional diary card reminder phone call on day 3.

Additionally, all subjects will receive, at a minimum, weekly phone calls or messages to assess for primary protocol-defined ILI symptoms during the first active ILI surveillance period, defined as from Day 1 through Day 181 or until the end of the regional seasonal influenza season, whichever is longer. The purpose of the ILI surveillance phone calls/message is to trigger a clinic or home visit to collect a nasopharyngeal swab.

Subjects will also be asked for the duration of the trial (through day 366) to contact the site at the onset of ILI symptoms to schedule a clinic visit for NP swab collection.

After an initial screening, subjects will be vaccinated with a single dose of aQIV or non-influenza comparator on Day 1. Subjects will be observed for at least 30 minutes post-vaccination on Day 1 for any immediate reactions. All subjects will receive a Subject Diary along with instructions to ensure proper completion and assessment on reactogenicity.

During the remaining follow-up period of the study, safety data including adverse events leading to withdrawal, AESI, NOCD and SAE and concomitant medication use related to these events will be captured via safety phone calls or clinic visits throughout the remainder of the trial (up to Day 366).

For further details please refer to section 3.0 of the protocol.

Table 3-1: Time and Events Table – Treatment Period

	Visit Type	Clinic Visit	Reminder Phone Call[b]	Safety Phone Call	Clinic Visit
	Study Day	**1**	**3**	**15**	**22**
	Visit Window (Days)	**N/A**	**+/- 1**	**+/- 3**	**-1 to +4**
	Visit Number	**1**		**2**	**3**
Study Event	**References in the protocol**				
Study Treatment					
Vaccination	Section 5.2	X			
Screening and Safety					
Informed Consent [a]	Section 5.1.1	X [f]			
Medical History	Sections 5.1.2	X [f]			
Physical Exam	Sections 5.1.2 and 5.3.1	X [c, f]			X
Exclusion/Inclusion Criteria	Section 4.0	X [f]			
Randomization	Section 5.1.4	X [f]			
30 Minutes Post Injection Assessment	Section 5.2.1	X			
Subject Diary Dispensed with Training[b]	Section 5.2.1	X			
Subject Diary Reminder Call[b]	Section 5.2.2		X		
Subject Diary Reviewed and Collected[b]	Section 5.3.1				X
Assess all AEs	Sections 7.1.1 and 7.1.3	X		X	X
Assess SAEs	Section 7.1.4	X		X	X

	Visit Type	Clinic Visit	Reminder Phone Call[b]	Safety Phone Call	Clinic Visit
	Study Day	**1**	**3**	**15**	**22**
	Visit Window (Days)	**N/A**	**+/- 1**	**+/- 3**	**-1 to +4**
	Visit Number	**1**		**2**	**3**
Study Event	**References in the protocol**				
Assess for NOCDs, AEs leading to withdrawal, ILI and AESIs	Sections 7.1.4.1 and 7.1.3	X		X	X
Assess Relevant Medications/ Vaccinations	Sections 5.1.2 and 6.5	X		X	X
Efficacy					
ILI Instruction Sheet Dispensed	Section 5.2.1	X			
ILI Booklet Dispensed with Instructions	Section 5.2.1	X			
Message/ Phone Call to Assess ILI	Section 5.3.3	X [d]			
Immunogenicity					
Serology Blood Draw[e]	Section 3.5	X [f]			X

Notes:

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

[b] Only applies to subjects who have been selected for participation in the solicited safety subset.

[c] Includes measurement of height and weight.

[d] Message/ phone call surveillance for primary protocol-defined ILI to be performed on a weekly basis from Study Day 1 through Study Day 181 or end of the influenza season, whichever is longer.

[e] Only applies to subjects selected for participation in the immunogenicity subset

[f] Procedures to be performed prior to vaccination.

Table 3-2: Times and Events Table – Follow-up Period

	Visit Type	**Safety Phone Call**	**Clinic Visit**	**Safety Phone Call**	**Clinic Visit**
	Study Day	**91**	**181**	**271**	**366**
	Visit Window (Days)	**+/- 3**	**+/- 7**	**+/- 3**	**-60 to +30**
	Visit Number	**4**	**5**	**6**	**7**
Study Event	**References in the protocol**				
Safety					
Physical Exam	Sections 5.1.2 and 5.3.1		X		X
Assess SAEs	Section 7.1.4	X	X	X	X
Assess for NOCDs, AEs leading to withdrawal, ILI and AESIs	Sections 7.1.4.1 and 7.1.3	X	X	X	X
Assess Relevant Medications/ Vaccinations	Sections 5.1.2 and 6.5	X	X	X	X
Efficacy					
Message/ Phone Call to Assess ILI	Section 5.3.3	X[a]			
Study Completion Procedures					
Study Termination [b]	Section 5.5				X

Notes:

[a] Message/ phone call surveillance for primary protocol-defined ILI to be performed on a weekly basis from Study Day 1 through Study Day 181 or end of the influenza season, whichever is longer.

[b] Subjects who terminate the study early are recommended to complete certain study-related procedures. See Section 5.5, Study Termination Visit for further details.

Table 3-3: Times and Events Table – ILI Visit Schedule

	Visit Type	**Clinic Visit**	**ILI Follow-up Safety Call**
	ILI Day	**1-4**	**30**
	Visit Window (Days)	**+3**	**+7**
	Visit Number	**n/a**	**n/a**
Study Event	**References in the protocol**		
NP Swab Specimen Collection	Section 5.4	X	
Assess ILI symptoms and Relevant Medications	Section 5.4	X	X
Assess for Medically-Attended Adverse Events	Section 5.4	X	X
Assess Relevant Medications	Section 5.4	X	
Physical Exam	Section 5.4	X	
ILI Booklet Reviewed and Collected	Section 5.4	X	

Notes:
[a] The Clinic Visit should occur as soon as possible within 3 days after the first day of onset of ILI symptoms (Day 1-4), but up to 6 days (window + 3)

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Approximately 10,620 subjects ≥ 65 years will be randomized to receive either aQIV or non-influenza comparator in a 1:1 allocation ratio, stratifying according to age (≥ 65 to 74 and ≥ 75 years), study site and comorbidity status (at risk/not at risk). Each randomized subject will be automatically assigned a unique Subject identification (ID) and Pack ID indicating the assigned treatment. The Subject ID will be the subject's unique identification number for all CRFs and associated study documentation that will be used for duration of the study.

