Statistical Analysis Plan, Protocol Number: V201_07
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STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 2b, Randomized, Observer-Blind, Antigen and Adjuvant Dose-Confirmation Clinical Study to Evaluate Safety and Immunogenicity of Different Formulations of MF59-Adjuvanted Quadrivalent Subunit Inactivated Cell-derived Influenza Vaccine (aQIVc) in Older Adults ≥50 Years of Age
Study Number:	V201_07
Protocol Version and Date:	Final 1.0, 01 JUN 22
Phase of Development:	Phase 2b
Plan Prepared by:	[redacted] *Version 1.0: Date (20 December 22)*
Approvers:	[redacted]

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APPROVALS

[redacted]

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[redacted]

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LIST OF ABBREVIATIONS

AE	*Adverse Event*
AESI	*Adverse Events of Special Interest*
ANCOVA	*Analysis of Covariance*
ANOVA	*Analysis of Variance*
aQIV	*Adjuvanted quadrivalent influenza vaccine*
aQIVc	*Adjuvanted quadrivalent influenza vaccine cell derived*
CRF	*Case Report Form*
CI	*Confidence Interval*
CSR	*Clinical Study Report*
DMC	*Data Monitoring Committee*
FAS	*Full Analysis Set*
GLM	*Generalized Linear Model*
GMFI	*Geometric Mean Fold Increase*
GMR	*Geometric Mean Ratios*
GMT	*Geometric Mean Titers*
eCRF	*electronic case report form*
eDiary	*Electronic diary card*
HA	*hemagglutinin*
HI	*Hemagglutination Inhibition*
ICF	*Informed Consent Form*
ICH	*International Council for Harmonisation*
IRT	*Interactive Response Technology*
LL	*lower limit*
LLOQ	*lower limit of quantitation*
MAAE	*medically-attended adverse event*
MedDRA	*Medical Dictionary for Regulatory Activities*
MN	*Microneutralization*
PD	*Protocol Deviation*
PPS	*Per Protocol Set*

QIV	*Quadrivalent Influenza Vaccine*
RCDC	*Reverse cumulative distribution curves*
SAE	*Serious Adverse Event*
SAP	*Statistical Analysis Plan*
SCR	*Seroconversion rate*
SD	*Standard Deviation*
SP	*Statistical Programmer*
SPR	*Seroprotection Rate*
SUSAR	*Suspected Unexpected Serious Adverse Reactions*
TFL	*Tables, Figures and Listings*
TOC	*Table of Content*
ULOQ	*upper limit of quantitation*

1. BACKGROUND AND RATIONALE

Influenza is an acute and highly contagious viral infection with global circulation. Globally, there are an estimated 1 billion cases of influenza disease every year, of which 3 to 5 million are severe cases, resulting in 290,000 to 650,000 influenza-related respiratory deaths (WHO 2019; Iuliano et al. 2018).

Vaccination is the primary method for preventing influenza and its potentially severe complications. Efficacy of the conventional influenza vaccines in the adult population is demonstrated to be high (Samuel and Miller 2019). In contrast, the efficacy in elderly 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 and Sullivan 2011). In view of the limitations of conventional influenza vaccines in older adults, there continues to be an unmet need for a new generation of influenza vaccines that provides more consistent and broader coverage against all seasonal virus subtypes and variants (Wong and Webby 2013; Reber et al. 2012).

The investigational vaccine in this study, MF59-Adjuvanted Quadrivalent Subunit Inactivated Cell-derived Influenza Vaccine (aQIVc), combines benefits of both cell-based and adjuvanted technologies to meet the unmet need of influenza prevention in older adults. The cell-derived antigen provides a better match to circulating wild-type strains, therefore addressing mismatch due to egg adaptation (Hedge 2015; Lambert and Fauci 2010), which could improve vaccine effectiveness. The effect of inclusion of MF59 adjuvant to the vaccine is expected to enable increased magnitude, breadth, and duration of immune response. Results of a dose-finding study with different dosages of antigen and adjuvant for the egg-based adjuvanted trivalent influenza vaccine (aTIV) indicate that the increase of the antigen and MF59 dose may be associated with a higher antigen-specific immune response (Otten et al. 2020).

The aim of this dose-confirmation study is to confirm immunogenicity and safety of the adjuvanted quadrivalent cell-based influenza (aQIVc) vaccine containing [redacted] of antigen per strain and [redacted] MF59 ([redacted] of squalene). Other formulations of (a)QIVc will also be included to confirm the incremental benefit of additional antigen and adjuvant which is expected to lead to the optimal dose-response elicited by aQIVc.

Immunogenicity and safety will be assessed in the overall study population (adults ≥50 years and above) and in the age subgroups ≥50 to 64 years and ≥65 years. Data from this study will be used to select the optimal dose to be tested in the pivotal Phase 3 immunogenicity and safety study in older adults.

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

2. OBJECTIVES

2.1 Primary Objective

2.1.1 Primary Immunogenicity Objective

1. To assess the immunogenicity of the different (a)QIVc formulations through pairwise comparisons and trend analysis as measured by hemagglutination inhibition (HI) assay* using cell-derived target viruses at Day 29 after vaccination.

* *In case of lack of HA agglutination for a specific strain using HI assay, immunogenicity for that strain will be assessed as measured by microneutralization (MN) assay as an acceptable alternative.*

2.1.2 Safety Objective

1. To assess the reactogenicity and safety of all (a)QIVc formulations and the comparator influenza vaccine.

2.2 Secondary Objective

2.2.1 Secondary Immunogenicity Objective

1. To assess the immunogenicity of the different (a)QIVc formulations through pairwise comparisons and trend analysis, as measured by MN assay using cell-derived target viruses at Day 29 after vaccination.

