NCT02915302

Safety and Immunogenicity of Fluzone® Quadrivalent Vaccine Administered to Healthy Children 6 to < 36 Months of Age

Phase IV, randomized, observer-blinded, 2-arm, multi-center trial to evaluate the safety and immunogenicity of 2 different dose levels of Fluzone® Quadrivalent vaccine in healthy children 6 to < 36 months of age

Clinical Trial Protocol, Amendment 1

Health Authority File Number:	BB-IND #: 14078
WHO Universal Trial Number (UTN):	U1111-1143-9273
Trial Code:	GRC88
Development Phase:	Phase IV
Sponsor:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
Investigational Product:	Fluzone® Quadrivalent, Influenza Vaccine (2016–2017 formulation)
Form/Route:	Liquid/Intramuscular
Indication For This Study:	To evaluate the safety and immunogenicity of 2 different dose levels of Fluzone Quadrivalent vaccine (2016–2017 formulation) in children 6 to < 36 months of age
Manufacturer:	Same as Sponsor
Coordinating Investigator:	To be determined
Sponsor's Responsible Medical Officers:	[redacted]
Product Safety Officer:	[redacted]
Clinical Trial Manager:	[redacted]
Version and Date of the Protocol:	Version 2.0 dated 13 June 2016

This protocol version 2.0 is the first amendment to the initial trial protocol version 1.0, dated 28 January 2015.

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Synopsis

Company:	Sanofi Pasteur
Licensed Product:	Fluzone® Quadrivalent, Influenza Vaccine (2016–2017 Formulation)
Active Substances:	Influenza virus surface antigens of the following strains: • A/California/7/2009 X-179A (H1N1) • A/Hong Kong/4801/2014 X-263B (H3N2) • B/Brisbane/60/2008 (Victoria lineage; B1) • B/Phuket/3073/2013 (Yamagata lineage; B2)

Title of the Trial:	Safety and Immunogenicity of Fluzone® Quadrivalent Vaccine Administered to Healthy Children 6 to < 36 Months of Age
Development Phase:	Phase IV
Coordinating Investigator:	To be determined
Trial Centers:	This will be a multi-center trial conducted in the United States. Investigators and sites are listed in the "List of Investigators, Trial Centers, and Sponsor's Personnel Involved in the Trial" document.
Planned Trial Period:	First Visit, First Subject: September 2016 Last Visit, Last Subject: February 2017
Trial Design and Methodology:	This will be a Phase IV, randomized, observer-blinded, 2-arm, multi-center trial of approximately 2190 subjects 6 to < 36 months of age. Using a pre-programmed interactive response technology (IRT) system, subjects will be randomly assigned in a 1:1 ratio to either 1 of the following groups: Group 1: 0.25 mL of Fluzone Quadrivalent vaccine (approximately 1095 subjects) Group 2: 0.5 mL of Fluzone Quadrivalent vaccine (approximately 1095 subjects) Enrollment will be stratified by age at each site so that approximately 50% of subjects at each site will be 6 to < 24 months of age and approximately 50% of subjects at each site will be 24 to < 36 months of age. If necessary to achieve 50% overall enrollment of subjects 6 to < 24 months of age, individual sites may be permitted to deviate from this ratio. All subjects will receive 1 intramuscular dose of Fluzone Quadrivalent vaccine during Visit 1. For subjects for whom 2 doses of influenza vaccine are recommended per the Advisory Committee on Immunization Practices (ACIP) guidance, a second dose of Fluzone Quadrivalent vaccine will be administered during Visit 2 (28 [window, 28–35] days after Visit 1). Blood specimens will be obtained from a planned subset of 1600 subjects randomly selected by an IRT system (half in Group 1 and half in Group 2) 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 event (AE) information will be collected for 7 days after each vaccination. Unsolicited AE information 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 subjects receiving 2 doses. Note: Any SAE that occurs after a subject has completed the study but that is likely to be related to the product is to also be reported to the Sponsor. The subject, Investigator, study site personnel, and Sponsor's clinical team members involved in the trial will be blinded to the vaccine dose administered, with the exception of 1 or more unblinded qualified study staff members who will administer the vaccine. The unblinded qualified study staff will not participate in the collection of safety data.
Early Safety Data Review:	This trial will not include an early review of safety data. However, it may be interrupted at any time if new data about the investigational product become available, and/or on advice of the Sponsor, the Institutional Review Board(s) (IRB[s]), or the Food and Drug Administration (FDA). If the trial is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators, the IRB(s), and the FDA of the reason for termination or suspension. If the trial is prematurely terminated for any reason, the Investigator will promptly inform the trial subjects' parents/guardians and should assure appropriate therapy for and follow-up of subjects.
Primary Objective:	To compare the rate of any fever (temperature ≥ 100.4°F [38.0°C]) following the 0.5-mL dose of Fluzone Quadrivalent vaccine to that following the 0.25-mL dose of Fluzone Quadrivalent vaccine during the 7 days after either vaccination (Dose 1 and Dose 2 combined) in subjects 6 to < 36 months of age. ***Hypothesis:*** During the 7 days after either vaccination (Dose 1 and Dose 2 combined), the 0.5-mL dose of Fluzone Quadrivalent vaccine will be non-inferior to the 0.25-mL dose of Fluzone Quadrivalent vaccine in terms of fever reaction (temperature ≥ 100.4°F [38.0°C]) in subjects 6 to < 36 months of age.
Primary Endpoints:	Rates of any fever (temperature ≥ 100.4°F [38.0°C]) during the 7 days after either vaccination (Dose 1 and Dose 2 combined) in each vaccine group.
Secondary Objective:	To compare antibody responses induced by the 0.5-mL dose of Fluzone Quadrivalent vaccine to those induced by the 0.25-mL dose of Fluzone Quadrivalent vaccine as assessed by geometric mean titer (GMT) ratios and seroconversion rate differences after the final vaccination in subjects 6 to < 36 months of age. ***Hypotheses:*** <u>Geometric mean titers</u>: For each of the 4 virus strains, the post-final vaccination GMTs in subjects 6 to < 36 months of age who receive the 0.5-mL dose of Fluzone Quadrivalent vaccine will be non-inferior to the post-final vaccination GMTs in those subjects who receive the 0.25-mL dose of Fluzone Quadrivalent vaccine. <u>Seroconversion</u>: For each of the 4 virus strains, the seroconversion rates in subjects 6 to < 36 months of age who receive the 0.5-mL dose of Fluzone Quadrivalent vaccine will be non-inferior to the seroconversion rates in those subjects who receive the 0.25-mL dose of Fluzone Quadrivalent vaccine.
Secondary Endpoints:	• Geometric mean titers: The hemagglutination inhibition (HAI) GMTs (for each of the 4 virus strains) at 28 (window, 28–35) days after the final vaccination.

	• Seroconversion rates: The percentages of subjects with either a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil), or a pre-vaccination titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-vaccination titer at 28 (window, 28–35) days after the final vaccination.
Observational Objectives:	***Safety*** To describe the safety of 2 different dose levels of the 2016–2017 formulation of Fluzone Quadrivalent vaccine in subjects 6 to < 36 months of age. ***Immunogenicity*** To describe the immunogenicity of 2 different dose levels of the 2016–2017 formulation of Fluzone Quadrivalent vaccine in subjects 6 to < 36 months of age. ***Serum Collection*** To submit available sera from approximately 30 subjects to the Center for Biologics Evaluation and Research for further analysis by the World Health Organization, the Centers for Disease Control and Prevention, and the FDA to support formulation recommendations for subsequent influenza vaccines.
Observational Endpoints:	***Safety*** 1) Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, and relationship to vaccination of any unsolicited systemic AEs reported in the 20 minutes after each 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 electronic case report form [eCRF]) occurring between Day 0 and Day 7 after each 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 between Day 0 and Day 7 after each vaccination. 4) Occurrence, nature (MedDRA preferred term), 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 between Visit 1 and Visit 2 for subjects receiving 1 dose or between Visit 1 and Visit 3 for subjects receiving 2 doses. 5) Occurrence, nature (MedDRA preferred term), time to onset, seriousness criteria, relationship to vaccination, outcome, and whether the SAE led to early termination from the study, of SAEs between Visit 1 and Visit 2 for subjects receiving 1 dose or between Visit 1 and Visit 3 for subjects receiving 2 doses. Adverse events of special interest 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), and anaphylaxis or other hypersensitivity/allergic reactions between Visit 1 and Visit 2 for subjects receiving 1 dose, or between Visit 1 and Visit 3 for subjects receiving 2 doses.

	Immunogenicity Immunogenicity will be evaluated in a subset of 1600 randomly selected subjects prior to vaccination on Day 0 (Visit 1) and at 28 (window, 28–35) days after the final vaccination using the HAI technique. For each influenza vaccine strain, HAI assay titers at pre-vaccination (Day 0) and at 28 (window, 28–35) days after the final vaccination will be determined in duplicate. The derived endpoints are: • Geometric means of HAI assay titers for individual subjects at pre-vaccination (Day 0) and at 28 (window, 28–35) days after the final vaccination. • Ratios of individual post-final vaccination titers divided by individual pre-vaccination titers. • Seroprotection: subjects with a titer ≥ 40 (l/dil) at pre-vaccination or at 28 (window, 28–35) days after the final vaccination. • Seroconversion: subjects with either a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil), or a pre-vaccination titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-vaccination titer at 28 (window, 28–35) days after the final vaccination. ***Serum Collection*** There are no observational endpoints or statistical methods for this objective.
Planned Sample Size:	This study will enroll approximately 2190 subjects 6 to < 36 months of age: Group 1 (0.25 mL of Fluzone Quadrivalent vaccine): approximately 1095 subjects Group 2 (0.5 mL of Fluzone Quadrivalent vaccine): approximately 1095 subjects
Schedule of Study Procedures:	***Vaccination*** All subjects will receive 1 intramuscular injection of Fluzone Quadrivalent vaccine (0.25 mL [Group 1] or 0.5 mL [Group 2]) at Visit 1. For subjects for whom 2 doses of influenza vaccine are recommended per ACIP guidance, a second intramuscular injection of influenza vaccine (same 0.25-mL or 0.5-mL dose as administered at Visit 1) will be administered at Visit 2. ***Blood Sampling*** A total of 2 blood samples of approximately 5 mL each will be collected from a planned subset of 1600 subjects randomly selected by an IRT system (half in Group 1 and half in Group 2). The first blood sample will be collected at Visit 1 prior to vaccination. The second blood sample will be collected at Visit 2, if no study vaccine is administered at Visit 2; or at Visit 3, if study vaccine is administered at Visit 2. Study sites, at their discretion, may apply a topical analgesic to the venipuncture site prior to obtaining sera. However, topical analgesic agents must not be applied at the site of vaccine administration. ***Collection of Safety Data*** Staff will observe subjects for 20 minutes following vaccine administration and will record the occurrence of any immediate unsolicited systemic AEs. Parents/guardians will record information about solicited injection site and