The Subject ID consists of a 7-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 Interactive Response Technology (IRT) service provider and approved by Seqirus or delegate.

One season in which at least 2800 subjects are anticipated to be enrolled will be selected for the collection of immunogenicity samples. Samples from first 2800 subjects participating in the selected season (1,400 from each treatment arm) will be collected at Day 1 (baseline) and Day 22 (Visit 3). In order to maintain the study blind equal numbers of subjects from both treatment groups will be asked to provide blood specimens. After all of the specimens have been obtained, a subset of subjects with both samples collected will be randomly selected on a 4:1 ratio (1,362 aQIV; 340 Boostrix) for the purpose of completing the analysis of the immunogenicity objectives. The selected subjects will constitute the Immunogenicity Set. The samples from the selected subjects will be randomized into batches for HI testing in such a way that both samples from one subject are contained within one batch, but the sequence of samples is random. See section 4.2 for discussion of maintaining the study blind with respect to the selected Immunogenicity Set subjects. Serum samples from subjects with RT-PCR confirmed influenza, along with a subset of subjects who did not have RT-PCR confirmed influenza, may be tested to estimate the correlate of protection. The serologic component of the correlate of protection will be measured using either a validated haemagglutination inhibition (HI) and/or other immunological assays (such as microneutralization).

Twenty percent of subjects (every 5th subject enrolled from each treatment arm) will be designated during randomization, across all sites and countries, for Solicited Safety Set, see section 7.5.

For further details please refer to Section 5.1.4 of the protocol.

4.1.1 Definition of Randomization/Vaccination Errors

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

- Subject was vaccinated with a vaccine different from the one assigned at randomization
- Subject was vaccinated with the correct vaccine but received a lower volume than planned
- Subject randomized in the wrong stratum.

Differentiation between incorrect stratification and mis-dosing:

A mis-dosing is defined as when a subject receives a vaccine other than the one assigned by randomization. Mis-dosing is a Clinical Study Report (CSR)-reportable Protocol Deviation (PD) and should be analyzed as randomized in Full Analysis Set (FAS), excluded from Per Protocol Set (PPS), analyzed as treated for Safety.

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

- Major stratification errors: 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: not having impact on dose/schedule administered. These will *not* be considered as CSR reportable PD.

Stratification error	FAS	PPS	Safety Set
Minor	Analyze as corrected	Analyze as corrected	Analyze as corrected
Major	Analyze as originally stratified	Exclude from analysis	Analyze as corrected

Please see section 7 of this document for a complete guidance on how vaccination errors are handled in the statistical analysis.

4.1.2 Forced Randomization

Forced randomization will not be utilized in this trial.

4.2 Blinding and Unblinding

Selection of the subject for inclusion in Immunogenicity Set (1,362 from aQIV; 340 from Boostrix arm) among approximately 2,800 subjects for which blood samples will be collected will be performed by the unblinded study statistician. The handling of all samples selected will be performed by unblinded team members. The list of subjects selected for the Immunogenicity Set will be considered as unblinded information and thus kept in the unblinded study area until the database is locked and study is officially unblinded.

Unblinded teams will be used for preparation of the reports for the DMC and the interim analysis, see sections 14 and 15. For details please refer to section 3.3 of the protocol.

If a subject is unblinded during the study, it is to be documented as a as CSR-reportable PD, except for subjects unblinded by Pharmacovigilance due to suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately.

The unblinded subject(s) are excluded from the PPS. Unblinded subjects will be included in the FAS and safety sets.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

Efficacy objectives:

Sample size and power consideration for the aQIV efficacy objective is based on criteria for demonstrating efficacy in placebo controlled studies provided in CBER guidance, specifically that the study should be powered to assess the lower bound of the two-sided 95% CI of vaccine effectiveness, anticipated to be substantially above zero. This study is planned using a group sequential design. The power of Cox Regression which is planned to be used for analysis is dependent on the overall number of influenza cases, so the sample sizes on number of subjects described in this section is provided more for operational reasons. Additional information on the planning of number of subjects that may need to be enrolled if it is decided to continue enrolment after interim analyses is described in Section 8.6.

For the primary efficacy objective: with 1-sided alpha of 2.5% and assuming an attack rate of influenza of 3.5% among subjects enrolled in the non-influenza comparator group and 1.4% among the aQIV group, i.e. 60% assumed vaccine efficacy for aQIV, 238 events (i.e. 4,860 subjects per group under the assumed ERs) are needed with 86.5% power to show the absolute efficacy of aQIV versus non-influenza comparator, in preventing first occurrence RT-PCR-confirmed influenza A and/or B, due to any strain of influenza regardless of antigenic match to the strains in aQIV, in subjects ≥ 65 years of age, using a margin of 40% for lower bound of the CI for absolute vaccine efficacy.

For the first secondary efficacy objective: with a group vaccine efficacy of 65% but expecting conservatively reduced event rates of influenza cases due to matched strains of 2.5% among subjects in the non-influenza comparator group and 0.9% among the aQIV group then 144 events has estimated power approximately 88.5% to show the absolute efficacy of aQIV versus non-influenza comparator, using a margin of 40% for lower bound of the CI.

