

STATISTICAL ANALYSIS PLAN

Study Title: A Phase 2, Randomized, Study to Evaluate Safety and Immunogenicity of One or Two Heterologous Booster Vaccinations With an MF59-adjuvanted, Cell Culture-derived H5N6 Influenza Vaccine in Adults Primed With MF59-adjuvanted, Cell Culture-derived H5N1 Influenza Vaccine or Unprimed.

Study Number: V89_18E1

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
aH5N1c	MF59-adjuvanted Monovalent A/H5N1 Influenza Vaccine
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
CBER	Center for Biologics Evaluation and Research
CI	Confidence Interval
CSR	Clinical Study Report
DMC	Data Monitoring Committee
EDC	Electronic Data Capture
eCRF	electronic Case Report Form
EMA	European Medicines Agency
FAS	Full Analysis Set
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
GMT _r	Geometric Mean Titer ratio
HA	Hemagglutinin
HI	Hemagglutination Inhibition
ICH	International Council for Harmonisation
ID	Identification
IRT	Interactive Response Technology
ITT	Intention-to-Treat
MAAE	Medically Attended Adverse Event(s)
MCAR	Missing Completely At Random
MedDRA	Medical Dictionary for Regulatory Activities
MF59	MF59C.1 adjuvant
MN	Microneutralization
NOCD	New Onset of Chronic Disease
PD	Protocol Deviation
PI	Principal Investigator

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PPS	Per Protocol Set
PT	Preferred Term (MedDRA)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCR	Seroconversion rate
SE	Standard Error
SD	Standard Deviation
SDA	Source Document Agreement
SOC	System Organ Class
SOP	Standard Operating Procedure
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reactions
TFL	Tables, Figures and Listings
TOC	Table of Content
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

This study is designed to investigate whether two priming doses of MF59-adjuvanted A/turkey/Turkey/1/2005 (H5N1, clade 2.2) cell culture-derived vaccine (aH5N1c) followed by one or two booster vaccinations with a MF59-adjuvanted H5N6 (A/Guangdong/18SF020/2018 (H5N6)-like CDC/CNIC [clade 2.3.4.4h]) cell culture-derived vaccine (aH5N6c) 3 weeks apart elicit immune responses to the antigens used for priming (H5N1) and boosting (H5N6) after first and second heterologous booster vaccination.

Seqirus recognizes the need for a viable approach to providing heterologous coverage protection to first responders (who will be on the front line of a pandemic response) during the interval between the declaration of a pandemic and the availability of a strain matched vaccine (typically around four months). Zoonotic (formerly known as pre-pandemic) vaccines can play an important role in managing the unpredictability of a pandemic.

An influenza A/turkey/Turkey/1/2005 NIBRG-23 strain (H5N1) cell culture-derived vaccine (AUDENZTM, Seqirus Inc.) combined with MF59C.1® oil-in-water emulsion adjuvant has been approved in the United States (US) for active immunization of persons 6 months and older for the prevention of disease caused by the influenza A virus H5N1 subtype contained in the vaccine. Two doses of this MF59-adjuvanted cell culture-derived H5N1 vaccine, given at a 21-day interval, produce antibody levels against the vaccine-homologous virus that meet US regulatory criteria, as well as substantial levels of cross-reactive antibodies against viruses of other H5N1 clades ([Frey et al. 2019](#); [Chantavanich et al. 2019](#)).

For further details on the background and rationale of the study, please refer to Section 1.0 of the protocol.

This statistical analysis plan describes the data and variables to be summarized and analyzed, including specifics of the statistical analyses to be performed and is based on protocol version Version 1: 17 DEC 21. Any deviations from the current statistical plan and changes in the conduct or planned analysis will be described and justified in the final Clinical Study Report (CSR).

It 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; FDA Center for Biologics Evaluation and Research (CBER) Guidance for Industry, May 2007, *Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines*; and *Guideline on Influenza Vaccines Non-clinical and Clinical Module*, 21 July 2016 (EMA/CHMP/VWP/457259/2014).

2. OBJECTIVES

2.1 Primary Objective

2.1.1 Primary Immunogenicity Objective

The primary immunogenicity objective is to assess immune responses against H5N6 (contained in the vaccine) as measured by hemagglutination inhibition (HI)¹ assay 1 week (Day 8) and 3 weeks (Day 22) after the first heterologous aH5N6c booster vaccination, and 3 weeks (Day 43) after the second heterologous aH5N6c booster or placebo vaccination.

2.2 Secondary Objectives

2.2.1 Secondary Immunogenicity Objectives

- a. To assess immune responses against H5N1 (contained in the priming vaccine) as measured by HI assay 1 week (Day 8) and 3 weeks (Day 22) after the first heterologous aH5N6c booster vaccination, and 3 weeks (Day 43) after the second heterologous aH5N6c booster or placebo vaccination.
- b. To assess persistence of immune response against H5N1 (contained in the priming vaccine) as measured by HI assay at Day 1 (pre-vaccination) and at Day 202, 6 months after the second heterologous aH5N6c booster or placebo vaccination.
- c. To assess persistence of immune response against H5N6 (contained in the booster vaccine) as measured by HI assay at Day 202, 6 months after the second heterologous aH5N6c booster or placebo vaccination.

2.2.2 Secondary Safety Objectives

To assess the safety and reactogenicity of aH5N6c vaccine.