	systemic reactions from Day 0 to Day 7 after each vaccination, and will record information about unsolicited non-serious AEs and SAEs from Visit 1 to Visit 2 for subjects receiving 1 dose of study vaccine and from Visit 1 to Visit 3 for subjects receiving 2 doses of study vaccine. • Staff will contact parents/guardians by telephone at Day 8 (window, Days 8–10) after any study vaccine administration to remind parents/guardians to record any AEs experienced and any concomitant medications taken from the most recent visit to the next visit in the diary card and to bring the diary card with them to the next visit. Parents/guardians will also be reminded to notify the site immediately if an SAE occurs. • Staff will review the safety data with parents/guardians at Visit 2 for subjects receiving 1 dose of study vaccine, or at Visit 2 and Visit 3 for subjects receiving 2 doses of study vaccine.
Duration of Participation in the Trial:	• For subjects receiving only 1 dose of Fluzone Quadrivalent vaccine: 28 (window, 28–35) days following the dose of influenza vaccine • For subjects receiving 2 doses of Fluzone Quadrivalent vaccine: 28 (window, 28–35) days following the second dose of influenza vaccine • No additional safety follow-up beyond Visit 2 (for subjects receiving 1 dose) or Visit 3 (for subjects receiving 2 doses) is planned
Licensed Product:	Fluzone Quadrivalent vaccine, No Preservative: Pediatric Dose (0.25-mL dose), 2016–2017 formulation or Fluzone Quadrivalent vaccine, No Preservative (0.5-mL dose), 2016–2017 formulation
Form:	Liquid – pre-filled syringes
Composition:	Each 0.25-mL dose contains 7.5 µg hemagglutinin (HA) of each antigen, and each 0.5-mL dose contains 15 µg HA of each antigen: • A/California/07/2009 X-179A (H1N1) • A/Hong Kong/4801/2014 X-263B (H3N2) • B/Brisbane/60/2008 (Victoria lineage; B1) • B/Phuket/3073/2013 (Yamagata lineage; B2)
Route:	Intramuscular
Batch Number:	TBD
Inclusion Criteria:	An individual must fulfill *all* of the following criteria in order to be eligible for trial enrollment: 1) Aged 6 to < 36 months of age on the day of first study vaccination (study product administration). 2) Born at full term of pregnancy (≥ 37 weeks) and/or with a birth weight ≥ 2.5 kg. **Note:** This inclusion criterion only applies to subjects 6 to < 12 months of age on the day of the first study visit. 3) Informed consent form has been signed and dated by the parent(s) or guardian(s). 4) Subject and parent/guardian are able to attend all scheduled visits and

	to comply with all trial procedures.
Exclusion Criteria:	An individual fulfilling *any* of the following criteria is to be excluded from trial enrollment: 1) Participation at the time of study enrollment (or in the 30 days preceding the first trial vaccination) or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure. **Note:** Subjects may be considered eligible for enrollment if no intervention for the other study occurred within the 30 days prior to the first study vaccination and none are planned before the subject would complete safety surveillance for the present study. 2) Receipt of any vaccine in the 30 days preceding the first trial vaccination, or planned receipt of any vaccine before Visit 2 for subjects receiving 1 dose of influenza vaccine or Visit 3 for subjects receiving 2 doses of influenza vaccine. 3) Previous vaccination against influenza (in the 2016–2017 season) with either the trial vaccine or another vaccine. 4) Receipt of immune globulins, blood, or blood-derived products in the past 3 months. 5) Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy, such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months). 6) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccine used in the trial or to a vaccine containing any of the same substances. **Note**: the list of vaccine components is included in the Prescribing Information. 7) Thrombocytopenia, which may be a contraindication for intramuscular vaccination, at the discretion of the Investigator. 8) Bleeding disorder, or receipt of anticoagulants in the 3 weeks preceding inclusion, contraindicating intramuscular vaccination. 9) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily. 10) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion. 11) Moderate or severe acute illness/infection (according to Investigator judgment) on the day of planned vaccination or febrile illness (temperature ≥ 100.4°F [38.0°C]). A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided. 12) Identified as a natural or adopted child of either the Investigator or an employee with direct involvement in the proposed study. 13) History of serious adverse reaction to any influenza vaccine.

	14) Personal history of GBS. 15) Any condition that in the opinion of the Investigator would pose a health risk to the subject if enrolled or could interfere with the evaluation of the vaccine. 16) Personal history of clinically significant developmental delay (at the discretion of the Investigator), neurologic disorder, or seizure disorder. 17) Known seropositivity for human immunodeficiency virus, hepatitis B, or hepatitis C. Note: Subjects enrolled into this study will not be prohibited from donating blood for non-interventional studies or other purposes.
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, median, and minimum and maximum), sex, race, and ethnic origin will be summarized, along with the number and description of protocol violations. Continuous variables will be summarized by descriptive statistics (e.g., means and standard deviations for the non-immunogenicity endpoints, and geometric means and their confidence intervals [CIs] for the immunogenicity endpoints); categorical variables will be summarized by frequency distributions (frequency counts, percentages, and their CIs for the main endpoints). ***Primary Objective: Safety*** The 0.5-mL dose of Fluzone Quadrivalent vaccine will be demonstrated as non-inferior to the 0.25-mL dose of Fluzone Quadrivalent vaccine in terms of fever reaction through an assessment of the rates of any fever (temperature ≥ 100.4°F [38.0°C]) during the 7 days following any dose (Dose 1 and Dose 2 combined). Non-inferiority will be demonstrated if the upper bound of the 2-sided 95% CI of the rate difference between Group 2 (subjects receiving 0.5 mL of vaccine) and Group 1 (subjects receiving 0.25 mL of vaccine) is < 5%. The 95% CI of the rate difference will be computed using the Wilson Score method without continuity correction. The Safety Analysis Set will be used for this objective. ***Secondary Objective: Immunogenicity*** **Geometric mean titers:** The statistical methodology will be based on a 2-sided 95% CI of the ratio of the GMTs (0.5-mL dose divided by 0.25-mL dose) at 28 (window, 28–35) days after the final vaccination. Non-inferiority for GMTs will be demonstrated if the lower limit of the 2-sided 95% CI of the GMT ratio is > 0.667 for each of the 4 virus strains. The 95% CI will be calculated using normal approximation of log-transformed titers. The Per-Protocol Analysis Set (PPAS) will be used as the primary analysis set for this objective. **Seroconversion**: The statistical methodology will be based on a 2-sided 95% CI of the difference in seroconversion rates (0.5-mL dose minus 0.25-mL dose) at 28 (window, 28–35) days after the final vaccination. Non-inferiority for seroconversion will be demonstrated if the lower limit of the 2-sided 95% CI is > -10% for the 4 strains. The 95% CI of the rate difference will be computed using the Wilson Score method without continuity correction. The PPAS will be used as the primary analysis set for this objective.

Observational Objectives

Safety

The Safety Analysis Set will be used for the safety analyses. All analyses will be descriptive; no hypotheses will be tested.

For the main parameters, 95% CIs of point estimates will be calculated using the exact binomial distribution (Clopper-Pearson method) for proportions.

Immunogenicity

The following immunogenicity parameters will be calculated for each influenza strain with 95% CIs of point estimates calculated using the exact binomial distribution (Clopper-Pearson method) for proportions and using normal approximation for GMTs:

- Geometric mean HAI assay titers at pre-vaccination (Day 0) and at 28 (window, 28–35) days after the final vaccination.
- Geometric means of the individual titer ratios of post-final vaccination/pre-vaccination.
- Seroprotection rates: The percentages of subjects with a titer ≥ 40 (l/dil) at pre-vaccination and at 28 (window, 28–35) days after the final vaccination.
- Seroconversion rates: The percentages of subjects with either a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil), or a pre-vaccination titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-vaccination titer at 28 (window, 28–35) days after the final vaccination.

Sample size determination

[redacted]

Table of Study Procedures

Trial Flow Chart for Subjects 6 to < 36 Months of Age: 2 or 3 Visits, 1 or 2 Vaccinations, 2 Groups

	All Subjects		Subjects Receiving 1 Dose of Influenza Vaccine	Subjects Receiving 2 Doses of Influenza Vaccine		
Visit Number	**Visit 1**	**Telephone Contact**	**Visit 2**	**Visit 2**	**Telephone Contact**	**Visit 3**
Trial 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	X					
Inclusion & Exclusion Criteria	X					
Demographic data	X					
Medical history	X					
Influenza vaccination history	X					
History-directed physical examination	X			X		
Temperature	X			X		
Allocation of subject number/Randomization	X					
Blood sample (BL) [a]	BL1		BL2			BL2
Vaccination[b,c]	X			X		
Immediate surveillance (20 minutes)	X			X		
Diary card (DC) provided	DC1			DC2		
Telephone contact[d]		X			X	
Diary card reviewed and collected			DC1	DC1		DC2
Interim history			X	X		X
Termination record[e]			X			X
Serious adverse events	To be reported throughout the study period					

[a] A blood sample, approximately 5 mL, will be collected from subjects randomly assigned to the immunogenicity subset 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).

[b] Group 1 will receive a 0.25-mL dose of Fluzone Quadrivalent vaccine at Day 0; Group 2 will receive a 0.5-mL dose of Fluzone Quadrivalent vaccine at Day 0.

[c] 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 (of the same volume as the first dose) will be administered approximately 28 days later during Visit 2.

[d] The subject's parent/guardian will be contacted by telephone on Day 8 after vaccination as a reminder to complete the diary card and to bring it with them to the next visit.

[e] 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.

List of Abbreviations

ACIP	Advisory Committee on Immunization Practices
AE	Adverse Event
AR	Adverse Reaction
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
CDM	Clinical Data Management
CI	Confidence Interval
CRA	Clinical Research Associate
CTA	Clinical Trial Agreement
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
FAS	Full Analysis Set
FDA	Food and Drug Administration
FVFS	First Visit, First Subject
GBS	Guillain-Barré Syndrome
GCI	Global Clinical Immunology
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
GPV	Global PharmacoVigilance
HA	Hemagglutinin
HAI	Hemagglutination Inhibition
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IIV3	Trivalent Inactivated Influenza Vaccine
IND	Investigational New Drug (application)
IRB	Institutional Review Board
IRT	Interactive Response Technology
LLT	Lowest Level Term
LLOQ	Lower Limit of Quantification
LVLS	Last Visit, Last Subject
MedDRA	Medical Dictionary for Regulatory Activities
NA	Neuraminidase
NACI	National Advisory Committee on Immunization
PICU	Pediatric Intensive Care Unit
PPAS	Per-Protocol Analysis Set

PSO	Product Safety Officer
RBC	Red Blood Cell
RMO	Responsible Medical Officer
SAE	Serious Adverse Event
TMF	Trial Master File
ULOQ	Upper Limit of Quantification
WHO	World Health Organization

1 Introduction

1.1 Background

This is a trial using the 2016–2017 formulation of quadrivalent inactivated influenza vaccine (Fluzone® Quadrivalent, Influenza Vaccine).

Influenza viruses types A and B belong to the genus *Orthomyxoviridae* and are characterized as enveloped, negative-strand, segmented 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) (2).

Influenza is transmitted through inhalation of virus-containing droplets from infected individuals. The incubation period is usually 1 to 2 days (3). The virus multiplies in the ciliated columnar epithelium of the upper- and lower-respiratory tract, causing cellular necrosis and sloughing (2). Virus shedding typically begins just before illness onset (within 24 hours), rapidly peaks, and remains elevated for 1 to 2 days before rapidly declining to low levels. Usually, virus shedding lasts a total of 5 to 10 days (3).

