STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 2, Randomized, Observer-Blind, Multicenter Study to Evaluate the Immunogenicity and Safety of Several Doses of Antigen and MF59 Adjuvant Content in a Monovalent H5N1 Pandemic Influenza Vaccine in Healthy Pediatric Subjects 6 Months to < 9 Years of Age
Study Number:	V87_30
Protocol Version and Date:	Version 5, 24 JUN 2020
Phase of Development:	Phase 2
Plan Prepared by:	[redacted], Global Director Biostatistics
Version and Date	Version 1.0, 06 MAY 2022
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LIST OF ABBREVIATIONS

AE	Adverse Event(s)
AESI	Adverse Event(s) of Special Interest
ANCOVA	Analysis of Covariance
CI	Confidence Interval
CSR	Clinical Study Report
CRA	Clinical Research Associate
DMC	Data Monitoring Committee
FAS	Full Analysis Set
GMT	Geometric Mean Titers
HA	Hemagglutinin Antigen
HI	Hemagglutination Inhibition
ICH	International Council for Harmonization
IM	intramuscular
IRT	Interactive Response Technology
LAR	Legally Acceptable Representative
MAAE	Medically Attended Adverse Event(s)
MCAR	Missing Completely At Random
MedDRA	Medical Dictionary for Regulatory Activities
MN	Microneutralization
NOCD	New Onset of Chronic Disease
PI	Principal Investigator
PD	Protocol Deviation
PPS	Per Protocol Set
PT	Preferred Term (MedDRA)
SAE	Serious Adverse Event(s)
SAP	Statistical Analysis Plan

SD	Standard Deviation
SE	Standard Error
SOC	System Organ Class (SOC)
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reaction(s)
TFL	Tables, Figures and Listings
TOC	Table of Content

1. BACKGROUND AND RATIONALE

Pandemic influenza differs from seasonal influenza by representing a novel influenza strain, to which humans are immunologically naive, and has the potential for heavily impacting a young otherwise healthy population. If sustained human-to-human transmission were to occur with a pandemic influenza strain, global spread is likely, and morbidity and mortality could be staggering if efforts to contain the spread were not implemented quickly. Vaccination is the best option by which spread of a pandemic virus could be minimized or prevented. Vaccination efforts would be further enhanced by identification of the fewest number of vaccinations and lowest antigen dose needed for protection of segments of the population.

The present study is a post authorization commitment in Europe. The purpose of this study is to provide additional clinical data on aH5N1 in children in anticipation of an avian influenza pandemic, as agreed in the pediatric investigational plan with EMA/Pediatric Committee. This is a pediatric dose-ranging study for aH5N1 to dosages with decreased content of HA antigen and/or MF59 adjuvant (versus the licensed dosage for adults). The study is designed to evaluate the safety and immunogenicity of two vaccinations using three different amounts of aH5N1 and two different dosages of MF59 adjuvant, administered 3 weeks apart.

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 V87_30 and is based on protocol version 5, 24 JUN 20.

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 Objectives

2.1.1 Primary Safety Objective

To evaluate the safety in each study vaccine group from Day 1 through Day 387, by total population and by age cohort.

2.1.2 Primary Immunogenicity Objective

To assess by total population and by age cohort, the antibody responses to each of the study vaccines prior to (Day 1) and at 3 weeks after the first or second vaccination (Day 22 or Day 43), as measured by HI and MN assays.

2.2 Secondary Objectives

2.2.1 Secondary Immunogenicity Objective(s)

To evaluate in each study vaccine group, by total population and by age cohort, the persistence of antibody responses to the H5N1 vaccine strain 6 months after the second vaccination (Day 202) as measured by HI and MN assays.

2.3 Exploratory Objectives

To further evaluate the antibody responses to seasonal, and/or homologous and/or heterologous pandemic influenza strain(s) by vaccine group on Days 1, 22, 43, and 202, as measured by HI, MN, or SRH assays (depending on availability of adequate sera and on assay availability).

3. STUDY DESIGN

This randomized, observer blind, multi-center clinical trial evaluates the immunogenicity in healthy children ages 6 months to <9 years of 6 vaccine regimens utilizing 6 different formulations including either 1.875, 3.75, or 7.5 µg HA of pandemic H5N1 influenza strain combined with either 0.125 mL or 0.25 mL MF59, in two intramuscular (IM) injections administered three weeks apart.