2.3 Exploratory Objectives

To further evaluate immune responses to seasonal and/or homologous and/heterologous pandemic influenza strain(s), such as measured by the HI, microneutralization (MN), and single radial hemolysis (SRH) (depending on availability of blood samples and on assay availability).

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

3. STUDY DESIGN

3.1 Overview of Study Design

This is a Phase 2, randomized, multi-center study in approximately 300 adults who received 2 doses of aH5N1c or placebo in and completed the parent study V89_18 in the <65 years of age cohort.

Eligible subjects, who received 2 doses of aH5N1c in the parent study V89_18 will be randomized in a 1:1 ratio to receive either two aH5N6c vaccinations, 3 weeks apart (Group 1) or an aH5N6c vaccination on Day 1 and saline placebo on Day 22 (Group 2). Eligible subjects, who received placebo in the parent study will receive two aH5N6c vaccinations, 3 weeks apart (Group 3), see table below.

Table 3-1: Study Design

Treatment group	Treatment in parent Study V89_18	Treatment schedule in extension study V89_18E1		Total planned (N)
		Day 1	Day 22	
Group 1	aH5N1c	aH5N6c	aH5N6c	100
Group 2	aH5N1c	aH5N6c	Placebo	100
Group 3	Placebo ²	aH5N6c	aH5N6c	100

Immunogenicity will be measured by HI assay. Blood samples for immunogenicity assessments will be collected from each subject on Day 1 (before vaccination), Day 8, Day 22 (before vaccination), Day 43, and Day 202. The total study duration will be approximately 7 months per subject.

For all subjects, study participation includes a total of 5 clinic visits, 2 diary completion reminder calls, and 2 safety telephone calls through the treatment and follow-up periods.

- Treatment period (Day 1 through Day 43): 4 clinic visits and 2 diary completion reminder calls.
- Follow-up period (Day 44 through Day 202): 1 clinic visit and 2 safety telephone calls.

A detailed schedule and listing of procedures are shown in Table 3-2, Time and Events Table.

For further details please refer to [section 3.0 of the protocol](#).

² In case insufficient numbers of subjects who received 2 doses of placebo in the V89_18 parent study can be included, A/H5 naïve subjects may be enrolled (i.e. did not receive an influenza H5 vaccine in the past or have a history of H5 influenza infection prior to enrollment).

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

Visit Type	Clinic Visit	Diary Reminder Phone Call	Clinic Visit	Clinic Visit	Diary Reminder Phone Call	Clinic Visit	Phone Call	Phone Call	Clinic Visit
Study Day ^a	1	V1+3 (Day 4)	V1+7 (Day 8)*	V1+21 (Day 22)	V3+3 (Day 25)	V3+21 (Day 43)*	V3+70 (Day 92)	V3+120 (Day 142)	V3+180 (Day 202)*
Visit Window (Days)	n/a	-1 to +1	0 to +2	-1 to +7	-1 to +1	-1 to +7	-7 to +7	-7 to +7	-14 to +14
Visit Number	1	n.a.	2	3	n.a.	4	5	6	7
Study Event									
Study Treatment									
Vaccination	X			X					
Screening and Safety									
Informed Consent ^b	X								
Medical History ^c	X								
Physical Exam ^d	X		X	X		X			X
Pregnancy Test ^e	X			X					
Exclusion/Inclusion Criteria	X			X					
Randomization	X								
30 Minutes Post Injection Assessment	X			X					
Subject Diary Dispensed with Training	X			X					
Subject Diary Reminder Call		X			X				
Subject Diary Reviewed and Collected			X			X			
Assess all AEs	X		X	X		X			
Assess SAEs	X		X	X		X	X	X	X
Assess AEs leading to withdrawal, and AESIs	X		X	X		X	X	X	X
Assess relevant medications	X		X	X		X	X	X	X
Immunogenicity									
Serology blood draw	X ^f		X	X ^f		X			X
Study Completion Procedure									
Study Completion ^g									X
Notes: *In the exceptional case that a clinic visit is not possible due to the site being closed, with appropriate sponsor approvals a home visit may be considered. ^a Visit 1 (vaccination visit) is the baseline for calculating visits 2 and 3; Visit 3 is the baseline for calculation of all following visits ^b Consent form should be signed prior to any procedures. The informed consent process may be conducted earlier, but within 10 days prior to Day 1; ^c Medical history includes existing comorbidities ^d A physical examination will be based on a review of systems, ie, a structured interview for complaints for each organ system; ^e A pregnancy test should be done for females of childbearing potential in order to rule out any pregnancy; ^f Blood sample for serology to be taken after temperature measurement, but prior to vaccination; ^g Subjects who terminate the study early will be requested to complete all safety-related Study Completion procedures.									

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Enrollment will be managed with Interactive Response Technology (IRT) system using the subject identification number (ID) assigned to the subject in the parent study V89_18. The Subject ID will be the subject's unique identification number for all eCRFs and associated study documentation that will be used for duration of the study. The list of randomization assignments is produced by the IRT service provider and approved by Seqirus according to applicable Seqirus Standard Operating Procedure (SOP).

Enrolled subjects who received 2 doses of aH5N1c in the parent study V89_18 will be randomized in a 1:1 ratio to receive either two aH5N6c vaccinations or an aH5N6c vaccination (Day 1) and saline placebo (Day 22). Enrolled subjects who received placebo in the parent study will receive two aH5N6c vaccinations. A validated randomization system will be used.

