STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 2, Randomized, Stratified, Observer-Blind Clinical Study to Evaluate Safety and Immunogenicity of the MF59-Adjuvanted Quadrivalent Subunit Inactivated Cell-derived Influenza Vaccine (aQIVc) in adults ≥50 years of age
Study Number:	V200_10
Protocol Version and Date:	1.0, 30 MAR 2020
Phase of Development:	Phase 2
Plan Prepared by:	[redacted]
Version and Date	Final 2.0, 16 MAR 2021
Approvers:	[redacted]

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
aQIV	MF59-adjuvanted Quadrivalent Subunit Inactivated egg-derived Influenza Vaccine
aQIVc	MF59-adjuvanted Quadrivalent Subunit Inactivated cell-derived Influenza Vaccine
CSR	Clinical Study Report
CRF	Case Report Form
CRO	Contract Research Organization
DMC	Data Monitoring Committee
FAS	Full Analysis Set
FDA	Food and Drug Administration
GMFI	Geometric Mean Fold Increase
GMR	Geometric Mean Ratios
GMT	Geometric Mean Titers
HI	Hemagglutination Inhibition
ICH	International Council for Harmonisation
IRT	Interactive Response Technology
IST	Internal Safety Team
LL	Lower Limit
LLOQ	Lower Limit of Quantification
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities

MN	microneutralization
PD	Protocol Deviation
PPS	Per Protocol Set
PT	preferred term
QIVc	Quadrivalent subunit inactivated cell-derived influenza vaccine
QIVr	Recombinant Quadrivalent influenza vaccine
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion rate
SD	standard deviation
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reactions
TFL	Tables, Figures and Listings
TOC	Table of Content

1. BACKGROUND AND RATIONALE

Vaccination is the primary method for preventing influenza and its severe complications. The efficacy in elderly individuals is lower due to the aging of the immune system and underlying medical conditions that can both increase the risk of influenza complications as well as interfere with immune response. 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 in older adults. The cell-derived antigen provides a better match to circulating strains, therefore addressing mismatch due to egg adaptation. The effect of inclusion of MF59 adjuvant to the vaccine is expected to enable increased magnitude of immune response by stimulating higher antibody responses at standard doses; increased breadth of immune response by expanding antibody repertoire, and increased duration of immune response with higher antibody response at 6 months after vaccination which is important in case of prolonged influenza seasons and/or late-season outbreaks.

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

This plan describes all details related to the statistical analysis of the data collected in the study V200_10 and is based on protocol version 30 March 2020.

This analysis plan is compliant with ICH Harmonized Tripartite Guideline, 5 February 1998, Statistical Principles for Clinical Trials, E9; World Health Organization, WHO Technical Report, Series No. 924. 2004, Annex 1: Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations; and FDA Center for Biologics Evaluation and Research (CBER) Guidance for Industry, May 2007, Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines.

2. OBJECTIVES

2.1 Primary Objective

2.1.1 Primary Immunogenicity Objective

1. To assess the immunogenicity of aQIVc in comparison with the QIVc, aQIV, and QIVr comparator vaccines as measured by HI assay* using *cell-derived* target viruses at 28 days after vaccination, in subjects 50-64 and ≥65 years of age and overall (adults ≥50 years).

* *In case of lack of 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.*

2.2 Secondary Objectives

2.2.1 Secondary Immunogenicity Objective(s)

1. To assess the immunogenicity of aQIVc in comparison with the QIVc, aQIV, and QIVr comparator vaccines as measured by HI assay* using *cell-derived* target viruses at 180 days after vaccination, in subjects 50-64 and ≥65 years of age and overall.
2. To assess the immunogenicity of aQIVc in comparison with the QIVc, aQIV, and QIVr comparator vaccines as measured by MN assay using *cell-derived* target viruses at 28 and 180 days after vaccination, in subjects 50-64 and ≥65 years of age and overall.

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

2.2.2 Secondary Safety Objectives

1. To assess the reactogenicity of aQIVc as compared to QIVc, aQIV, and QIVr in subjects 50-64 and ≥65 years of age and overall for 7 days after vaccination.
2. To assess the safety of study vaccines in subjects 50-64 and ≥65 years of age and overall for 180 days after vaccination.