There is considerable variation in the severity of illness in different individuals, partly due to age, general health, and immune status relative to previous influenza infections. The classic symptoms include rapid onset (12 hours or less) of malaise, fever, myalgia, headache, and a non-productive cough or sore throat. Most symptoms last several days, but malaise and cough may last for a week or more (3). Complications of influenza include primary viral pneumonia, secondary bacterial pneumonia, and exacerbation of underlying medical conditions such as chronic obstructive pulmonary disease and congestive heart failure.

While influenza affects all age groups, the elderly and persons with underlying health problems are at increased risk for complications. Members of high-risk groups who become ill with influenza are more likely than the general population to require hospitalization. Among infants and younger children, estimated rates of influenza-associated hospitalization are substantially higher than among older children and are similar to rates for other groups considered at higher risk for influenza-related complications, including persons aged ≥ 65 years (1).

Antigenic variation is an important feature of the influenza virus. The viral HA and NA surface antigens are subject to continuous and sequential evolution within immune or partially immune populations. Antigenic drift results from mutation(s) affecting the RNA segment coding for either HA or NA, but more commonly HA. As a result, there is alteration in protein structure involving 1 or a few amino acids, resulting in minor changes in antigenicity. Antigenic variants within a subtype (e.g., H1 or H3) emerge and through natural selection gradually become the more predominant circulating virus strain, while the preceding antigenic variant is suppressed by specific immunity in the population. In contrast to antigenic drift, *antigenic shift* represents the emergence of completely new subtypes, typically through gene reassortment with other circulating strains and acquisition of antigenically different gene sequences. Antigenic shift occurs at irregular intervals and may lead to pandemics (2) (3). While influenza B appears to be more genetically stable than influenza A, the dominant circulating B strain typically varies from

season to season. For over a decade, both Yamagata and Victoria lineages have co-circulated during each season with varying prevalence (4). The large antigenic divergence between the 2 influenza B lineages limits antigenic cross-reactivity, and immunity to influenza viruses of 1 B lineage may not provide adequate protection against viruses of the other lineage. Accordingly, switching from a trivalent vaccine to a quadrivalent vaccine is expected to prevent additional morbidity and mortality associated with influenza B (4). With this in mind, Fluzone Quadrivalent vaccine was developed.

1.1.1 Epidemiology

Influenza is noted for occurring in epidemics. Typically, localized influenza epidemics begin abruptly, peak in 2 to 3 weeks, and last 5 to 6 weeks. The first sign of influenza in the community is usually reports of increased numbers of children with febrile respiratory illness, although a nursing home outbreak may be the first indication. Outbreaks in children are usually followed by the occurrence of influenza-like illness among adults. Following this is an increase in hospital admissions for pneumonia, exacerbation of chronic obstructive pulmonary disease, croup, and congestive heart failure. Increased absenteeism from school and the workplace occurs as a late indicator. Finally, an increased number of deaths due to pneumonia and influenza are a highly specific indicator of influenza. However, due to the reporting delay and time course from infection to death, this indicator lags behind the others (3).

As with other viral respiratory infections, influenza is a seasonal disease. In the Northern Hemisphere, influenza is most likely to occur from November to April, and in the Southern Hemisphere from May to October. In tropical regions, it is more endemic, with periods of increased activity occurring more than once a year.

The public health impact of influenza is dramatic. Estimated rates of influenza-associated hospitalization are substantially higher among infants and younger children than among older children and are similar to rates for other groups considered at higher risk for influenza-related complications, including persons aged ≥ 65 years. During 1993–2008, the estimated rate of influenza-associated hospitalizations was 91.5 per 100,000 among children aged < 1 year and 21.9 per 100,000 for children aged 1 through 4 years. Annual hospitalization rates for influenza decrease with increasing age, ranging from 240–720 hospitalizations per 100,000 children aged < 6 months to approximately 20 hospitalizations per 100,000 children aged 2 through 5 years. Hospitalization rates for children aged < 5 years with high-risk medical conditions are higher, with estimates of 250–500 hospitalizations per 100,000 children in some studies (1).

In the United States, death associated with laboratory-confirmed influenza virus infection among children aged < 18 years has been a nationally reportable condition since 2004. Since reporting began, the annual number of influenza-associated pediatric deaths during regular influenza seasons has ranged from 34 deaths during the 2011–2012 season to 149 deaths during the 2012–2013 season (1) (5). However, between 15 April 2009 and 02 October 2010 (the period of the 2009 H1N1 influenza pandemic), 348 deaths attributed to laboratory-confirmed 2009 H1N1 influenza occurred among children aged < 18 years, the majority of whom had 1 or more underlying medical conditions previously associated with conferring a greater risk for influenza complications (1) (5).

Based on current understanding, the epidemiology of influenza B is characterized by major epidemics every 2–4 years. It causes infections in all age groups, including children, young adults, and the elderly. While influenza affects all age groups, young children remain at increased risk for complications and are more likely than the general population to require hospitalization. Influenza B has been associated with myalgia, myositis, pneumonia, and leukopenia in children (6) (7) (8). Across all ages, the burden of disease from influenza B is less than that from A/H3N2 but greater than that from A/H1N1. Overall, it is a significant cause of absenteeism, clinic visits, hospitalizations, and deaths (4).

1.1.2 Prevention and Control of Infection

Currently, the most effective measure for reducing the impact of influenza is to vaccinate persons at risk each year before the onset of the influenza season, especially persons at high risk for influenza-related complications. The Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC) recommends that all eligible persons 6 months of age and older receive annual vaccination against influenza (9).

Influenza vaccine has been effective in reducing influenza-related morbidity and mortality. The effectiveness of the influenza vaccine in preventing or attenuating influenza illness depends in part on the age and immune competence of the vaccine recipient and on the similarity between the virus strains present in the vaccine and those circulating in the community. Most vaccinated children and young adults develop high post-vaccination hemagglutination inhibition (HAI) antibody titers. These antibodies are protective against illness caused by strains similar to those in the vaccine or the related variants that may emerge during outbreak periods.

1.1.3 Advisory Committee on Immunization Practices Recommendations

Because children 6 through 23 months of age are at substantially increased risk for influenza-related hospitalizations, and children 24 through 59 months of age are at increased risk for influenza-related clinic and emergency department visits, ACIP has recommended annual vaccination of all eligible children in these age groups. In recent years, ACIP has further expanded the targeted age groups, and now recommends that all eligible people 6 months of age and older receive annual influenza vaccination (1) (9). The ACIP continues to emphasize the importance of vaccinating persons ≥ 6 months of age who have high-risk medical conditions (1).

If a child 6 months through 8 years of age is receiving influenza vaccine for the first time, based on ACIP recommendations, 2 doses of influenza vaccine should be administered during the current season. This recommendation is based on studies demonstrating that vaccine effectiveness is lower among children who have never received influenza vaccine previously or who received only 1 dose in their first year of vaccination than it is among children who received 2 doses in their first year of being vaccinated. Children 6 months through 8 years of age who are adequately primed, based on influenza vaccination history, should receive 1 dose during the current season as per ACIP recommendations (1) (9).

1.2 Background of the Investigational Product

Vaccine Testing and Release

Before being released for clinical use, the 2016–2017 formulation of Fluzone Quadrivalent vaccine will have passed all approved release-testing requirements.

Previous Clinical Experience

Fluzone vaccine was licensed in the United States in 1947 as a whole-virus preparation and it has been available since 1980 as a split-virus preparation. Numerous clinical trials have demonstrated its safety, immunogenicity, and effectiveness. Clinical trials, in which Fluzone vaccine was used as a comparator, have also demonstrated the safety and immunogenicity of Fluzone Quadrivalent vaccine. Fluzone Quadrivalent vaccine was first licensed in the United States by the Food and Drug Administration (FDA) on 07 June 2013 in STN: BL103914/5574 for use in persons 6 months of age and older.

1.3 Potential Benefits and Risks

1.3.1 Potential Benefits to Subjects

The benefit to children participating in this trial is the receipt of the 2016–2017 formulation of Fluzone Quadrivalent vaccine.

1.3.2 Potential Risks to Subjects

The most frequent side effect of influenza vaccination is pain or tenderness at the injection site that usually resolves within 3 days. Injection site reactions are generally mild.

Systemic findings such as crying, irritability, or fever (young children); malaise or myalgia (older children); and other systemic symptoms can occur following vaccination and most often affect persons who have had no prior exposure to the vaccine antigens (e.g., young children) (10). These reactions usually begin 6 to 12 hours after vaccination and usually resolve within 3 days. A previous trial of children 6 to 36 months of age has been published and demonstrates the safety of influenza vaccination in this age group (11).

Immediate allergic reactions (e.g., hives, angioedema, allergic asthma, and systemic anaphylaxis) occur rarely after influenza vaccination. These reactions probably result from hypersensitivity to some vaccine component; the majority is most likely related to residual egg protein (1).

Guillain-Barré syndrome (GBS) is a very rare, acute, and frequently severe polyneuropathy characterized by ascending fulminant muscle paralysis. The 1976 swine influenza vaccine was associated with an increased frequency of GBS. Among persons who received the swine influenza vaccine in 1976, the rate of GBS that exceeded the background rate was < 10 cases/1,000,000 persons vaccinated. Evidence for a causal relationship of GBS with subsequent vaccines prepared from other influenza viruses is unclear. Obtaining strong epidemiologic evidence for such a possible limited increase in risk is difficult for a condition as rare as GBS, which has an annual incidence of 10–20 cases per 1,000,000 adults and stretches the limits of epidemiologic investigation.

The reasons why swine influenza vaccine triggered GBS in 1976 to 1977 have never been discovered. In subsequent annual influenza vaccine programs in the United States, from 1977 to 1991, the overall relative risk estimates for GBS after influenza vaccination were slightly elevated but were not statistically significant in any of the studies. However, in a study of the 1992–1993 and 1993–1994 seasons, the overall relative risk for GBS was 1.7 (95% confidence interval [CI]: 1.0–2.8; P=0.04) during the 6 weeks after vaccination, representing approximately 1 additional case of GBS for each 1,000,000 persons vaccinated. The combined number of GBS cases peaked 2 weeks after vaccination. A meta-analysis provided a similar risk estimate for GBS following receipt of 2009 influenza A (H1N1) monovalent inactivated influenza vaccine (12). Thus, investigations to date indicate that there is no substantial increase in GBS associated with influenza vaccines (other than the swine influenza vaccine in 1976) and that, if influenza vaccine does pose a risk, it is probably slightly more than 1 additional case per 1,000,000 persons vaccinated. Even if GBS were a true side effect of vaccination in the years after 1976, the estimated risk for GBS (approximately 1 additional case/1,000,000 persons vaccinated) is substantially less than the risk for severe influenza, which could be prevented by vaccination in all age groups and especially in persons aged ≥ 65 years and those who have medical indications for influenza vaccination.

Neurological disorders temporally associated with influenza vaccination such as myelitis (including encephalomyelitis and transverse myelitis), optic neuritis/neuropathy, partial facial paralysis, and brachial neuritis have been reported. However, no cause and effect has been established. Almost all persons affected were adults, and the described clinical reactions began as soon as a few hours and as late as 2 weeks after vaccination. Full recovery was almost always reported.