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

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

For further details please refer to [section 5.1.4 of the protocol](#).

4.1.1 Definition of Randomization/Vaccination Errors

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

- Subject was administered a vaccine different from the one assigned at randomization
- Subject was administered the correct vaccine but received a lower volume

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 treated for Safety.

Please see [section 7](#) of this document for a complete guidance on how vaccination errors are handled in the statistical analysis of immunogenicity and safety.

4.1.2 Forced Randomization

Not applicable.

4.2 Blinding and Unblinding

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

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

All personnel involved in processing samples and performing laboratory assays and other personnel who are directly involved in the conduct of the study or in the analysis of the final study results, or have contact with study centers, will remain blinded to the treatment codes until the database has been locked for final analysis.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

The sample size was chosen to provide sufficient power to enable reliable descriptive statistics and detection of differences between treatment groups. No formal power calculations were performed for these analyses.

The table below shows the statistical power to demonstrate the GMT ratio superiority (margin of 1) assuming that the true GMT ratios between two groups are 2, 3 or 4 and that the null GMT ratio is 1 under a two-sided test with 5% level of significance, with sample sizes of 50 and 100 evaluable subjects per group and a standard deviation of 0.5 to 1 for the log₁₀ HI titers:

Table 5-1: Power calculation comparing GMT between treatment groups using GMT ratio

SD	Sample Size					
	50	100	50	100	50	100
	GMT _r =2		GMT _r =3		GMT _r =4	
0.50	84.6	98.9	99.7	100	100	100
0.60	70.0	94.2	97.6	100	98.9	100
0.70	56.7	85.7	92.1	99.8	98.9	100
0.80	46.1	75.4	83.9	98.7	96.1	100
0.90	38.1	65.3	74.7	96.2	91.2	99.7
1.0	31.9	56.3	65.6	91.9	84.6	98.9

Thus with 100 evaluable subjects (sample size=112 with 10% drop off rate), the power to detect the differences in GMT using GMT ratio between any two groups is at least 85.7% with SD of the log₁₀ HI titer not greater than 0.7.

With a Safety Population of 100 evaluable subjects, AEs with population rates of 1 in 50 have an 86.5% probability of being detected. Events with population rates of 1 in 20 have a 99.3% chance of being observed with n=100. Events with population rates of 1 in 34 have a 94.7% chance of being observed with n=100.

Sample size calculations were performed using PASS 2021.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

Major 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 major and minor.

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

Major 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.

6.2 Determination of Protocol Deviations

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

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

Prior to database lock and unblinding, all PDs will have been reviewed and categorized regularly throughout the study. A Blinded Data Review Meeting will take place to discuss and confirm assignment of subjects to analysis sets based on the final protocol deviations. Decisions will be captured in a Data Review Report which is including an overview of analysis sets. This will be signed off by at least the Sponsor / CRO Biostatistician and the Clinical Scientist prior to unblinding.

Classification of protocol deviations (and associated actions for analysis sets) that can only be assessed after unblinding, e.g., administration of study drug from the wrong treatment arm, will be discussed as much as possible before unblinding considering all potential scenarios. However, the final assessment can only be confirmed based on the unblinded locked database. Therefore, final classification of such PDs will be carried out after unblinding. Documentation will be signed off again by at least the Sponsor / CRO Biostatistician and the Clinical Scientist.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events will be directly measured by the subject and will not be subject to a reconciliation process, even if they are biologically implausible.

Therefore, these implausible measurements will be removed from the analysis but included in listings. Implausible measurements are summarized in the table below:

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	$\leq 33^{\circ}\text{C}$ or $\geq 42^{\circ}\text{C}$
Erythema	Measurements ≥ 900 mm Measurements < 0 mm
Induration	Measurements ≥ 500 mm Measurements < 0 mm
Ecchymosis	Measurements ≥ 500 mm Measurements < 0 mm

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). Data for any subject not randomized after enrollment will be displayed under "not randomized" as treatment group.

7.2 All Exposed Set

All subjects in the All Enrolled Set who receive a study vaccination. In case of vaccination error, subjects will be analyzed as "treated".

7.3 Full Analysis Set (FAS), Immunogenicity Set

All subjects in the All Enrolled Set who are randomized, received at least one dose of study vaccination and provided immunogenicity data on Day 1 and on at least one post-vaccination assessment. The post-vaccination assessments are Day 8, Day 22, Day 43 and Day 202.

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). Any subject who received the wrong vaccination will not be excluded from the FAS.

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

7.4 Per Protocol Set (PPS), Immunogenicity Set

All subjects in the FAS Immunogenicity who:

- Correctly receive both vaccinations (i.e., receive the vaccine to which the subject is randomized and at the scheduled time points)
- Have no major PD leading to exclusion (See SAP Section 6 Determination of Protocol Deviations) as defined prior to unblinding or analysis.
- Have immunogenicity results (of the same strain, H5N6 or H5N1) on Day 1 (before first vaccination) and at least one of post-vaccination visits (Day 8, Day 22 or at Day 43) within the defined window around the planned visits.

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

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

4 subsets of PPS will also be defined per objective and timepoint:

- PPS-1 will include all subjects in PPS with a Day 8 immunogenicity result who have no major PD prior to Day 8 blood sample collection.
- PPS-2 will include all subjects in PPS with a Day 22 immunogenicity result who have no major PD prior to Day 22 blood sample collection
- PPS-3 will include all subjects in PPS with a Day 43 immunogenicity result who have no major PD prior to Day 43 blood sample collection
- PPS-4 will include all subjects in PPS with a Day 202 immunogenicity result who have no major PD prior to Day 202 blood sample collection.