2.3 Exploratory Objectives

1. The immunogenicity of aQIVc in comparison with the QIVc, aQIV and QIVr comparator vaccines as measured by HI assay may be assessed using *egg-derived* target viruses at 28 days after vaccination, in subjects 50-64 and ≥65 years of age and overall.
2. To explore the comparability of the cell-based and egg-based titers as measured by HI assay and the association with the vaccine or other subject characteristics.
3. Additional exploratory immunogenicity analyses may be conducted to further characterize the immune response of aQIVc.

3. STUDY DESIGN

The study is a phase 2, stratified, randomized, observer-blind, multicenter study to evaluate the immunogenicity and safety of aQIVc influenza vaccine compared with three other influenza vaccines (aQIV, QIVc and QIVr) in subjects ≥ 50 years of age.

Approximately 480 subjects ≥ 50 years, 120 per vaccine group, will be randomized to receive either one of the four vaccines in 1:1:1:1 allocation ratio, stratifying according to age (≥ 50 to 64 and ≥ 65 years), and previous vaccine history (previous 3 years yes or no). The distribution of all enrolled subjects across the two age subgroups should be about 50% in each. Approximately 40% of subjects in each age subgroup for each treatment group should not have been vaccinated with an influenza vaccine within the 3 previous influenza seasons.

Each subject will have two periods of study participation: Treatment Period (Day 1 to Day 29) and Follow-up Period (Day 30 to Day 181). The study procedures consist of three clinic visits (on Days 1, 29 and 181), and two safety phone calls on Days 7 and 91 (Table 1).

After an initial screening, subjects will be vaccinated with a single dose of aQIVc, aQIV, QIVc or QIVr on Day 1. All subjects will receive a Subject eDiary along with instructions to ensure proper completion and assessment on reactogenicity. Solicited AEs will be recorded daily for 7 consecutive days (or longer if the events are not resolved) following vaccination (Day 1 to Day 7).

Unsolicited AEs will be collected for 28 days after vaccination. SAEs, MAAEs, AEs leading to study withdrawal, and AESIs will be collected during the entire study period. These data will be captured by interviewing the subject during the clinic visits and safety phone calls and by review of available medical records. Subjects will be instructed to call the site in the event of any AE which they perceive as being of concern during the entire study period.

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

Table 1 Time and Event Schedule

Visit Type		Clinic Visit	Safety Phone Call	Clinic Visit	Safety Phone Call	Clinic Visit
Study Day		1	7	29	91	181
Visit Window (Days)		n/a	-1/+3 day	-7/+3 days	+/-7 days	+/-7 days
Visit Number		1	2	3	4	5
Study Event						
Study Treatment						
Vaccination		X				
Screening and Safety						
Informed Consent		X[a]				
Medical History[b]		X				
Pregnancy test[c]		X				
Physical Exam		X				
Targeted Physical Exam[d]		X		X		X
Measuring body temperature		X				
Exclusion/Inclusion Criteria		X				
Randomization		X				
30 Minutes Post Injection Assessment		X				
Train and Dispense Subject eDiary		X				

	Visit Type	Clinic Visit	Safety Phone Call	Clinic Visit	Safety Phone Call	Clinic Visit
	Study Day	**1**	**7**	**29**	**91**	**181**
	Visit Window (Days)	**n/a**	**-1/+3 day**	**-7/+3 days**	**+/-7 days**	**+/-7 days**
	Visit Number	**1**	**2**	**3**	**4**	**5**
Study Event						
Review of eDiary data and compliance		Ongoing during eDiary use				
Assess unsolicited AEs		X	X	X		
Assess SAEs		X	X	X	X	X
Assess for AEs leading to withdrawal, medically attended AEs and AESIs		X	X	X	X	X
Assess relevant medications and vaccinations		X	X	X	X	X
Immunogenicity						
Serology blood draw		X		X		X
Study Completion/termination						X[e]

Notes: [a] Consent form(s) should be signed prior to performing any procedures. The informed consent process may be conducted earlier, but within 10 days prior to Day 1. [b] Medical history includes existing comorbidities. [c] A pregnancy test should be done for females of childbearing potential in order to rule out any pregnancy. [d] Based on specific complaints as indicated by the subject. [e] Subjects who terminate the study early will be requested to complete all safety-related Study Completion procedures. Abbreviations: AE = adverse event; AESI = adverse event of special interest; n/a = not applicable; SAE = serious adverse event.