In a study of the 2010–2011 influenza season, the CDC found that there was a risk of fever-associated seizure (convulsion) occurring on the day of influenza vaccination and for 1 day after vaccination in children 6 months through 4 years of age. The risk was higher among children who received concomitant inactivated influenza vaccine and 13-valent pneumococcal conjugate vaccine and peaked at approximately age 16 months. The magnitude of the increased risk was less than 1 episode per 1,000 children immunized. A similar risk was found during the 2011–2012 season (in which the formulation of the influenza vaccine used was the same as that used during the 2010–11 season); however, an increased risk for febrile seizures following influenza vaccination was not observed during the 2012–13 influenza season. No increased risk was found for children older than 4 years of age. After taking into consideration benefits and risks of vaccination, no policy change was recommended for use of inactivated influenza vaccine or 13-valent pneumococcal conjugate vaccine (1).

There may be other risks not yet identified.

Please refer to the US Prescribing Information for other adverse events (AEs).

1.4 Rationale for the Trial

The ACIP recommends that children 6 through 35 months of age receive influenza vaccine with 7.5 µg HA of each influenza viral strain, in a volume of 0.25 mL compared to 15 µg HA per strain in a 0.5-mL dose administered to persons 3 years of age and older. This recommendation is based primarily on the results of large multicenter trials of monovalent and bivalent whole-virus

influenza vaccines conducted in children during the 1970s (13). These studies demonstrated that adverse reactions (ARs) were problematic in children administered whole-virus vaccines and downward adjustment of the dose resulted in improved tolerability. However, the introduction of split-virus and subvirion influenza vaccines, which are less reactogenic than whole-virus vaccines, may have eliminated the necessity for dose adjustments in this young age group.

The dose administered to young children may impact the level of protection provided by influenza vaccines. Clinical studies have shown that vaccine effectiveness may be lower in very young children compared with older children. A recent large randomized trial compared rates of laboratory-confirmed influenza among 4707 children aged 6 through 71 months who received inactivated vaccine, MF59-adjuvanted inactivated vaccine, or a non-influenza control vaccine (14). All study participants received 2 doses 28 days apart: children 6 through 35 months of age received 0.25 mL per dose and children 36 through 71 months of age received 0.5 mL per dose. During the 2 seasons of this study (2007–2008 and 2008–2009), overall efficacy across all age groups of non-adjuvanted inactivated vaccine versus control vaccine against all strains was 43% (95% CI: 15%–61%) (14). By age group, efficacy rates were 11% (95% CI: -89%–58%) among children 6 through 23 months of age, 40% (95% CI: -6%–66%) among those 6 through 35 months of age, and 45% (95% CI: 6%–68%) among those 36 through 71 months of age.

A 2012 systematic review of published studies estimated vaccine effectiveness among healthy children was 40% (95% CI: 6%–61%) for those aged 6 through 23 months and 60% (95% CI: 30%–78%) for those aged 24 through 59 months (15). During the 2010–2011 season, when all 3 vaccine virus strains appeared antigenically similar to circulating strains, vaccine effectiveness was assessed among children in a large multi-site observational study (16). Vaccine effectiveness among children aged 6 months through 2 years was 58% (95% CI: 31%–74%) and the effectiveness among children aged 3 through 8 years was 69% (95% CI: 56%–77%).

To evaluate whether a higher dose might induce a more robust antibody response (and potentially offer improved protection) among children 6 through 35 months of age, investigators have evaluated the safety and immunogenicity of a full dose (15 µg HA per strain in 0.5-mL volume) versus a half dose (7.5 µg HA per strain in 0.25-mL volume).

Skowronski and colleagues evaluated 252 previously unimmunized children 6 through 23 months of age who were randomly assigned to receive 2 doses administered 4 to 6 weeks apart of full-dose (15 µg HA per strain) or half-dose (7.5 µg HA per strain) trivalent inactivated influenza vaccine (IIV3) during the 2008–2009 season (17). Children administered full-dose IIV3 responded with higher geometric mean HAI antibody titers to all 3 vaccine components compared to those who received half-dose IIV3. In addition, seroprotection and seroconversion rates were higher among children 6 through 11 months of age who received the full-dose vaccine. For example, seroprotection rates ranged between 40% and 55% in the half-dose group and between 70% and 75% in the full-dose group. Rates of fever were not higher among full- versus half-dose recipients. Rates of injection site reactions were lower in the half-dose group, but the differences between groups were not statistically significant.

Esposito and colleagues investigated full-dose (15 µg HA per strain) versus half-dose (7.5 µg HA per strain) virosomal-adjuvanted subunit IIV3 (2008–2009 season) administered 4 weeks apart to 65 previously unvaccinated children 6 through 35 months of age (18). Following the first dose, HAI titers were significantly higher in the full-dose group compared with the half-dose group for

all 3 antigens. No significant differences in HAI titers between groups were noted 4 weeks after the second administration, but HAI titers were significantly higher among the full-dose recipients 6 months post-vaccination for 2 of the strains (H1N1 and H3N2). There was no increase in systemic or injection site reactions in the full-dose group versus the half-dose group. A subsequent Phase III study conducted by these same investigators during the 2010–2011 season further supported that a single 0.5-mL dose of the virosomal-adjuvanted subunit influenza vaccine could effectively and safely provide long-term immunogenicity to all 3 influenza strains in unprimed children 6 through 35 months of age (19).

Langley and colleagues studied 374 children 6 through 35 months of age who were randomly assigned 1:1 to receive either a full-dose (15 µg HA per strain) or half-dose (7.5 µg HA per strain) of IIV3 during the 2008–2009 season (20). One or 2 doses were administered to primed and unprimed children, respectively. Geometric mean HAI antibody titers and seroprotection and seroconversion rates were higher among children 6 through 23 months of age who received full-dose IIV3 versus half-dose IIV3, although the differences were not statistically significant. In general, differences of these endpoints between the full- and half-dose groups were greater for the younger children 6 through 23 months of age compared to the older cohort 24 through 35 months of age. There were no statistically significant differences in reactogenicity between the 2 dose groups. Fever was not reported more frequently in children who received the full-dose IIV3 versus those who received the half-dose IIV3.

Finally, Halasa and colleagues enrolled 243 children 6 through 35 months of age during the 2010–2011 and 2011–2012 seasons and randomly assigned them 2:1 to receive either full-dose (15 µg HA per strain) or half-dose (7.5 µg HA per strain) IIV3 (1 or 2 doses depending on vaccination history) (13). Among primed subjects, the geometric mean HAI antibody response to H1N1 was significantly higher in the full-dose group. A similar higher response to H1N1 in the full-dose versus half-dose group was observed among naïve subjects 12 through 35 months of age, although the difference was not statistically significant. No immunologic differences between groups were noted for the other antigens in the primed or naïve cohorts. No significant differences between the full- and half-dose groups were observed for systemic and injection site reactions among the primed and naïve cohorts for the 2 seasons combined.

In summary, vaccine efficacy and effectiveness studies have generally demonstrated that inactivated influenza vaccines reduce influenza disease in children, but in some studies the effectiveness in children 6 through 35 months of age is lower than that in children 3 years of age and older. The practice of administering half-dose IIV (7.5 µg HA per strain; 0.25 mL) to young children originates from experience with whole-virus vaccine, which was reactogenic and frequently caused fever in young children. Split-virus and subvirion influenza vaccines are less reactogenic and data from the studies cited above support the safety of the full-dose IIV (15 µg HA per strain; 0.5-mL dose) in this population. Further, data from recent studies suggest that in children 6 through 35 months of age, full-dose influenza vaccine induces generally higher antibody responses compared to those induced by half-dose vaccine, without causing materially higher rates of systemic or injection site reactions. In most studies, differences in antibody responses between the full- and half-dose groups were greatest in the youngest age cohorts (i.e., 6- through 11-month-old or 6- through 23-month-old children).

Full-dose influenza vaccine is now recommended for all children, including those 6 through 35 months of age, by health authorities in Canada (21), the United Kingdom, and Finland (13). In

2012, when the Canadian National Advisory Committee on Immunization (NACI) first recommended a full 0.5-mL dose for children 6 through 35 months of age, it stated (22):

> "Infants and toddlers have a high burden of illness and their response to [IIV3] is not as robust as with older children. Published and unpublished evidence suggests moderate improvement in antibody response in infants, without an increase in reactogenicity, with the use of full vaccine doses. In light of this evidence, NACI recommends that children 6 to 35 months of age should be given a full dose (0.5 mL) of [IIV3] instead of the previously recommended half dose (0.25 mL). This recommendation applies whether the child is being given one dose of [IIV3] or a two dose series."

Moreover, although influenza vaccine coverage among young children continues to improve, coverage is not yet at desired levels. During the 2013–2014 season, 70.4% of children 6 months through 4 years of age received at least 1 dose of an influenza vaccine (23). However, coverage rates with 2 doses of influenza vaccine are far lower. During the 2011–2012 season, 59.2% and 40.5% of children 6 through 23 months and 24 through 59 months of age, respectively, received at least 1 dose of influenza vaccine, but only 42.9% and 29.0% of children in the same age cohorts were fully vaccinated (24). Similarly, during the 2012–2013 season, 65.9% and 45.9% of children 6 through 23 months and 24 through 59 months of age, respectively, received at least 1 dose of influenza vaccine, but only 45.5% and 31.4% to 33.6% (depending on the definition of unprimed children for whom 2 doses were recommended) of children in the same age cohorts were fully vaccinated (25). Ferdinands and colleagues recently underscored the importance of being fully vaccinated by demonstrating that such children were 74% (95% CI: 19%–91%) or 82% (95% CI: 23%–96%) less likely to be admitted to a pediatric intensive care unit (PICU) for influenza compared to PICU controls or community controls, respectively. In stark contrast, receipt of 1 dose of vaccine among children for whom 2 doses were recommended was not protective (26).

[redacted]

Based on the foregoing, there is a strong rationale to allow use of the 0.5-mL dose for young children. Consequently, the purpose of the study described herein is to describe the safety and immunogenicity of the 0.5-mL dose (15 µg HA per strain) of Fluzone Quadrivalent vaccine in children 6 through 35 months of age, with the intent to modify the vaccine's Prescribing Information (Dosage and Administration) to show the 0.5-mL dose of Fluzone Quadrivalent vaccine as indicated for all ages 6 months and older, including children 6 months through 8 years of age (who might receive 1 or 2 doses as recommended by the ACIP).

[redacted]

2 Trial Objectives

2.1 Primary Objective

To compare the rate of any fever (temperature ≥ 100.4°F [38.0°C]) following the 0.5-mL dose of Fluzone Quadrivalent vaccine to that following the 0.25-mL dose of Fluzone Quadrivalent vaccine during the 7 days after either vaccination (Dose 1 and Dose 2 combined) in subjects 6 to < 36 months of age.

The endpoints for the primary objective are presented in Section 9.1.1.1.

2.2 Secondary Objective

To compare antibody responses induced by the 0.5-mL dose of Fluzone Quadrivalent vaccine to those induced by the 0.25-mL dose of Fluzone Quadrivalent vaccine as assessed by geometric mean titer (GMT) ratios and seroconversion rate differences after the final vaccination in subjects 6 to < 36 months of age.