Assuming a drop-out rate of 10%, approximately 10,692 subjects ≥ 65 years, 5,346 per vaccine group will be randomized to receive either aQIV or non-influenza comparator (Boostrix®) in a 1:1 allocation ratio, stratifying by age (≥ 65 to 74 and ≥ 75 years of age), study site and by low vs high risk of influenza complications (score <50 vs ≥ 50).

Immunogenicity objective:

Anti-HA antibody will be measured using a validated haemagglutination inhibition (HI) assay with egg-derived HA antigens from homologous strains of the virus.

The immunogenicity objective is considered as secondary and so no alpha adjustment for multiplicity will be done for the analysis therefore a significance level of 5% (2-sided) or 2.5% (1-sided) was used for sample size calculation. One season in which at least 2800 subjects are anticipated to be enrolled will be selected for the collection of immunogenicity samples. Samples from approximately 2800 subjects participating in the immunogenicity season will be obtained. In order to maintain the study blind blood samples from an equal number of subjects participating in both treatment groups will be collected at Day 1 (baseline) and Day 22 (visit 3).

After all of the specimens have been obtained a subset of samples from those collected will be randomly selected on a 4:1 ratio for analysis. In total approximately 1,702 samples are planned to be included the analysis (1,362 aQIV; 340 Boostrix). The details and the guidelines regarding the selection process of these samples can be found in the SAP.

Following assumptions from the V70_27 study in elderly adult subjects were used: specifically after aTIV vaccination, seroconversion rates, H1N1: 77%, H3N2: 74% and B: 47%, and percentages of subjects with a HI titer ≥ 40, H1N1: 91%, H3N2: 99%, B: 64%.

With samples from at least 1,362 enrolled subjects (1,226 evaluable subjects assuming a rate of 10% for missing data in the aQIV group) there will be enough power to evaluate achievement of CBER criteria with all four strains contained in the vaccine (see Table 5-1).

Table 5-1: Power to assess CBER criteria for subjects ≥ 65 years of age

Strain	π_i (proportion in aQIV, based on data of V70_27)	π_0 (CBER threshold)	Sample size for aQIV	Marginal Power
Proportion of subjects with Seroconversion				
H1N1	77%	30%	1226	99%
H3N2	74%	30%	1226	99%
B strains	47%	30%	1226	99%
Proportion of subjects with HI titer ≥ 1:40				
H1N1	91%	60%	1226	99%
H3N2	99%	60%	1226	99%
B strains	64%	60%	1226	82%

Safety objectives:

With 4,860 evaluable subjects in each treatment group, the probability of detecting a rare safety event which occurs at 0.001 (1/1000) rate will be 99%. With 1,000 evaluable subjects in each treatment group of the Solicited Safety Set, the probability of detecting an event which occurs at a rate of 0.002 will be ≥86%, assuming a drop-out/missing data rate of up to 5% .

Sample size calculations were performed using nQuery 7.0 and SAS 9.2.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

CSR reportable PD are defined in accordance with International Conference on Harmonization (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 and a pre-specified list of protocol deviations will be included in the Protocol Deviation Specification document included in appendix A of this Statistical Analysis Plan.

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

- 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

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

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

Prior to unblinding, designated study staff will develop a memo that describes the PDs that led to exclusions from analysis sets. This memo will be signed off by at least the Biostatistician and the Clinical Program Medical or Scientific Lead (CPML/CPSL) and will be included in the trial master file.

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

6.2 Determination of Protocol Deviations

A set of listings will be provided to the Clinical Program Medical or Scientific Lead and the Global Clinical Operations Lead for review on an ongoing basis during the study.

The listings will be programmed following the Protocol Deviation Specification list presented in table in section 7.7.

The review will also include protocol deviations captured reported in monitoring reports.

After the review, the Clinical Program Medical or Scientific Lead and the Global Clinical Operation Lead will provide the Biostatistician with:

- An assessment of CSR reportable PDs based on blinded clinical data review.
- An assessment of subjects without PDs (e.g., premature withdrawals due to adverse event, withdrawal of consent) who should be excluded from an analysis set.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events will be directly measured by the subject and will not be subject to a reconciliation process, even if they are biologically implausible. Therefore these implausible measurements will be removed from the analysis but included in listings. Implausible measurements are summarized in the table below:

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 33°C or ≥ 42°C
Erythema	Measurements: ≥ 900 mm or < 0 mm
Induration	Measurements: ≥ 500 mm or < 0 mm
Ecchymosis	Measurements: ≥ 500 mm or < 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, receive a subject ID, regardless of the subject's randomization and vaccination status in the trial.

Demography and baseline characteristics tables will be produced on the All Enrolled Set, Full Analysis set, Per Protocol Set and Overall Safety Set.

7.2 Exposed Set

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

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

Full Analysis Set (FAS)

All subjects in the All Enrolled Set who are randomized and received a study vaccination (demography)

Full Analysis Set Efficacy:

All subjects in the All Enrolled Set who are randomized and received a study vaccination, are under observation for at least 21days post-vaccination and provide efficacy data.

Full Analysis Set Immunogenicity:

A randomly selected sample of 1,702 subjects including subjects from both treatment arms (1,362 Aqiv; 340 Boostrix), in the All Enrolled Set who are randomized, received a study vaccination, and provide immunogenicity data at Days 1 and 22, see Section 4.1.

See section 4.1 for details on how to handle subjects randomized in the wrong stratum.

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

7.4 Per Protocol Set (PPS)

All subjects in the FAS Efficacy / Immunogenicity who:

- Correctly received the vaccine (i.e., receive the vaccine to which the subjects is randomized to receive).

- Had no CSR-reportable PD leading to exclusion (see section 6.2) as defined prior to unblinding or analysis.
- Are not excluded due to other reasons defined prior to unblinding (see section 6.2)

In case of vaccination error, the subject is excluded from the PPS. If a subject receives a vaccine, labelled for another subject but the same as the one the subject was randomized to, the subject will not be removed from the PPS.

