NCT Number: NCT03617523

Safety and Immunogenicity of Fluzone® Quadrivalent, Flublok® Quadrivalent, and Fluzone® High-Dose, Influenza Vaccines, 2018–2019 Formulations

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

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	GRC90
Development Phase:	Phase IV
Sponsor:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
Investigational Product(s):	Fluzone® Quadrivalent, Influenza Vaccine (2018–2019 formulation) Flublok® Quadrivalent, Influenza Vaccine (2018–2019 formulation) Fluzone® High Dose, Influenza Vaccine (2018–2019 formulation)
Form / Route:	Liquid/Intramuscular
Indication For This Study:	To evaluate the safety and immunogenicity of the 2018–2019 formulations of Fluzone Quadrivalent vaccine in children 6 months to < 9 years of age and adults 18 to < 65 years of age, Flublok Quadrivalent vaccine in adults 18 to < 65 years of age, and Fluzone High-Dose vaccine in adults ≥ 65 years of age, with all study vaccines given by the intramuscular route
Version and Date of the SAP core body part:	Version 1, 22Mar2019

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List of Abbreviations

ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event
AESI	Adverse Event of Special Interest
AR	Adverse Reaction
BL	Blood Sample
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
eCRF	Electronic Case Report Form
CSR	Clinical Study Report
D	Day
DC	Diary Card
dil	Dilution
FAS	Full Analysis Set
FDA	Food and Drug Administration
GBS	Guillain-Barré Syndrome
GM	Geometric Mean
HA	Hemagglutinin
IRT	Interactive Response Technology
LLOQ	Lower Limit of Quantification
MD	Missing Data
MedDRA	Medical Dictionary for Regulatory Activities
NA	Neuraminidase
PPAS	Per-Protocol Analysis Set
PT	Preferred Term
Q1; Q2; Q3	First Quartile; Second Quartile (median); Third Quartile
RCDC	Reverse Cumulative Distribution Curve
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SafAS	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class (primary)
ULOQ	Upper Limit of Quantification
V	Visit
WHO	World Health Organization

1 Introduction

GRC90 is a study using the 2018–2019 formulations of quadrivalent inactivated influenza vaccine (Fluzone® Quadrivalent, Influenza Vaccine), recombinant influenza vaccine (Flublok® Quadrivalent, Influenza Vaccine), and high-dose trivalent inactivated influenza vaccine (Fluzone® High-Dose, Influenza Vaccine).

Influenza viruses types A and B belong to the genus *Orthomyxoviridae* and are characterized as enveloped, negative-strand, segmented ribonucleic acid (RNA) viruses. The viral envelope contains 2 virus-coded glycoprotein spikes, the hemagglutinin (HA) and neuraminidase (NA) proteins, which are key antigens in the host response to influenza virus in both natural infection and vaccination. A third protein, M2, is a minor envelope component of the A-strain viruses (1).

Vaccination with influenza vaccine is the primary method for preventing influenza and its severe complications. It has been shown to be effective in reducing influenza-associated morbidity and mortality in groups at increased risk for influenza-related complications such as infants and young children and persons 50 years of age and older. Of note, immune responses to the vaccine are lower in seniors than those in young healthy adults (2). Strategies to improve immune responses to the vaccine in the elderly population could provide significant additional reductions in influenza-associated morbidity and mortality. One approach is to increase the dose of HA in inactivated vaccines. Previous studies evaluating the immune responses in terms of hemagglutination inhibition (HAI) antibodies with higher doses of HA per strain in different influenza vaccines support a dose-response effect (3).

Fluzone Quadrivalent vaccine contains 4 influenza strains (A/H1N1, A/H3N2, and 2 B strains [1 each from the Yamagata and Victoria lineages]). In pre-licensure studies, Fluzone Quadrivalent vaccine induced antibody responses that were comparable to those induced by trivalent Fluzone vaccine with respect to the strains contained in each vaccine. Pre-licensure studies also demonstrated that the safety profile of Fluzone Quadrivalent vaccine was similar to that of trivalent Fluzone vaccine. Accordingly, Fluzone Quadrivalent vaccine offers the possibility of protecting against both B lineages simultaneously, without compromising vaccine safety (4) (5).