The endpoints for the secondary objective are presented in Section 9.2.2.1.

2.3 Observational Objectives

2.3.1 Safety

To describe the safety of 2 different dose levels of the 2016–2017 formulation of Fluzone Quadrivalent vaccine in subjects 6 to < 36 months of age.

The endpoints for the observational safety objective are presented in Section 9.3.1.2.

2.3.2 Immunogenicity

To describe the immunogenicity of 2 different dose levels of the 2016–2017 formulation of Fluzone Quadrivalent vaccine in subjects 6 to < 36 months of age.

The endpoints for the observational immunogenicity objective are presented in Section 9.3.2.1.

2.3.3 Serum Collection

[redacted]

There are no endpoints for the serum collection objective.

3 Investigators and Trial Organization

This trial will be conducted in approximately 38 centers in the United States. Details of the trial centers, the Investigators at each center, and the Coordinating Investigator(s) are provided in the "List of Investigators and Centers Involved in the Trial" document.

The Sponsor's Responsible Medical Officers (RMOs) (the persons authorized to sign this protocol and any amendments on behalf of the Sponsor) are [redacted] or such delegate(s) as may be identified in their absence.

4 Institutional Review Board

Before the investigational product can be shipped to the investigational site and before the inclusion of the first subject, this protocol, the informed consent form(s) (ICF[s]), subject recruitment procedures, and any other written information to be provided to subjects must be approved by, and/or receive favorable opinion from, the appropriate Institutional Review Board(s) (IRB[s]).

In accordance with Good Clinical Practice (GCP) and local regulations, each Investigator and/or the Sponsor are responsible for obtaining this approval and/or favorable opinion before the start of the trial. If the protocol is subsequently amended, approval must be re-obtained for each substantial amendment. Copies of these approvals, along with information on the type, version number, and date of document, and the date of approval, must be forwarded by the Investigator to

the Sponsor together with the composition of the IRB(s) (the names and qualifications of the members attending and voting at the meetings).

The Investigator will submit written summaries of the status of the trial to the IRB(s) annually, or more frequently if requested. All serious adverse events (SAEs) occurring during the trial that are related to vaccination will be reported by the Investigator to the IRB(s), according to IRB policy.

5 Investigational Plan

5.1 Description of the Overall Trial Design and Plan

5.1.1 Trial Design

This will be a Phase IV, randomized, observer-blinded, 2-arm, multi-center trial to evaluate the safety and immunogenicity of 2 different dose levels of Fluzone Quadrivalent vaccine in approximately 2190 healthy children 6 to < 36 months of age.

5.1.2 Justification of the Trial Design

See Section 1.4 for the justification for the selection of subjects. The trial will be conducted in a modified double-blind (i.e., observer-blinded) fashion, with both the Investigators and the subjects unaware of which vaccine is being administered, to secure objective collection and assessment of safety data.

5.1.3 Trial Plan

Using a pre-programmed interactive response technology (IRT) system, subjects will be randomly assigned in a 1:1 ratio to either 1 of the following groups:

- Group 1: 0.25 mL of Fluzone Quadrivalent vaccine (approximately 1095 subjects)
- Group 2: 0.5 mL of Fluzone Quadrivalent vaccine (approximately 1095 subjects)

Enrollment will be stratified by age at each site so that approximately 50% of subjects at each site will be 6 to < 24 months of age and approximately 50% of subjects at each site will be 24 months to < 36 months of age. If necessary to achieve 50% overall enrollment of subjects 6 to < 24 months of age, individual sites may be permitted to deviate from this ratio.

All subjects will receive 1 intramuscular dose of Fluzone Quadrivalent vaccine (0.25 mL [Group 1] or 0.5 mL [Group 2]) during Visit 1. For subjects for whom 2 doses of influenza vaccine are recommended per ACIP guidance, a second dose of Fluzone Quadrivalent vaccine (same 0.25-mL or 0.5-mL dose as administered at Visit 1) will be administered during Visit 2 (28 [window, 28–35] days after Visit 1).

Blood specimens will be obtained from a planned subset of 1600 subjects randomly selected by an IRT system (half in Group 1 and half in Group 2) 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 AE information will be collected for 7 days after each vaccination. Unsolicited AE information and SAE information will be collected from Visit 1 to Visit 2 for subjects receiving 1 dose or from Visit 1 to Visit 3 for subjects receiving 2 doses.

Note: Any SAE that occurs after a subject has completed the study but that is likely to be related to the product is to also be reported to the Sponsor.

5.1.4 Visit Procedures

All subjects, Investigators and study site staff, and Sponsor personnel involved in the clinical trial will be blinded to the vaccine administered, with the exception of unblinded qualified study staff who will administer the vaccine. The unblinded qualified study staff will not participate in the collection of safety data.

Medical procedures (injections, examinations, etc.) must be conducted by appropriately licensed or credentialed study site staff working within the scope of their licenses/credentials.

Visit 1 (Day 0): Inclusion and Vaccination

The Investigator or delegate will:

1) Explain the trial, including but not limited to its objectives and design and the potential risks and benefits of participating, to the subject's parent/guardian, and answer any questions the subject or subject's parent/guardian may have.
2) Obtain a written informed consent from the subject's parent/guardian. The Investigator or delegate will also sign and date the ICF, retain the original, and give a copy of the signed and dated form to the subject's parent/guardian.
3) Check eligibility of the subject by reviewing applicable inclusion and exclusion criteria.
4) Collect relevant demographic information (date of birth, sex, race, and ethnic origin).
5) Obtain significant medical history (see Section 5.2.6 for details).
6) Collect influenza vaccination history to determine vaccination schedule (1 dose versus 2 doses) per ACIP recommendations in effect during the study.
7) Perform a directed physical examination, if indicated, based on medical history.
8) Measure the temperature by the preferred route (i.e., rectal; see Section 9.3.1.3.2) and record this information in the source document. If the subject has a temperature ≥ 100.4°F (≥ 38.0°C) defer enrollment until the subject has been afebrile for at least 24 hours (see Section 5.2.7.1).
9) If subject meets all inclusion and no exclusion criteria, connect to the IRT system to enter required data to receive subject number and vaccine dose identification number, and to determine if subject has been assigned to the immunogenicity subset.
10) If subject is randomly assigned to the immunogenicity subset, obtain a pre-vaccination blood sample (approximately 5 mL; see Section 7.1 for detailed instructions regarding the collection of blood samples).

The unblinded qualified study staff member will:

11) Prepare the study product according to the group assignment and based on the information provided in Section 6.1.1.2.

12) Within 30 minutes of removing the assigned vaccine from the refrigerator, inject 1 dose of Fluzone Quadrivalent vaccine (0.25 mL [Group 1] or 0.5 mL [Group 2]) intramuscularly into the anterolateral muscle of the thigh or the deltoid muscle, as appropriate.

The Investigator or delegate will:

13) Observe subject for 20 minutes following the injection for the occurrence of allergic and anaphylactic reactions and immediate injection site and systemic reactions.

14) Provide Diary Card 1 to the subject's parent/guardian to record solicited injection site and systemic reactions from Day 0 to Day 7 post-vaccination, and unsolicited AEs, SAEs, as well as any concomitant medications (see Section 6.7) from Visit 1 to Visit 2.

15) Provide the subject's parent/guardian with a digital thermometer and flexible ruler along with instructions for their use.

16) Schedule Visit 2. Remind the subject's parent/guardian that they will be contacted by phone on or about Day 8 post-vaccination to remind them to complete the diary card and to bring the completed diary card with them to Visit 2.

17) Remind the subject's parent/guardian to notify the site immediately if an SAE occurs.

18) Complete the relevant electronic case report form (eCRF) pages for this visit.

Telephone Contact – Visit 1 + 8 (window, 8–10) days

Eight days after the study vaccination at Visit 1, a delegated staff member from the study site will telephone the subject's parent/guardian to perform the following:

1) Remind the subject's parent/guardian to record on the diary card any AEs and any concomitant medications (see Section 6.7) from Visit 1 until Visit 2.

2) Remind the subject's parent/guardian to notify the site immediately if an SAE occurs. If an SAE occurs, follow the instructions in Section 10 for reporting it.

3) Confirm the date of the appointment of Visit 2, instruct the subject's parent/guardian to return at this time, and to bring the completed diary card with them to the study site.

Note: If Day 8 falls on a weekend or a holiday, the telephone call may be placed preferably on the following business day. If the subject's parent/guardian is not available, the study staff should document the attempts to make contact.

Visit 2 (Visit 1 + 28 [window, 28–35] days) – Subjects Receiving 1 Dose of Influenza Vaccine - Collection of Safety Information and Blood Sample

The Investigator or delegate will:

1) Review Diary Card 1 with the subject's parent/guardian for accuracy and collect it as the source document.

2) Review interim health history, including any AEs, medications, or therapy that occurred since vaccination.

3) If subject is part of the immunogenicity subset, obtain blood sample (approximately 5 mL; see Section 7.1 for detailed instructions regarding the collection of blood samples).

4) Complete the relevant eCRF pages for this visit as well as the termination record in the eCRF.

Visit 2 (Visit 1 + 28 [window, 28–35] days) – Subjects Receiving 2 Doses of Influenza Vaccine[a] - Second Vaccination and Collection of Safety Information

The Investigator or delegate will:

1) Review Diary Card 1 with the subject's parent/guardian for accuracy and collect it as the source document.

2) Review interim health history, including any AEs, medications, or therapy that occurred since vaccination.

3) Perform a history-directed physical examination.

4) Measure the temperature by the preferred route (i.e., rectal; see Section 9.3.1.3.2) and record this information in the source document. If the subject has a temperature ≥ 100.4°F (≥ 38.0°C) defer enrollment until the subject has been afebrile for at least 24 hours (see Section 5.2.7.1).

5) Contact the IRT system to have vaccine dose identification number assigned.

The unblinded qualified study staff member will:

6) Prepare the study product according to the information provided in Section 6.1.1.2.

7) Within 30 minutes of removing the vaccine from the refrigerator, inject subject with the same dose of vaccine administered to the subject during Visit 1 (i.e., either 0.25 mL or 0.5 mL) intramuscularly into the anterolateral muscle of the thigh or the deltoid muscle, as appropriate.

The Investigator or delegate will:

8) Observe subject for 20 minutes following the injection for the occurrence of allergic and anaphylactic reactions and immediate injection site and systemic reactions.

9) Provide Diary Card 2 to the subject's parent/guardian to record solicited injection site and systemic reactions from Day 0 to Day 7 post-vaccination, and unsolicited AEs, SAEs, as well as any concomitant medications (see Section 6.7) from Visit 2 to Visit 3.

10) Schedule Visit 3. Remind the subject's parent/guardian that they will be contacted by phone on or about Day 8 post-vaccination to remind them to complete the diary card and to bring the completed diary card with them to Visit 3.

11) Remind the subject's parent/guardian to notify the site immediately if an SAE occurs.

12) Complete the relevant eCRF pages for this visit.

[a] As per ACIP guidance.