If a subject received the correct study vaccine (dose, batch) but from another ongoing study at the site, then the subject should be excluded from the PPS.

See section 4.1 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.

Primary vaccine efficacy analyses will be based on the Efficacy/immunogenicity FAS, and repeated, as a sensitivity analysis, on the Efficacy/immunogenicity PPS.

7.5 Safety Set

Solicited Safety Set

All designated subjects (i.e., a sample of 1053 subject from each treatment arm) in the Exposed Set, with solicited safety assessments beyond 30 minutes (e.g., use—or lack of use—of analgesics/antipyretics).

Unsolicited Safety Set

All subjects in the Exposed Set with unsolicited adverse event data.

A record of safety assessment performed at a specific time point, with confirmation of no AE, is considered as adverse event data hence subject is to be included.

Overall Safety Set

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

Subjects providing only 30 minute post-vaccination safety data will be reported separately in a 30 minute post-vaccination safety analysis and excluded from all other 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).

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

In case a subject is randomized in the wrong stratum:

- Subject will be reassigned to the correct stratum for solicited and unsolicited 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

None

7.7 Overview of Analysis Sets by PD

See Protocol Deviation Specification document included in Appendix A.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The estimate of the hazard ratio and the respective estimate for relative vaccine efficacy (rVE) and pertaining 2-sided 95% confidence intervals will be calculated based on the Cox proportional hazards model with vaccine group as the main effect. Randomization stratifying factors (ie, by age (cohorts 65 to 74 years and 75 years and above), study site, and risk for complications from influenza (assessment score < 50 or ≥50) will be modelled as random effects as suggested by Kahan and Morris (2012). In case of computational difficulties due to small number of events (for example, in a subgroup analysis) an unadjusted hazard ratio will be estimated and reported. Small sites (<30 subjects) will be grouped within country.

The log-transformed antibody titer at day 1, Day 22, Day 181 and Day 366 will be analyzed, in key analyses, using an Analysis of Covariance (ANCOVA) model which includes the site effect (with small sites grouped within country), an effect defined by the log-transformed pre-vaccination antibody titer, age stratification and comorbidity (<50, ≥50) . 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.

8.2 Handling of Dropouts, Missing Data

First-line analyses will be without missing values.

To minimize the effect of dropouts and missing data the study period will be divided into time intervals for statistical analysis of safety, immunogenicity, and efficacy.

8.2.1 Safety Data

For unsolicited adverse events, the entire study period will be divided into the following intervals: Day 1 – Day 22; Day 23 – Day 181; Day 182 – Day 366, and Day 1 – Day 366.

For solicited adverse events, the solicited study period 30 min – Day 7 will be divided into: 30 min, 6h – Day 3, Day 4 – Day 7, and overall interval: 6h – Day 7.

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

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 objectives will be analyzed using 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.

8.2.3 Efficacy Data

Efficacy data will be analyzed using both the FAS (Efficacy) and the PPS, 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 (e.g., ILI swabs collected without any RT-PCR assessment record), then the analyses 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 with vaccine group as a categorical variable and a newly created variable describing the missing information as dependent variable (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 Multicenter Studies

Vaccine group effects will be investigated first using a linear model which allows for center differences, but does not consider vaccine-by-center interaction, i.e., the model only considers effects for center and vaccine. A center effect will be included in all analyses of the primary and secondary objective*s*.

To achieve a sufficient number of subjects for the statistical analysis, centers will be grouped according to a higher level factor, country. This factor will be used in the statistical models instead of center.

The analysis of vaccine group effect split by country will be provided in Section 14 of the CSR for the primary objectives. If significant vaccine effects are found in the trial, there will be an exploration of the heterogeneity of vaccine group effects across countries.

Results of vaccine by country interaction analysis will be provided in Appendix 16.1.9 of the CSR.

8.4 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.

8.5 Immunogenicity Subsets

For the overall study - Approximately 10,620 subjects ≥ 65 years will be randomized to receive either aQIV or non-influenza comparator in a 1:1 allocation ratio, stratifying according to age (≥ 65 to 74 and ≥ 75), study site and comorbidity status (at risk/not at risk).

Within this randomization, a subset of 1702 subjects will be allocated for immunogenicity testing in a 4:1 ratio from either aQIV or non-influenza comparator , as described in Section 4.1.

8.6 Subgroups

Unadjusted efficacy analysis will be performed by stratifying for the following subgroups:

- Age at enrollment categorized as 65-74, 75-84, and ≥ 85 years.
- Comorbidity/risk (yes/no) defined as assessment score < 50 or ≥50 based on scale described in the Section 5.1.2 of the protocol (Hak, 2004);
- Previous influenza vaccination in the past 5 years (yes/no);
- Smoking status (yes/no);
- Sex;
- Race;
- Country;
- By season, if applicable.

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

Unadjusted immunogenicity analysis will be performed by stratifying for the following subgroups:

- Age at enrollment categorized as 65-74, 75-84, and ≥ 85 years.
- Comorbidity/risk (yes/no) defined as assessment score < 50 or ≥50 based on scale described in the Section 5.1.2 of the protocol (Hak, 2004);
- Previous influenza vaccination in the past 5 years (yes/no);
- Sex;
- Race;

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

- Age at enrollment categorized as 65-74, 75-84, and ≥ 85 years.
- Sex;
- Race;
- Country

8.7 Data Transformation

Distributions of antibodies are generally skewed to the right and approximately log-normally distributed (Nauta, 2010). 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.

8.8 Derived and Computed Variables

Demographics

In the case that Age and/or Body Mass Index must be recomputed by standard software

Age will be calculated in years using the following formula:

Round [(Date of Visit 1 – Date of Birth + 1) / 365.25]

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

Mass (kg) / Height^2 (m^2)

Immunogenicity

Values below the limit of quantification will be set to half that limit.