A total number of 420 subjects will be enrolled to assure at least 150 evaluable subjects for each of the age cohorts as required by the pediatric investigational plan. Subjects <9 years will be randomized to receive either one of the six formulations in 1:1:1:1:1:1 allocation ratio, stratifying according to age cohort (6 months to < 36 months and 3 years to < 9 years of age). The distribution of all enrolled subjects across the two age cohorts should be about 50% in each.

Subjects in each study vaccine group will be scheduled to receive 2 injections of aH5N1 vaccine 3 weeks (21 Days) apart. Each subject will have two periods of study participation: Treatment Period (Day 1 to Day 43 – i.e. 21 days after second injection) and Follow-up Period (Day 44 to Day 387 – i.e. 12 months after second injection).

Blood samples for serology assessments will be collected from each subject on Day 1 (before randomization), Day 22 (before vaccination), Day 43, and Day 202.

Subject Diary Cards will be provided to subjects for recording of local and systemic reactions (solicited adverse events) for 7 consecutive days after each vaccination. All unsolicited adverse events (AEs) will be collected during the treatment period.

In the follow-up period (Day 44 through Day 387), only the following subset of unsolicited AEs and the associated concomitant medications/vaccinations will be collected and documented in the subject's eCRFs: all serious AEs (SAEs), New Onset of Chronic Disease (NOCD), AEs leading to study withdrawal, and Adverse Events of Special Interest (AESIs) (as described in section 7.1 of the current protocol). These data will be captured by interviewing the subject's parent(s) or legally authorized representative (LAR) and/or by reviewing the available medical records.

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

Table 1 Time and Events Table

		Treatment period					Follow-up period			
	Visit Type	**clinic visit**	**Diary reminder phone call**	**clinic visit**	**Diary reminder phone call**	**clinic visit***	**safety phone call**	**safety phone call**	**clinic visit***	**safety phone call**
	Study Day[a]	**1**	**V1 + 4**	**V1 + 21**	**V2 + 4**	**V2 + 21**	**V2 + 70**	**V2 + 130**	**V2 + 180**	**V2+ 365**
	Visit Window (days)	**-10/0**	**-1/+1**	**-1 to +7**	**-1/+1**	**-1 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**
	Visit Number	**V1**	**n/a**	**V2**	**n/a**	**V3**	**V4**	**V5**	**V6**	**V7**
Study event										
study treatment										
vaccination		**X**		**X**						
screening and safety										
informed consent[b]		**X**								
demographics[c]		**X**								

		Treatment period					Follow-up period			
	Visit Type	**clinic visit**	**Diary reminder phone call**	**clinic visit**	**Diary reminder phone call**	**clinic visit***	**safety phone call**	**safety phone call**	**clinic visit***	**safety phone call**
	Study Day[a]	**1**	**V1 + 4**	**V1 + 21**	**V2 + 4**	**V2 + 21**	**V2 + 70**	**V2 + 130**	**V2 + 180**	**V2+ 365**
	Visit Window (days)	**-10/0**	**-1/+1**	**-1 to +7**	**-1/+1**	**-1 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**
	Visit Number	**V1**	**n/a**	**V2**	**n/a**	**V3**	**V4**	**V5**	**V6**	**V7**
Study event										
review of systems[c]		X		X		X			X	
clinical signs[c]		X		X[d]						
medical history[c, e]		X								
physical exam[c, f]		X		X		X			X	
exclusion/inclusion criteria[g]		X		X						
randomization[h]		X								
30 minutes post injection assessment		X		X						
Subject Diary Card dispensed with training		X		X						