7.5 Safety Set

Solicited Safety Set

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

Unsolicited Safety Set

All subjects in the All Exposed Set with unsolicited AE data. A record of safety assessment performed at a specific time point, with confirmation of no AE, is considered as AE 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 post-vaccination safety data will also be reported separately in a 30-minute post-vaccination safety analysis.

In case of vaccination error, subjects will be analyzed as “treated” (i.e., according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized).

7.6 Other Analysis Set

Not applicable.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

Adjusted estimates of geometric mean titers (GMTs), and their associated 95% confidence intervals (CIs) at Day 8, Day 22, Day 43 and Day 202 will be determined using analysis of covariance (ANCOVA) with factors for vaccine group (Group 1, Group 2, Group 3), gender and a covariate for the effect defined by the log-transformed pre-vaccination antibody titer. Geometric mean titers (GMTs) for Day 1 will be determined using a similar ANCOVA model, including factors for vaccine group (Group 1, Group 2, Group 3) and gender.

The main analysis of binary immunogenicity endpoints (i.e., percentages of subjects with seroconversion, percentage of subjects with a titer greater than 1:40) will not be adjusted for any of the covariates.

8.2 Handling of Dropouts, Missing Data

Immunogenicity Data

Missing immunogenicity values are considered ‘missing completely at random (MCAR)’ and therefore will not contain information that impact the result of the analysis (i.e., not informative). Imputation methods will therefore not be used. Visit-specific per-protocol sets will be used to minimize the effect of dropouts and missing data on the statistical analysis of immunogenicity.

Values below the HI limit of quantification (recorded as “< 10”) will be set to half that limit (i.e. 5) and the values higher than the upper LOQ will be set as the upper value, if any.

Safety Data

No imputation of missing solicited or unsolicited AEs will be used. The percentage of subjects with missing solicited AE assessments and missing safety phone calls or safety assessment, including subjects who discontinued the study, will be reported for each time period. The number of days and number of subjects with missing diary data will be tabulated by vaccine group.

8.3 Multicenter Studies

There will be no adjustment for multiple centers.

8.4 Multiple Comparisons and Multiplicity

There is no need for adjustment for multiplicity and multiple comparisons.

8.5 Immunogenicity Subsets

Not applicable.

8.6 Subgroups

Primary and secondary analyses of immunogenicity endpoints will be performed by stratifying for the following subgroups:

- baseline HI (or MN) titer $<1:10$ or $\geq 1:10$;
- with and without seasonal influenza vaccine in the last 12 months;
- age (18 to <50 and ≥ 50 years of age);
- gender;
- race;
- ethnicity;
- center.

Safety analysis will be stratified by the following subgroups:

- subjects by age (18 to <50 and ≥ 50 years of age);
- gender;
- race;
- ethnicity.

8.7 Data Transformation

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

8.8 Derived and Computed Variables

Demographics

Age will be calculated in years using the following formula:

$$\text{Age (years)} = (\text{Date of Visit 1} - \text{Date of Birth} + 1) / 365.25, \text{ round to smallest integer}$$

Body Mass Index (BMI, kg/m²) will be calculated using the following formula:

$$\text{BMI} = \text{Weight (kg)} / \text{Height}^2 (\text{m}^2)$$

Immunogenicity

Values below the HI (or MN) limit of quantification (LOQ; recorded as “< 10”) will be set to half that limit (i.e. 5) and the values higher than the upper LOQ (ULOQ) will be set as the ULOQ.

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

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

= 0, otherwise

HI (or MN) antibody titer $\geq 1:40$ is defined as binary variable for subjects with non-missing values as:

= 1, if achieving a HI (or MN) antibody titer $\geq 1:40$

= 0, otherwise

Seroconversion based on **HI (or 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 or equal the LLOQ (1:10), or a post-vaccination titer $\geq 1:40$ for subjects with pre-vaccination titer below the LLOQ (1:10))

= 0, otherwise

Geometric Mean Titer

The GMT will be calculated using the following formula:

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

where t_1, t_2, \dots, 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 \log_{10} -transformed titers, respectively.

Geometric Mean Fold Increase

Geometric mean fold increase (GMFI) measures the changes in HI (or MN) titers *within* subjects.

The GMFI will be calculated using the following formula:

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

where, for n subjects, v_{ij} and v_{ik} are observed immunogenicity titers for subject i at time-points j and k , $j \neq k$. The 95% confidence intervals for GMFI will be calculated as $10^{\{M - t_{0.975, n-1} SE\}}$, $10^{\{M + t_{0.975, n-1} SE\}}$; where M and SE are the means and standard error of \log_{10} -transformed GMT fold increase, respectively.

Duration in the Study

Time under observation for safety is defined in days as:

$$\text{Last visit date (visit 7)}^3 - \text{First vaccination date} + 1$$

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

Solicited Adverse Events

Body temperature will be converted to Celsius prior to applying grading.

For details see section 13.2.

Unsolicited Adverse Events

All adverse events will be characterized according to the date of occurrence related to the treatment period (first vaccination to 21 days post vaccination 2) as follows:

- **Pre-vaccination (will be mapped to Medical History):** start date before the first study vaccination.
- **Emergence after vaccination:** all other cases.