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Approximately 480 subjects ≥ 50 years will be randomized to receive either aQIVc or one of 3 comparator vaccines in a 1:1:1:1 allocation ratio, stratifying according to age (50 to 64 and ≥ 65 years) and previous vaccination (in 3 years before yes versus no). The number of subjects randomized will be an equal split between the age subgroups. Approximately 40% of subjects in each age subgroup for each treatment group should not have been vaccinated within the 3 previous influenza seasons.

The subject will be randomized in the IRT/RTSM system. The subject will receive a unique Subject ID that will be used for all eCRFs and associated study documentation for the duration of the study. The list of randomization assignments is produced by the service provider (IQVIA) and approved by the Sponsor according to the applicable Sponsor's Standard Operating Procedure (SOP).

4.1.1 Definition of Randomization/Vaccination Errors

The list below provides categories for errors that may occur during vaccination.

Randomization errors:

- Administered wrong kit (subject was vaccinated with a vaccine different from the one assigned at randomization).

Vaccination errors:

- Administered only part of the study vaccine.
- Incorrect vaccine location.
- Administered expired vaccine.
- Administered temperature deviated vaccine.

Stratification error:

- Subject randomized in the wrong stratification stratum.

Randomization and Vaccination errors are considered as major (CSR-reportable) protocol deviations. Stratification errors will not be considered as CSR-reportable PDs as there will no impact on the actual dose administered. Subjects will be included in analysis according to actual age subgroup and actual previous vaccination history.

4.1.2 Forced Randomization

Forced randomization will not be utilized in this trial

4.2 Blinding and Unblinding

The study is designed as an observer-blind study. Unblinded teams will be used for preparation of the safety reports for the Independent Safety Team (IST) and Data Monitoring Committee (DMC).

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.

A final analysis on the primary and the secondary objectives including all immunogenicity and safety data collected up to including the Day 29 Visit will be conducted on cleaned and locked data. Access to information about study groups will be limited by biostatisticians and programmers in charge of statistical analysis. No individual listings and unblinding data will be generated at this stage until the completion of the study.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

This study is an exploratory Phase 2 study without any formal hypothesis testing. The total sample size of 480 subjects has been determined by the feasibility of conducting the study in one season.

This sample size of n=108 per vaccine group – assuming 10% drop-out rates – is enough to obtain preliminary immunogenicity data.

For example, assuming a SD of log10-transformed HI titres as 0.45:

- In pairwise dose-group comparisons with n=108 per dose group it would be feasible to detect a difference of 1.49 in the GMT ratio with statistical power of 80% with the two sample T-test at significance level of 0.05.

- With n=108 per dose group; the precision of the GMT ratio expressed as 95% CI will be from 0.76 to 1.32 multiplied by the observed GMT estimate.

Table 2 illustrates the effects which can be detected depending (80% power and the two-sided significance level of 0.05) on different values for the inter-subject variation, as well as the size of the 95% two-sided confidence interval indicating the precision of the GMT ratio estimates.

For example, assuming a SD of log10-transformed HI titres as 0.45:

- In pairwise dose-group comparisons with n=108 per dose group it would be feasible to detect a difference of 1.49 in the GMT ratio with statistical power of 80% with the two sample T-test at significance level of 0.05.

- With n=108 per dose group; the precision of the GMT ratio expressed as 95% CI will be from 0.76 to 1.32 multiplied by the observed GMT estimate.

