

NCT number: NCT03308825

## Safety and Immunogenicity of Fluzone® Quadrivalent and Fluzone® High-Dose, Influenza Vaccines, 2017–2018 Formulations

Phase IV, multi-center, open-label trial to assess the safety and immunogenicity of Fluzone® Quadrivalent vaccine in children and adults and Fluzone® High-Dose vaccine in adults

### Statistical Analysis Plan (SAP) - Core Body Part

<b>Trial Code:</b>	GRC73
<b>Development Phase:</b>	Phase IV
<b>Sponsor:</b>	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
<b>Investigational Product(s):</b>	Fluzone® Quadrivalent, Influenza Vaccine (2017–2018 formulation) Fluzone® High-Dose, Influenza Vaccine (2017–2018 formulation)
<b>Form / Route:</b>	Liquid/Intramuscular
<b>Indication For This Study:</b>	To evaluate the safety and immunogenicity of the 2017–2018 formulations of Fluzone Quadrivalent vaccine (intramuscular route) in children 6 months to < 9 years of age and adults 18 to < 65 years of age, and Fluzone High-Dose vaccine (intramuscular route) in adults ≥ 65 years of age
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## **List of Abbreviations**

AE	Adverse Event
AR	Adverse Reaction
BL	Blood Sample
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CSR	Clinical Study Report
dil	Dilution
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
FDA	Food and Drug Administration
GBS	Guillain-Barré Syndrome
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
LLOQ	Lower Limit of Quantification
MD	Missing Data
MedDRA	Medical Dictionary for Regulatory Activities
PPAS	Per-Protocol Analysis Set
PT	Preferred Term
RCDC	Reverse Cumulative Distribution Curve
SAE	Serious Adverse Event
SafAS	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
ULOQ	Upper Limit of Quantification
WHO	World Health Organization

## 1 Introduction

This is a trial using the 2017–2018 formulations of quadrivalent inactivated influenza vaccine (Fluzone® Quadrivalent, Influenza Vaccine) and high-dose trivalent inactivated influenza vaccine (Fluzone® High-Dose, Influenza Vaccine). Fluzone Quadrivalent vaccine contains 4 influenza strains (A/H1N1, A/H3N2, and 2 B strains [1 each from the Victoria and Yamagata lineages]). Fluzone High-Dose vaccine contains 60 µg hemagglutinin (HA) per virus strain per dose, which is 4 times the amount of HA per strain per dose in Fluzone Quadrivalent vaccine. It was developed for use in the elderly to elicit enhanced immune responses against influenza through the use of higher antigen content.

During this study, Fluzone Quadrivalent or Fluzone-High-Dose vaccine will be administered in accordance with the age indications and guidance specified in the respective Prescribing Information for each vaccine.

The objectives of this study are to describe the safety and immunogenicity of Fluzone Quadrivalent vaccine in children 6 to <36 months and 3 to < 9 years of age, and in adults 18 to < 65 years of age, and to describe the safety and immunogenicity of Fluzone High-Dose vaccine in adults ≥ 65 years of age. An additional objective of the study is to submit sera from selected subjects to the Center for Biologics Evaluation and Research (CBER) for further analysis by the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA) to support formulation recommendations for subsequent influenza vaccines.

Fluzone Quadrivalent and Fluzone High-Dose vaccines are registered internationally. In the past, annual clinical study reports (CSRs) have been requested by certain national health authorities to demonstrate the safety and immunogenicity of Fluzone (trivalent) and Fluzone Quadrivalent vaccines in support of their registration outside the United States. The CSR for the trial described herein will be used to honor requests for information regarding the safety and immunogenicity of the vaccines evaluated in this trial, as appropriate.

## 2 Trial Objectives

### 2.1 Observational Objectives

#### 2.1.1 Safety

To describe the safety of the 2017–2018 formulation of Fluzone Quadrivalent vaccine in children 6 to <36 months of age and 3 to < 9 years of age, and in adults 18 to < 65 years of age, and the safety of the 2017–2018 formulation of Fluzone High-Dose vaccine in adults ≥ 65 years of age.

The endpoints for the observational safety objective are presented in Section 4.3.1.2.

### **2.1.2 Immunogenicity**

To describe the immunogenicity of the 2017–2018 formulation of Fluzone Quadrivalent vaccine in children 6 to <36 months of age, 3 to < 9 years of age, and in adults 18 to < 65 years of age, and the immunogenicity of the 2017–2018 formulation of Fluzone High-Dose vaccine in adults  $\geq$  65 years of age.

The endpoints for the observational immunogenicity objective are presented in Section 4.3.2.1.

### **2.1.3 Serum Collection**

To submit available sera from approximately 120 subjects (30 subjects from each age group) to CBER for further analysis by the WHO, the CDC, and the FDA to support formulation recommendations for subsequent influenza vaccines.

There are no endpoints for the serum collection objective.

## **3 Description of the Overall Trial Design and Plan**

### **3.1 Trial Design**

### **3.2 Trial Plan**

This is a Phase IV, multi-center, open-label study of a planned 240 subjects to describe the safety and immunogenicity of Fluzone Quadrivalent vaccine in children 6 months to < 9 years of age, adults 18 to < 65 years of age, and to describe the safety and immunogenicity of Fluzone High-Dose vaccine in adults  $\geq$  65 years of age. Each subject will be assigned to the appropriate age group based on the subject's age at the time of enrollment. An approximately equal number of subjects from each group will be enrolled at each site.