		Treatment period					Follow-up period			
	Visit Type	**clinic visit**	**Diary reminder phone call**	**clinic visit**	**Diary reminder phone call**	**clinic visit***	**safety phone call**	**safety phone call**	**clinic visit***	**safety phone call**
	Study Day[a]	**1**	**V1 + 4**	**V1 + 21**	**V2 + 4**	**V2 + 21**	**V2 + 70**	**V2 + 130**	**V2 + 180**	**V2+ 365**
	Visit Window (days)	**-10/0**	**-1/+1**	**-1 to +7**	**-1/+1**	**-1 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**
	Visit Number	**V1**	**n/a**	**V2**	**n/a**	**V3**	**V4**	**V5**	**V6**	**V7**
Study event										
Subject Diary Card reminder call[i]			X		X					
Subject Diary Card reviewed and collected[j]				X		X				
assess all AEs		X		X		X				
assess SAEs		X		X		X	X	X	X	X
assess NOCDs, AEs leading to withdrawal, AESIs		X		X		X	X	X	X	X
assess relevant medications[k]		X		X		X	X	X	X	X
Immunogenicity										

		Treatment period					Follow-up period			
	Visit Type	**clinic visit**	**Diary reminder phone call**	**clinic visit**	**Diary reminder phone call**	**clinic visit***	**safety phone call**	**safety phone call**	**clinic visit***	**safety phone call**
	Study Day[a]	**1**	**V1 + 4**	**V1 + 21**	**V2 + 4**	**V2 + 21**	**V2 + 70**	**V2 + 130**	**V2 + 180**	**V2+ 365**
	Visit Window (days)	**-10/0**	**-1/+1**	**-1 to +7**	**-1/+1**	**-1 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**
	Visit Number	**V1**	**n/a**	**V2**	**n/a**	**V3**	**V4**	**V5**	**V6**	**V7**
Study event										
serology blood draw[l]		**X[m]**		**X**		**X**			**X**	
Study termination/completion										
Study completion[n]										**X**

		Treatment period					Follow-up period			
	Visit Type	**clinic visit**	**Diary reminder phone call**	**clinic visit**	**Diary reminder phone call**	**clinic visit***	**safety phone call**	**safety phone call**	**clinic visit***	**safety phone call**
	Study Day[a]	**1**	**V1 + 4**	**V1 + 21**	**V2 + 4**	**V2 + 21**	**V2 + 70**	**V2 + 130**	**V2 + 180**	**V2+ 365**
	Visit Window (days)	**-10/0**	**-1/+1**	**-1 to +7**	**-1/+1**	**-1 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**	**-7 to +7**
	Visit Number	**V1**	**n/a**	**V2**	**n/a**	**V3**	**V4**	**V5**	**V6**	**V7**
Study event										

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 visit 2 and 3; Visit 2 is the baseline for calculation of all following visits; [b] Confirm consent form (and if applicable assent form) signed prior to any procedures. The informed consent process may be conducted earlier, but within 10 days prior to Day 1. [c] Pre-vaccination procedures may occur within 10 days prior to or within the Day 1 visit, if procedures are performed prior to Day 1 inclusion/exclusion criteria have to be rechecked, and the subject's eligibility must be confirmed prior to the Day 1 vaccination; [d] clinical signs includes weight, height and temperature measurement before the 1st vaccination, and only temperature measurement before 2nd vaccination; [e] Including prior and concomitant medications. [f] A physical examination will be based on a review of systems, ie, a structured interview for complaints for each organ system; [g] To be checked prior to each vaccination; [h] Randomization only to be done if baseline blood sample has been collected. [i] A reminder call after each vaccination should occur between 3 to 5 days following vaccination; [j] If a clinic visit is not possible, review should be completed upon return of the Subject Diary card. [k] 24 hours prior to vaccination antipyretic medication is not allowed; [l] Blood sample for serology to be taken after temperature measurement, but prior to vaccination; [m] Subjects must provide a baseline blood sample after informed consent, but before randomization, up to 10 days prior to the 1st vaccination. [n] Subjects who terminate the study early should complete an early termination visit when possible,

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

Approximately 420 subjects ≥ 6 months and < 9 years will be randomized to receive either one of 6 formulations of aH5N1 vaccine in a 1:1:1:1:1:1 allocation ratio, stratifying according to site and age cohort (6 months to < 36 months and 3 years to < 9 years). The number of subjects randomized will be approximately in an equal split between the age subgroups.

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 and approved by the Sponsor according to the applicable Sponsor's Standard Operating Procedure (SOP).