2.3 Exploratory Objective

1. Additional exploratory immunogenicity analyses may be conducted to further characterize the immune response of the study vaccines.

3. STUDY DESIGN

This is a Phase 2b, randomized, stratified, controlled, observer-blind, clinical study in approximately 1000 male and female adults aged 50 years and older (approximately equally split between two age groups; ≥50 to 64 and ≥65 years of age) who are healthy or have co-morbidities which increase their risk of complications from influenza infection.

Blinding: Observer-blind study.

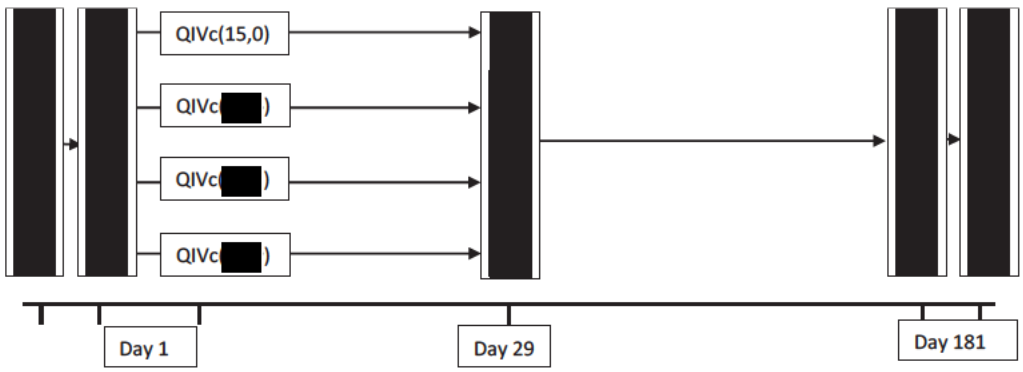
Duration of the study: Approximately 6 months for each subject in a single influenza season.

Vaccination schedule: Single dose vaccination on Day 1.

Investigational Vaccines: The study will use 3 investigational formulations of (a)QIVc (see Table 3-1)

Comparator Vaccine: QIVc, containing 15 µg HA/strain (non-adjuvanted) in a total volume of 0.5 mL.

4 parallel doses will be included:
QIVc (15, 0)
QIVc HD ([redacted])
aQIVc HD ([redacted])
aQIVc HD ([redacted])



Treatment groups: All treatment groups will include approximately 250 subjects aged ≥50 years of age (see Table 3-1). All subjects will receive one dose of study vaccine at Day 1

Table 3-1 Overview of study vaccines

Vaccine	Type	HA antigen content/ strain (µg)	MF59 squalene content (mg)	Total injected volume (mL)	Presentation/ mixing
QIVc ([redacted])*	Investigational	[redacted]	[redacted]	[redacted]	[redacted]
aQIVc ([redacted])*	Investigational	[redacted]	[redacted]	[redacted]	[redacted]
aQIVc ([redacted])	Investigational	[redacted]	[redacted]	[redacted]	[redacted]
QIVc	Comparator	15	0	0.5	PFS

* aQIVc ([redacted]) will be obtained by 'bed-side' mixing of QIVc and aQIVc ([redacted]), whereas QIVc ([redacted]) will be obtained by 'bed-side' mixing of [redacted] of QIVc

Blood sample schedule: A blood sample (approximately 10 mL) will be collected from all subjects on Day 1 (prior to vaccination) and Day 29.

Data collection: electronic Case Report Form (eCRF) and electronic Diary (eDiary).

Study periods: The study has a treatment period (Day 1 to Day 29) and a follow-up period (Day 30 to Day 181).

Study clinic visits: Two clinic visits for each subject: at Day 1 and at Day 29.

Safety phone call: A safety phone call on Day 8 will be conducted to review the eDiary data as well as to collect any unsolicited AEs, and their related medications and any vaccinations. A second and third safety phone call will be conducted at Day 91 and at the end of study (Day 181) to collect any serious adverse events (SAEs), non-serious Medically-attended Adverse Events (MAAEs), AEs leading to withdrawal and/or AESIs, together with associated medications and vaccinations.

Reactogenicity data collection: Solicited AEs, occurring at the day of study vaccination and the following 6 days (Day 1 through Day 7, or longer if the events are not resolved [maximum up to Day 14]), will be recorded daily using an eDiary as completed by the subject.

Safety data collection: All unsolicited AEs (including SAEs, non-serious MAAEs, AEs leading to study withdrawal, and/or AESIs) will be collected until Day 29 after vaccination. During the follow-up period (from Day 30 onwards), only SAEs, non-serious MAAEs, AEs leading to study withdrawal, and/or AESIs will be collected. 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 cell-derived target virus, will be performed in all subjects on serum samples collected on Days 1 and 29.

- MN assay for homologous vaccine strains, using cell-derived target virus, will be performed in all subjects on serum samples collected on Days 1 and 29.