Table 2 Detectable GMT ratios (80% power, 5% two-sided test)

SD #	Log10 GMT Differences that can be detected	GMT ratios can be detected	95% confidence interval (times the GMT ratio)
0.45	0.172	1.49	0.76 to 1.32
0.50	0.191	1.55	0.73 to 1.36
0.55	0.211	1.62	0.71 to 1.40
0.60	0.230	1.70	0.69 to 1.45
0.65	0.250	1.77	0.67 to 1.49
0.70	0.268	1.85	0.65 to 1.54
0.75	0.287	1.94	0.63 to 1.59
0.80	0.306	2.02	0.61 to 1.64

Notes: # range of values of the standard deviation of the log10 titers based on studies V7P38 and V70P3 (data on file). Abbreviations: GMT= geometric mean titer. SD = Standard deviation.

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.

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 Biostatistician and the Clinical Program Director (CPD) 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 captured reported in monitoring reports. After the review, the Clinical Program Director 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.

7. ANALYSIS SET

7.1 All Enrolled Set

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

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

7.2 All Exposed Set

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

7.3 Full Analysis Set (FAS), Immunogenicity

All subjects in the All Enrolled Set who are randomized, received study vaccination and provided immunogenicity data on Day 1 and Day 29 assessment.

In case of vaccination error, subjects in the FAS sets will be analyzed "as-treated" (i.e., according to the vaccine the subject received).

If a subject is unblinded before the completion of the Day 29 visit, he/she will be included in the FAS.

7.4 Per Protocol Set (PPS), Immunogenicity

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subject is randomized).
- Have no CSR-reportable PD leading to exclusion (i.e. impacting the results) as defined prior to unblinding.
- Have immunogenicity assessments within the window of +/- 7 days around the Day 29 visit, and Day 1 sample taken within 3 days before vaccination.

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 is unblinded during the study (except for SUSAR), he/she will be excluded from the PPS.

7.5 Safety Set

Solicited Safety Set

All subjects in the All Exposed Set with any solicited adverse event data collected on the electronic diary, including temperature measurements or use of analgesics/antipyretics.

Unsolicited Safety Set

All subjects in the All Exposed Set with results of the unsolicited adverse event assessments recorded. 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 or in the Unsolicited Safety Set.

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).

7.6 Other Analysis Set

For the analysis of the immunogenicity data collected at Day 181 assessment, subsets of the FAS and PPS, immunogenicity will be used. Subjects will be only included if there was Day 181

immunogenicity data collected and disallowed concomitant medication impacting the results was not used.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The log-transformed antibody titers at Day 29 and Day 181 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 in previous 3 years, yes). Summary tables will show both adjusted and unadjusted GMTs for each vaccine group and adjusted and unadjusted GMT ratio for aQIVc versus each of the other three vaccine groups.

The main analysis of binary immunogenicity endpoints ((i.e., percentages of subjects with seroconversion and with 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. Sensitivity analysis may be done to include the stratification factors age and previous vaccination history as covariates in a generalized linear model.

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

Solicited adverse events are collected using electronic diaries from Day 1 to Day 7 post-vaccination. If data have not been recorded for all 7 days, the assessment is considered missing and excluded from the safety analysis. In case, at least one day is filled in but other days are missing the presence or absence of the event will be based on the available data. If more than 5% of the subjects have incomplete diary data, additional tables for solicited adverse events will be created based on complete diary card data. The number of days and number of subjects with missing data will be tabulated by treatment group.

8.3 Multicenter Studies

Stratification is used on two important factors related to the immunogenicity endpoints: age and previous vaccination history. These factors will be included in the statistical model.

Centers will not be used as stratification factor and will not be included in the statistical analysis.

8.4 Multiple Comparisons and Multiplicity

This a phase 2, exploratory study and no adjustment will be applied for multiple endpoints and multiple comparisons.

8.5 Subgroups

All analyses (safety and immunogenicity) will be done by the age subgroups 50-64 years or ≥65 years.

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

- Influenza vaccination history previous 3 years as yes and no;
- Comorbidity risk (yes/no) defined as Hak score < 50 or ≥50;
- Sex;

Exploratory interaction analyses may be conducted to support consistency or lack of consistency of the results among the subgroups.