The following 6 formulations will be used:

Arm	Description
A	1.875 µg + 50% MF59 0.25 mL
B	3.75 µg + 50% MF59 0.25 mL
C	7.5 µg + 50% MF59 0.25 mL
D	1.875 µg + 100% MF59 0. 5 mL
E	3.75 µg + 100% MF59 0.5 mL
F	7.5 µg + 100% MF59 0.5 mL

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 administration
- Administered expired vaccine.
- Administered temperature deviated vaccine.

Stratification error:

- Subject randomized in the wrong stratification stratum.

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

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. Vaccine preparation and administration should be completed by the designated unblinded team members. Any other subject related assessments should be performed by the principal investigator (PI) and/or blinded staff members as applicable. Sponsor personnel will remain blinded.

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) may be excluded from the Per Protocol Set (PPS). Unblinded subjects will be included in the Full Analysis Set (FAS) and safety sets.

After the final data has been collected and the database has been locked, unblinding will take place to generate the study results.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

This is a Phase 2, dose-ranging study without inferential hypothesis testing. The total sample size to be randomized is 420 subjects equally divided for two age cohorts. This number of subjects should provide sufficiently accurate estimates of the Geometric Mean Titers (GMT) to evaluate the pediatric dose. Assuming an exclusion rate of up to 14% of subjects from the analysis, around 180 subjects per age cohort will be included in the analysis. With equal allocation to one of six vaccine groups, we expect at least 60 subjects per vaccine group and at least 30 per vaccine group and age cohort to be evaluable for the statistical analysis. No formal power calculations have been done. However, the accuracy of the estimates of the GMT's can be illustrated by the length of the 95% confidence intervals (CI). Assuming a Standard Deviation (SD) of log10- transformed Hemagglutination Inhibition (HI) titers as 0.7 (based on studies V87_25 and V87_26 in healthy elderly):

- With n=30 per dose group per age cohort; the 95% CI will be from 0.56 to 1.78 times the GMT estimate

- With n=60 per dose group; the 95% CI will be from 0.67 to 1.50 times from the GMT estimate.

In pairwise dose-group comparisons with n=60 per dose group it would be feasible to detect a difference of 2.5 in the GMT ratio with statistical power of 80% with the two sample T-test at significance level of 0.05.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

The 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.

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.

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.

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 Clinical Research Associates (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 PDs. These listings will be provided for review on an ongoing basis during the study.

This review will also include PDs captured reported in monitoring reports. The PD review output will include:

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

Prior to unblinding, all reportable PDs will be evaluated, and the PDs that led to exclusions from analysis sets will documented and signed off by at least the Biostatistician and the Clinical Scientist.

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6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic AEs 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 2 Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 33°C or ≥ 42°C
Erythema	For subjects ≥ 6 years: ≥ 900 mm and for subjects < 6 years: ≥ 450 mm Measurements < 0 mm
Induration	For subjects ≥ 6 years: ≥ 500 mm and for subjects < 6 years: ≥ 250 mm Measurements < 0 mm
Ecchymosis	For subjects ≥ 6 years: ≥ 500 mm and for subjects < 6 years: ≥ 250 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).

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 at least one dose of study vaccination.

7.3 Full Analysis Set (FAS), Immunogenicity

All subjects in the All Enrolled Set who are randomized, received at least one dose of study vaccination and provided immunogenicity data at any data points.

In case of randomization error, subjects in the FAS sets will be analyzed "as-randomized" (i.e., according to the vaccine the subject is assigned to per randomization).

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 and at the scheduled time points)
- Have no CSR-reportable PD leading to exclusion as defined prior to unblinding.
- Are not excluded due to other reasons defined prior to unblinding or analysis (see section 6.2)
- Have immunogenicity results on Day 1 (before first vaccination) and Day 22 or at Day

43 within the defined window around the planned visits.

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 Exposed Set with any solicited AE data collected, including temperature measurements or use of analgesics/antipyretics.

Unsolicited Safety Set

All subjects in the Exposed Set with results of the unsolicited AE assessments recorded. 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.

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 actual received as first dose, rather than the vaccine to which the subject may have been randomized).

All immunogenicity analyses (primary, secondary, and exploratory) will be performed on the PPS Immunogenicity. The primary immunogenicity analyses will be also performed based on the FAS Immunogenicity if the percentage of subjects excluded from the PPS immunogenicity is greater than 5%. All solicited safety analyses will be performed in solicited safety set. All unsolicited safety analyses will be performed on the unsolicited safety set.