The rate of seroconversion is defined as the percentage of subjects with either a prevaccination HI titer < 1:10 and a postvaccination HI titer ≥ 1: 40 or a prevaccination HI titer ≥ 1:10 and a ≥ 4-fold increase in postvaccination HI titer.

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

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

Geometric Mean Ratio

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

The GMR will be calculated using the following formula:

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

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

Duration in the Study

Duration in the study is defined in days as:

[Last visit date (visit 15)[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 date of injection of study vaccine.
- **Emergence during vaccination phase**: start date on or after the date of injection of study vaccine or, adverse event increase in severity, including to "serious" adverse event.

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

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 vaccination (i.e., year or year & month is/are before the 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 (PT) 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 as possibly related, probably related or unknown/ missing.

Safety Laboratory Data

Not Applicable

Pre-study, Concomitant and Post-Vaccination Medications

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

A **post-vaccination medication** is a medication used only after study vaccination date + 366 days / study termination date.

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 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.

8.9 Analysis Software

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

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

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

The time the subjects are under observation will be summarized by vaccine group and overall and by study period using summary statistics (mean, standard deviation (SD), minimum, median, maximum)

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

In general, all tables presented in CSR section 14.1 should show a Total column across vaccine groups.

10.1 Demographics

Age, height, weight, body mass index will be summarized by reporting the mean, standard deviation, median and range, and will be calculated by vaccine group, country and overall.

In addition, the frequencies of age categories will be reported as 65-74 years old vs. ≥ 75 years old (age as a randomization stratum) and 65-74 vs 75-84 vs. ≥ 85 years old. The number and percentages of subjects by sex, country, age categories (for posting), ethnic origin, race, entry criteria fulfilled, by previous vaccination (in past 5 years), smoking status and comorbidity will be presented by vaccine group and overall. Demographic data will be tabulated for the All Enrolled, FAS (demography), PPS and Safety sets.

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 preferred term (PT) by vaccine group and overall. Medical history data will be tabulated for the All Enrolled, FAS (demography), PPS and Safety Sets.

11. IMMUNOGENICITY ANALYSIS

Where applicable, immunogenicity objectives will be evaluated using Center for Biologics Evaluation and Research (CBER) Guidance Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines (CBER 2007).

For the elderly population the CBER criteria are as follows:

- the lower bound of the two-sided 95% confidence interval (CI) for the percent of subjects achieving seroconversion for HI antibody should meet or exceed 30%
- the lower bound of the two-sided 95% CI for the percent of subjects achieving an HI antibody titer ≥ 1:40 should meet or exceed 60%.

Both endpoints should be met for each strain included in the vaccine.

11.1 Blood Samples

The numbers and percentages of subjects with blood draws will be summarized overall and by vaccine group where applicable. Data will be tabulated for the immunogenicity subset.

11.2 Primary Objectives Analysis

Not Applicable

11.3 Secondary Objectives Analysis

The following secondary immunogenicity analyses will be performed:

1. Geometric mean HI titer (GMT) on Days 1 and 22.

2. Geometric Mean Ratios (GMRs) AT Day 22/ Day 1.

3. Percentage of subjects achieving seroconversion (defined as: HI ≥1:40 for subjects negative at baseline [<1:10]; or a minimum 4-fold increase in HI titer for subjects positive at baseline [HI≥1:10]) on Day 22.

4. Percentage of subjects with HI titer ≥ 1:40 on Day 22.

5. Reverse cumulative distribution plots will be generated in order to display the distribution of the antibody responses at Day22.

CBER requirements for each vaccine group translate into the following hypothesis:

$$H_{0k}^{(SC)}: \pi_{ik} - \pi_0 \leq 0 \quad \text{vs.} \quad H_{1k}^{(SC)}: \pi_{ik} - \pi_0 > 0$$

$$H_{0k}^{(SP)}: \tau_{ik} - \tau_0 \leq 0 \quad \text{vs.} \quad H_{1k}^{(SP)}: \tau_{ik} - \tau_0 > 0,$$

assuming that responses Y_{ij} , Z_{ij} are independent identically distributed Bernoulli variables B(1, π_{ik}) and B(1, τ_{ik}), where i denotes vaccine group; j=1,..,n_i denotes subject, k denotes strain, π_{ik} and τ_{ik} represent the population proportions of subjects achieving seroconversion and post-vaccination HI titer ≥ 1:40, respectively, π_0 and τ_0 denote the thresholds for seroconversion ($\pi_0 = 0.3$) and the threshold for proportion of subjects with HI titer ≥ 1:40 ($\tau_0 = 0.6$).

All hypothesis related to CBER criteria will be tested on unadjusted two-sided 5% significance level.

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.

All statistical analyses for HI titers will be performed on the logarithmically (base 10) transformed values. Individual HI titers below detection limit (for example <10) will be set to half of that limit (5).

Estimates for GMTs, GMRs and pertaining 2-sided 95% CIs will be calculated assuming log-normal distribution of the titers and will be completed by providing minimum, maximum and median titers for each vaccine group.

Binary data (i.e., percentages of subjects with seroconversion and with titer ≥ 1:40) will be summarized using unadjasted estimates and will be reported together with 2-sided 95% Clopper and Pearson Cis (Clopper and Pearson 1934). Differences of proportions will be reported with 95% CI calculated using Miettinen and Nurminen's method (Miettinen and Nurminen 1985)'s method.

No multiplicity adjustment to the CI levels will be implemented.

Reverse Cumulative Distribution Curve will be generated in order to display the distribution of the antibody responses at Day 22.

11.4 Exploratory Objectives Analysis

The following exploratory immunogenicity analyses will be performed:

1. Geometric mean MN titer (GMT) on Days 1 and 22.

2. Ratio of Geometric mean MN titer (GMTr) of aQIV versus Non-influenza comparator at Day 22.

3. Percentage of subjects with a four-fold rise in MN antibody titer at Day 22.

4. Reverse cumulative distribution plots will be generated in order to display the distribution of the MN antibody responses at Day22.