- Group 1: Children 6 to < 36 months of age assigned to receive a 0.25-mL pediatric dose of Fluzone Quadrivalent vaccine (60 subjects planned)
- Group 2: Children 3 to < 9 years of age assigned to receive a 0.5-mL dose of Fluzone Quadrivalent vaccine (60 subjects planned)
- Group 3: Adults 18 to < 65 years of age assigned to receive a 0.5-mL dose of Fluzone Quadrivalent vaccine (60 subjects planned)
- Group 4: Adults  $\geq$  65 years of age assigned to receive a 0.5-mL dose of Fluzone High-Dose vaccine (60 subjects planned)

All subjects will receive an intramuscular dose of their assigned vaccine during Visit 1. For subjects 6 months to < 9 years of age for whom 2 doses of influenza vaccine are recommended per guidance from the Advisory Committee on Immunization Practices, a second dose of the same volume of Fluzone Quadrivalent vaccine as the first dose will be administered at Visit 2 (28 [window, 28–35] days after Visit 1).

Subjects 6 months to < 9 years of age (Group 1 and Group 2): blood specimens will be obtained from all subjects prior to the first vaccination and 28 (window, 28–35) days following the final vaccination (Visit 2 for subjects receiving 1 dose; Visit 3 for subjects receiving 2 doses) and assayed for immunogenicity. Solicited adverse reaction (AR) information will be collected for 7 days after each vaccination. Unsolicited non-serious adverse event (AE) and serious adverse event (SAE) information will be collected from Visit 1 to Visit 2 for subjects receiving 1 dose or from Visit 1 to Visit 3 for those subjects receiving 2 doses.

Subjects ≥ 18 years of age (Group 3 and Group 4): blood specimens will be obtained from all subjects prior to vaccination and 21 (window, 21–28) days post-vaccination (Visit 2). Solicited AR information will be collected for 7 days after vaccination. Unsolicited non-serious AE and SAE information will be collected from Visit 1 to Visit 2.

***Pregnancy Testing***

A urine human chorionic gonadotropin pregnancy test or a serum pregnancy test supplied by Sanofi Pasteur will be used to test females of child-bearing potential for pregnancy prior to vaccination. Subjects will not participate in the study if the initial pregnancy test is positive.

**Table 3.1: Study Procedures**

**Trial Flow Chart for Subjects 6 Months to < 9 Years of Age: 2 or 3 Visits, 1 or 2 Vaccinations**

Visit Number	All Subjects		Subjects Receiving 1 Dose of Influenza Vaccine	Subjects Receiving 2 Doses of Influenza Vaccine		
	Visit 1	Telephone Contact		Visit 2	Telephone Contact	Visit 3
Trial Timelines	<b>Day 0</b>	<b>Visit 1 + 8 days</b>	<b>Visit 1 + 28 days</b>	<b>Visit 1 + 28 days</b>	<b>Visit 2 + 8 days</b>	<b>Visit 2 + 28 days</b>
Time Windows	--	<b>+ 8 to 10 days</b>	<b>+ 28 to 35 days</b>	<b>+ 28 to 35 days</b>	<b>+ 8 to 10 days</b>	<b>+ 28 to 35 days</b>
Informed consent/assent <sup>a</sup>	X					
Inclusion & exclusion criteria	X					
Demographic data	X					
Medical history	X					
Influenza vaccination history	X					
History-directed physical examination	X			X		
Temperature <sup>b</sup>	X			X		
Review contraindications for vaccination				X		
Allocation of subject number	X					
Blood sample (BL) <sup>c</sup>	BL1		BL2			BL2
Vaccination <sup>d</sup>	X			X		
Immediate surveillance (20 minutes)	X			X		
Diary card (DC) provided	DC1			DC2		
Telephone contact <sup>e</sup>		X			X	
Diary card reviewed and collected			DC1	DC1		DC2
Interim history			X	X		X
Termination record <sup>f</sup>			X			X
Serious adverse events	To be reported throughout the study period					

<sup>a</sup> Informed consent form will be signed and dated by parent(s) or guardian(s) for subjects 6 months to < 9 years of age and assent form will be signed and dated by subjects 7 to < 9 years of age.

<sup>b</sup> The preferred route for this study is rectal for subjects 6 to < 36 months of age, and oral for subjects 3 to < 9 years of age. The axillary route may be used when a rectal or oral temperature cannot be obtained.

<sup>c</sup> A blood sample, approximately 5 mL, will be collected from all subjects at Visit 1, prior to vaccination, and at either Visit 2 (for subjects receiving 1 influenza vaccine dose) or at Visit 3 (for subjects receiving 2 influenza vaccine doses).

- <sup>d</sup> One or 2 doses of influenza vaccine will be administered per guidance from the Advisory Committee on Immunization Practices in effect during the study. If 2 doses of influenza vaccine are indicated, 1 dose will be administered during Visit 1 and the second dose (same 0.25-mL or 0.5-mL volume of Fluzone Quadrivalent vaccine as administered at Visit 1) will be administered approximately 28 days later during Visit 2.
- <sup>e</sup> The subject's parent/guardian will be contacted by telephone on Day 8 (window, Days 8–10) after vaccination as a reminder to complete the diary card and to bring it with them to the next visit.
- <sup>f</sup> The termination form will be completed at Visit 2 for subjects receiving 1 dose of influenza vaccine or at Visit 3 for subjects receiving 2 doses of influenza vaccine.