Flublok Quadrivalent vaccine is a recombinant HA influenza vaccine indicated for active immunization against disease caused by influenza A subtype viruses and type B viruses contained in the vaccine for adults 18 years of age and older. Flublok Quadrivalent vaccine contains 45 µg HA per virus strain per dose, 3 times the amount of HA compared to standard-dose quadrivalent inactivated influenza vaccine (SD-IIV4). This higher dose of antigen may be a particular advantage to older adults whose immune response to influenza vaccines can be suboptimal. In fact, in an efficacy study of approximately 9000 adults 50 years of age and older, Flublok Quadrivalent vaccine, compared to a SD-IIV4 comparator, demonstrated 30% to 43% greater efficacy in preventing laboratory-confirmed, protocol-defined influenza-like-illness caused by any viral type or subtype (6).

Fluzone High-Dose vaccine contains 60 µg HA per virus strain per dose, which is 4 times the amount of HA per strain per dose in Fluzone vaccine. It was developed for use in the elderly to

elicit enhanced immune responses against influenza through the use of higher antigen content. A large-scale efficacy trial, which was conducted during 2 influenza seasons (2011–2012 and 2012–2013) and involved more than 30,000 persons, showed that Fluzone High-Dose vaccine was 24.2% more effective than Fluzone vaccine in preventing laboratory-confirmed symptomatic influenza in persons 65 years of age and older. The results of the study met the FDA-agreed criteria for demonstrating superiority of Fluzone High-Dose vaccine compared with Fluzone vaccine for prevention of influenza disease in older adults (7).

During this study, Fluzone Quadrivalent, Flublok Quadrivalent, or Fluzone-High-Dose vaccine will be administered according to the guidelines in the Prescribing Information and only to persons for whom it is indicated.

The objectives of this study are to describe the safety and immunogenicity of Fluzone Quadrivalent vaccine in children 6 months to < 9 years of age and adults 18 to < 65 years of age, Flublok Quadrivalent vaccine in adults 18 to < 65 years of age, and Fluzone High-Dose vaccine in adults ≥ 65 years of age. An additional objective of the study is to provide available sera collected from 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. In addition, available sera from adult subjects may be further analyzed by the Sponsor to assess breadth of immune response induced by the study vaccines.

2 Trial Objectives

Safety

To describe the safety of the 2018–2019 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; the safety of the 2018–2019 formulation of Flublok Quadrivalent vaccine in adults 18 to < 65 years of age; and the safety of the 2018–2019 formulation of Fluzone High-Dose vaccine in adults ≥ 65 years of age.

Immunogenicity

To describe the immunogenicity of the 2018–2019 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; the immunogenicity of the 2018–2019 formulation of Flublok Quadrivalent vaccine in adults 18 to < 65 years of age; and the immunogenicity of the 2018–2019 formulation of Fluzone High-Dose vaccine in adults ≥ 65 years of age.

Serum Collection

To provide available sera (collected from subjects before vaccination [Blood Sample 1] and after final vaccination [Blood Sample 2]) to CBER for further analysis by the WHO, the CDC, and the FDA to support formulation recommendations for subsequent influenza vaccines. In addition, available sera from adult subjects may be further analyzed by the Sponsor to assess breadth of immune response induced by the study vaccines.

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

GRC90 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 and adults 18 to < 65 years of age, Flublok Quadrivalent vaccine in adults 18 to < 65 years of age, and Fluzone High-Dose vaccine in adults ≥ 65 years of age.

Using a pre-programmed interactive response technology (IRT) system, each subject will be assigned to a vaccine 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.

Using the IRT system, subjects 18 to < 65 years of age will be randomly assigned to either Group 3 or Group 4. Enrollment in Group 3 and Group 4 will be stratified by age into 2 subgroups at each site so that approximately 50% of subjects at each site will be 18 to < 50 years of age and approximately 50% of subjects at each site will be 50 to < 65 years of age, so that an equal proportion of subjects within each age subgroup will receive either Fluzone Quadrivalent or Flublok Quadrivalent vaccine. If necessary to achieve 50% overall enrollment of subjects 50 to < 65 years of age and assignment of Fluzone Quadrivalent and Flublok Quadrivalent vaccines in a 1:1 ratio within each age subgroup (i.e., 18 to < 50 years of age and 50 to < 65 years of age), individual sites will be permitted to deviate from these ratios.