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

Table 3-2: Times and Events Table(s)

Visit Type		Clinic Visit	Safety Phone Call	Clinic Visit*	Safety Phone Call	Safety Phone Call
Study Day		1	8	29	91	181
Visit Window (Days)		n/a	+3 day	+7 days	-14/+14 days	-14/+14 days
Visit Number		1	2	3	4	5
Study Event	**Protocol References**					
Screening and Randomization						
Informed Consent	Section 5.1.1	X				
Medical History	Section 5.1.2	X				
Physical Exam	Section 5.1.2	X				
Targeted Physical Exam	Section 5.4.1			X		
Measuring body temperature	Section 5.1.2	X				
Pregnancy test	Section 5.1.2	X				
Exclusion/Inclusion Criteria	Section 4	X				
Randomization	Section 5.1.4	X				
Vaccination	Section 5.2	X				
30 Minutes Post Injection Assessment	Section 5.3	X				
Train and Dispense Subject eDiary	Section 5.3	X				
Review of eDiary data and compliance	Section 3.6.2	Ongoing during eDiary use				
Assess unsolicited AEs	Section 7.1.2	X	X	X		
Assess SAEs	Section 7.1.4	X	X	X	X	X
Assess for AEs leading to withdrawal, non-serious medically-attended AEs and AESIs	Section 7.1.4.1	X	X	X	X	X
Collect all previous and relevant concomitant medications and vaccinations	Section 6.5	X	X	X	X	X
Immunogenicity						
Serology blood draw	Section 5.1.5	X[g]		X		
Study Completion/termination	Section 5.6					X[h]

Abbreviations: AE = adverse event; AESI = adverse event of special interest; MAAE = medically-attended adverse event; N/A = not applicable; SAE = serious adverse event.

* Visit window for serology blood draw at clinical Visit 3: -7 days to +21 days

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Subjects will be randomly assigned in an equal ratio to receive one of the 4 treatment groups. An Interactive Response Technology (IRT) will be used for subject randomization, with stratification factors for age group (≥50 to 64 and ≥65 years of age) and history of any influenza vaccination within 3 previous influenza seasons (yes/no).

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:

- Subjects got vaccinated with a vaccine different from the one assigned at randomization
- Subjects got vaccinated with the correct vaccine but containing a lower volume
- Subject is randomized/allocated to an age group which is not the correct one
- Subject is randomized/allocated to a vaccination history stratum which is not the correct one
- Subject receive expired vaccine or vaccine with temperature deviation from instruction
- Administration of vaccine in different location (i.e., leg instead of arm) or different route (IV instead of IM)

Differentiate between incorrect stratification and misrandomizations.

A misrandomization is defined as a subject receiving a vaccine other than the one assigned by randomization. Misrandomization 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) 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: 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. Note that major stratification error is not applicable for this study, because vaccine dosage or schedule does not depend on stratification.
- 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 randomized	excluded	Analyze as exposed

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

Subject receiving expired vaccine or an administration with incorrect route or location will be considered major deviations (i.e. CSR-reportable) and eliminated by PPS (but included in FAS and Safety set)

4.1.2 Forced Randomization

Not applicable.

4.1.3 Blinding and Unblinding

The study is designed as an observer-blind study. Observer-blind means that during the study, designated and trained unblinded nurse(s), physician(s), or other qualified healthcare professional(s) will be responsible for administering the study vaccines to the subjects. They will be instructed not to reveal the identity of the study vaccines either to the subject or to the investigative site personnel (i.e., investigator and study nurse) involved in the conduct of the trial, except in an emergency if unblinding in IRT is not possible.

For further details please refer to section 3.5. of the protocol. For unblinding due to interim analysis please refer to section 14 of this document.

If a subject is unblinded during the study, it is to be documented as a 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

The total sample size of 1000 subjects, allocated to one of the 4 vaccine groups in 1:1:1:1 ratio, in this exploratory Phase 2b study has been based on feasibility and to provide information for further development. The power calculations are provided to illustrate what differences can be detected.

Anticipating 6% of the subjects will be non-evaluable over the course of the study (due to being lost to follow-up, having insufficient samples, incomplete laboratory results, or protocol violations, etc.), approximately 235 evaluable subjects for each vaccine group will be included in the statistical analysis. Based on data from Seqirus study V201_01, it is assumed that the within group standard deviations for the log10 HI titers for all strains are about 0.65 or lower.

Effect size:

Table 5-1 shows the minimum effect size needed to detect statistical significance in the GMT ratio of (a)QIVc formulations versus QIVc at different levels of significance (ie, α-level) and for different sample sizes. It shows that 235 subjects provide 80% power to confirm that at least one dose is higher than QIVc when the true GMT ratio is at least 1.46, assuming that log10 Day 29 HI titers will be normally distributed with a standard deviation (SD) equal to 0.65, and at 0.05 (2-sided) significance level.

Table 5-1 Required Minimal Treatment Effect by Study Size

		Sample Size		
		200	250	300
α (2-sided)	Power	GMT Ratio	GMT Ratio	GMT Ratio
0.025	80%	1.59	1.51	1.46
	85%	1.64	1.55	1.49
	90%	1.70	1.60	1.54
0.05	80%	1.52	1.46	1.41
	85%	1.57	1.50	1.44
	90%	1.63	1.55	1.49
0.1	80%	1.45	1.40	1.36
	85%	1.50	1.43	1.39
	90%	1.55	1.48	1.43

Trend analysis:

Three adjuvant doses ([redacted]) are included in the evaluation of dose response in terms of HI and MN GMTs at 29 days post vaccination, which leads to the following vaccine groups to be included in the trend analysis: QIVc ([redacted]), aQIVc ([redacted]), and aQIVc ([redacted]).

Assuming that adjuvant dose-response follows a linear trend and assuming the same SD for all strains and doses equal to 0.65 (on log10 HI data at Day 29), and minimum effect size equal to approximately 29% in terms of GMT ratio (i.e. =1.29), 235 subjects in each dose group provide >80% power to detect a slope >0 with a 0.05 (1-sided) significant test (Table 5-2).