**Trial Flow Chart for Subjects  $\geq 18$  Years of Age: 2 Visits, 1 Vaccination**

Visit Number	Visit 1	Telephone Contact	Visit 2
<b>Trial Timelines</b>	<b>Day 0</b>	<b>Visit 1 + 8 days</b>	<b>Visit 1 + 21 days</b>
<b>Time Windows</b>	--	<b>+ 8 to 10 days</b>	<b>+ 21 to 28 days</b>
Informed consent	X		
Inclusion & exclusion criteria	X		
Demographic data	X		
Medical history	X		
Influenza vaccination history (previous season)	X		
History-directed physical examination	X		
Temperature <sup>a</sup>	X		
Urine or serum pregnancy test <sup>b</sup>			
Allocation of subject number	X		
Blood sample (BL) <sup>c</sup>	BL1		BL2
Vaccination	X		
Immediate surveillance (20 minutes)	X		
Diary card (DC) provided	X		
Telephone contact <sup>d</sup>		X	
Diary card reviewed and collected			X
Termination record			X
Serious adverse events	To be reported throughout the study period		

<sup>a</sup> The preferred route for this trial for subjects  $\geq 18$  years of age is oral.

<sup>b</sup> Only for women of child-bearing potential.

<sup>c</sup> A blood sample, approximately 10 mL, will be collected at Visit 1 and Visit 2.

<sup>d</sup> Subjects will be contacted via telephone on Day 8 (window, Days 8–10) as a reminder to complete the diary card and to bring it with them to Visit 2

## 4 Endpoints and Assessment Methods

### 4.1 Primary Endpoints and Assessment Methods

#### 4.1.1 Safety

There are no primary objectives for safety.

#### 4.1.2 Immunogenicity

There are no primary objectives for immunogenicity.

#### 4.1.3 Efficacy

No clinical efficacy data will be obtained in the trial.

### 4.2 Secondary Endpoints and Assessment Methods

There are no secondary objectives in this study.

### 4.3 Observational Endpoints and Assessment Methods

#### 4.3.1 Safety

The observational safety objective is presented in Section 2.1.1.

##### 4.3.1.1 Safety Definitions

The following definitions are taken from the International Conference on Harmonisation E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

##### *Adverse Event:*

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Therefore an AE may be:

- A new illness
- The worsening of a concomitant illness
- An effect of the vaccination, including the comparator

- A combination of the above

All AEs include serious and non-serious AEs.

Surgical procedures are not AEs; they are the action taken to treat a medical condition. It is the condition leading to the action taken that is the AE (if it occurs during the study period).

Pre-existing medical conditions are not to be reported as AEs. However, if a pre-existing condition worsens in frequency or intensity, or if in the assessment of the treating physician there is a change in its clinical significance, this change should be reported as an AE (exacerbation). This applies equally to recurring episodes of pre-existing conditions (e.g., asthma) if the frequency or intensity increases post-vaccination.

***Serious Adverse Event:***

*Serious* and *severe* are not synonymous. The term *severe* is often used to describe the intensity of a specific event as corresponding to Grade 3. This is not the same as *serious* which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations.

An SAE is any untoward medical occurrence that at any dose

- Results in death
- Is life-threatening<sup>a</sup>
- Requires inpatient hospitalization or prolongation of existing hospitalization<sup>b</sup>
- Results in persistent or significant disability/incapacity<sup>c</sup>
- Is a congenital anomaly/birth defect
- Is an important medical event<sup>d</sup>

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<sup>a</sup> The term “life-threatening” refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

<sup>b</sup> All medical events leading to hospitalizations will be recorded and reported as SAEs, with the exception of: hospitalization planned before inclusion into the study or out-patient treatment with no hospitalization.

<sup>c</sup> “Persistent or significant disability or incapacity” means that there is a substantial disruption of a person’s ability to carry out normal life functions.

<sup>d</sup> Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the health of the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, the development of drug dependency or drug abuse, or new-onset diabetes or autoimmune disease.

Additionally, the following important medical events are to be considered as SAEs

New onset of Guillain-Barré syndrome (GBS), encephalitis/myelitis (including transverse myelitis), neuritis (including Bell's palsy, optic neuritis, and brachial neuritis), thrombocytopenia, vasculitis, convulsions (including febrile convulsions [children only]), and anaphylaxis or other hypersensitivity/allergic reactions.

***Adverse Reaction:***

All noxious and unintended responses to a medicinal product related to any dose should be considered ARs.

(The phrase “responses to a medicinal product” means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility)

***Unexpected Adverse Reaction:***

An unexpected AR is an AR, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).

The following additional definitions are used by Sanofi Pasteur:

***Solicited Reaction:***

A solicited reaction is an event that is prelisted in the electronic case report form (eCRF). The assessment of these AEs post-vaccination is mandatory. A solicited reaction is defined by a combination of:

- Symptom and
- Onset post-vaccination

Examples of solicited reactions include injection site pain from Day 0 through Day 7 post-vaccination, or headache from Day 0 through Day 7.

A solicited reaction is therefore an AR observed and reported under the conditions (symptom and onset) prelisted (i.e., solicited) in the eCRF and considered as related to vaccination.