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

³ or premature discontinuation date (in case of withdrawal from the study)

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 treatment period using the following rules:

- If the partial end date is before ($<$) the first vaccination (i.e., year or year & month is/are before the study vaccination year or year & month) then the AE is pre-vaccination.
- If the partial start date is equal or, after (\geq) the first study vaccination and prior to Day 43 (i.e., year or year & month is/are after or the same as the first study injection year or year & month) then the adverse event is emergent during treatment period.
- If the partial start date is after ($>$) Day 43 (or 21 days post vaccination 2) (i.e., year or year & month is/are after Day 43 (or date of 21 days post-vaccination 2) year or year & month) then the adverse event is emergent after treatment period.

All AEs emergent during treatment period will be categorized as occurring during the period of 21 days following the last vaccination based on the start date. If start date is missing or incomplete, events will be counted as yes during the period of 21 days following the last vaccination.

Adverse events that **do not** meet any of the following criteria SAE, AESI, or AE leading to withdrawal, and had a start date more than 21 days after the last vaccination are not to be recorded. However, if recorded, these AEs should be flagged (i.e. **exclusion flag**), excluded from analysis and listed separately.

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

Multiple AEs with the same Preferred Term (PT) for the same subject are counted only once.

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

Prior and Concomitant Medications and Vaccines

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

- **Prior medication and vaccines:** start and end date before the date of injection of study vaccine.
- **Concomitant medications and vaccinations:** start date before first vaccination but continued after vaccination or start date after vaccination. The period Day 1- 43 (i.e. ending 21 days after the last study vaccination) will be labeled if the start date is on or before Day 43.

When start and/or end dates of a medication intake are missing, the medication is considered as concomitant with the study vaccination schedule. Concomitant medication associated with AEs excluded described above will be flagged for exclusion from all medication summary.

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 randomized subjects will be accounted for in this study. The numbers and percentages of subjects in each analysis set, study withdrawals, age subgroups, and major protocol deviations will be presented for exposed and enrolled sets. Number of subjects per site will be presented by treatment group and overall for the enrolled and exposed set.

The time the subjects (in safety set) are under observation will be summarized by treatment group and overall using summary statistics (mean, standard deviation (SD), minimum, median, maximum).

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

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

10.1 Demographics

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

The frequencies and percentages of subjects by gender, ethnicity, race, and previous influenza vaccination (in the past 12 months) will be presented by treatment group and overall. Demographic data will be tabulated for the All Enrolled, FAS, PPS, and Overall Safety Set.

10.2 Medical History

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

11. IMMUNOGENICITY ANALYSIS

11.1 Blood samples

For each visit, the number and percentages of subjects with and without blood draws will be summarized overall and by vaccine group. Data will be tabulated for the all enrolled set.

11.2 Primary Objectives Analysis

The primary immunogenicity objective is to assess immune responses against H5N6 (contained in the vaccine) as measured by hemagglutination inhibition (HI)⁴ assay 1 week (Day 8) and 3 weeks (Day 22) after the first heterologous aH5N6c booster vaccination, and 3 weeks (Day 43) after the second heterologous aH5N6c booster or placebo vaccination.

The primary immunogenicity endpoints are the humoral immune responses in terms of HI antibody response against the booster vaccine strain (A/H5N6):

- Geometric mean titer (GMT) of HI (or MN) antibodies on Day 1, Day 8, Day 22, and Day 43;
- Geometric mean fold increase (GMFI): The geometric mean of the fold increase of postvaccination HI (or MN) titers over the pre-vaccination HI (or MN) titer (Day 8/Day 1, Day 22/Day 1, Day 43/Day 1).
- Seroconversion rate (SCR) defined as the percentage of subjects with either a pre-vaccination HI (or MN) titer $<1:10$ and a postvaccination HI (or MN) titer $\geq 1:40$, or with either a pre-vaccination HI (or MN) titer $\geq 1:10$ and a ≥ 4 -fold increase in postvaccination HI (or MN) titer on Day 8, Day 22 and Day 43;
- The percentage of subjects with a HI (or MN) titer $\geq 1:40$ on Day 1, Day 8, Day 22, and Day 43.

The derived variables are:

- Geometric mean titer ratios (GMTr) of HI (or MN) antibodies between vaccine arms (Group 1/Group 2, Group 1/Group 3 and Group 2/Group 3) on Day 1, Day 8, Day 22, and Day 43 for A/H5N6;
- The inter-group differences between vaccine arms (Group 1 - Group 2, Group 1 - Group 3 and Group 2 - Group 3) in the SCRs at Day 8, Day 22, and Day 43 for A/H5N6.
- The inter-group differences between vaccine arms (Group 1/Group 2, Group 1/Group 3 and Group 2/Group 3) in the percentage of subjects with HI (or MN) titer $\geq 1:40$ on Day 1, Day 8, Day 22, and Day 43 for A/H5N6.

⁴ In case of lack of agglutination or agglutination mediated through neuraminidase for a specific strain using HI assay, immunogenicity for that strain will be assessed as measured by MN assay as an acceptable alternative.

No formal statistical hypothesis testing will be performed for the primary immunogenicity objective.

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

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

In addition, reverse cumulative distribution plots will be generated to display the distribution of the H5N6 antibody titers by visit (Day 1, Day 8, Day 22 and Day 43) 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.