Table 5-2: Power analysis assuming linear trend and different means level (k)

A	Power	n per group	Number of Doses	K (means multiplier)	Effect Size	GMTs ratio
0.05	51.37%	150	3	1	0.09	1.23
	59.24%	150	3	1.1	0.10	1.26
	66.75%	150	3	1.2	0.11	1.29
	73.65%	150	3	1.3	0.12	1.32
	63.46%	200	3	1	0.09	1.23
	71.72%	200	3	1.1	0.10	1.26
	78.95%	200	3	1.2	0.11	1.29
	84.97%	200	3	1.3	0.12	1.32
	70.47%	235	3	1	0.09	1.23
	78.46%	235	3	1.1	0.10	1.26
	85.03%	235	3	1.2	0.11	1.29
	90.10%	235	3	1.3	0.12	1.32
	73.13%	250	3	1	0.09	1.23
	80.91%	250	3	1.1	0.10	1.26
	87.12%	250	3	1.2	0.11	1.29
	91.77%	250	3	1.3	0.12	1.32

Pairwise comparison (Tukey-Kramer)

Simulations were performed to evaluate the power to reject at least one null hypothesis of equality between groups when a multiplicity adjustment is performed.

Table 5-3: Summary of Simulations of 4 Groups. MC Procedure: Tukey-Kramer M.C. Test

Power	n	N	Power	SD	FWER		SD
Any Pairs	Grp Sample	Total Sample	All Pairs	H1	Actual	Target	
0.721	200	800	0	0.653	0.049	0.05	0.65
0.799	235	940	0	0.648	0.054	0.05	0.65
0.819	250	1000	0	0.653	0.049	0.05	0.65

With a sample size of 235 subjects, we can achieve ~80% power to conclude that at least one difference between group mean will be >0 using Tukey-Kramer Multiple Comparison test, with a target family-wise error rate of 0.05 and actual family-wise error rate 0.049. Simulations were done with 3000 runs of Monte Carlo samples from the null distribution of normal data with equal mean and alternative distribution of normal data with mean equal to 0.14, 0.15 and 0.16, and common standard deviations equal to 0.65.

Safety analysis:

Table 5.4 shows the probability to observe an adverse event with low frequency (i.e., less than 1%). Two-hundred fifty subjects per treatment group give >70% probability to detect an event with a frequency of 1 in 200 and 92% probability to detect an adverse event with a frequency of 1 in 100.

Table 5-4 Probability to Observe an Adverse Event With Predefined Frequency

AE frequency	Sample Size		
	250	200	190
10%	99.9%	99.9%	99.9%
5%	99.9%	99.9%	99.9%
1%	91.9%	86.6%	85.2%
0.5%	71.4%	63.3%	61.4%

Power was computed using PASS 15.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

CSR reportable PDs 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.

Seqirus's standard Protocol Deviation Specification Document lists all the pre-specified observable and programmable PDs, including their classification, categories, sub categories 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 PP analysis set. The number of subjects in any and by PD category will be summarized by study treatment and overall. Individual subject listings will be provided sorted by subject and by PD category.

Prior to unblinding, all reportable PDs will be evaluated, and 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 Lead Biostatistician, Lead Data manager, Global Clinical Operations Lead and the Clinical Scientist and will be included in the trial master file

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. Observable PDs are identified by CRAs during monitoring or other team members.

A set of listings will be programmed following the Protocol Deviation Specification List to determine and categorize the protocol deviations. These listings will be provided for review on an ongoing basis during the study.

This review will also include protocol deviations reported in monitoring reports. After the review, the Clinical Scientist and the Global Clinical Operation Lead will provide the Biostatistician with:

• An assessment of CSR reportable PDs based on blinded clinical data review.

6.3 Exclusions of Individual Values for Safety Analysis

Local and systemic adverse events will be directly measured by the subject and will not be subject to a reconciliation process. The adverse events mentioned in Table 6.3-1 will be collected using ediary, which does not allow reporting a measurement beyond the limit mentioned in the table.

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 92°F or ≥ 108°F
Erythema	>500 mm
Induration	>500 mm

7. ANALYSIS SET

7.1 All Enrolled Set

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

Demographic and baseline characteristics tables as well as subject listings will be produced on the All Enrolled Set.

7.2 Exposed Set

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

7.3 Full Analysis Set (FAS), Immunogenicity Set

All subjects in the All Enrolled Set who are randomized, receive study vaccination and provide immunogenicity data at the Day 1 and Day 29 assessment.

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

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

To Note: FAS is defined for each immunogenicity assay. Number of subjects included in FAS-HI may differ from number of subjects included in FAS-MN.

7.4 Per Protocol Set (PPS), Immunogenicity Set

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subject is randomized and at the scheduled time points)
- Have blood samples taken at Day 1 and within the window of -7 to +21 days around the Day 29 visit
- Have no CSR-reportable PD leading to exclusion (see section 6.2) as defined prior to *unblinding*.
- Are not excluded due to other reasons defined prior to unblinding or analysis (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.

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 SUSAR), he/she will be excluded from the PPS.

7.5 Safety Set

For all safety sets, subjects will be analyzed "as treated" (i.e., according to the vaccine a subject received, rather than the vaccine to which the subject may have been randomized).

Solicited Safety Set

All subjects in the Exposed Set with any solicited adverse event data and/or indicators of solicited adverse events (e.g., use of analgesics/antipyretics).

Unsolicited Safety Set

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

(A 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 minutes postvaccination safety data will be reported separately in a 30 minutes postvaccination 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).

In case a subject is randomized in the wrong stratum subject will be reassigned to the correct stratum for solicited and unsolicited safety sets.

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

Out of window AEs and reason from exclusions of these AEs for the analysis of the Safety data will be presented as separate data listings.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The log-transformed antibody titers at Day 29 will be analyzed using an Analysis of Covariance (ANCOVA) model which includes the log-transformed pre-vaccination antibody titer, age subgroup stratification (≥50 – 64, ≥65 years) and previous vaccination history stratification (no, yes). Summary tables will show both adjusted and unadjusted GMTs for each vaccine group and adjusted and unadjusted GMT ratio for the different vaccine groups.