***Unsolicited Adverse Event/Adverse Reaction:***

An unsolicited AE is an observed AE that does not fulfill the conditions prelisted in the eCRF in terms of diagnosis and/or onset post-vaccination (i.e., excluding solicited reactions). For example, if headache from Day 0 through Day 7 is a solicited reaction (i.e., prelisted in the eCRF), then a headache starting on Day 7 is a solicited reaction, whereas headache starting on Day 8 post-vaccination is an unsolicited AE.

Unsolicited non-serious AEs, by definition, do not include SAEs.

**Injection Site Reaction:**

An injection site reaction<sup>a</sup> is an AR at and around the injection site. Injection site reactions are commonly inflammatory reactions.

**Systemic Adverse Event:**

Systemic AEs are all AEs that are not injection site reactions. They therefore include systemic manifestations such as headache, fever, as well as localized or topical manifestations that are not associated with the vaccination site, e.g., erythema that is localized but that is not at the injection site).

**Adverse Events of Special Interest:**

Adverse events of special interest are AEs that are considered by the Sponsor to be relevant for the monitoring of the safety profile of the investigational vaccine.

#### 4.3.1.2 Safety Endpoints

The observational endpoints for the evaluation of safety are:

- 1) Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term [PT]), duration, intensity, and relationship to vaccination of any unsolicited systemic AEs reported in the 20 minutes after vaccination.
- 2) Occurrence, time to onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited injection site reactions (prelisted in the subject's diary card and eCRF) occurring from Day 0 through Day 7 after vaccination.
- 3) Occurrence, time to onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited systemic reactions (prelisted in the subject's diary card and eCRF) occurring from Day 0 through Day 7 after vaccination.
- 4) Occurrence, nature (MedDRA PT), time to onset, duration, intensity, action taken, relationship to vaccination (for systemic AEs only), and whether the event led to early termination from the study, of unsolicited AEs from Visit 1 through Visit 2 for subjects receiving 1 dose or from Visit 1 through Visit 3 for subjects receiving 2 doses.
- 5) Occurrence, nature (MedDRA PT), time to onset, seriousness criteria, relationship to vaccination, outcome, and whether the SAE led to early termination from the study, of SAEs from Visit 1 through Visit 2 for subjects receiving 1 dose or from Visit 1 through Visit 3 for subjects receiving 2 doses.

Adverse events of special interest will be captured as SAEs. These include new onset of GBS, encephalitis/myelitis (including transverse myelitis), neuritis (including Bell's palsy, optic neuritis, and brachial neuritis), thrombocytopenia, vasculitis, convulsions (including febrile convulsions [children only]), and anaphylaxis or other hypersensitivity/allergic reactions from Visit 1 through

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<sup>a</sup> All injection site AEs are considered to be related to vaccination and therefore are all *injection site reactions*.

Visit 2 for subjects receiving 1 dose of vaccine, or from Visit 1 through Visit 3 for subjects receiving 2 doses of vaccine.

#### **4.3.1.3 Safety Assessment Methods**

See Section 9.3.1.3 of the protocol version 1.0, 24 April 2017.

#### **4.3.2 Immunogenicity**

The observational immunogenicity objective is presented in Section 2.1.2.

##### **4.3.2.1 Immunogenicity Endpoints**

Immunogenicity will be evaluated in all subjects by analyzing sera collected prior to vaccination on Day 0 (Visit 1) and after final vaccination (28 [window, 28–35] days post-final vaccination for subjects 6 months to < 9 years of age [Visit 2 for subjects receiving 1 dose; Visit 3 for subjects receiving 2 doses]; or 21 [window, 21–28] days post-vaccination for subjects  $\geq$  18 years of age) using the hemagglutination inhibition (HAI) assay technique.

For each influenza vaccine strain (i.e., 3 strains for Fluzone High-Dose vaccine [a trivalent vaccine] and 4 strains for Fluzone Quadrivalent vaccine), HAI assay titers at pre-vaccination (Day 0) and after the final vaccination will be determined in duplicate.

The derived endpoints<sup>a</sup> are:

- Geometric means of HAI assay titers at pre-vaccination and post-final vaccination
- Ratios of post-final vaccination titers divided by pre-vaccination titers
- Seroprotection: a titer  $\geq$  40 (1/dilution [dil]) at pre-vaccination and at post-final vaccination
- Seroconversion: either a pre-vaccination titer  $<$  10 (1/dil) and a post-final vaccination titer  $\geq$  40 (1/dil), or a pre-vaccination titer  $\geq$  10 (1/dil) and a  $\geq$  4-fold increase in post-final vaccination titer

##### **4.3.2.2 Immunogenicity Assessment Methods**

See Section 9.3.2.2 of the protocol for immunogenicity assessment methods.

#### **4.3.3 Efficacy**

No clinical efficacy data will be obtained in the trial.