- Group 1: Children 6 to < 36 months of age assigned to receive a 0.25-mL dose of Fluzone Quadrivalent vaccine (30 subjects planned)
- Group 2: Children 3 to < 9 years of age assigned to receive a 0.5-mL dose of Fluzone Quadrivalent vaccine (30 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 18 to < 65 years of age assigned to receive a 0.5-mL dose of Flublok Quadrivalent vaccine (60 subjects planned)
- Group 5: Adults ≥ 65 years of age assigned to receive a 0.5-mL dose of Fluzone High-Dose vaccine (60 subjects planned)

3.2 Trial Plan

All subjects will receive either a 0.25-mL (subjects 6 to < 36 months of age [Group 1]) or a 0.5-mL (subjects 3 to < 9 years of age and ≥ 18 years of age [Groups 2, 3, 4, and 5]) intramuscular injection of study vaccine based on their assigned group at Visit 1. For subjects 6 months to < 9 years of age for whom 2 doses of influenza vaccine are recommended per the Advisory Committee on Immunization Practices (ACIP) guidance, a second intramuscular injection of Fluzone Quadrivalent vaccine (same 0.25-mL or 0.5-mL volume as administered at Visit 1) 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 (approximately 5 mL) will be obtained from all subjects prior to the first vaccination at Visit 1 (Day 0) and 28 (window, 28–35) days following the final vaccination (Visit 2, if no study vaccine is administered at Visit 2; or Visit 3, if a second dose of study vaccine is administered at Visit 2) and assayed for immunogenicity. Staff will observe subjects for 20 minutes following study vaccine administration and will record the occurrence of any immediate unsolicited systemic adverse events (AEs). Subjects' parents/guardians will record information about solicited injection site and systemic reactions from Day 0 through Day 7 post-vaccination, and will record information about unsolicited non-serious AEs and serious adverse event (SAEs) from Visit 1 through Visit 2 for subjects receiving 1 dose of study vaccine, and from Visit 1 through Visit 3 for subjects receiving 2 doses of study vaccine.

Subjects ≥ 18 years of age (Group 3, Group 4, and Group 5): blood specimens (approximately 20 mL) will be obtained from all subjects prior to vaccination at Visit 1 and 21 (window, 21–28) days post-vaccination (Visit 2). Staff will observe subjects for 20 minutes following study vaccine administration and will record the occurrence of any immediate unsolicited systemic AEs. Subjects will record information about solicited injection site and systemic reactions from Day 0 through Day 7 post-vaccination, and will record information about unsolicited non-serious AEs and SAEs from Visit 1 through Visit 2.

Table 3.1: Study procedures 1 (Subjects 6 Months to < 9 Years of Age)

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

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

[a] 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.

[b] 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.

[c] 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).

[d] One or 2 doses of influenza vaccine will be administered according to the Advisory Committee on Immunization Practices guidance 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.

[e] 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.

[f] 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.

Table 3.2: Study procedures 2 (Subjects ≥ 18 Years of Age)

Study Flow Chart for Subjects ≥ 18 Years of Age: 2 Visits, 1 Vaccination

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

[a] The preferred route for this study for subjects ≥ 18 years of age is oral.

[b] Only for women of child-bearing potential.

[c] A blood sample, approximately 20 mL, will be collected at Visit 1 and Visit 2.

[d] 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

See Section 9.1 of the protocol.

4.2 Secondary Endpoints and Assessment Methods

See Section 9.2 of the protocol.

4.3 Observational Endpoints and Assessment Methods

See Section 9.3 of the protocol.

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

For measurable injection site reactions (erythema/swelling):

- For subjects 6 months to < 9 years of age
  Grade 1: > 0 to < 25 mm
  Grade 2: ≥ 25 to < 50 mm
  Grade 3: ≥ 50 mm
- For subjects 18 years of age and older
  Grade 1: ≥ 25 to ≤ 50 mm
  Grade 2: ≥ 51 to ≤ 100 mm
  Grade 3: > 100 mm

For measurable systemic reactions (i.e., fever):

- For subjects 6 months to < 36 months of age
  Grade 1: ≥ 38.0°C to ≤ 38.5°C, or ≥ 100.4°F to ≤ 101.3°F
  Grade 2: > 38.5°C to ≤ 39.5°C, or > 101.3°F to ≤ 103.1°F
  Grade 3: > 39.5°C, or > 103.1°F

- For subjects 3 to < 9 years of age and 18 years of age and older
  Grade 1: ≥ 38.0°C to ≤ 38.4°C, or ≥ 100.4°F to ≤ 101.1°F
  Grade 2: ≥ 38.5°C to ≤ 38.9°C, or ≥ 101.2°F to ≤ 102.0°F
  Grade 3: ≥ 39.0°C, or ≥ 102.1°F

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

1) Solicited reactions (except fever/pyrexia) with an investigator presence recorded as "No" and with all daily records missing then all daily intensities will be derived as None.
2) For a temperature partially missing after decimal point, the data will be analyzed replacing "MD" (missing data) by zero. For example, a "39.MD" daily temperature will be considered as "39.0°C" at the time of analysis.
3) For non-measurable solicited reactions, the daily intensities will correspond to the 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 that a reaction that is too large to measure (non-measurable, "NM") is Grade 3. Note the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement > 0 mm but < 25 mm in adults).