Analysis of binary immunogenicity endpoints (i.e., percentages of subjects with seroconversion and with HI titer ≥1:40) 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 exact 95% CIs.

To note: Each of the 4 strains will be analyzed separately.

8.2 Handling of Dropouts, Missing Data

The distribution of subjects with reasons for missing immunogenicity values will be described by vaccine group. Key baseline characteristics, such as age and previous vaccination history, will be compared between the subjects with immunogenicity values and those who have missing data.

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

Solicited adverse events are collected using electronic diaries from Day 1 to Day 7 post-vaccination. If no data are recorded for all 7 days, the assessment is considered missing and excluded from the safety analysis. In case of partial reporting, presence or absence of the event will be based on the available data. If more than 5% of the subjects have incomplete diary data, a selection of summary tables for solicited adverse events may be repeated based on complete diary card data. The number of days and number of subjects with missing data will be tabulated by vaccine group.

8.3 Multicenter Studies

There will be no adjustment for multiple centres.

8.4 Multiple Comparisons and Multiplicity

Because of the descriptive nature of the objectives in this study, adjustment for multiple comparisons will not be applied and differences observed will be indicative of possible effect to be confirmed in the successive phase.

Nevertheless, for the pairwise comparisons a second line analysis will be performed adjusting confidence interval with the method of Tukey-Kramer (Tukey et al. 1985)

8.5 Immunogenicity/Safety/Other Subsets

Not applicable.

8.6 Subgroups

Primary and secondary analyses of immunogenicity and safety endpoints will be done for the total population and by age subgroup (50 to 64 years and ≥ 65 years)

Immunogenicity analysis of the GMTs, GMFI, GMTs ratios, SCR rate, and percentage of subjects with post vaccination titer ≥1:40 will be additionally performed in the following subgroups:

- Previous influenza vaccination history (not previously vaccinated; previously vaccinated)
- Baseline HI titer (<1:10; ≥1:10) (for primary analysis)
- Baseline MN titer (<LLOQ; ≥LLOQ) (for secondary analysis)

For the purpose of the specific analysis, when using ANCOVA model (i.e. GMT analysis), the factor used for stratifying the population will not be included in the GLM model as covariate.

8.7 Data Transformation

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

Integer [(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

Discrete endpoint titres will be reported, including those above the ULOQ. Range extension activity using pooled, high-titred serum samples from the study will be conducted to extend the ULOQ to allow titres to fall within the validated assay range, and thus their use in the statistical analysis.

However, if a range extension cannot be performed (e.g. insufficient sera available or if the range extension fails), at the time of statistical analysis the titres above the ULOQ will therefore be set as equal to the original validated ULOQ value.

Values below the lower limit of quantification will be set to half that limit (i.e., LLOQ/2).

Seroconversion based on **HI** antibodies is defined as binary variable for subjects with non-missing pre-vaccination- and post-vaccination values, as:

= 1, if seroconverted (defined as either a pre-vaccination titer < 1:10 and a postvaccination titer ≥1:40, or a pre-vaccination titer ≥ 1:10 and a ≥4-fold increase in post-vaccination titer).

= 0, otherwise

Seroconversion based on **MN** antibodies is defined as binary variable for subjects with non-missing pre-vaccination- and post-vaccination values, as:

= 1, if seroconverted (defined as either a pre-vaccination MN titer < LLOQ and a post-vaccination MN titer ≥1:(4xLLOQ), or a pre-vaccination MN titer ≥LLOQ and a ≥4-fold increase in post-vaccination MN titer.

= 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% 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.

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. If the start date is before the date of injection of study vaccine or indicated as on injection day but before injection, these events will not be considered as unsolicited adverse events and will instead be mapped to the medical history.

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

When start and/or end dates of an adverse event are only partially known, adverse events will be categorized as unsolicited adverse event unless the partial end date is before the vaccination date.

All unsolicited adverse events will be categorized as occurring during the treatment period of 28 days following vaccination (period Day 1 to Day 29) based on the start date. If start date is missing, events will be counted as yes during the period of 28 days following vaccination.

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.

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.

Durations:

Study duration or duration of an AE will be computed in days using the following formula:
[date of AE - (date of vaccination +1)]

Prior and Concomitant Medications

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

- **Pre-vaccination**: start date before the date of injection of study vaccine.
- **Concomitant:** start date before vaccination but continued after vaccination or start date after vaccination. Concomitant medication during the period Day 1- 29 will be labeled if in addition, the start date is on or before the Day 29. If start date and/or stop date is missing or incomplete, medication is considered concomitant unless the information on the stop date is unambiguous before the vaccination date.

8.9 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 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 be presented. 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 - (date of vaccination +1)) the subjects are under observation for safety will be summarized by vaccine group and overall using summary statistics (mean, standard deviation, minimum, median, maximum).

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

All tables related to baseline characteristics will include a 'Total' column across vaccine group and will be performed for All Enrolled Set, FAS, PPS and for safety set.

10.1 Demographics

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

The frequencies and percentages of subjects by sex, age categories, ethnic origin, race, entry criteria fulfilled, plasma donation and history of previous influenza vaccination will be presented by vaccine group and overall.

The frequencies of age categories will be reported as 50 – 64 and ≥ 65 years old (age as a randomization stratum) and 50-64, 65-74, and 75-84, and ≥ 85 years old.

10.2 Medical History

The frequencies and percentages of subjects with medical history will be presented by MedDRA system organ class and preferred term, by vaccine group and overall.

11. IMMUNOGENICITY ANALYSIS

The measures of immunogenicity used in this study are standard, i.e., widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response).

The immunogenicity analysis will be descriptive in nature and focus on the estimation of the treatment effects of the different aQIVc formulations. For the primary immunogenicity objectives, HI antibody responses, using a cell-derived target virus, will be evaluated for all strains. In case of a lack of HA agglutination for a specific strain using the HI assay, immunogenicity for that strain will be assessed as measured by the MN assay.