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<sup>a</sup> Post-final vaccination in each of the derived endpoints is defined as 28 (window, 28–35) days post-final vaccination for subjects 6 months to < 9 years of age, and 21 (window, 21–28) days post-vaccination for subjects  $\geq$  18 years of age

## 4.4 Derived Endpoints: Calculation Methods

### 4.4.1 Safety

#### 4.4.1.1 Solicited Reactions

##### 4.4.1.1.1 Daily Intensity

All daily intensities will be derived from daily records for solicited reactions according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

For the derivation of daily intensities, the following sequential steps will be applied:

- 1) For solicited reactions (except fever/pyrexia) for which an Investigator records their presence as “No” and for which all daily records are missing, all daily intensities will be derived as None
- 2) For a temperature with partially missing data (MD) after the decimal point, the data will be analyzed by replacing “MD” with zero. For example, a “39.MD°C” daily temperature will be considered as “39.0°C” in the analysis
- 3) For non-measurable solicited reactions, daily intensities will correspond to daily records reported in the clinical database. For measurable solicited reactions, the daily measurements reported in the clinical database will be converted based upon the intensity scales defined in the protocol; this assumes a reaction that is too large to measure (non-measurable, “NM”) is Grade 3

Note: The maximum intensity during the ongoing period will be derived from the record of the maximum intensity/measurement after the end of the solicited period following the rule described above.

##### 4.4.1.1.2 Maximum Overall Intensity

Maximum overall intensity will be derived from the daily intensities computed as described in Section 4.4.1.1.1 and will be calculated as the maximum of the daily intensities during the period considered.

##### 4.4.1.1.3 Presence

Presence will be derived from the maximum overall intensity during the period considered:

None: No presence

Grade 1, Grade 2, or Grade 3: Presence

Missing: Missing presence

Subjects with at least 1 non-missing presence for a specific endpoint will be included in the analysis. Conversely, those with all missing presence will not be included in the analysis of the endpoint.

#### 4.4.1.1.4 Time to Onset

Time to onset will be derived from the daily intensities computed as described in Section 4.4.1.1.1. It will correspond to the first day with an intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (i.e., reaction occurs during 2 separate periods of time intervened by at least 1 daily intensity of Missing or None) then the time to onset is the first day of the first occurrence. For subjects who received 2 doses, the worst-case scenario will be used for the post-any dose summary (i.e., the shorter time to onset [latency] period will be used if present for both doses, or the available time to onset [latency] period will be used if present for either dose).

#### 4.4.1.1.5 Number of Days of Occurrence

Number of days of occurrence during the period considered will be derived from the daily intensities computed as described in Section 4.4.1.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. The number of days of occurrence during the solicited period with a specified intensity may also be derived. For subjects who received 2 doses, the worst-case scenario will be used for the post-any dose summary (i.e., the larger number of days will be used if present for both doses, or the available number of days will be used if present for either dose).

#### 4.4.1.1.6 Overall Number of Days of Occurrence

If a reaction is ongoing at the end of the solicited period, then the overall number of days of occurrence will be derived from the daily intensities and the stop date of the reaction after the end of the solicited period. The overall number of days of occurrence will be:

$$(\text{stop date} - \text{last vaccination date}) + (\text{number of days of occurrence within the solicited period}) - \text{length of the solicited period} + 1$$

If the stop date is missing or incomplete (contains MD), the overall number of days of occurrence will be considered as Missing. For subjects who received 2 doses, the worst-case scenario will be used for the post-any dose summary (i.e., the larger number of days will be used if present for both doses, or the available number of days will be used if present for either dose).

#### 4.4.1.1.7 Ongoing

Ongoing will be derived from the last daily intensity of the solicited period computed as described in Section 4.4.1.1.1 and the maximum intensity during the ongoing period. The Investigator's ongoing flag is not used because the measurement would determine the ongoing status of the reaction.

If the last daily intensity of the solicited period is at least Grade 1 and the maximum intensity in the ongoing period is also at least Grade 1, then the reaction is considered ongoing. In all other cases, the reaction will not be considered as ongoing.

#### 4.4.1.2 Unsolicited Non-serious AEs

##### 4.4.1.2.1 Intensity

Intensity for unsolicited non-serious AEs will be derived according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

If the unsolicited non-serious AE is measurable and its PT is part of the list of solicited reactions, then the measurement will be derived based upon and following the same rule as the intensity scales defined in the protocol for that measurable injection site or systemic reaction.

Intensity for other unsolicited non-serious AEs will correspond to the value reported in the eCRF. The maximum intensity will correspond to the highest intensity for a unique term.

##### 4.4.1.2.2 Last Vaccination

The last vaccination before an unsolicited non-serious AE is derived from the visit numbers provided in the clinical database and will be calculated as follows:

- If an unsolicited non-serious AE has a non-missing visit number, the visit number will be used to determine the last vaccination before the unsolicited non-serious AE
- If the visit number is missing, then the start date of the unsolicited non-serious AE will be used to determine the last vaccination before the unsolicited non-serious AE

##### 4.4.1.2.3 Time to Onset

Time to onset will be derived from the start date of the unsolicited non-serious AE provided in the clinical database and the date of last vaccination:

start date of the unsolicited non-serious AE - date of previous vaccination

The time to onset will be considered as missing only if 1 or both of the dates are missing or partially missing.

Adverse events with a missing time to onset will be considered to have occurred just after the vaccination indicated by the visit number and will be included in tables. For subjects who received 2 doses, the worst-case scenario will be used for the post-any dose summary (i.e., the shorter time to onset [latency] period will be used if present for both doses or the available time to onset [latency] period will be used if present for either dose).

Note: Unsolicited non-serious AEs that occurred before vaccination (negative time to onset) will not be included in the analysis, but will be listed separately.