Exploratory objectives related to the MN-assay will be evaluated using methods for lognormal and binomial data as described in the Section 11.3.

Additionally, an estimate of the serologic correlate of protection on the basis of HI or other immunological responses including MN, measured three weeks after vaccination against the respective strain.

Evaluation of a Serological Predictor of Protection Against Influenza

The immunogenicity data of the blood draw 21 days after vaccination from all subjects reporting an influenza case and subjects randomized from the population without an influenza case serving as the control group will be used to evaluate the relationship between antibody levels tested with the HI assay and clinical protection from influenza.

The Prentice criterion will be used to assess, whether an immunologic correlate of protection can be determined.

To accommodate the criterion a linear logistic regression model will be fitted with vaccine group included as independent predictor and incidence of influenza as dependent variable to show that the observed vaccine effect be explained in a statistical model using immunologic data.

A second logistic regression model will be fitted, adjusted for log-transformed antibody titer and vaccine group to determine the effect of antibody titer on the incidence of influenza. Then, the relationship between the occurrence of influenza and antibody titer level will be modeled using the logistic regression model advocated by Dunning that accommodates both antibody titers and factors independent of antibody titers.

In this model, the probability that a subject develops influenza is the probability that the subject is susceptible multiplied by the probability that susceptible individuals develop disease. Susceptibility is characterized by the probability l, and the probability that a subject with titer t is protected is represented by a 2-parameter logit function. Analyses

will be conducted to determine the level of HI (or other assays that might be performed) antibody titer associated with 50%, 60%, 70%, 80% and 90% probability of protection.

12. EFFICACY ANALYSIS

12.1 Primary Objectives Analysis

The primary absolute efficacy objective will be evaluated using following null (H_0) and alternative (H_1) hypotheses:

$$H_0: 1 - HR = VE \leq 0.4 \qquad \text{versus} \qquad H_1: 1 - HR = VE > 0.4,$$

where HR is a hazard ratio of aQIV versus non-influenza comparator and VE is vaccine efficacy. The primary objective will be demonstrated 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 40%. In case an interim analysis is 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.

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

The HR and the related 95% CI of HR, for onset of first RT-PCR confirmed influenza will be estimated by a proportional hazards regression model with treatment effect as a fixed effect and stratifying covariates as a 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 8.1for further discussion of the covariates utilised).

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 2-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.

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

Primary analysis will take into account only first ILI episode for a subject. As exploratory evaluation, Cox PH model might be fitted taking into account recurrent ILI events.

In addition VE estimate estimated from Cox proportional model, a simple unadjusted estimate of VE will be also presented as $1-\pi_{aQIV}/\pi_{Comp}$ along with 95% exact unconditional confidence interval; where π_{aQIV} and π_{Comp} will be attack rates for each group estimated as a binomial variable.

12.2 Secondary Objectives Analysis

Hypotheses for testing secondary objectives are formulated in the similar way as for the primary efficacy objective. If an interim analysis is performed and the trial doesn't stop, then all the subsequent secondary 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% at the final analysis.

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. For non-key secondary objectives, if the percentage of vaccinated subject excluded from the FAS is greater than 5%, a secondary analysis based on the PPS will be performed to complement the FAS.

Similarly to the primary efficacy objectives, a Cox PH model will be used to estimate absolute vaccine efficacy VE for each secondary objective 1-4.

Criteria for evaluation : if the lower limit of the 95% two-sided confidence interval for VE estimate is greater than 40% then the secondary efficacy objectives will be demonstrated.

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 adverse events.

- Medically attended AE within 30 days of first-occurrence RT-PCR confirmed ILI
- Unsolicited adverse events.
- SAEs, AE leading to withdrawal, NOCD, AESI

13.1 Analysis of Extent of Exposure

The numbers and percentages of subjects with vaccinations will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled Set.

13.1.1 Safety Completeness Analysis

Solicited adverse events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed 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 diary card (including implausible values) except "Not done/unknown".

Four summaries will be produced:

1. The frequencies of subjects who provide diary cards by vaccine group.
2. For each solicited adverse event, the frequencies of subjects with valid data will be presented by vaccine group and timepoint: 30 min, 6h –Day 3, Day 4-7, 6h - Day 7, Day 1 – 7 (with/without 30 mins).
3. For each type of solicited adverse event (local, systemic) and indicators of solicited adverse events, such as analgesic use, the frequencies of subjects with valid data by vaccine group, aggregated over time points: 30 min, 6h –Day 3, Day 4-7, 6h - Day 7, Day 1 – 7 (with/without 30 mins).
4. For each solicited adverse event, the frequencies of subjects with valid data by vaccine group, aggregated over time points: 30 min, 6h –Day 3, Day 4-7, 6h - Day 7, Day 1 – 7 (with/without 30 mins).
5. For the corresponding percentages, the denominator will be the respective numbers of exposed subjects, i.e., subjects who received a vaccination and were still in-study for that time point or time interval, irrespective of whether a diary card was present or not.

All analyses will be based on the 'as treated' Solicited Safety Set.

13.2 Solicited Local and Systemic Adverse Events

For details please refer to section 7.1.1 of the protocol.

Only solicited local and systemic adverse events reported in the diary card will be analyzed. Implausible measurements will not be taken into consideration in the analysis but listed in the Appendix (see section 6.3).

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 separately for 30 minutes and based on three intervals: 6h - Day 3, Day 4 - 7 and 6h - Day 7, each without 30 minutes data. In addition solicited adverse events ongoing after Day 7 will be presented as unsolicited AE.