Telephone Contact – Visit 2 + 8 (window, 8–10) days

Eight days after the study vaccination at Visit 2 (for subjects receiving a second vaccination), a delegated staff member from the study site will telephone the subject's parent/guardian to perform the following:

1) Remind the subject's parent/guardian to record on the diary card any AEs and any concomitant medications (see Section 6.7) from Visit 2 until study termination Visit 3.
2) Remind the subject's parent/guardian to notify the site immediately if an SAE occurs. If an SAE occurs, follow the instructions in Section 10 for reporting it.
3) Confirm the date of the appointment of Visit 3. Instruct the subject's parent/guardian to return at this time and to bring the completed diary card with them to the study site.

Note: If Day 8 falls on a weekend or a holiday, the telephone call may be placed preferably on the following business day. If the subject's parent/guardian is not available, the study staff should document the attempts to make contact.

Visit 3 (Visit 2 + 28 [window, 28–35] days) – Subjects Receiving 2 Doses of Influenza Vaccine[a]- Collection of Safety Information and Blood Sample

The Investigator or delegate will:

1) Review Diary Card 2 with the subject's parent/guardian for accuracy and collect it as the source document.
2) Review interim health history, including any AEs, medications, or therapy that occurred since vaccination.
3) If subject is part of the immunogenicity subset, obtain blood sample (approximately 5 mL; see Section 7.1 for detailed instructions regarding the collection of blood samples).
4) Complete the relevant eCRF pages for this visit as well as the termination record in the eCRF.

Collection of Diary Cards

If the subject's parent/guardian does not return for Visit 2 or Visit 3, and the diary card is not received at the site, site personnel will contact the subject's parent/guardian by telephone. During the telephone call, the subject's parent/guardian will be reminded to return the diary card to the study site. Telephone calls will be recorded in the subject's source documents. If study personnel are unable to contact the subject's parent/guardian by telephone with 3 attempts, study personnel will follow instructions given in Section 5.2.9.

SAEs and AEs That Are Related to Vaccination or That Led to Discontinuation:

At any time during the study, a subject who experiences an SAE or an AE must be followed if *either* of the following is true:

- The SAE or AE is considered by the Investigator to be related to vaccination and is not resolved by the end of the subject's participation in the trial

[a] As per ACIP guidance

- The subject has been discontinued from the trial because of the SAE or AE

Any such subject must be followed until the condition resolves, becomes stable, or becomes chronic, unless the subject's parent/guardian specified that they do not want to be contacted for follow-up and it is documented in the source document.

5.1.5 Planned Trial Calendar

The following dates are approximate. The actual dates may differ as, for example, the trial will not start until all the appropriate regulatory and ethical approvals have been obtained.

Planned trial period - FVFS (first visit, first subject) to LVLS (last visit, last subject): September 2016 to February 2017

Planned end of trial: February 2017

Planned date of final clinical study report: within approximately 10 months of study completion.

5.1.6 Early Safety Data Review

This trial will not include an early review of safety data. However, it may be interrupted at any time if new data about the investigational product become available, and/or on advice of the Sponsor, the IRB(s), or the FDA.

If the trial is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators, the IRB(s), and the FDA of the reason for termination or suspension. If the trial is prematurely terminated for any reason, the Investigator will promptly inform the trial subjects' parents/guardians and should assure appropriate therapy for and follow-up of subjects.

5.2 Enrollment and Retention of Trial Population

5.2.1 Recruitment Procedures

Subjects may be recruited from the general population. The site will ensure that any advertisements used to recruit subjects (informational brochures, parent letters, posters, and other advertisements) are submitted to Sanofi Pasteur prior to submission to the IRB for approval.

5.2.2 Informed Consent Procedures

Informed consent is the process by which a subject's parent/guardian voluntarily confirms his or her willingness to participate in a particular trial. Informed consent must be obtained before any study procedures are performed. The process is documented by means of a written, signed, and dated ICF.

In accordance with GCP, prior to signing and dating the consent form, the subject's parent/guardian must be informed by appropriate study personnel about all aspects of the trial that are relevant to making the decision to participate, and must have sufficient time and opportunity to ask any questions.

The actual ICF used at each center may differ, depending on local regulations and IRB requirements. However, all versions must contain the standard information found in the sample ICF provided by the Sponsor. Any change to the content of the ICF must be approved by the Sponsor and the IRB prior to the form being used.

If new information becomes available that may be relevant to the subject's parent's/guardian's willingness to continue participation in the trial, this will be communicated to him/her in a timely manner. Such information will be provided via a revised ICF or an addendum to the original ICF.

Informed consent forms will be provided in duplicate, or a photocopy of the signed consent will be made. The original will be kept by the Investigator, and the copy will be kept by the subject's parent/guardian.

Documentation of the consent process is to be recorded in the source documents.

5.2.3 Screening Criteria

There are no screening criteria other than the inclusion and exclusion criteria.

5.2.4 Inclusion Criteria

An individual must fulfill *all* of the following criteria in order to be eligible for trial enrollment:

1) Aged 6 to < 36 months of age on the day of first study vaccination (study product administration).[a]
2) Born at full term of pregnancy (≥ 37 weeks) and/or with a birth weight ≥ 2.5 kg.

   Note: This inclusion criterion only applies to subjects 6 to < 12 months of age on the day of the first study visit.
3) Informed consent form has been signed and dated by the parent(s) or guardian(s).
4) Subject and parent/guardian are able to attend all scheduled visits and to comply with all trial procedures.

5.2.5 Exclusion Criteria

An individual fulfilling *any* of the following criteria is to be excluded from trial enrollment:

1) Participation at the time of study enrollment (or in the 30 days preceding the first trial vaccination) or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure.

   Note: Subjects may be considered eligible for enrollment if no intervention for the other study occurred within the 30 days prior to the first study vaccination and none are planned before the subject would complete safety surveillance for the present study.

[a] "6 to < 36 months" means from the first day of the 6th month after birth to the day before the 36th month

2) Receipt of any vaccine in the 30 days preceding the first trial vaccination, or planned receipt of any vaccine before Visit 2 for subjects receiving 1 dose of influenza vaccine or Visit 3 for subjects receiving 2 doses of influenza vaccine.
3) Previous vaccination against influenza (in the 2016–2017 season) with either the trial vaccine or another vaccine.
4) Receipt of immune globulins, blood, or blood-derived products in the past 3 months.
5) Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy, such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months).
6) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccine used in the trial or to a vaccine containing any of the same substances.

   Note: The list of vaccine components is included in the Prescribing Information and in Section 6.1.1.1.
7) Thrombocytopenia, which may be a contraindication for intramuscular vaccination, at the discretion of the Investigator.
8) Bleeding disorder, or receipt of anticoagulants in the 3 weeks preceding inclusion, contraindicating intramuscular vaccination.
9) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily.
10) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion.[a]
11) Moderate or severe acute illness/infection (according to Investigator judgment) on the day of planned vaccination or febrile illness (temperature ≥ 100.4°F [38.0°C]). A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided.
12) Identified as a natural or adopted child of either the Investigator or an employee with direct involvement in the proposed study.
13) History of serious ARs to any influenza vaccine.
14) Personal history of GBS.
15) Any condition that in the opinion of the Investigator would pose a health risk to the subject if enrolled or could interfere with the evaluation of the vaccine.
16) Personal history of clinically significant developmental delay (at the discretion of the Investigator), neurologic disorder, or seizure disorder.

[a] Chronic illness may include, but is not limited to, cardiac disorders, renal disorders, autoimmune disorders, diabetes, psychomotor diseases, and known congenital or genetic diseases

17) Known seropositivity for human immunodeficiency virus, hepatitis B, or hepatitis C.

Note: Subjects enrolled into this study will not be prohibited from donating blood for non-interventional studies or other purposes.

5.2.6 Medical History

Prior to enrollment, subjects will be assessed for pre-existing conditions and illnesses, both past and ongoing. Any such conditions will be documented in the source document. Significant medical history (reported as diagnosis), including conditions for which the subject is or has been followed by a physician, or conditions that could resume during the course of the study or lead to an SAE or to repetitive outpatient care will be collected in the eCRF. The significant medical history section of the eCRF contains a core list of body systems and disorders that could be used to prompt comprehensive reporting, as well as space for the reporting of specific conditions and illnesses.

For each condition, the data collected will be limited to:

- Diagnosis (this is preferable to reporting signs and symptoms[a])
- Presence or absence of the condition at enrollment

Dates, medications, and body systems are not to be recorded, and the information collected will not be coded. Its purpose is to assist in the later interpretation of safety data collected during the trial.

5.2.7 Contraindications for Subsequent Vaccinations

5.2.7.1 Temporary Contraindications

Should a subject experience 1 of the conditions listed below, the Investigator will postpone further vaccination until the condition is resolved. Postponement must still be within the timeframe for vaccination indicated in the Table of Study Procedures.

- Febrile illness (temperature ≥ 100.4°F [≥ 38.0°C]) or moderate or severe acute illness/infection on the day of planned vaccination, according to Investigator judgment

5.2.7.2 Definitive Contraindications

Should a subject experience 1 or more of the conditions listed below after the first dose of study vaccine, the Investigator will not administer the second dose of vaccine that has been allocated for use in the study (i.e., study vaccine); however, the Investigator may administer a second dose of licensed, non-study influenza vaccine in accordance with standard clinical care.

Definitive contraindications include:

- An anaphylactic or other significant allergic or serious reaction to the previous dose of vaccine.

[a] Investigators are highly encouraged to evaluate signs and symptoms to establish and report a diagnosis whenever feasible; reporting only signs and symptoms in lieu of a unifying diagnosis is strongly discouraged

- Receipt of any non-study vaccine (including a non-study dose of 2016–2017 influenza vaccine), immune globulins, blood, or blood-derived products between Visit 1 and Visit 2.
- Bleeding disorder, receipt of anticoagulants, or thrombocytopenia, which may be a contraindication for intramuscular vaccination, at the discretion of the Investigator.
- Development of any condition that in the opinion of the Investigator would pose a health risk to the subject or could interfere with the evaluation of the study vaccine (including GBS, clinically significant developmental delay, neurologic disorder, seizure disorder, human immunodeficiency virus, hepatitis B, or hepatitis C).
- Development of an immunodeficiency; or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy; or receipt of long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks).
- Adverse events that are considered a contraindication for further participation in the trial.
- Poor or non-compliance by the subject. In cases where a subject is outside the recommended visit schedule, the subject may be continued in the study as a protocol violator and will be analyzed accordingly.

Subjects will not be withdrawn due to a definitive contraindication but will be followed up for safety and possibly immunogenicity assessment.

5.2.8 Conditions for Withdrawal

Parents/guardians will be informed that they have the right to withdraw their child from the trial at any time. A subject may be withdrawn from the study for any of the following:

- At the discretion of the Investigator or Sponsor due to safety concerns (withdrawal) without the parent's/guardian's permission
- At the request of the parent/guardian (dropout)

The following will result in automatic withdrawal or exclusion of a subject from the study:

- Significant non-compliance with the protocol, based on the Investigator's judgment

The reason for a withdrawal or dropout should be clearly documented in the source documents and on the eCRF.

The Investigator must determine whether voluntary withdrawal is due to safety concerns (in which case, the reason for discontinuation will be noted as "SAE" or "other AE" as appropriate) or for another reason.