7.6 Other Analysis Set

Not applicable.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The main statistical analysis includes descriptive statistics for the overall population <9 years of age. Subgroup analyses will be done by age cohort (6 months to < 36 months, 3 to < 9 years). Summary tables will show unadjusted GMTs for each vaccine group by assessment.

Adjusted GMTs will be calculated based on the log-transformed antibody titers at Day 22 and Day 43 using an Analysis of Covariance (ANCOVA) model which includes the log-transformed pre-vaccination antibody titer, age cohort and vaccine group.

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 country, 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 AEs are collected using diaries from Day 1 to Day 7 post-vaccination. If the data have not been recorded for all 7 days, the assessment is considered missing and excluded from analysis. In case, at least one day is filled in but other days are missing the presence or absence of the event will be based on the available data. If more than 10% 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 vaccine group.

8.3 Multicenter Studies

The study will be conducted in two countries with a total of around 7 sites. The randomization will be stratified by site and age cohort. The effect of country and site will be explored as subgroup analysis provided that the number of enrolled subjects is sufficient.

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 cohorts (6 months to < 36 months or 3 to < 9 years). Descriptive immunogenicity analysis of the GMTs will be performed by stratifying for the following subgroups:

- Sex;
- Country;
- Site.

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 for the statistical analysis:

Age will be calculated in months using the following formula:

Integer [12*(Date of Visit 1 – Date of Birth + 1) / 365.25]

Age will be calculated in years using the following formula:

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

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

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

Immunogenicity

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

Seroconversion based on **HI** 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 ≥ 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 AEs will be characterized according to the date of occurrence related to the vaccination as follows:

- **Pre-vaccination (will be mapped to MH)**: start date before the date of first injection of study vaccine or indicated as on injection day but before injection.
- **Emergence during vaccination phase**: all other cases.

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

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

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

All AEs emergent during vaccination phase will 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 meet none of the following criteria SAE, AESI, NOCD, 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 (PT) for a reported AE 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 first injection of study vaccine.
- **Concomitant:** start date before vaccination but continued after vaccination or start date after vaccination. The period Day 1- 43 (i.e. 21 days after the last study vaccination) will be labeled if the start date is on or before Day 43.

8.8 Analysis Software

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

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

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

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

In addition, the frequency of age categories will be reported as 6 to < 36 months and 3 to < 9 years (age cohort). The number and percentages of subjects by sex, country, ethnic origin, race, and previous influenza vaccine (within the past 2 years) 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 (site*age subgroup) 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 CRF 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 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 each of the 6 vaccine formulations.

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 all enrolled set.

11.2 Primary Objectives Analysis

The primary endpoints in terms of measures of immunogenicity, as determined by the HI and MN assay against the H5N1 pandemic influenza homologous strain include the following:

- Geometric Mean Titer (GMT) on Day 1, Day 22 (3 weeks after the first vaccination) and Day 43 (3 weeks after the second vaccination).
- Geometric mean ratios calculated as follows: Day 22/day 1 or Day 43/Day 1 as determined by HI and MN assays against the homologous H5N1 pandemic influenza strain.
- Percentage of subjects with seroconversion on Day 22 and Day 43.
- Percentages of subjects with HI or MN titers ≥ 1:40 at Day1, Day 22 and Day 43.

In addition, reverse cumulative distribution plots will be generated to display the distribution of the antibody responses by visit (Day1, Day 22, Day 43 and Day 202) for each of the vaccine groups. The x-axis represents the immunogenicity values, and the scale of the axis is original scale. 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.

The primary immunogenicity objective analyses will be performed on the PPS Immunogenicity, and will be also performed based on the FAS Immunogenicity if the percentage of subjects excluded from the PPS immunogenicity is greater than 5%.

Geometric Mean Titer

Summary statistics (geometric mean, minimum, median, maximum) of the titers will be presented by assessment (Day 1, Day 22, Day 43 or Day 202) and vaccine group for the overall group and separate by each age subgroup.