Note: The maximum intensity on the ongoing period is 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 is calculated as the maximum of the daily intensities over the period considered.

4.4.1.1.3 Presence

Presence is derived from the maximum overall intensity on 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 without a non-missing presence will not be included in the analysis of the endpoint.

4.4.1.1.4 Time of Onset

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

Note: If a reaction is not continuous (i.e., reaction occurs over 2 separate periods of time intervened by at least 1 daily intensity Missing or None) then the time of 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 period will be used if present for both doses, or the available time to onset period will be used if present for either dose).

Categories for time of onset are as follows:

- D0–D3
- D4–D7

4.4.1.1.5 Number of Days of Occurrence

Number of days of occurrence over the period considered is 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. Number of days of occurrence on 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).

Categories for number of days of occurrence during the solicited period are as follows:

- 1–3 days
- 4–7 days
- 8 days

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 is 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 is:

- (stop date – last vaccination date) + (number of days of occurrence within the solicited period) – length of the solicited period + 1

If the stop date is missing or incomplete (contains missing data), 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).

Categories for overall number of days of occurrence are as follows

- 1–3 days
- 4–7 days
- ≥ 8 days
- Missing

4.4.1.1.7 Ongoing

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

Note: The intensity could be considered None (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement > 0 mm but < 25 mm in adults). If the last daily intensity of the solicited period is at least Grade 1 and maximum intensity on the ongoing period is also at least Grade 1, then the reaction is considered ongoing. In any other cases, the reaction will not be considered as ongoing.

4.4.1.2 Unsolicited Non-serious AEs

4.4.1.2.1 Presence

An observation will be considered an event if it has at least a verbatim term and is not a Grade 0 intensity event. Grade 0 events should be included in the listing "Unsolicited non-serious adverse events not included in the safety analysis."

4.4.1.2.2 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 preferred term (PT) is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule as the intensity scales defined in the protocol for that measurable injection site or systemic reaction. Note the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement > 0 mm but < 25 mm in adults).

Intensity for the other unsolicited non-serious AEs will correspond to the value reported in the (electronic) case report form (CRF).

The maximum intensity corresponds to the highest intensity for a unique term.

4.4.1.2.3 Last Vaccination

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

- If an unsolicited non-serious AE has a non-missing visit number, the visit number should 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 should be used to determine the last vaccination before the unsolicited non-serious AE

4.4.1.2.4 Time of Onset

Time of onset is 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 of onset should be considered as missing only if 1 or both of the dates are missing or partially missing.

An AE with missing time of onset will be considered to have occurred just after the vaccination indicated by the visit number and will be included in these 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 period will be used if present for both doses or the available time to onset period will be used if present for either dose).

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

Time of onset will be displayed as follows:

- D0–D3
- D4–D7
- D8–D14
- ≥ D15
- Missing

4.4.1.2.5 Duration

Duration is 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 should 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).

Duration will be displayed by period as following:

- 1–3 days
- 4–7 days
- 8–14 days
- ≥ 15 days
- Missing

4.4.1.3 SAEs

4.4.1.3.1 Last Vaccination

Last vaccination before an SAE is derived from the visit number provided in the clinical database and is calculated as follows:

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

4.4.1.3.2 Time of Onset

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

An SAE with missing time of onset will be considered to have occurred just after the vaccination indicated by the visit number, so will be included in these tables.

Note: SAEs that occurred before initial vaccination with study vaccine (negative time of onset) will not be included in analysis, but will be listed separately.

4.4.1.3.3 Duration

Duration will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.1.2.5.

4.4.1.4 Other Safety Endpoints

4.4.1.4.1 Pregnancy

This information will be summarized as collected. No derivation or imputation will be done.

4.4.1.4.2 Action Taken

This information will be summarized as collected, including missing observations. No derivation or imputation will be done.

4.4.1.4.3 Seriousness

This information will be summarized as collected. No derivation or imputation will be done.

4.4.1.4.4 Outcome

This information will be summarized as collected. No derivation or imputation will be done.