For erythema and induration, and ecchymosis recorded originally as diameters (mm), the following categorization will be used to summarize the data:

Type I: none (0 mm), any (1-24 mm, 25-50 mm, 51-100 mm, >100 mm)

Type II: Grade 0 (< 25 mm), any (25-50 mm, 51-100 mm, >100 mm)

Body temperature will be broken down by route of measurement according to the recommendations of the Brighton collaboration as well as CBER and will be summarized according to the 3 schemes described below:

- Brighton:
- <38.0,
- 38.0 - 38.4
- 38.5 - 38.9
- 39.0 - 39.4
- 39.5 - 39.9
- 40.0 – 40.4
- 40.5 – 40.9
- ≥41.0°C

- CBER: 38.0–38.4, 38.5-38.9, 39.0-40.0, >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 a systemic adverse event.

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 30 min measurement (after 30 minutes).
3. Solicited adverse events, maximum event severity by event and interval 6h - Day 3, Day 4 -7, and 6h - Day 7, each without 30 min.
4. Duration of solicited adverse events, including ongoing AE after Day 7.
5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval 6h-Day 3, Day 4-7 and 6h-Day 7, each 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 at which the respective solicited adverse event first occurred. For erythema, induration, and ecchymosis the following threshold will be used: ≥ 1 mm. The summary will provide the frequencies and percentages of subjects with first onset of each solicited adverse events by vaccine group and by each time point.

The following example is used to illustrate how the onset data is selected:

Suppose four subjects, who receive vaccination A, have the following post-vaccination data for solicited adverse event XY

Table 13.2-1: Example for Time to First Onset of Solicited Adverse Events

Vaccination	Subject Number	6 Hours	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	…	Not done

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 and ≥ 1 mm for erythema and induration and ecchymosis. If a solicited adverse event continues beyond Day 7 the period after Day 7 is added.

The following example is used to illustrate how the duration is calculated:

Suppose six (6) subjects, who received a vaccination have the post-vaccination solicited adverse event data shown in the table below. In addition, there are unsolicited adverse event reports indicating that the adverse event in subject 003 and 006 continued until Day 12 and Day 8, respectively. For subject 003 the number of days is calculated as 6+5 and for subject 006 as 3+1. Missing values ('Missing') are not taken into consideration

Table 13.2-2: Example for Number of Days With Solicited Adverse Events

Subject Number	6 Hours	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of days
001	None	Severe	Moderate	None	None	None	None	2
002	Mild	None	None	Moderate	Moderate	Moderate	Missing	4
003	Moderate	Mild	None	Severe	Severe	Severe	Mild[a]	11

Subject Number	6 Hours	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of days
004	None	None	None	None	None	None	Not done	0
005	None	Mild	Mild	Missing	Missing	Missing	Missing	2
006	Severe	None	Mild	None	None	None	Severe[b]	4

[a] continued until Day 12; [b] continued until Day 8

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", ≥ 1 mm for erythema, ecchymosis and induration) for the respective event and "none" otherwise. 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, 6h - Day 3, Day 4 -7, and 6h - Day 7.

13.3 Unsolicited Adverse Events

If the reporting source is collected in the study, specify the following: 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 (medical records, study specific worksheet).

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. Safety data including medically-attended AE within 30 days after first occurrence, and RT-PCR confirmed ILI, adverse events leading to withdrawal, AESI, NOCD and SAE up to Day 366 will be captured. The original verbatim terms used by investigators to identify adverse events in the 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 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:

- Onset between Day 1 and Day 22.
- Onset between Day 23 and Day 181.
- Onset between Day 182 and Day 366
- Onset between Day 1 and Day 366

A number of adverse events typically captured on diary cards will be reported as unsolicited adverse events. Unsolicited adverse events corresponding to local and systemic solicited events (for example, injection site pain, fatigue, etc.), which occur in the 7 days following vaccination, will be presented by treatment group.

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.
- Medically-attended AE within 30 days after first occurrence RT-PCR confirmed ILI
- Unsolicited adverse events leading to premature withdrawal from study.
- Unsolicited adverse events leading to new onset of chronic disease.
- Unsolicited adverse events of special interest.

Solicited adverse events continuing beyond Day 7 will be coded by MedDRA and will be reported into the respective unsolicited adverse events.

13.4 Combined Solicited and Unsolicited Adverse Events

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

13.5 Clinical Safety Laboratory Investigations

Not Applicable.

13.6 Concomitant Medication

The numbers and percentages of subjects reporting concomitant medications will be tabulated overall and by vaccine group and study period (treatment / Follow-up). Medications (generic drug name) will be coded using the World Health Organization (WHO) Drug dictionary (see section 8.7 for definition).

14. INTERIM ANALYSIS

14.1 Interim Analysis

As the circulation of influenza viruses is seasonal and the rates of influenza are difficult to predict, this study is group sequentially designed with maximally 2 interim analyses planned over the course of the study. The goal of 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 or for futility after observing at least 50% of planned RT-PCR confirmed influenza cases.

The data that will be included for the interim analysis will be derived from the EDC forms (Demographics, Vaccination, ILI Report) and external lab data from [redacted] (ILI Swab form). For the purpose of the interim analysis, the data are not required to be completely clean, frozen and locked but as clean as possible:

1) A snapshot would be acceptable for the purpose of this interim analysis.
2) EDC data part of the Interim Analysis should be 100% SV complete.
3) There should be no open / unanswered queries and should be considered clean following the ongoing data review process.
4) Lab data reconciliation EDC <> Laboratory Operations Management System (Lomas) should have no discrepancies.
5) Lab data [redacted] <> Lomas should have no discrepancies.

Any exceptions to the above requirements are to be reviewed by the study team and confirmed acceptable prior to taking the interim data snapshot. Freezing and Locking of the forms in Inform is not required. PI signatures are also not required.The first interim analysis will be performed after observing approximately 50% of planned RT-PCR confirmed influenza cases. In addition, DMC may request an additional interim analysis if deemed necessary.