For the secondary immunogenicity objectives, MN antibody responses using cell-derived homologous target viruses for all vaccine strains.

All immunogenicity analyses will be evaluated based on both, PPS and FAS Immunogenicity.

11.1 Blood samples

The frequencies and percentages of subjects with blood draws will be summarized overall and by vaccine group. Data will be tabulated for the enrolled set.

11.2 Primary Objectives Analysis

Primary Objective: To assess the immunogenicity of the different (a)QIVc formulations through pairwise comparisons and trend analysis as measured by hemagglutination inhibition (HI) assay using cell-derived target viruses at Day 29 after vaccination.

Study is descriptive and there is no formal statistical hypothesis testing. Immunogenicity in terms of HI antibody response will be evaluated for each homologous vaccine strain using cell-derived target virus via the following measures together with their relative 95% CI:

- Geometric Mean Titer (GMT): Geometric mean of HI antibodies at Day 1 and Day 29.
- Geometric Mean Fold Increase (GMFI): The Geometric mean of the fold increase in serum HI titer post-vaccination (Day 29) compared to pre-vaccination (Day 1).
- Percentages of subjects with HI titers ≥1:40 at Day 1 and Day 29.
- Percentage of subjects with seroconversion (defined as a ≥4-fold increase in titer post-vaccination in those with pre-vaccination titer equal to or above the LLOQ (1:10), or a post-vaccination titer ≥1:40 for subjects with baseline titer below the LLOQ (1:10) for HI antibodies at Day 29).
- GMT ratio: All pairwise Geometric mean of HI antibodies titer ratio
- Reverse cumulative distribution curves of HI titers by time-point at Day 1 and Day 29

<u>Geometric Mean Titer and GMR at Day 29:</u>

Summary statistics (i.e., geometric mean, 95% confidence interval of GMT, minimum, median, maximum) of the HI titers will be presented by strain, assessment (Day 29) and vaccine group.

It is assumed that for each strain $\log_{10}$-transformed Y_{ij}, i=(QIVc(15,0), QIVc([redacted]), aQIVc([redacted]), aQIVc([redacted])), *j=1,..,n,* are identical, independent normal distributed random variables: $\log_{10}$-transformed $Y_{ij} \sim N(\mu_i, \sigma^2)$ with μ_i, and σ^2 unknown. μ_i represents the mean antibody level in vaccine group i and σ^2 the variance.

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

Adjusted estimate of GMT will be derived using ANCOVA; A general linear model (GLM) with $\log_{10}$-transformed Day 29 titers as the outcome variable and vaccine groups, $\log_{10}$ pre-vaccination titer, age subgroup and previous

vaccination history as covariate will be applied. From this model, adjusted differences in the least square means (on the log scale) will be produced with 95% confidence limits, for QIVc([redacted]) versus QIVc(15,0) via contrast statement. The estimated difference and the confidence limits will be back-transformed to obtain an adjusted GMT ratio with 95% confidence limits.

From the same GLM model, a trend analysis to detect adjuvant effect will be conducted using linear contrast for the QIVc([redacted]), aQIVc([redacted]) and aQIVc([redacted]) vaccine group. Dose response analysis will be performed with an alpha level of 0.05 (one-sided) and an increasing trend or dose-response relationship will be assessed using p-values (<0.05) for all four strains.

Once trend is established, the pairwise comparisons using the same ANCOVA least-square means model as specified above, will be assessed to understand the GMT ratio of one formulation over another.

As the inflation of probability of the type 1 error increases with the increase in the number of comparisons an adjustment for multiplicity is proposed as second line analysis where confidence intervals, each pairwise comparisons will be computed using the Tukey-Kramer method (Tukey et al. 1985).

The following SAS codes will be used for the ANCOVA model:

```
/* QIVc(15,0) vs QIVc([redacted]), trend analysis and all comparisons
 without multiplicity adjustment */;
proc glm data=<adis>;
 where substr(paramn,1,5) = 'Log10' and AVISITN NE 1,
 by PARAM;
 class TRT01A PREVAC AGEGR;
 model AVAL = TRT01A BASE PREVAC AGEGR / solution clparm;
 contrast 'Adjuvant Trend Test' group 0 -1 0 1;
 contrast '(15,0) ([redacted])' group -1 1 0 0;
 lsmeans TRT01A/CL pdiff tdiff stderr alpha=0.05;
 ods output contrasts = _ctr lsmeancl=_mcl LSMeanDiffCL=_dcl;
run;

/* Slope of the trend analysis outcome */;
data slope ;
 * Arm ([redacted]) coded as group 2, Arm ([redacted]) coded as group 4;
 set _dcl (where = (i = 2 & j =4)) ;
 slope = -Difference/2 ;
 LCL = -UpperCL/2 ;
 UCL = -LowerCL/2 ;
run;

/* all comparisons adjusted for multiplicity with Tukey-Kramer method*/;
proc glm data=<adis>;
 where substr(paramn,1,5) = 'Log10' and AVISITN NE 1,
 by PARAM;
 class TRT01A PREVAC AGEGR;
 model AVAL = TRT01A BASE PREVAC AGEGR / solution clparm;
 lsmeans TRT01A/CL pdiff ADJUST=Tukey tdiff stderr alpha=0.05;
 ods output lsmeancl=_mcl LSMeanDiffCL=_dcl diff = _diff_adj;
run;
```

where AVAL represents the log10-transformed antibody value of the immunogenicity variable at Day 29, TRT01A indicates the vaccine group, BASE the log10-transformed antibody baseline value of the immunogenicity variable and PARAM the different vaccine strain.