8.6 Data Transformation

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

8.7 Derived and Computed Variables

Demographics

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)

The total HAK score will be derived by the sum of all scores (see protocol appendix 1). The total HAK score will be categorized as a score < 50 or ≥ 50.

Immunogenicity

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

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

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

= 0, otherwise

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

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

= 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 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 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 pre-vaccination and the event will be mapped to the medical history.

- If the partial start date is equal or after (≥) the first study vaccination (i.e., year or year and month is/are after or the same as the first study injection year or year and month) then the adverse event is emergent during vaccination phase.

All adverse events emergent during vaccination phase will categorized as occurring during the period of 28 days following vaccination based on the start date. If start date is missing or incomplete, 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.

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. The period Day 1- 29 will be labeled if in addition, the start date is on or before Day 29.

8.8 Analysis Software

All analyses will be performed using SAS Software version 9.5 or higher.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All randomized 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 minus date of vaccination plus 1) the subjects are under observation for safety will be summarized by vaccine group and overall.

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

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

10.1 Demographics

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

In addition, 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. The number and percentages of subjects by sex, ethnic origin, race, previous vaccination history (in past 3 years), and comorbidity risk (Hak score <50 and ≥50) will be presented by vaccine group and overall.

Demographic data will be tabulated for the All Enrolled, FAS, PPS and Safety sets.

The distribution across all stratification factors (age subgroup * previous vaccination history) will be presented by vaccine group and overall. This table will be presented based on the IRT data and – if different – also for the actual eCRF data.

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, PPS and Safety Sets.

11. IMMUNOGENICITY ANALYSIS

The immunogenicity analysis will be descriptive in nature and focus on the estimation of the treatment effects of aQIVc versus each of the three other vaccines.

11.1 Blood samples

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

11.2 Primary Objectives Analysis

The primary endpoints in terms of HI antibody response against homologous cell-derived vaccine strains (A/H1N1, A/H3N2, B/Yamagata and B/Victoria) are defined as:

- Geometric Mean Titer (GMT): Geometric mean of HI antibodies at Day 29 and Day 1;
- Geometric Mean Fold Increase (GMFI): The Geometric mean of the fold increase in serum HI GMTs post-vaccination (Day 29) compared to pre-vaccination (Day 1);
- Percentages of subjects with HI titers ≥ 1:40 at Day 29 and Day 1;
- Percentage of subjects with seroconversion (defined as a ≥ 4-fold increase in titer post-vaccination in those with pre-vaccination titer 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).

Note that the HI assay could be replaced by the MN assay due to a lack of agglutination.

In addition, reverse cumulative distribution plots will be generated to display the distribution of the antibody responses at Day1 and Day 29 for each of the vaccine groups. 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.

No hypothesis testing will be applied.

Geometric Mean Titer

Summary statistics (geometric mean, 95% confidence interval of GMT, minimum, median, maximum) of the titers will be presented by strain, assessment (Day 1 or Day 29) and vaccine group.

The analysis model used for the comparison of the HI (or MN) GMT between aQIVc and the other three vaccine groups will be done using a general linear model on log-transformed (base 10) Day 29 titers as the outcome variable and as covariates: treatment groups (aQIVc, QIVc, aQIV, and QIVr), log-transformed pre-vaccination titer, age subgroup and previous vaccination history. From this model, adjusted differences in the least square means (on the log scale) will be produced with 95% confidence limits for aQIVc versus QIVc, for aQIVc versus aQIV and for aQIVc versus QIVr. The estimated difference and the confidence limits will be back-transformed to obtain an *adjusted GMT ratio* with 95% confidence limits. Each of the four strains will be analyzed separately.

The same analysis model, except excluding the factor age subgroup, will be run for each age subgroup separately and and present all adjusted GMT ratios with 95% confidence intervals for each age subgroup.

Additional exploratory analysis will be considered using age as continuous covariable including the assessment of interaction between age and treatment group.

Unadjusted GMT ratio will be based on a simple ANOVA model on the log-transformed titers and the treatment group.

Geometric Mean Fold Increase

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 models as mentioned above on log-transformed (base 10) (Day 29 titers/Day 1 titers) as the outcome variable but excluding the log-transformed pre-vaccination titer.