Adjusted GMTs will be calculated based on the log₁₀-transformed antibody titers at Day 8, Day 22 and Day 43 using an Analysis of Covariance (ANCOVA) model which includes the vaccine group (Group 1, Group 2, Group 3), gender and log₁₀-transformed pre-vaccination antibody titer. From the model, an adjusted difference in least square means (on the log scale) will be produced with 95% confidence limits. The analysis model for Day 1 adjusted GMTs will be performed using the same model as mentioned, however, using the log-transformed (base 10) Day 1 GMT as the outcome variable and without the pre-vaccination antibody titer as a covariate. The estimated difference and the confidence limits will be back transformed to obtain an adjusted GMT ratio with 95% confidence limits.

Unadjusted estimates for GMTs, GMFIs and pertaining 2-sided 95% CIs will be calculated assuming a log-normal distribution of the titers and will be completed by providing minimum, maximum and median titers for each study group for each treatment group, for each assessment time-point.

The number and proportion of subjects achieving the binary endpoints (percentage of subjects with seroconversion and with titer $\geq 1:40$) will be summarized by assessment (Day 1, Day 8, Day 22, Day 43) and vaccine group. These summaries will be reported together with the associated two-sided 95% CIs for the proportion according to Clopper-Pearson.

The binary endpoints (percentage of subjects with seroconversion and with titer $\geq 1:40$) at Day 1, Day 8, Day 22 and Day 43 will be compared between treatment groups by the differences of proportions with 95% CI using the Miettinen and Nurminen method without adjustment for covariate.

In addition, SCRs and the percentages of subjects achieving HI antibody titer $\geq 1:40$ results collected at Day 8, Day 22, and Day 43 will also be evaluated against the age appropriate Center for Biologics Evaluation and Research (CBER) criteria, CBER Guidance Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines (United States Food and Drug Administration (FDA), 2007):

- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 30% for adults ≥ 65 years of age and 40% for adults < 65 years of age.
- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving an HI antibody titer $\geq 1:40$ should meet or exceed 60% for adults ≥ 65 years of age and 70% for adults < 65 years of age.

11.3 Secondary Objectives Analysis

The secondary immunogenicity objectives are:

- To assess immune responses against H5N1 (contained in the priming vaccine) as measured by HI (or MN) assay 1 week (Day 8) and 3 weeks (Day 22) after the first heterologous aH5N6c booster vaccination, and 3 weeks (Day 43) after the second heterologous aH5N6c booster or placebo vaccination.
- To assess persistence of immune response against H5N1 (contained in the priming vaccine) as measured by HI (or MN) assay at Day 1 (pre-vaccination) and at Day 202, 6 months after the second heterologous aH5N6c booster or placebo vaccination.
- To assess persistence of immune response against H5N6 (contained in the booster vaccine) as measured by HI (or MN) assay at Day 202, 6 months after the second heterologous aH5N6c booster or placebo vaccination.

The secondary immunogenicity endpoints are:

- (a) the immune responses to the aH5N1c vaccine in terms of HI (or MN) antibody responses against the A/H5N1 strain contained in the priming vaccine by:
- GMT of HI (or MN) titer on Day 1, Day 8, Day 22, Day 43 and Day 202;
 - GMFI: The geometric mean of the fold increase of postvaccination HI (or MN) titers over the pre-vaccination HI (or MN) titer against H5N1 (Day 8/Day 1, Day 22/Day 1, Day 43/Day 1, Day 202/Day 1).
 - SCR defined as the percentage of subjects with either a pre-vaccination HI (or MN) titer $< 1:10$ and a postvaccination HI (or MN) titer $\geq 1:40$, or with either a pre-vaccination HI (or MN) titer $\geq 1:10$ and a ≥ 4 -fold increase in postvaccination HI (or MN) titer against H5N1 on Day 8, Day 22, Day 43, and Day 202;
 - The percentage of subjects with a titer $\geq 1:40$ against H5N1 on Day 1, Day 8, Day 22, Day 43, and Day 202.
- (b) the immune responses to the aH5N6c vaccine in terms of HI (or MN) antibody responses against the A/H5N6

strain contained in the vaccine by:

- GMT: Geometric mean of HI (or MN) titers at Day 202.
- GMFI: The Geometric mean of the fold increase in serum HI (or MN) titer 6 months (Day 202) after the second vaccination compared to pre-vaccination (Day 1).
- Percentages of subjects with HI (or MN) titers $\geq 1:40$ at Day 202.
- Percentage of subjects with seroconversion (defined as a ≥ 4 -fold increase in titer postvaccination in those with pre-vaccination titer $\geq 1:10$, or a postvaccination titer $\geq 1:40$ for subjects with baseline titer $< 1:10$) for HI (or MN) antibodies at Day 202.

The derived variables are:

- GMTr of HI (or MN) titres between vaccine arms (Group 1/Group 2, Group 1/Group 3 and Group 2/Group 3) on Day 1, Day 8, Day 22, Day 43 and Day 202 for A/H5N1, and on Day 202 for A/H5N6;
- The inter-group differences between vaccine arms (Group 1 - Group 2, Group 1 - Group 3 and Group 2 - Group 3) in the SCRs at Day 8, Day 22, Day 43, and Day 202 for A/H5N1, and at Day 202 for A/H5N6.
- The inter-group differences between vaccine arms (Group 1 - Group 2, Group 1 - Group 3 and Group 2 - Group 3) in the percentage of subjects with a titer $\geq 1:40$ on Day 1, Day 8, Day 22, Day 43, and Day 202 for A/H5N1, and Day 202 for A/H5N6.