Geometric Mean Titer and GMR at Day 1:

HI titer reported at baseline (Day1) will presented via summary statistics (i.e., geometric mean, 95% confidence interval of GMT, minimum, median, maximum) by vaccine group.

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

In addition, adjusted GMT and vaccine difference (i.e., GMR) at Day 1 will be computed based on GLM model with log_{10}-transformed Day 1 titers as the outcome variable and vaccine groups, age subgroup and previous vaccination history as covariates.

The following SAS code will be used for the ANOVA model:

```
proc glm data=<adis>;
 where substr(paramn,1,5) = 'Log10' and AVISITN = 1,
 by PARAM;
 class TRT01A PREVAC AGEGR;
 model AVAL = TRT01A PREVAC AGEGR / solution clparm;
 lsmeans TRT01A/CL stderr alpha=0.05;
 ods output lsmeancl=_mcl LSMeanDiffCL=_dcl;
run;
```

where AVAL represents the log10-transformed antibody value of the immunogenicity variable at Day 1 and PARAM the different vaccine strain.

Geometric Mean Fold Increase at Day 29:

Summary statistics (geometric mean, 95% confidence interval of GMT, minimum, median, maximum) of the relative increase in titers will be presented by assessment (Day 29), strain and vaccine group.

The analysis model for the fold increase in titers will be done using the same ANOVA model as mentioned above on log10- (Day 29 titers/Day 1 titers) as the outcome variable and vaccine groups, age subgroup and previous vaccination history as covariates.

The following SAS code will be used for the ANOVA model:

```
proc glm data=<adis>;
 by PARAM;
 class TRT01A PREVAC AGEGR;
 model AVAL = TRT01A PREVAC AGEGR / solution clparm;
 lsmeans TRT01A/CL stderr alpha=0.05;
 ods output lsmeancl=_mcl LSMeanDiffCL=_dcl;
run;
```

where AVAL represents the ratio between the log10-transformed antibody value Day 29/Day 1 and PARAM the different vaccine strain.

Analysis of binary endpoints:

The number and proportion of subjects achieving the binary endpoints (seroconversion or HI titer >=1:40) will be summarized by assessment (Day 29), strain and vaccine group. For each of the influenza vaccine strains and for each of the vaccine groups, summaries will include the associated two-sided 95% confidence intervals according to Clopper-Pearson.

The seroconversion rates will be compared between each formulation using the Miettinen and Nurminen method. The results will be presented as the difference in the percentage of subjects with 95% confidence intervals.

RCDC:

Reverse cumulative distribution plots will be generated to display the distribution of the antibody responses at Day 1 and Day 29 for each of the vaccine formulations. The x-axis represents the immunogenicity values, and the scale of the axis is logarithmic. The y-axis represents the percentage of subjects having at least that immunogenicity value. Due to the discrete values of antibody response, the plot will show a step-wise function. The figures begin at 100%, and then descends to the lowest point on the curve, which is the percentage of subjects having an immunogenicity value equal to the highest observed value.

11.3 Secondary Objectives Analysis

Secondary Immunogenicity Objective: To assess the immunogenicity of the different (a)QIVc formulations through pairwise comparisons and trend analysis as measured by MN assay using cell-derived target viruses at Day 29 after vaccination.

The same statistical models planned to be used for the primary objective will be applied for the secondary objective.

11.4 Exploratory Objectives Analysis

Exploratory Immunogenicity Objective:

- Percentages of subjects with HI titers ≥1:80 at Day 1 and Day 29
- Percentages of subjects with HI titers ≥1:160 at Day 1 and Day 29
- Percentages of subjects with HI titers ≥1:320 at Day 1 and Day 29

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 adverse events and indicators of solicited adverse events.
- Unsolicited adverse events.
- Serious AEs, non-serious MAAEs, AESIs and AEs leading to withdrawal from study.

13.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled 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 on day 1 and then daily until day 7 using structured eDiaries. The analyses of solicited adverse events will be done separately for the first 30 minutes (subjects observed at site immediately after vaccination) and based on following intervals: Day 1-3, Day 4-7, Day 1-7 by maximal severity and by vaccine group. In addition, solicited adverse events ongoing at day 7 will be collected until Day 8 and reported as well on time period Day 8-14 by maximal severity and by vaccine group.

The severity of solicited local AEs, will be summarized according to their grading.

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

Type I: *none (< 25 mm), any (≥100 mm)*

Type II: *Grade 0 (< 25 mm), mild [25-50 mm], moderate [51-100 mm], severe (>100 mm)*

Solicited local AEs measuring less than 25 mm will not be considered an AE.

Body temperature will be collected in Fahrenheit (°F) and converted in Celsius (°C) for the purpose of the analysis and reporting.

Body temperature will be used to define fever:

Any fever: [≥38°C), mild [38°C-38.4°C], moderate [38.5°C-38.9°C], severe [≥ 39°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.

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

- by 0.5 °C increments:
  - <36.0,
  - 36.0 - 36.4
  - 36.5 - 36.9
  - 37.0 - 37.4
  - 37.5 - 37.9
  - 38.0 - 38.4
  - 38.5 - 38.9
  - 39.0 - 39.4
  - 39.5 - 39.9
  - ≥40.0°C
- by 1.0 °C increments: <36.0, ≥36.0-<37.0, ≥37.0-<38.0, ≥ 38.0-<39.0, ≥39.0-<40, ≥40°C
- <38.0, ≥38.0 °C

The analyses of solicited local and systemic adverse events will be based of solicited safety set and encompass summaries of the data by vaccine group, overall and by age subgroup.:

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events (excluding 30 min measurement).
3. Solicited adverse events, maximum event severity by event and time interval (Day 1-3, Day 4-7, Day 1-7 and Day 8-14) (excluding 30 min measurement)
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 (Day 1-3, Day 4-7, Day 1-7 and Day 8-14) (excluding 30 min measurement).