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 binary endpoints (i.e. seroconversion or titer ≥1:40) will be compared between aQIVc and each of three other vaccine group using the Miettinen and Nurminen method without adjustment for the age subgroup. The results will be presented as the difference in the percentage of subjects with 95% confidence intervals. Additional supportive analyses will be done using generalized linear models with factors for vaccine group, age subgroup as well as previous vaccination history. Adjusted differences between vaccine groups with 2-sided 95% CI will be calculated based on the model. The same analyses will be run by age group separately excluding the factor age subgroup.

11.3 Secondary Objectives Analysis

The secondary endpoints in terms of HI antibody response against homologous cell-derived vaccine strains (A/H1N1, A/H3N2, B/Yamagata and B/Victoria) at Day 181, are:

- GMT: Geometric mean of HI antibodies at Day 181;
- GMFI: The Geometric mean of the fold increase in serum HI GMTs post-vaccination (Day 181) compared to pre-vaccination (Day 1);
- Percentages of subjects with HI titers ≥ 1:40 on Day 181.

In addition, the humoral immune response in terms of MN antibody response against homologous cell-derived vaccine strains (A/H1N1, A/H3N2, B/Yamagata and B/Victoria) at Days 29 and 181:

- GMT: Geometric mean of MN antibodies at Days 29 and 181;
- GMFI: The Geometric mean of the fold increase in serum MN GMTs post-vaccination (Day 29 and Day 181) compared to pre-vaccination (Day 1);
- Percentages of subjects with seroconversion (defined as ≥ 4-fold increase for subjects with pre-vaccination MN titers ≥ Lower Limit of Quantitation (LLOQ) or as ≥ 4*LLOQ for subjects with pre-vaccination MN titer <LLOQ) at Day 29.

The analysis of the secondary immunogenicity endpoints will be conducted in the same way as described for the primary immunogenicity endpoints.

11.4 Exploratory Objectives Analysis

Post hoc decision will be made to measure titers on Day 1 and Day 29 assessments by the HI assay using *egg*-derived target viruses. These data will be subject to an exploratory analysis along the same methods as for the primary immunogenicity endpoints. Additional analyses may be done on the comparability of the cell and based titers. Details on this exploratory analysis will be included in a separate statistical analysis plan. The results may be presented in an addendum to the CSR.

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;
- Unsolicited adverse events;
- Serious AEs, MAAEs, AESIs and AEs leading to withdrawal from study.

13.1 Analysis of Extent of Exposure

The frequencies of subjects with vaccinations will be summarized overall and by vaccine group and by age group. Data will be tabulated for the All Exposed Set.

13.1.1 Safety Completeness

Analysis Solicited Adverse Events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed the electronic diaries. The analysis will show the number of subjects with results by solicited adverse event and day.

Three summaries will be produced:

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

For the corresponding percentages, the denominator will be the respective number of subjects vaccinated (All Exposed Set).

All analyses will be based on the ‘as treated’ analysis set.

13.2 Solicited Local and Systemic Adverse Events

Each solicited AE is to be assessed for Day 1 to Day 7 according to a defined severity grading scale; see specifics of the solicited event and grading system below in Table 3.

Table 3 Severity Grading for Solicited Local and Systemic Adverse Events

		Any Event		
Type	**Solicited Event**	**Grade 1/Mild**	**Grade 2/Moderate**	**Grade 3/Severe**
Local	Injection site pain	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Erythema	25-50 mm	51-100 mm	> 100 mm
	Induration	25-50 mm	51-100 mm	> 100 mm
Systemic	Loss of appetite	Eating less than usual with no effect on normal activity	Eating less than usual /interfered with normal activity	Not eating at all
	Nausea	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Fatigue	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Myalgia	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Arthralgia	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Headache	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Chills	No interference with daily activity	Interferes with daily activity	Prevents daily activity
	Fever	38.0 - 38.4 °C 100.4 – 101.1 °F	38.5 – 38.9 °C 101.2 – 102.0 °F	≥39.0 °C ≥102.1 °F

Note: presence of an event on a day is defined as grade 1, 2 or 3; absence is defined as non-missing and not present. Erythema and Induration: grading will be derived from the actual measurements in mm; Fever will be derived from the actual temperature converted to °C in one decimal.