No formal statistical hypothesis testing will be performed for the secondary immunogenicity objectives.

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

Reverse cumulative distribution plots will be generated to display the distribution of the H5N1 antibody titers by visit (Day 1, Day 8, Day 22, Day 43 and Day 202) and Day 202 visit for H5N6 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.

Adjusted GMTs will be calculated based on the log₁₀-transformed antibody titers at Day 8, Day 22, Day 43 and Day 202 using an Analysis of Covariance (ANCOVA) model which includes the vaccine group (G1, G2, G3), log₁₀-transformed pre-vaccination antibody titer, and gender. The analysis model for Day 1 adjusted GMTs will be performed using the same model as mentioned, however, using the log-transformed (base 10) Day 1 GMT as the outcome variable and without the pre-vaccination antibody titer as a covariate. GMT ratios between groups and pertaining 2-sided CIs will be calculated based on these models.

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

The number and proportion of subjects achieving the binary endpoints (percentage of subjects with seroconversion and with titer $\geq 1:40$) will be summarized by assessment (Day 1, Day 8, Day 22, Day 43 and Day 202) and vaccine group. These summaries will be reported together with the associated two-sided 95% CIs for the proportion according to Clopper-Pearson.

The binary endpoints (percentage of subjects with seroconversion and with titer $\geq 1:40$) at Day 1, Day 8, Day 22, Day 43 and Day 202 will be compared between treatment groups by the differences of proportions with 95% CI using the Miettinen and Nurminen method without adjustment for covariate.

In addition, SCRs and the percentages of subjects achieving HI antibody titer $\geq 1:40$ results collected at Day 8, Day 22, and Day 43 will also be evaluated against the Center for Biologics Evaluation and Research (CBER) criteria, CBER Guidance Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines (United States Food and Drug Administration (FDA), 2007):

- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving seroconversion for HI antibody should meet or exceed 30% for adults ≥ 65 years of age and 40% for adults < 65 years of age.
- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving an HI antibody titer $\geq 1:40$ should meet or exceed 60% for adults ≥ 65 years of age and 70% for adults < 65 years of age.

11.4 Exploratory Objectives Analysis

A post hoc decision will be made if other measurements are required. If adequate sera are available and depending on assay availability, antibody responses and their persistence to seasonal and/or homologous and/or heterologous pandemic influenza strains as measured by HI, MN and SRH assays may be described at Days 1, 22, 43, and 202 in the same manner as for primary and secondary immunogenicity endpoints. The same statistical model as the one used for the primary analysis will be applied. The results will be included in a separate report.

12. EFFICACY ANALYSIS

12.1 Primary Objectives Analysis

Not Applicable.

12.2 Secondary Objectives Analysis

Not Applicable.

12.3 Exploratory Objectives 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:

- Solicited local and systemic AEs for 7 days following each vaccination (Day 1 through Day 7; and Day 22 through Day 28);
- All unsolicited AEs for 21 days following each vaccination (Day 1 through Day 43);
- SAEs, AEs leading to withdrawal from the study, AESIs and MAAEs as collected from Day 1 through Day 202.

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 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 diary cards, irrespective of severity. The analysis will show the number of subjects with valid data by solicited adverse event and time point. Valid data in the context of the safety completeness analysis are all data entered in the diary card excluding implausible values) except “Not done/unknown”.

Following summaries will be produced:

- The frequencies of subjects who provide valid data on the diary cards by vaccine group for any vaccination and by first and second vaccination separately.
- For each solicited adverse event, the frequencies of subjects with valid data will be presented by vaccine group and timepoint: Day 1-3, Day 4-7, and Day 1-7, for any vaccination and by first and second vaccination separately.
- For each type of solicited adverse event (local, systemic) and indicators of solicited adverse events, such as analgesic use the frequencies of subjects with valid data by vaccine group, aggregated over time points: Day 1-3, Day 4-7, and Day 1-7, for any vaccination and by first and second vaccination separately.

For the corresponding percentages, the denominator will be the respective numbers of exposed subjects, i.e., subjects who received a vaccination and were still in-study for that time point or time interval, irrespective of whether a diary card was present or not.

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

13.2 Solicited Local and Systemic Adverse Events

Only solicited local and systemic adverse events reported in the diary card will be analyzed using solicited safety set per treatment group. Implausible measurements will not be taken into consideration in the analysis but listed in the Appendix of the CSR (see section 6.3 of this document).

Solicited adverse events will be collected starting Day 1 and then daily until Day 7 using structured diaries. The analyses of solicited adverse events will be done for any vaccination and by first and second vaccination separately, for intervals: Day 1-3, Day 4-7, and Day 1-7. In addition, solicited adverse events ongoing after Day 7 will be recorded by the investigator in the AE CRF and presented in the evaluation of unsolicited AE and will be categorized as reactogenicity in AE domain.

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

Grade 0 (<25 mm), any (25-50 mm [Grade I], 51-100 mm [Grade II], >100 mm [Grade III]).