Withdrawn subjects will not be replaced.

5.2.9 Lost to Follow-up Procedures

In the case of subjects who fail to return for a follow-up examination, documented reasonable effort (i.e., documented telephone calls and certified mail) should be undertaken to locate or recall them, or at least to determine their health status while fully respecting their rights. These efforts should be documented in the eCRF and in the source documents.

5.2.10 Classification of Subjects Who Discontinue the Trial

For any subject who discontinues the trial prior to completion, the most significant reason for early termination will be checked in the eCRF. Reasons are listed below from the most significant to the least significant (refer to the eCRF completion guidelines for additional details and examples):

- **Serious adverse event:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an SAE, as defined in Section 9.3.1.1.
- **Other adverse event:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an AE other than an SAE, as defined in Section 9.3.1.1.
- **Non-compliance with protocol:** To be used when the Investigator withdraws a subject from the study because of failure to follow protocol guidelines, including the case when it is retrospectively discovered that a subject did not fulfill the eligibility criteria. The Investigator will provide a comment as to the specific cause of non-compliance.
- **Lost to follow-up:** To be used when the Investigator withdraws a subject from the study because of failure to establish contact, as outlined in Section 5.2.9. The Investigator will provide documentation that contact was attempted (i.e., return of unsigned certified letter receipt).
- **Voluntary withdrawal not due to an adverse event:** To be used when a subject drops out of the study for any reason other than those listed above.

5.2.11 Follow-up of Discontinuations

The site should complete all scheduled safety follow-ups and contact the parent/guardian of any subject who has prematurely terminated the trial because of an SAE, other type of AE, non-compliance with the protocol, or loss of eligibility, including definitive contraindications.

For subjects where the reason for early termination was loss to follow-up or if the subject's parent/guardian withdrew informed consent and specified that they do not want to be contacted again and it is documented in the source document, the site will not attempt to obtain further safety information.

For subjects where the reason for early termination is voluntary withdrawal, the site will attempt to contact the subject's parent/guardian except if they specified that they do not want to be contacted again and it is documented in the source document.

5.3 Safety Emergency Call

If, as per the Investigator's judgment, a subject experiences a medical emergency, the Investigator may contact the Sponsor's RMOs for advice on trial-related medical questions or problems. The RMOs will be available 24 hours a day, 7 days a week, as needed. Contact information for the RMOs is provided in the Operating Guidelines.

Making an emergency call does not replace the need to report an SAE. The Investigator is still required to follow the protocol-defined process for reporting SAEs to the Global PharmacoVigilance (GPV) Department (Please refer to Section 10).

5.4 Modification of the Trial and Protocol

Any amendments to this trial plan and protocol must be discussed with and approved by the Sponsor. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Sponsor, and the amended version of the protocol will replace the earlier version. All substantial amendments (e.g., that affect the conduct of the trial or the safety of subjects) require IRB approval and must also be forwarded to regulatory authorities.

An administrative amendment to a protocol is one that modifies some administrative or logistical aspect of the trial but does not affect its design or objectives or have an impact on the subjects' safety. Regulatory authorities need only be notified about administrative changes. Administrative changes do not require IRB approval; however, the IRB(s) must be notified whenever one is made.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which IRB approval has already been given, are not initiated without IRB review and approval, except to eliminate apparent immediate hazards to subjects.

5.5 Interruption of the Trial

The trial may be discontinued if new data about the investigational product resulting from this or any other trials become available; or for administrative reasons; or on advice of the Sponsor, the Investigators, and/or the IRB(s). If the trial is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the regulatory authorities, and IRB(s) of the reason for termination or suspension, as specified by the applicable regulatory requirements.

The Investigator shall promptly inform the trial subjects' parents/guardians and should assure appropriate therapy for and follow-up of subjects.

6 Vaccines Administered

Subjects will be administered 1 of the following vaccines:

- Fluzone Quadrivalent vaccine, No Preservative: Pediatric Dose (0.25-mL dose), 2016–2017 formulation
- Fluzone Quadrivalent vaccine, No Preservative (0.5-mL dose), 2016–2017 formulation

One or 2 doses of influenza vaccine will be administered according to ACIP 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 will be administered approximately 28 days later, during Visit 2.

6.1 Identity of the Investigational Product

6.1.1 Identity of Trial Product

The 2016–2017 formulation of Fluzone Quadrivalent vaccine (0.25-mL and 0.5-mL syringe presentations) is a sterile suspension prepared from the allantoic fluid of chicken embryos infected with specific influenza virus strains. The virus-containing fluid is harvested and the virus inactivated with formaldehyde. The influenza virus is concentrated and purified in a linear sucrose density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted producing a split antigen. The split antigen is then suspended in sodium phosphate-buffered isotonic sodium chloride solution. Antibiotics are not used in the manufacture of the vaccine. Fluzone Quadrivalent vaccine is essentially clear and slightly opalescent in color.

6.1.1.1 Composition

Each 0.25-mL and 0.5-mL dose of vaccine contains 7.5 µg and 15 µg HA, respectively, of each antigen:

- A/California/07/2009 X-179A (H1N1)
- A/Hong Kong/4801/2014 X-263B (H3N2)
- B/Brisbane/60/2008 (Victoria lineage; B1)
- B/Phuket/3073/2013 (Yamagata lineage; B2)

6.1.1.2 Preparation and Administration

Fluzone Quadrivalent vaccine is a liquid preparation; as such, no diluent is required. This product is provided in 0.25-mL or 0.5-mL, pre-filled, single-dose syringes. The vaccine will be administered intramuscularly into the anterolateral muscle of the thigh or the deltoid muscle, as appropriate. The vaccine must be administered within 30 minutes of removing the assigned vaccine from the refrigerator.

Prior to administration, all study products must be inspected visually for cracks, broken seals, correct label content (see Section 6.3.1), and extraneous particulate matter and/or discoloration, whenever solution and container permit. If any of these conditions exists, the vaccine must not be administered; another dose is to be used, and the event is to be reported to the Sponsor.

Subjects must be kept under observation for 20 minutes after each vaccination to ensure their safety, and any reactions during this period will be documented in the eCRF. Appropriate medical equipment and emergency medications, including epinephrine (1:1000), must be available on site in the event of an anaphylactic or other immediate allergic reaction.

6.1.1.3 Dose Selection and Timing

Fluzone Quadrivalent vaccine will be administered as a single 0.25-mL (Group 1) or 0.5-mL (Group 2) dose given on Day 0 at Visit 1. For subjects for whom 2 doses are recommended per ACIP guidance, a second dose of the same volume as the first dose will be administered at Visit 2 (28 [window, 28–35] days after Visit 1).

6.1.2 Identity of Control Products

Not applicable.

6.2 Identity of Other Products

Not applicable.

6.3 Product Logistics

6.3.1 Labeling and Packaging

All study vaccine will be supplied with investigational labeling.

6.3.2 Product Shipment, Storage, and Accountability

6.3.2.1 Product Shipment

The Clinical Logistics Coordinator will contact a qualified unblinded qualified study staff member to determine the dates and times of delivery of products.

Each vaccine shipment will include a temperature-monitoring device to verify maintenance of the cold chain during transit. On delivery of the product to the site, the person in charge of product receipt will follow the instructions given in the Operating Guidelines, including checking that the cold chain was maintained during shipment (i.e., verification of the temperature recorders). If there is an indication that the cold chain was broken, this person should immediately quarantine the product, alert the Sanofi Pasteur representative, and request authorization from Sanofi Pasteur to use the product.

6.3.2.2 Product Storage

The Investigator will designate unblinded qualified study staff to be responsible for product administration.

At the site, products must be kept in a secure place with restricted access. Vaccines will be stored in a refrigerator at a temperature ranging from +2°C to +8°C. The vaccines must not be frozen. The temperature must be monitored and documented (see the Operating Guidelines) for the entire time that the vaccine is at the trial site. In case of accidental freezing or disruption of the cold chain, vaccines must not be administered and must be quarantined, and the authorized designee should contact the Sanofi Pasteur representative for further instructions.

6.3.2.3 Product Accountability

The Authorized qualified study staff will maintain records of product delivery to the trial site, product inventory at the site, the dose(s) given to each subject, and the disposal of or return to the Sponsor of unused doses.

The necessary information on the product labels is to be entered into the source document and the eCRF. If applicable, information may also be entered into the subject's vaccination card.

The Sponsor's monitoring staff will verify the trial site's product accountability records against the record of administered doses in the CRFs and the communication from the IRT system.

In case of any expected or potential shortage of product during the trial, the authorized designee should alert the Sanofi Pasteur representative as soon as possible, so that a shipment of extra doses can be arranged.

6.3.3 Replacement Doses

If a replacement dose is required (e.g., because the syringe broke or particulate matter was observed in the syringe), the site personnel must either contact the IRT system to receive the replacement dose allocation or follow the instructions given in the Operating Guidelines.

6.3.4 Disposal of Unused Products

Unused or wasted products will be returned to the Sponsor in accordance with the instructions in the Operating Guidelines. Product accountability will be verified throughout the trial period.

6.3.5 Recall of Products

If the Sponsor makes a decision to launch a retrieval procedure, the Investigator(s) will be informed of what needs to be done.

6.4 Blinding and Code-breaking Procedures

The study will be performed in an observer-blinded fashion:

- Unblinded qualified study staff members who are not involved with safety evaluation and other trial procedures will prepare and administer the vaccine.
- Blinded Investigators and study staff who conduct safety assessments will not know which vaccine is administered.
- Subjects will not be informed as to which vaccine will be administered.

The place for storage of the product will be separate from the place where the preparation and injection is performed. The Investigator and study staff responsible for safety assessment will not be present during vaccination, but will be available in case of an emergency (e.g., anaphylactic shock).

The IRT system vendor will be responsible for providing the vaccine dose identification number to be received by the enrolled subject. The subject, the Investigator, and study staff members who collect the safety data and laboratory personnel who analyze the blood samples will all be blinded to the group assignment. The individual responsible for preparing/administering vaccine will not be authorized to collect any safety/serology data.

Code-breaking procedures:
The code may be broken by the Investigator only in the event of an SAE and if identification of the vaccine received could influence the treatment of the SAE. Code-breaking should be limited, to the extent possible, to the subject(s) experiencing the SAE.

The blind can be broken by the Investigator or a sub-investigator (medical doctor only[a]), by contacting the IRT system as explained in the code-breaking procedures described in the Operating Guidelines. Once the emergency has been addressed by the site, the Investigator must notify 1 of the Sanofi Pasteur RMOs if a subject's code was broken. All contact attempts with the Sponsor prior to unblinding are to be documented in the source documents.
A request for the code to be broken may be made:

- by the GPV Department for reporting to health authorities in the case of an SAE, as described in the International Conference on Harmonisation (ICH) E2A. In this case, the code will be broken only for the subject(s) in question. The information resulting from code-breaking (i.e., the subject's vaccine dose/group assignment) will not be communicated to either the Investigator or the immediate team working on the study, except for the GPV representative.

The IRB must be notified of code-breaking. All documentation pertaining to the event must be retained in the site's study records and in the Sanofi Pasteur files. Any intentional or unintentional code-breaking must be reported, documented, and explained, and the name of the person who requested it must be provided to the Sponsor.