Other Indicators of Reactogenicity

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

The analyses will be based on the solicited safety set and encompass various summaries of the data by vaccine group, overall and by age subgroup.

1. Overall summary of subjects with solicited adverse events
2. Local Solicited adverse events, maximum event severity by event and time interval
3. Systemic Solicited adverse events, maximum event severity by event and time interval
4. Number of days of solicited adverse events, including ongoing AE after Day 7
5. Daily reports of subjects with solicited adverse events.
6. Day of first onset of solicited adverse events
7. Ongoing adverse events at Day 7
8. Distribution of maximum temperature
9. Other use of analgesics/antipyretics.

For each of the time points or time intervals presented in the summaries, only subjects with at least one valid observation (i.e., any non-missing values) for the solicited adverse events in the interval of interest will be considered. Subjects without valid data will be removed from the denominator to prevent a downward bias (towards zero).

All tables are run by vaccine group, overall and by age subgroup.

Overall summary of subjects with solicited adverse events.

Any solicited adverse event presence is defined as at least one day recorded a presence of a local or a systemic adverse event. No solicited adverse event is defined as for all days 'No' for all pre-defined solicited adverse events.

Note that, where applicable, missing values for all days for all events will be excluded from % calculations.

The use of analgesics/antipyretics will be considered in this summary as a separate category under "other", however will be considered as part of reactogenicity.

Local Solicited adverse events, maximum event severity by event and time interval

The **maximum event severity** will be defined if there is at least one non- missing observation within this time interval. Each subject's data will be aggregated across the time points of the interval and summarized according to the maximal severity observed for each local adverse event, followed by a summary across subjects for each vaccine. Subjects with missing values at each of the requested time points, will be excluded.

The time intervals will be Day 1 to Day 7, Day 1 to Day 3 and Day 4 to Day 7. A summary table will be created with the frequency using only any local solicited AE and severe local solicited events.

Systemic adverse events, maximum event severity by event and interval

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

Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. If a solicited adverse event continues beyond day 7 the period after day 7 is added.

The frequency distribution of the number of days (including mean and median values) will be provided in a summary table by vaccine and by adverse event.

Daily reports of solicited adverse event

For each of the time points only subjects with at least one non-missing observation for the solicited adverse event in the interval of interest will be considered. Subjects with missing 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, and time point (i.e. by day).

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 day at which the respective solicited adverse event first occurred. 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, as well as mean and median day of onset.

Adverse events ongoing at Day 7

For each of the solicited adverse events, the number and proportion of subjects in the solicited safety set reported the event ongoing at Day 7 will be summarized.

Distribution of maximum temperature

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

Use of analgesics/antipyretics

The use of antipyretics and analgesics will be summarized as "other" by type of use (prophylactic versus treatment) as the number and percentage of subjects reporting use.

13.3 Unsolicited Adverse Events

This analysis applies to all AE's occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in the AE eCRF, with a start date on (but with onset after vaccination) or after the date of vaccination. AE starting prior to study vaccination will only be listed.

The original verbatim terms used by investigators to identify adverse events in the eCRFs will be mapped to preferred terms using the MedDRA dictionary.

The adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. All reported AEs, as well as AEs judged by the investigator as at

least possibly related to study vaccine, will be summarized according to system organ class and the preferred term within system organ class. These summaries will be presented by the vaccination group. When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

The assignment to safety follow-up time intervals will be done by day of onset and not by days ongoing/persisting.

The summaries will be presented by period of onset (Day 1 to Day 29, Day 30 to end of study or Day 1 to end of study). An overview summary will be created by time -period with number and % of subjects with the following categories:

- AE
- AE by maximum severity
- Related (i.e. at least possible) AE
- SAEs
- Related SAEs
- AESIs
- MAAEs
- AEs leading to withdrawal from study
- Deaths.