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 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 (i.e. by day).

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 and induration, following threshold will be used: ≥ 25 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.

For each vaccination the first onset of the adverse event will be used for each subject.

For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, 'not done' is treated identical to 'missing'.

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 solicited adverse event is defined irrespective of severity. This means at least 'mild' solicited adverse event that are assessed qualitatively and ≥ 25 mm for erythema and induration. If a solicited adverse event continues beyond day 7 the period after day 7 is added.

The frequency distribution of the number of days will be provided in a summary table by vaccine and by solicited 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" (≥25 mm for erythema 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.

The use of antipyretics and analgesics will be summarized as "other" by type of use ('for prevention' or 'for treatment') as the number and percentage of subjects reporting use.

13.3 Unsolicited Adverse Events

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 up to 29 days after vaccination. Unsolicited AEs define as serious (SAE), or medical attended AE (MAAE), or leading to withdrawal or AEs of special interest (AESI) will be reported in eCRF until study completion (i.e., up to 6 months after vaccination)

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 29.
- Onset between Day 30 and Day 181(i.e., Study Termination).

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited AE.
- Possibly or probably related unsolicited AEs.
- Serious AEs.
- Possibly or probably related serious AEs.
- Unsolicited AEs leading to death.
- Unsolicited AEs leading to premature withdrawal from study.
- Unsolicited AEs leading to hospitalization.
- Unsolicited AEs of special interest.
- Unsolicited non-serious MAAEs.

Solicited adverse events continuing beyond day 14 will be captured and followed as unsolicited AE in eCRF and coded by MedDRA.

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

All tables will be produced overall and by age subgroup.

13.4 Combined Solicited and Unsolicited Adverse Events

A summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. 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 frequencies and percentages of subjects reporting concomitant medications will be tabulated overall and by vaccine group. Medications (generic drug name) will be coded using the WHODRUG dictionary (see section 8.7 for definition).

The frequencies and percentages of subjects reporting medication started before study and still ongoing at enrolment (pre-trial), concomitant medications during exposure (Day 1 – 29) and during follow up (from Day 30 to end of study) will be tabulated overall and by vaccine group, using all randomized set.

14. INTERIM ANALYSIS

Study design foresees two time periods: form Day 1 to Day 29 (treatment period), and from Day 30 to Day 181 (follow up). An interim analysis including all immunogenicity data as well as all safety data collected from Visit 1 (Day 1) to Visit 3 (Day 29) will be performed.

This early read-out will be done to inform phase 3 clinical development.

14.1 Interim Analysis

Interim analysis will include all immunogenicity and safety data collected from Day 1 up to Day 29 and associated primary objective analysis will be conducted on cleaned and locked data. No individual listings and unblinded single data will be generated at this stage. Access to information about treatment allocation will be limited by a biostatistician and statistical programmers in charge of statistical analysis. Other study team members will be group-unblinded and a 'first interpretable results report' will be produced.

The interim analysis is to be considered final with regards to immunogenicity and safety up to day 29. No decisions on the continuation of the study will be made based on the interim results, hence no statistical penalties will be applied.

Once all safety data up to day 181 will be collected, a full database lock and final and complete analysis will be performed. No changes are expected from the interim immunogenicity and safety analysis, nevertheless analysis populations may differ.

14.1.1 Futility Analysis

Not Applicable.

15. DATA MONITORING COMMITTEES

A Data Monitoring Committee (DMC) will not be used for this study.

16. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

The list of tables, listings and figures will be developed based on the final version 1.0 of this statistical analysis plan. This will be documented as part of the TLF shells.

Numbering will follow ICH E3 guideline on clinical study report

17. REFERENCES

Clinical Study Protocol V201_03 FINAL VERSION 1.0: 31 Mar 2022

Clopper CJ, Pearson ES. The use of confidential or fiducial limits illustrated in the case of the binomial. Biometrika 1934; 26:404-413.

ICH E3. Structure and content of clinical study reports, CPMP/ICH/137/95, July 1996.

ICH E9. Statistical Principles for Clinical Trials, CPMP/ICH/363/96, September 1998.

Iuliano AD, Roguski KM, Chang HH, Muscatello DJ, et al. Estimates of global seasonal influenza-associated respiratory mortality: A modeling study. Lancet. 2018 Mar;391(10127):1285-130

Miettinen O., Nurminen M. Comparative analysis of two rates. Statistics in Medicine 1985; 4(2):213-226.

Nauta J. Statistics in Clinical Vaccine Trials. 2010. Heidelberg.

Otten G, Matassa V, Ciarlet M, Leav B. A phase 1, randomized, observer blind, antigen and adjuvant dosage finding clinical trial to evaluate the safety and immunogenicity of an adjuvanted, trivalent subunit influenza vaccine in adults ≥65 years of age. Vaccine. 2020;38:578-587.

Reber AJ, Chirkova T, Kim JH, Cao W et al. Immunosenescence and challenges of vaccination against influenza in the aging population. Aging and Disease. 2012;3:68-90.

Sasaki S and Sullivan M. Limited efficacy of inactivated influenza vaccine in elderly individual is associated with decreased production of vaccine-specific antibodies. J Clin Invest. 2011;121(8):3109-3119.

Tukey JW, Ciminera JL and Heyse JF. Testing the statistical certainty of a response to increasing doses of a drug. Biometrics. 1985;41:295-30.

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

Wong SS and Webby RJ. Traditional and new influenza vaccines. Clin Microbiol Rev. 2013;26:476-492.

World Health Organization (WHO) 2019. WHO launches new global influenza strategy. March 2019.