##### 4.4.1.2.4 Duration

Duration will be derived from the start and stop dates of the unsolicited non-serious AE provided in the clinical database:

stop date of unsolicited non-serious AE - start date of unsolicited non-serious AE + 1

The duration will be considered as missing only if 1 or both of the start and stop dates of the unsolicited non-serious AE is missing or partially missing. For subjects who received 2 doses, the worst-case scenario will be used for the post-any dose summary (i.e., the longer duration will be used if present for both doses or the available duration will be used if present for either dose).

#### **4.4.1.3 Serious Adverse Events**

##### **4.4.1.3.1 Last Vaccination**

The last vaccination before an SAE will be derived from the last visit number provided in the clinical database and will be calculated as follows:

- If an SAE has a non-missing visit number, the visit number will be used to determine the last vaccination before the SAE
- If the visit number is missing, then the start date of the SAE will be used to determine the last vaccination before the SAE

##### **4.4.1.3.2 Time to Onset**

Time to onset will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.1.2.3.

##### **4.4.1.3.3 Duration**

Duration will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.1.2.4.

#### **4.4.2 Immunogenicity**

##### **4.4.2.1 Computed Values for Analysis**

To appropriately manage extreme values (< LLOQ [lower limit of quantification] and  $\geq$  ULOQ [upper limit of quantification]) for analysis purposes, the following computational rules will be applied to the values provided in the clinical database for each blood sample drawn, as appropriate:

If a value is < LLOQ, then use the computed value LLOQ/2

If a value is between  $\geq$  LLOQ and < ULOQ, then use the value

If a value is  $\geq$  ULOQ, then use the value ULOQ

#### 4.4.2.2 Seroprotection<sup>a</sup>

If the computed value is  $\geq 40$  (1/dil) at pre-vaccination or at post-final vaccination, then the derived seroprotection indicator will be “Yes” for that test at pre-vaccination or at post-vaccination, otherwise seroprotection will be “No” – for pre-vaccination or for post-vaccination as indicated by the data. Note: If the computed value is missing, seroprotection will be missing.

#### 4.4.2.3 Fold-rise<sup>a</sup>

The derived endpoint fold-rise will be driven by both baseline and post-final vaccination computed values and will be computed as follows. Generally, for extreme values, this algorithm minimizes the numerator and maximizes the denominator.

If the baseline computed value is < LLOQ and the post-final vaccination computed value is < LLOQ then the fold-rise will be 1

If the baseline computed value is  $\geq$  LLOQ and the post-final vaccination computed value is  $\geq$  LLOQ then the fold-rise will be post-final vaccination computed value/baseline computed value

If the baseline computed value is  $\geq$  LLOQ and the post-final vaccination computed value is < LLOQ then the fold-rise will be (LLOQ/2)/baseline computed value

If the baseline computed value is < LLOQ and the post-baseline computed value is  $\geq$  LLOQ then the fold-rise will be post-final vaccination computed value/LLOQ

Any titer reported as > ULOQ will be converted to ULOQ before the fold-rise calculation

#### 4.4.2.4 Seroconversion<sup>a</sup>

If a pre-vaccination titer is < 10 (1/dil) and a post-final vaccination titer is  $\geq 40$  (1/dil), or a pre-vaccination titer is  $\geq 10$  (1/dil) and there is a  $\geq 4$ -fold increase in post-final vaccination titer, then the derived seroconversion indicator will be “Yes” for that test, otherwise seroconversion will be set to “No”.

### 4.4.3 Efficacy

Not applicable.

### 4.4.4 Derived Other Variables

#### 4.4.4.1 Age for Demographics

To calculate age in months at enrollment for subjects 6 months to <36 months of age, the following algorithm will be used:

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<sup>a</sup> Post-final vaccination is defined as 28 (window, 28–35) days post-final vaccination for subjects 6 months to < 9 years of age, and 21 (window, 21–28) days post-vaccination for subjects  $\geq$  18 years of age.

- The difference in years will be calculated by subtracting the year of birth from the year of the enrollment visit; this difference will be multiplied by 12 to get the difference in months
- The month of birth will be subtracted from the month of the enrollment visit
- The values calculated above will be added to obtain a provisional age in months
- It will be determined when the day of the enrollment visit (e.g., the 31st day of the month) occurred relative to the day of birth (e.g., the 28th day of the month). The final age in months will be determined as follows:
  - a) If the day of the enrollment visit  $\geq$  day of birth, then the sum calculated above will be considered the final age in months; otherwise,
  - b) If the day of the enrollment visit  $<$  day of birth, then the sum calculated above will be reduced by 1 month to obtain the final age in months

To calculate age in years at enrollment for subjects  $\geq$  3 years of age, the following algorithm will be used:

- The difference in years will be calculated by subtracting the year of birth from the year of the enrollment visit.

Age in years will also be calculated using the following formula:

$[(\text{date of the first vaccination}) - (\text{date of birth}) + 1]/365.25$ , rounding to the first decimal place.

#### **4.4.4.2 Duration of a Subject in the Trial**

The duration of a subject in the study will be computed as follows:

maximum (date of visit, date of termination form) - date of Visit 1 + 1.

#### **4.4.4.3 Duration of the Study**

The duration of the study will be computed as follows:

maximum of all subjects (date of visit, date of termination form) - minimum for all subjects (date of Visit 1) + 1.

## **5 Statistical Methods and Determination of Sample Size**

The statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics platform using SAS® Version 9.2 software or later.

The results of the statistical analysis will be available in the final CSR.

For descriptive purposes, the statistics in [Table 5.1](#) will be presented.