- If the number of first-occurrence RT-PCR confirmed influenza cases from 21 through 180 days after vaccination or until the end of the influenza season, whichever is longer, is less than or equal to 119, no interim analysis for efficacy and futility will be done and the study will be continued because the probability to make a conclusion for futility or efficacy is too low.
- If the number of first-occurrence RT-PCR confirmed influenza cases from 21 through 180 days after vaccination or until the end of the influenza season, whichever is longer, is greater or equal to 120 but less than 238 unblinded interim analyses for efficacy and futility will be performed by the DMC (see below for description).

- If the number of first-occurrence RT-PCR confirmed influenza cases from 21 through 180 days after vaccination or until the end of the influenza season, whichever islonger, is greater or equal to 238 (targeted number of cases to be able to evaluate the primary objective) the trial will be stopped, data will be unblinded and the final analysis will be performed by the Sponsor. If the trial proceeds to the final analysis (upon reaching 238 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 confirmed influenza A and or B will be assess the one-sided 0.025 alpha level.

If 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 one-sided 0.025 and no further enrollment is needed. However if 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 additionally enrolled. The DMC should not be influenced by the results of the efficacy testing at the interim analysis 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:

$$N_{total} = (C_{planned} - C_{observed}) / ER,$$

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. 238 cases, $C_{observed}$ is the number of cases observed at current stage, and ER is the observed total attack rate for both groups combined. A drop-out rate of 10% should be added.

For the analysis of early stopping for futility and for efficacy, an error-spending function will be applied to provide statistical stopping rules for efficacy (α-boundaries) and futility (β-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 scale (SAS/STAT 9.2 user's guide, the SEQDESIGN Procedure)

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. Using the same error-spending-function applied on the overall type 2 error of 0.1 (Power 90%), β-boundaries are calculated, i.e. the adjusted probabilities for

the type 2 error β, and if the p-value is higher than the β-boundaries, the trial stops early for futility at this stage.

In other words the trial stops early for efficacy if data collected at this stage allows to demonstrate primary efficacy objective (reject null-hypothesis); and the trial stops early for futility, if the data provides sufficient evidence that the test vaccine is not good as expected (the alternative hypotheses of absolute vaccine efficacy over the protocol-specified margin is not true, i.e VE<0.4). 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:

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

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

With a specified type 1 error, α, and information level t at stage k, the cumulative error is αE(t; α) (as per page 69 of SAS/STAT 9.2 User's guide, the SEQDESIGN Procedure)

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 by the DMC, particularly if the first interim analysis is conducted soon after 119 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.

Table 1 shows the needed total number of cases at each stage of the two-stage design together with boundaries for early stopping for futility or 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 119 confirmed ILIs) the trial would stop early for futility at this stage in case a p-value higher than 0.35022 is observed, or it would stop early for efficacy if the p-value is lower than 0.00625. 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 1: Example of a Two Stages Group-Sequential Design

	Stage 1 First Interim	**Stage 2 Final**
Number of Events	119	238
Alpha Boundary on p-value scale (equivalent to Type 1 error, 1-sided), early stopping for efficacy	0.00625	0.02275
Beta Boundary on p-value scale (equivalent to Type 2 error, 1-sided), early stopping for futility	0.35022	0.02275

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. Another interim look at the data with appropriate adjustment of type 1 error might be recommended before reaching the targeted 238 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). 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.

For the complete list of tables, figures and listings (TLFs) for interim analysis, please refer to the Table of Contents (TOC) for Interim Analysis stored in the eTMF.

14.1.1 Futility Analysis

To overcome multiplicity testing problems an error-spending function (implemented in SAS® PROC SEQDESIGN) will be applied to provide statistical stopping rules for futility (β-boundaries) for all interim analyses, if necessary, based on the information accumulated until that specific interim stage, i.e., based on the accumulated variance of the parameter of the parameter of interest. Using the same power error-spending function applied on the overall type 2 error of 0.1 (power 90%), β-boundaries are calculated, i.e., the adjusted probabilities for the type 2 error β, and if the p-value is higher than β-boundaries, the trial stops early for futility at the stage. Further details are contained in the DMC charter.

15. DATA MONITORING COMMITTEES

An independent 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.

Unblinded interim analyses for efficacy and futility will be executed by the DMC, if the number of first-occurrence RT-PCR confirmed influenza cases from 21 through 180 days after vaccination or until the end of the influenza season, whichever is longer, is ≥ 120 and < 238. In addition, the DMC will also review blinded, and if requested, unblinded safety data at pre-specified intervals during the study. Another interim analysis might be recommended before target 238 cases are observed. Any unblinded data will be reviewed in closed sessions of the DMC, without participation of the Sponsor. All descriptions of these closed sessions will be unavailable to the Sponsor until study unblinding has occurred. All reports, following open sessions of blinded data review will be available to the Sponsor as appropriate. DMC recommendations will be expressed clearly to the Sponsor, at minimum in written communication.

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

Peer review of analyses should be performed in accordance with the applicable procedures for validation of SAS programs used in clinical data analysis.

The following minimum analyses are identified as key analyses to be peer reviewed by a biostatistician independent from the study:

- Primary efficacy analysis.

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

For the complete list of tables, listings and figures (TLFs), please refer to the Table of Contents (TOC) stored in the eTMF. Note that a listing of individual efficacy/immunogenicity lab data (see "Other TOC" tab in the TOC) will be created for each center, to allow data dissemination to the study subjects. This listing will show the following data:

- Subject ID
- Actual vaccine(s) received by the subject
- Lab results for immunogenicity/efficacy at each visit.

This listing is mentioned in the tab "Other TOC" in the TOC and will not be part of the CSR stat package.

18. LAYOUT SPECIFICATIONS FOR TABLES, LISTINGS AND FIGURES

All TLFs are to include the following header:

Seqirus UK Ltd	Vaccine: aQIV
Final Report: Study V118_18	Single Dose in Healthy Adults

In all tables, listings and figures, vaccine groups will be labeled as “aQIV” and “Boostrix”,

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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