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

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

Body temperature will be broken down by route of measurement according to the recommendations of the Brighton collaboration and will be summarized according to the 2 schemes described below:

- by 0.5 °C increments:
 - <36.0,
 - ≥36.0 - <36.5,
 - ≥36.5 - <37.0,
 - ≥37.0 - <37.5,
 - ≥37.5 - <38.0,
 - ≥38.0 - <38.5,
 - ≥38.5 - <39.0,
 - ≥39.0 - <39.5,
 - ≥39.5 - <40.0,
 - ≥40.0°C
- <38.0, ≥38.0 °C

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

Table 13.2-1: Severity Grading for Solicited Local and Systemic Adverse Events

	Solicited Event	Grade 1/Mild	Grade 2/Moderate	Grade 3/Severe
Local reactions	Injection site pain	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
	Injection site induration / erythema / ecchymosis	2.5-5 cm	5.1-10 cm	>10 cm
Systemic reactions	Fever	38.0–38.4°C 100.4-101.1°F	38.5–38.9°C 101.2-102°F	39.0–40°C 102.1-104°F
	Nausea	Nausea present but not interfering with oral intake	Nausea leading to decreased oral intake	Nausea leading to minimal or no oral intake
	Myalgia	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
	Arthralgia	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
	Headache	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
	Fatigue	No interference with activity	Some interference with activity	Significant, prevents daily activity
	Chills	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
	Loss of appetite	Loss of appetite without decrease in oral intake	Decreased oral intake without weight loss	Decreased oral intake with weight loss
	Malaise	No interference with activity	Some interference with activity	Significant, prevents daily activity

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

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events.
3. Solicited adverse events, maximum event severity by event and interval Day 1-3, Day 4-7, and Day 1-7.
4. Duration of solicited adverse events.
5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval Day 1-3, Day 4-7 and Day 1-7.
6. Solicited adverse events ongoing at Day 7 for each of the solicited AEs

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

Level 1: Daily reports of solicited adverse event

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

Level 2: Time of first onset of solicited adverse events

The **time of first onset** is defined, for each subject, for each solicited adverse event, as the time point at which the respective solicited adverse event first occurred. For erythema, induration and ecchymosis the following threshold will be used: ≥ 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.

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

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

For each vaccination the first onset of the adverse event will be used for each subject. For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, ‘not done’ is treated identical to ‘missing’. A mock-up table is shown in Table 13.2-2 below.

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

Vaccine group A

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

N: no. of subjects with data at a time point across all vaccinations.

n: no. of subjects with data at a time point for that specific vaccination.

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

Level 4: Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. 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 for each vaccination and by adverse event.

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

The **occurrence of at least one solicited adverse event** is defined as “any” for a subject if he/she reports greater than “none” for qualitatively assessed solicited systemic AEs and/or ≥ 25 mm for erythema, ecchymosis and induration for the respective event and “none” otherwise. The occurrence of at least one solicited adverse event (i.e., none versus any) will be summarized by category (i.e., local, systemic, any), by vaccine group, by vaccination (after each vaccination and after any vaccination) and by time interval.

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval Day 1 – 3, Day 4 – 7, Day 1 – 7.

Level 6: Solicited adverse events ongoing at Day 7

For each of the solicited AEs, the number of subjects reported the event ongoing at Day 7 will be summarized.

For details please refer to [section 7.1.1 of the protocol](#).

13.3 Unsolicited Adverse Events

This analysis applies to all AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in AE eCRF, with a start date on or after the date of first vaccination. The original verbatim terms used by investigators to identify AEs in the eCRFs will be mapped to preferred terms using the MedDRA dictionary. The AEs will then be grouped by MedDRA preferred terms (PT) into frequency tables according to system organ class (SOC).

All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine, will be summarized according to SOC and PT within SOC. These summaries will be presented by vaccination group and by interval of study observation. 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 time intervals will be done by day of onset and not by days ongoing/persisting. The summaries will be presented by SOC and PT according to the different periods of onset:

- Immediate post-vaccination AEs (reported within 30 minutes after study vaccination)
- Onset between Day 1 and Day 22 (or prior to Vaccination 2).

- Onset between Day 23 (vaccination 2) and Day 43 (or 21 days post-vaccination 2).
- Onset between Day 1 and Day 43 (or 21 days post-vaccination 2).
- Onset between Day 44 and last visit.
- Onset between Day 1 and last visit.

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited adverse event.
- Possibly or probably related unsolicited adverse events.
- Unsolicited adverse events leading to death.
- Serious adverse events.
- Possibly or probably related serious adverse event.
- Unsolicited adverse events leading to premature withdrawal from study.
- Unsolicited adverse events of special interest (AESI), see Appendix A of the protocol.
- Medically attended adverse events.

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

A summary of subjects with solicited and unsolicited non-serious treatment emergent adverse events reported by >5% of subjects sorted by system organ class and preferred term will be provided for clinicaltrials.gov and EudraCT.eu posting purposes.

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

13.4 Clinical Safety Laboratory Investigations

Not Applicable.

13.5 Concomitant Medication

The frequencies and percentages of subjects reporting medications and vaccines taken prior or during the study are categorized as prior and/or concomitant and will be tabulated overall and by vaccine group. Medications (generic drug name) will be coded using the WHODRUG dictionary (see section 8.8 for definition).

14. INTERIM ANALYSIS

14.1 Interim Analysis

There are no planned interim analyses for this study.

15. DATA MONITORING COMMITTEES

Not applicable.

16. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

This list of tables will be defined later combined with the table's shells.

17. REFERENCES

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