**Table 5.1: Descriptive Statistics Produced**

<b>Baseline characteristics and follow-up description</b>	<b>Categorical data</b>	Number of subjects Percentage of subjects
	<b>Continuous data</b>	Mean, standard deviation (SD), quartiles, minimum, and maximum
<b>Clinical safety results</b>	<b>Categorical data</b>	Solicited: Number and percentage (95% confidence intervals [CIs]) of subjects Unsolicited: Number and percentage (95% CIs) of subjects, and number of events
<b>Immunogenicity results</b>	<b>Categorical data (seroprotection, seroconversion, cutoff)</b>	Number and percentage (95% CIs) of subjects
	<b>Continuous data (titer / data)</b>	$\text{Log}_{10}$ : Mean and SD Anti- $\text{Log}_{10}$ (work on $\text{Log}_{10}$ distribution, and anti- $\text{Log}_{10}$ applied): Geometric mean, 95% CI of the geometric mean, quartiles, minimum, and maximum. Graphical representation by reverse cumulative distribution curves (RCDCs)

The confidence interval (CI) for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe [1]; i.e., using the inverse of the beta integral with SAS®).

For immunogenicity results, assuming that  $\text{Log}_{10}$  transformation of the titers/data follows a normal distribution, at first, the mean and the 95% CI will be calculated on  $\text{Log}_{10}$  (titers/data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide geometric means (GMs) and their 95% CI.

## 5.1 Statistical Methods

Summaries of the recruitment and baseline demographic characteristics of the study subjects will be presented. The number of subjects enrolled and their age at enrollment (mean [with standard deviation (SD)], quartiles, minimum and maximum), sex, race, and ethnic origin will be summarized, along with the number and description of protocol deviations.

### 5.1.1 Hypotheses and Statistical Methods for Primary Objectives

There are no primary objectives for this study.

### 5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

There are no secondary objectives for this study.

### 5.1.3 Statistical Methods for Observational Objectives

For the main safety and immunogenicity parameters, 95% CIs of point estimates will be calculated using the normal approximation for quantitative data and the exact binomial distribution (Clopper-Pearson method) for proportions.

#### 5.1.3.1 Safety Analysis

The safety analysis will report the occurrence of solicited reactions and the incidence of unsolicited AEs between Visit 1 and Visit 2 (or between Visit 1 and Visit 3 for those subjects receiving 2 doses).

To avoid any under-estimation of the incidences, the number of subjects with documented safety will be used as denominator for the frequencies.

- For solicited reactions, the denominator will be the total number of subjects who have non-missing data for the endpoint considered
- For unsolicited AEs, the denominator will be the total number of subjects who were vaccinated at the dose analyzed

The safety tables will be produced using a subject approach; i.e., number of subjects who experienced at least 1 safety event during a considered period.

The 2-sided 95% CIs for the percentages will be calculated using the exact binomial method (Clopper-Pearson method).

#### *Solicited reactions*

The solicited injection site reactions and the solicited systemic reactions will be presented separately (except in summary tables).

Solicited reactions will be presented according to their nature (MedDRA [the latest version at database lock] PT), intensity (Grade 1, 2, or 3), time to onset, and number of days of occurrence.

#### *Unsolicited AEs*

Unsolicited AEs included in the analysis will be summarized in the safety overview and analyzed according to their nature (MedDRA [the latest version at database lock] system organ class [SOC] and PT classification) and relationship to the vaccination.

#### *Injection Site Reactions*

For each treatment group, the number and percentage of subjects experiencing any injection site reaction after injection will be calculated.

The description of injection site reactions will be presented according to:

- Solicited injection site reactions
  - All solicited injection site reactions that occur each day within 7 days after injection will be analyzed.
- Unsolicited injection site reactions

All unsolicited injection site reactions between Visit 1 and Visit 2 (or between Visit 1 and Visit 3 for those subjects receiving 2 doses) will be described according to the type of events.

### ***Systemic Events and Reactions***

For each treatment group, the number and percentage of subjects experiencing any unsolicited immediate systemic event in the 20 minutes after injection will be calculated.

In addition, the description of systemic events will be structured according to:

- **Solicited systemic reactions**

All solicited systemic reactions that occur each day within 7 days after injection will be analyzed.

- **Unsolicited systemic events**

All unsolicited systemic events between Visit 1 and Visit 2 (or between Visit 1 and Visit 3 for those subjects receiving 2 doses) will be described according to the type of events.

### ***Serious Adverse Events***

The number and percentage of subjects with SAEs after vaccination between Visit 1 and Visit 2 (or between Visit 1 and Visit 3 for those subjects receiving 2 doses) in each treatment group will be calculated by outcome, seriousness, and relationship to vaccination.

#### **5.1.3.2 Immunogenicity Analysis**

All analyses will be descriptive; no hypotheses will be tested. The following immunogenicity parameters<sup>a</sup> will be calculated for each influenza strain with 95% CIs:

- Geometric mean HAI assay titers at pre-vaccination and post-final vaccination
- Geometric means of the individual titer ratios of post-final vaccination/pre-vaccination
- Seroprotection rates: The percentages of subjects with a titer  $\geq 40$  (1/dil) at pre-vaccination and at post-final vaccination
- Seroconversion rates: The percentages of subjects with either a pre-vaccination titer  $< 10$  (1/dil) and a post-final vaccination titer  $\geq 40$  (1/dil), or a pre-vaccination titer  $\geq 10$  (1/dil) and a  $\geq 4$ -fold increase in post-final vaccination titer

## **5.2 Analysis Sets**

Three analysis sets will be used: the Safety Analysis Set, the Full Analysis Set (FAS), and the Per-Protocol Analysis Set (PPAS).