4.4.1.4.5 Causality

This information will be summarized as collected. An adverse reaction (AR) is defined as an unsolicited non-serious AE or an SAE with causality to the vaccine. Missing causality (relationship) will be handled as described in Section 5.3.1.2.

4.4.1.4.6 AEs Leading to Study Discontinuation

A flag is available in the clinical database for all AEs in order to identify AEs leading to discontinuation.

In general, the items that are counted are:

- Disposition table: A subject who has, on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" is checked
- Safety overview table: A subject who has either on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" is checked or lists an AE on an AE page (solicited, unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated
- SOC/PT table: An event (solicited, unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated

4.4.1.4.7 AEs of Special Interest

Adverse events of special interest (AESIs) will be captured as SAEs. These include 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 from Visit 1 through Visit 2 for subjects receiving 1 dose of vaccine, or from Visit 1 through Visit 3 for subjects receiving 2 doses of vaccine.

4.4.2 Immunogenicity

4.4.2.1 Computed Values for Analysis

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

In order to appropriately manage replicate values for analysis purposes, the individual GM of all values will be computed for each BL after managing extreme values as described:

- If a value is < LLOQ, then the computed value LLOQ/2 will be used
- If a value is between ≥ LLOQ and < ULOQ, then the value will be used
- If a value is ≥ ULOQ, then the value ULOQ will be used

4.4.2.2 Seroprotection

If the computed value is ≥ 40 (l/dilution [dil]) at pre-vaccination or at post-final vaccination (28 [window, 28–35] days following the final vaccination for subjects 6 months to < 9 years of age; and 21 [window, 21–28] days post-vaccination for subjects ≥ 18 years of age), then the derived seroprotection indicator will be “Yes” for that test, otherwise seroprotection will be "No". Note: If the computed value is missing, seroprotection will be missing.

4.4.2.3 Fold-rise

The derived endpoint fold-rise is driven by both baseline and post-baseline computed values and is 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-baseline computed value is < LLOQ then the fold-rise is 1
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is ≥ LLOQ then the fold-rise is post-baseline computed value / baseline computed value
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is < LLOQ then the fold-rise is (LLOQ/2)/baseline computed value
- If the baseline computed value is < LLOQ and the post-baseline computed value is ≥ LLOQ then the fold-rise is post-baseline computed value / LLOQ

Note: If baseline or post-baseline is missing, then fold-rise is missing.

4.4.2.4 Seroconversion

If a pre-vaccination titer is < 10 (1/dil) and a post-final vaccination titer is ≥ 40 (1/dil), or a pre-vaccination titer is ≥ 10 (1/dil) and there is a ≥ 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

The age of a subject in the study is collected as the calendar age in months for children under 2 years old, and in years for subjects 2 years and older at the time of inclusion. The calendar age is the age computed automatically in the CRF, and presented as an integer.

4.4.4.2 Duration of a Subject in the Trial

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

Maximum (date of visit, date of termination form) - (date of visit 1 of that subject) + 1

4.4.4.3 Duration of the Study

The duration of the study is computed in days as follows:

Maximum of all subjects (date of last 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 9.4 software or later.

The results of the statistical analysis will be available in the final clinical study report (CSR).

For descriptive purposes, the following statistics will be presented:

Table 5.1: Descriptive statistics produced

Baseline characteristics and follow-up description	**Categorical data**	Number of subjects. Percentage of subjects.
	Continuous data	Mean, standard deviation (SD), quartiles, minimum, and maximum.
Clinical safety results	**Categorical data**	Solicited: Number and percentage (95% CIs) of subjects. Unsolicited: Number and percentage (95% CIs) of subjects, and number of events.
Immunogenicity results	**Categorical data (seroprotection, seroconversion, cutoff)**	Number and percentage (95% CIs) of subjects.
	Continuous data (titer / data†)	Log10: Mean and standard deviation. Anti-Log10 (work on Log10 distribution, and anti-Log10 applied): Geometric mean (GM), 95% CI of the GM, quartiles, minimum, and maximum. Graphical representation by a reverse cumulative distribution curve (RCDC).

The confidence interval (CI) for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (8), i.e., using the inverse of the beta integral with SAS®.

For immunogenicity results, assuming that Log_{10} transformation of the titers/data follows a normal distribution, at first, the mean and the 95% CI will be calculated on 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 and their 95% CI.

5.1 Statistical Methods

Summaries of baseline demographic characteristics of the study subjects will be presented. The number of subjects enrolled and their age at enrollment (mean, median, and 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 of proportions 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

All analyses will be descriptive; no hypotheses will be tested.

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).