The following summaries will be produced by SOC and Preferred Term and by time-period (Day 1 to Day 29, Day 30 to end of study or Day 1 to end of study):

- AEs
- AEs by maximum severity
- AEs that are possibly or probably related to the vaccine
- SAEs
- SAEs that are possibly or probably related to the vaccine

- AESIs
- MAAEs
- AEs leading to withdrawal
- Deaths.

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 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 based on the Overall Safety set.

Pausing rules

Review of safety data will be conducted at an ongoing basis to check if the pre-defined pausing rules have been met. A summary table will be created with number and % of subjects who met one of the criteria by treatment based on the Overall Safety set.

- Any SAE that cannot be reasonably attributed to a cause other than vaccination, according to the Investigator's assessment, within 28 days post-vaccination (i.e. probable).
- Severe systemic hypersensitivity such as anaphylaxis, within 24 hours after study vaccination.
- Any Grade 3 (severe) local solicited AE lasting ≥2 consecutive days, within 7 days postvaccination.
- Any Grade 3 (severe) systemic solicited AE lasting ≥2 consecutive days, within 7 days postvaccination.
- Any severe unsolicited AE that cannot be reasonably attributed to a cause other than vaccination (i.e. probable), within 7 days postvaccination.

13.5 Clinical Safety Laboratory Investigations

Not applicable.

13.6 Concomitant Medication

Medications and vaccines taken prior or during the study are categorized as pre-trial and/or concomitant. In addition, a subset of concomitant medications is defined for the period Day 1 to 29 (see section 8.7 for definition).

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

The frequencies and percentages of subjects reporting pre-trial, concomitant medications (Day 1 – 29 and Day 1 to end of study) will be tabulated by vaccine group.

14. INTERIM ANALYSIS

There are no planned interim analyses for this study.

The statistical analyses will be performed stepwise in two parts, as follows:

1. A final analysis including all immunogenicity and safety data collected from Visit 1 to Visit 3 (Day 29) and associated primary and secondary objectives will be conducted on cleaned and locked data. No individual listings and unblinding data will be generated at this stage. Access to information about study groups will be limited by a biostatistician in charge of statistical analysis.

2. The analysis of immunogenicity and safety data collected between Visit 3 and Visit 5 will be performed after all data is available. The results of this analysis will cover the entire study duration. All results will be presented in the clinical study report and will include individual data listings and unblinded information.

15. DATA MONITORING COMMITTEES

An internal safety team (IST) – independent of the study team - will be installed to monitor the safety data collected during the enrollment of the subjects in the study. Details are included in the IST charter. In addition, upon request an independent DMC will be asked to review the safety data and make recommendations for the conduct of the trial. Further details are described in the DMC charter.

16. CHANGES TO PLANNED ANALYSIS

Compared to the final protocol, one optional exploratory analysis has been added on the comparability of the cell-based and egg-based titers. Details will be described in a separate analysis plan. For the primary endpoints, the GMT and the % of subjects with HI >=1:40, Day 1 was added to assure that we include the pre-vaccination results.

The statistical analysis plan was amended before unblinding. The following changes since version 1.0 16 Jun 2020 were made:

Section	Change	Rationale
8.7	Unsolicited adverse events before vaccination will be mapped to medical history	This was requested by the US regulatory agency (FDA CBER).
11.2	Analysis of GMT ratio: Adjusted estimates of GMT ratio by age subgroup will be calculated using the same model excluding the factor age subgroup.	All subgroup analyses, including the age subgroup, will be conducted in a similar way by subgroup.
11.2	Analysis of binary endpoints: Additional supportive analyses will be done using generalized linear models without the factor for pre-vaccination titer.	Factor pre-vaccination titer was removed from the model due expected convergence issue leading to non-estimable treatment effects.
13.2	Clarification added that the temperature will be converted to °C in one decimal.	Needed for programming to apply the grading for the temperature

17. 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.

18. REFERENCES

Clinical Study Protocol V200_10 FINAL VERSION 1.0: MARCH 2020

ICH. Statistical Principles for Clinical Trials, Guideline E9, 1998.

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.

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