The analysis model for the GMT will be done using a general linear model on log-transformed (base 10) Day 22 or Day 43 titers as the outcome variable and as covariates: formulation, log-transformed pre-vaccination titer and age subgroup. From this model, adjusted differences in the least square means (on the log scale) will be produced with 95% confidence limits for each formulation versus the standard formulation F with highest dose. The estimated difference and the confidence limits will be back-transformed to obtain an *adjusted GMT ratio* with 95% confidence limits.

Potential interaction between age stratum and treatment effect will be examined by including an interaction term in the model and present the results of the overall interaction test. The analysis will also be done for each age subgroup separately.

Geometric Mean Ratios (GMRs)

Summary statistics (geometric mean, Coefficient of variation, minimum, median, maximum) of the relative increase in titers will be presented by assessment (Day 22 and Day 43) and vaccine group overall and by age subgroup.

The analysis model for the GMR in titers will be done using the same models as mentioned on log-transformed (base 10) (Day 22 titers/Day 1 titers and Day 43 titers/Day 1 titers) as the outcome variable excluding the pre-vaccination titer as covariate.

Analysis of binary endpoints

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

The binary endpoints (i.e. percentage of subjects with seroconversion or with titer ≥1:40) will be compared between each of formulations versus formulation F (highest dose) expressed by the differences of proportions with 95% CI using the Miettinen and Nurminen method without adjustment for the age stratum.

11.3 Secondary Objectives Analysis

The secondary endpoints are measures of persistence of antibody responses on Day 202 to study vaccine after primary vaccinations, as determined by the HI and MN assays against the H5N1 pandemic influenza homologous strain:

- Geometric mean titers on Day 1 and Day 202 (6 months after the second vaccination) as determined by HI and MN assays.
- Geometric mean ratios calculated as follows: Day 202/Day 1 as determined by HI and MN assays.
- Percentage of subjects achieving seroconversion on Day 202.
- Percentage of subjects with a titer of ≥1:40 on Day 202.

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

A *post hoc* decision will be made if other measurements will be made. 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 results will be included in a separate report. An additional exploratory analysis will be conducted making use of the factorial design of the study and assess the impact of the different factors (dose of MF59 and amount of antigen) on the titers and their GMT. For that we use an analysis of covariance model on log transformed (base ten) Day 22 or Day 43 HI (or MN) titers as the outcome variables and terms for covariates: age cohort, dose of MF59 adjuvant, amount of antigen and Day 1 pre-vaccination HI (or MN) titer. Potential covariate interaction effects will also be examined in this model.

12. EFFICACY ANALYSIS

Not applicable

13. SAFETY ANALYSIS

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

- Vaccine exposure
- Solicited local and systemic AEs
- Unsolicited AEs
- Serious AEs, NOCD, AEs leading to withdrawal, and AESIs.

13.1 Analysis of Extent of Exposure

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

13.1.1 Safety Completeness

Analysis Solicited Adverse Events

The safety completeness analysis on solicited AEs aims to identify subjects who completed the Subject Diary Cards.

Summaries will be produced:

1. The frequencies of subjects who provided data on the diary cards by vaccine group for any vaccination and by first and second vaccination separately.

2. For each solicited AE – including use of antipyretics/analgesics, frequency of the number of days with valid data on the diary by vaccine group for any vaccination and by first and second vaccination separately.
   .

For the corresponding percentages, the denominator will be the respective numbers of exposed subjects. All analyses will be based on the 'as treated' analysis set.

13.2 Solicited Local and Systemic Adverse Events

Different diary cards have been used for subjects <3 years and subjects >=3 years.
Each solicited AE is to be assessed for 7 days following each vaccination 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		
Age group	**Type**	**Solicited Event**	**Grade 1/Mild**	**Grade 2/Moderate**	**Grade 3/Severe**
Overall	**Local**	Injection site tenderness or pain	No interference with daily activity	Interferes with daily activity	Prevents daily activity
		Ecchymosis	11-25 mm	26-50 mm	>50 mm
		Erythema	11-25 mm	26-50 mm	>50 mm
		Induration	11-25 mm	26-50 mm	>50 mm
< 3 years	**Systemic**	Change of eating habits	Eating less than normal for 1 - 2 feeds / meals	Missed 1 or 2 feeds / meals	Missed more than 2 feeds /meals
>= 3 years		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
Overall		Vomiting	1 - 2 times in 24 hours	3 - 5 times in 24 hours	6 or more times in 24 hours or requires intravenous hydration