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<sup>a</sup> Post-final vaccination in each of the immunogenicity parameters is defined as 28 (window, 28–35) days post-final vaccination for subjects 6 months to  $< 9$  years of age, and 21 (window, 21–28) days post-vaccination for subjects  $\geq 18$  years of age

### 5.2.1 Safety Analysis Set

The Safety Analysis Set is defined as those subjects who have received at least 1 dose of the study vaccine.<sup>a</sup> Subjects 6 months to < 9 years of age will have their safety analyzed after “any dose” according to the study vaccine they received as the first dose. Safety analysis after each dose will be assessed in the subset of the Safety Analysis Set having received that dose. All subjects will have their safety data analyzed after each vaccination according to the vaccine they actually received for that dose. Safety data recorded for a vaccine other than the assigned study vaccine will be excluded from the study statistical summaries and listed separately.

### 5.2.2 Full Analysis Set

The FAS is defined as those subjects who have received at least 1 dose of the study vaccine and have a valid post-final vaccination serology result for at least 1 strain.

### 5.2.3 Per-Protocol Analysis Set

The PPAS is a subset of the FAS. The subjects presenting with at least 1 of the following relevant protocol deviations will be excluded from the PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least 1 of the protocol specified exclusion criteria
- Subject did not complete the vaccination schedule including a partially administered vaccination/dose
- Subject did not complete the second dose of study vaccine with a definitive contraindications as defined in the protocol after the first dose
- Subject received a vaccine dose other than the one that he/she was assigned to receive, or was assigned by mistake to another age group
- Preparation and/or administration of vaccine was not done as per-protocol
- Subject did not provide a post-dose serology sample in the proper time
- Subject received a protocol-prohibited therapy/medication/vaccine that might impact antibody response to the study vaccine
- Any other deviation identified during conduct of the study and judged by the clinical team during data review as having a potential impact on the assessment of immunogenicity

### 5.2.4 Populations Used in Analyses

Baseline and demographic analyses will be performed on all enrolled subjects.

The safety analyses will be performed on the Safety Analysis Set.

The immunogenicity analyses will be performed on both the FAS and PPAS.

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<sup>a</sup> for which safety data are scheduled to be collected.

## 5.3 Handling of Missing Data and Outliers

### 5.3.1 Safety

In all subject listings, partial and missing data will be clearly indicated as missing. No search for outliers will be performed.

#### 5.3.1.1 Immediate

For unsolicited non-serious systemic AEs, a missing response to the “Immediate” field is assumed to have occurred after the 20-minute surveillance period and will not be imputed.

#### 5.3.1.2 Causality

Unsolicited AEs and SAEs with missing causality will be considered as related to vaccination.

#### 5.3.1.3 Measurements

Missing measurements (for temperature or length) will remain missing and will not be imputed. Nevertheless, the following rule will be applied: If the measurement (temperature or length) is missing the value after the decimal point, the data will be analyzed by replacing “MD” with zero. For example, a “102.MD°F” daily temperature will be considered as “102.0°F” during the analysis.

#### 5.3.1.4 Intensity

No replacement of the missing qualifier will be done. For analysis, the missing class for the AE qualifier will be provided in the descriptive tables.

#### 5.3.1.5 Start Date and Stop Date

No replacement of missing dates will be performed.

For instance:

- If the onset of an AE cannot be calculated due to a missing start date, then the onset will be considered as missing
- If the stop date of an unsolicited AE is missing, then the duration of that AE will be considered as missing
- If the stop date of a solicited reaction continuing after the solicited period is missing or incomplete, then the number of days of occurrence of that solicited event will be considered as missing

All missing and partially missing dates will be listed where appropriate.

### **5.3.2 Immunogenicity**

For the computations, LLOQ and ULOQ will be managed as follows:

If a titer is < LLOQ, then LLOQ/2 will be used.

If a titer is  $\geq$  LLOQ and < ULOQ, then the titer itself will be used.

If a titer is  $\geq$  ULOQ, then ULOQ will be used.

Missing data will not be imputed. No search for outliers will be performed.

### **5.4 Interim/Preliminary Analysis**

No interim analyses are planned.

### **5.5 Determination of Sample Size and Power Calculation**

The study will enroll approximately 240 subjects: approximately 60 subjects 6 to < 36 months of age will be administered Fluzone Quadrivalent vaccine (Group 1), approximately 60 subjects 3 to < 9 years of age will be administered Fluzone Quadrivalent vaccine (Group 2), approximately 60 subjects 18 to < 65 years of age will be administered Fluzone Quadrivalent vaccine (Group 3), and approximately 60 subjects  $\geq$  65 years of age will be administered Fluzone High-Dose vaccine (Group 4).

No study power was calculated for this study. All analyses will be descriptive.

### **5.6 Data Review for Statistical Purposes**

A review of the data is anticipated through the data review process led by Clinical Data Management before database lock.

### **5.7 Changes in the Conduct of the Trial or Planned Analyses**

Not applicable.

## **6 References List**

- 1 Newcombe R.G. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Stat Med*. 1998;17(8): 857-72.