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 with 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 (Medical Dictionary for Regulatory Activities [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

All analyses will be descriptive; no hypotheses will be tested. Data will be summarized and presented for each age group.

The following immunogenicity parameters[a] 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 titer ratios (post-final vaccination divided by pre-vaccination).
- Seroprotection rates: The percentages of subjects with a titer ≥ 40 (l/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 ≥ 40 (1/dil), or a pre-vaccination titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-final vaccination titer

5.2 Analysis Sets

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

5.2.1 Safety Analysis Set

The SafAS is defined as those subjects who have received at least 1 dose of the study vaccine[b]. Subjects will have their safety data analyzed after "any dose" according to the vaccine they actually received as the first dose. Safety analysis after each dose will be assessed in the subset of the SafAS having received that dose. Subjects will have their safety data analyzed after each vaccination according to the vaccine they actually received for that dose.

5.2.2 Full Analysis Set

The FAS is defined as the subset of subjects who received at least 1 dose of the study vaccine and have a valid post-vaccination blood sample 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:

- Subjects did not provide a post-dose serology sample

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[a] 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 ≥ 18 years of age.

[b] for which safety data are scheduled to be collected

- 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
- Subject received a vaccine or dose other than the one that he/she was assigned to receive
- Preparation and/or administration of vaccine was not done as per-protocol
- Subject did not receive vaccine in the proper time window
- Subject did not provide a post-dose serology sample in the proper time window

  The proper time window for Group 1 and Group 2 is 28–35 days after final vaccination, and for Group 3, Group 4 and Group 5, the proper time window is 21–28 days post-vaccination.
- 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

In addition to the reasons listed above, subjects will also be excluded from the PPAS if their post-vaccination serology sample did not produce a valid test result for any antigen.

5.2.4 Other Analysis Set

Enrolled subjects

Enrolled subjects are subjects for whom a CRF has been created.

5.2.5 Populations Used in Analyses

Baseline and demographic analyses will be performed on all enrolled subjects and/or the analysis sets described above, as appropriate.

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

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

5.3 Handling of Missing Data and Outliers

5.3.1 Safety

No imputation of missing data will be conducted. All subjects with safety data and all safety data recorded in the CRFs will be included in the safety analyses. No search for outliers will be performed. In all subject listings, partial and missing data will be clearly indicated as missing.

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.

For SAEs recorded within 24 hours, missing or partially missing elapsed time from last vaccination will remain missing and not be imputed. Such SAEs will not be considered as immediate.

5.3.1.2 Causality

Missing causality (relationship) for unsolicited non-serious AEs and SAEs will be considered at the time of analysis as related to vaccination.

5.3.1.3 Measurements

Partially missing temperatures will be handled as described in Section 4.4.1.1.1.

5.3.1.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.4.1.1.1. For unsolicited non-serious AEs, missing intensities will remain missing and will not be imputed.

5.3.1.5 Start Date and Stop Date

Missing or partially missing start dates for unsolicited AEs will remain missing and not be imputed. If either the start or stop date is missing or partially missing, the time of onset will be considered to be missing. Nevertheless, unsolicited AEs with missing time of onset will be included in analyses according to the visit at which the AEs were collected.

Missing or partially missing stop dates for AEs (solicited reactions and unsolicited AEs) will remain missing and not be imputed.

5.3.1.6 Action Taken

Missing actions taken will remain missing and not be imputed.

5.3.2 Immunogenicity

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

In order to appropriately manage extreme values (undetectable responses < LLOQ and ≥ upper limit of quantitation [ULOQ]), the computational rule described in section 4.4.2.1 is applied.

The derived endpoint of fold-rise is computed as described in Section 4.4.2.3 for extreme values.

5.3.3 Efficacy

Not applicable

5.4 Interim/Preliminary Analysis

No planned interim /preliminary analyses are planned.

5.5 Determination of Sample Size and Power Calculation

The study will enroll approximately 240 subjects: approximately 30 subjects 6 to < 36 months of age will be administered Fluzone Quadrivalent vaccine (Group 1), approximately 30 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), approximately 60 subjects 18 to < 65 years of age will be administered Flublok Quadrivalent vaccine (Group 4), and approximately 60 subjects ≥ 65 years of age will be administered Fluzone High-Dose vaccine (Group 5).

No sample size calculations were performed. Only descriptive statistical analyses will be conducted in this study.

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. This review of the data includes a statistical review.

5.7 Changes in the Conduct of the Trial or Planned Analyses

Not applicable.

6 References List

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