			Any Event		
Age group	**Type**	**Solicited Event**	**Grade 1/Mild**	**Grade 2/Moderate**	**Grade 3/Severe**
Overall		Diarrhea	2-3 loose stools in 24 hours	4-5 loose stools in 24 hours	6 or more loose stools in 24 hours or requires intravenous hydration
< 3 years		Irritability	Requires more cuddling and is less playful than usual	More difficult to settle	Unable to console
< 3 years		Sleepiness	Shows an increased drowsiness	Sleeps through feeds / meals	Sleeps most of the time and it is hard to arouse him / her
>= 3 years		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
		Malaise	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Overall		Shivering/Chills	No interference with daily activity	Interferes with daily activity	Prevents daily activity
Overall		Fever	38.0 - 38.9 °C	39 – 39.9 °C	≥40.0 °C

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

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 encompass various summaries of the data by vaccination and – where applicable - combined across the two vaccinations using the solicited safety set:

1. Overall summary of subjects with solicited AEs
2. Solicited local AEs, maximum event severity by event and interval
3. Solicited systemic AEs, maximum event severity by event and interval
4. Number of days of solicited AEs, including ongoing AE after Day 7
5. Daily reports of subjects with solicited AEs, only per vaccination
6. Day of first onset of solicited AEs, only per vaccination
7. Solicited AEs ongoing 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 but excluding "Not done/unknown" and implausible 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 age stratum and vaccine group. The local solicited AEs and the common systemic solicited AEs (as defined in Table 3) are also presented combining the two age strata.

Overall summary of subjects with solicited adverse events.

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

The use of analgesics/antipyretics will be considered as a separate category under "other".

Solicited local 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 local AE, followed by a summary across subjects for each vaccine. Subjects without any solicited AEs in the interval, i.e., missing values at each of the requested time points, will be removed from the denominator.

The time intervals will be 30 minutes, Day 1 to Day 7, Day 1 to Day 3 and Day 4 to Day 7. A summary tables will be created with the frequency using only any and severe events.

Solicited 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 AE is defined irrespective of severity. If a solicited AE continues beyond day 7 the period after day 7 is added.

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

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 AE in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero). Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited AE, vaccination number and time point.

Time of first onset of solicited adverse events

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

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

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

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.

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.

Other use of analgesics/antipyretics

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

13.3 Unsolicited Adverse Events

This analysis applies to all ongoing solicited AEs beyond seven days after the vaccination, all unsolicited 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 AEs in the eCRFs will be mapped to PTs using the MedDRA dictionary.

The AEs will then be grouped by MedDRA PTs into frequency tables according to SOC. All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine, will be summarized according to SOC and the PT within SOC. These summaries will be presented by the 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 time intervals will be done by day of onset and not by days ongoing/persisting.

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

- From Day 1 to 21 days after the last vaccination:
    - AE
    - AE by maximum severity
    - Related (i.e. at least possible) AE
    - SAEs
    - Related SAEs
    - AESIs
    - NOCD
    - AEs leading to withdrawal (from the study or the second vaccination)
    - Deaths.

- From Day 1 to end of study:
    - SAEs
    - Related SAEs
    - AESIs
    - NOCD
    - AEs leading to withdrawal (from the study or the second vaccination)
    - Deaths.

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.

All tables will be produced overall and by age subgroup. The overview summary as well as the summary of AEs and SAEs will be also be presented by the other subgroups.

13.4 Clinical Safety Laboratory Investigations

Not applicable

13.5 Concomitant Medication

Medications and vaccines taken prior or during the study are categorized as prior and/or concomitant. In addition, a subset of concomitant medications is defined for the period Day 1 to 43 (see section 8.7 for definition).

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

The frequencies and percentages of subjects reporting prior, concomitant medications (Day 1 – 43 and Day to end of study) will be tabulated by vaccine group.

14. INTERIM ANALYSIS

There are no planned interim analyses for this study.

15. DATA MONITORING COMMITTEES

Not applicable

16. CHANGES TO PLANNED ANALYSIS

Not applicable.

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

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

18. REFERENCES

Clinical Study Protocol V87_30 Version 5, 24-JUN-2020

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Seqirus Signature Page

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