6.5 Randomization and Allocation Procedures

The Sponsor or designee will supply a centralized, computer-generated randomization code. Randomization of subjects will be performed with the permuted block method with stratification by site, age group, and vaccination schedule. Each subject who meets the inclusion/exclusion criteria will be randomly assigned according to his/her age group and vaccination schedule, to 1 of the 2 groups via an IRT system, according to a 1:1 ratio.

Before vaccination, authorized qualified study staff will contact the IRT system, enter identification and security information, and confirm a minimal amount of data in response to IRT system prompts. The IRT system will then state the vaccine dose identification number and whether the subject will be included in the immunogenicity subset. Subject numbers and vaccine dose identification numbers will be recorded on the eCRFs. The full procedures for randomization are detailed in the Operating Guidelines.

If the dose initially allocated for vaccination cannot be used (e.g., because the syringe broke or particulate matter was observed), authorized qualified study staff will obtain a replacement dose (see Section 6.3.3).

[redacted]

[a] according to local regulations

Subject numbers should not be reassigned for any reason.

6.6 Treatment Compliance

The following measures will ensure that the vaccine doses administered comply with those planned, and that any non-compliance is documented so that it can be accounted for in the data analyses:

- All vaccinations will be administered and recorded by unblinded qualified trial personnel
- Authorized qualified study staff will maintain accountability records of product delivery to the trial site, product inventory at the site, and the disposal of unused or wasted doses

6.7 Concomitant Medications and Other Therapies

At the time of enrollment, ongoing medications, including other therapies (e.g., blood products), should be recorded in the source document as well as new medications prescribed for new medical conditions/AEs during trial participation.

Documentation in the eCRF of concomitant medication will be limited to specific categories of medication of interest beginning on the day of vaccination. This may include medications of interest that were started prior to the day of vaccination.

Reportable medications will be collected in the eCRF from the day of vaccination to the end of the solicited and unsolicited follow-up period (i.e., between Visit 1 and Visit 2 for subjects receiving 1 dose of study vaccine or Visit 1 and Visit 3 for subjects receiving 2 doses of study vaccine) as they may impact the response to the vaccination and impact the consistency of the information collected on concomitant medications at any vaccination.

The "reportable" medications are classified according to 2 categories. These are:

- Category 1 antipyretics, analgesics, non-steroidal anti-inflammatory drugs, corticosteroids, and other immune modulators.

  Note: inhaled and topical steroids should not be captured.

- Category 2: Other reportable medications as specified in the protocol (i.e., medications related to exclusion criteria) and other medications as noted here. All non-study vaccines, immune globulins, and blood or blood-derived products are included in this category. Other medications include topical analgesics applied to the site of the blood draw and allergy hyposensitization therapy received within 30 days before study vaccination and through the Day 28 (window, 28–35 days) visit. These therapies are permissible but nonetheless should be recorded as Category 2 medications. Topical analgesics should NOT be applied at the site of vaccination; however, if they are applied inadvertently to the vaccination site, they should be recorded as a Category 1 medication in this specific instance, not as a Category 2 medication.

The information reported in the eCRF for each reported medication will be limited to:

- Trade name
- Given as treatment or as prophylaxis
- Medication category

- Start and stop dates

Dosage and administration route will not be recorded. Homeopathic medication will not be recorded. Topical medication, except for topical analgesics applied to the site of the blood draw or inadvertently applied to the vaccination site (see categorization instructions above), will not be recorded.

The fact that a medication was given in response to an AE will be captured in the "Action Taken" column of the AE only. No details will be recorded in the concomitant medication module of the eCRF unless the medication received belongs to 1 of the prelisted categories. Medications will not be coded.

7 Management of Samples

From subjects randomly assigned to the immunogenicity subset, blood samples for the assessment of antibody responses will be collected at Visit 1 and at Visit 2 for subjects receiving 1 dose of study vaccine, and at Visit 1 and Visit 3 for subjects receiving 2 doses of study vaccine. See the Table of Study Procedures and Section 5.1.3 for details of the sampling schedule.

7.1 Sample Collection

From subjects randomly assigned to the immunogenicity subset, at Visit 1 and Visit 2 (or Visit 1 and Visit 3 for subjects receiving 2 doses of vaccine), approximately 5 mL of blood will be collected in tubes provided by or recommended by the Sponsor. Immediately prior to the blood draw, the staff member performing the procedure will verify the subject's identity and will attach the pre-printed label that contains that subject's number and the sampling stage to the tube. It is preferred that the blood be taken from the limb opposite to the one that will be used for vaccination, but it is not required.

7.2 Sample Preparation

Detailed instructions on how to prepare blood samples for assessment of antibody response are contained in the Operating Guidelines provided to the site. An overview of the procedures is provided here.

Following the blood draw, the tubes are to be left undisturbed, positioned vertically and not shaken, for a minimum of 1 hour and a maximum of 24 hours to allow the blood to clot. Samples can be stored at room temperature for up to 2 hours; beyond 2 hours, they must be refrigerated at a temperature of +2°C to +8°C up to a maximum of 24 hours. The samples are then centrifuged, and the serum is transferred to the appropriate number of aliquoting tubes. These tubes are pre-labeled with adhesive labels that identify the study code, the subject's number, and the sampling stage.

The subject's number and the date of sampling, the number of aliquots obtained, and the subject's parent's/guardian's consent for future use of his/her samples are to be specified on a sample identification list. These previous items, as well as the date and time of preparation, are to be

recorded in the source document. Space is provided on the sample identification list to record comments regarding the quality of samples.

7.3 Sample Storage and Shipment

During storage, serum tubes are to be kept in a freezer whose temperature is set and maintained at -20°C or below. The temperature will be monitored and documented on the appropriate form during the entire trial. If it rises above -10°C for any period of time, the Clinical Logistics Coordinator must be notified. See the Operating Guidelines for further details.

Samples will be shipped to Global Clinical Immunology (GCI) at Sanofi Pasteur. The address is provided in the Operating Guidelines.

Shipments to the laboratory (GCI) will be made only after appropriate monitoring and following notification of the Clinical Logistics Coordinator. Sera will be shipped frozen, using dry ice to maintain them in a frozen state, in the packaging container provided by the carrier. Again, temperatures will be monitored. Shipments must be compliant with the International Air Transport Association (IATA) 602 regulations.

[redacted]

7.4 Future Use of Stored Serum Samples for Research

Any unused part of the serum samples will be securely stored at the Sanofi Pasteur serology laboratory (GCI) for at least 5 years after the last license approval in the relevant market areas has been obtained for the vaccine being tested.

Subjects' parents/guardians will be asked to indicate in the ICF whether they will permit the future use of any unused stored serum samples for other tests. If they refuse permission, the samples will not be used for any testing other than that directly related to this study. If they agree to this use, they will not be paid for giving permission. Anonymity of samples will be ensured. The aim of any possible future research is unknown today and may not be related to this particular study. It may be to improve the knowledge of vaccines or infectious diseases or to improve laboratory methods. Human genetic tests will never be performed on these samples without individual informed consent.

8 Clinical Supplies

Sanofi Pasteur will supply the trial sites with protocols, ICFs, eCRFs, diary cards, and other trial documents, as well as with the following trial materials: all study vaccines and injection materials, blood collection tubes, cryotubes, cryotube storage boxes, cryotube labels, temperature recorders, shipping containers, rulers, and digital thermometers.

The means for performing electronic data capture (EDC) will be defined by Sanofi Pasteur. If a computer is provided by Sanofi Pasteur, it will be retrieved at the end of the trial.

The Investigator will supply all phlebotomy (except for the blood collection tubes, which are provided by the Sponsor) and centrifugation equipment, and biohazard and/or safety supplies. The biohazard and safety supplies include needles and syringes, examination gloves, laboratory coats, sharps disposal containers, and absorbent countertop paper. The site will ensure that all biohazard wastes are autoclaved and disposed of in accordance with local practices. The Investigator will also supply appropriate space in a temperature-monitored refrigerator for the storage of the products and for the blood samples, and appropriate space in a temperature-monitored freezer for serum aliquots.

In the event that additional supplies are required, study staff must contact Sanofi Pasteur, indicating the quantity required. Contact information is provided in the Operating Guidelines. They must allow approximately 1 week for an order to be filled and to have the supplies sent to their site.

9 Endpoints and Assessment Methods

9.1 Primary Endpoints and Assessment Methods

9.1.1 Safety

The primary safety objective is presented in Section 2.1.

9.1.1.1 Safety Endpoints

Rates of any fever (temperature ≥ 100.4°F [38.0°C]) during the 7 days after either vaccination (Dose 1 and Dose 2 combined) in each vaccine group.

9.1.1.2 Safety Assessment Methods

The safety assessment methods for the primary endpoints are described in Section 9.3.1.3.

9.1.2 Immunogenicity

There are no primary objectives for immunogenicity.

9.1.3 Efficacy

No clinical efficacy data will be obtained in the trial.

9.2 Secondary Endpoints and Assessment Methods

9.2.1 Safety

There are no secondary objectives for safety.

9.2.2 Immunogenicity

The secondary immunogenicity objective is presented in Section 2.2.

9.2.2.1 Immunogenicity Endpoints

- Geometric mean titers: The HAI GMTs (for each of the 4 virus strains) at 28 (window, 28–35) days after the final vaccination.
- Seroconversion rates: The percentages of subjects with either a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil), or a pre-vaccination titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-vaccination titer at 28 (window, 28–35) days after the final vaccination.

9.2.2.2 Immunogenicity Assessment Methods

Antibodies to Influenza Viruses

Anti-influenza antibodies will be measured by HAI performed by Sanofi Pasteur's GCI department or an external testing laboratory under GCI's supervision. Serum samples and quality control sera (sheep and/or human sera) will be incubated with Sigma Type III NA to eliminate non-specific inhibitors. Adsorption of spontaneous anti-species agglutinins will be performed by incubating the serum samples and quality control sera with a red blood cell (RBC) suspension. Following this, the mixtures will be centrifuged and the supernatants containing the treated sera will be collected for testing. Ten 2-fold dilutions (starting at 1/10) of the treated serum samples and quality control sera will be incubated with a previously titrated influenza virus solution at a concentration of 4 hemagglutination unit (HAU)/25 µL. Influenza virus solution will not be added to the serum control wells containing only serum and RBCs. The mixture will be incubated and a RBC suspension will be added. Following incubation, the results will be read. The endpoint of the assay is the highest serum dilution in which complete inhibition of hemagglutination occurred. Each serum sample will be titrated in independent duplicates, and the 2 values, which do not differ by more than one 2-fold dilution, will be reported. The GMT between the 2 values will be calculated after the release of the data to the Clinical Data Management (CDM) platform. The lower limit of quantification (LLOQ) for HAI is set at 10, the lowest serum dilution used in the assay i.e., 1/10. Titers below this level are reported as < 10.

9.2.3 Efficacy

No clinical efficacy data will be obtained in the trial.

9.3 Observational Endpoints and Assessment Methods

9.3.1 Safety

The observational safety objective is presented in Section 2.3.1.

9.3.1.1 Safety Definitions

The following definitions are taken from the ICH 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 trial 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[a]

[a] 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.

- Requires inpatient hospitalization or prolongation of existing hospitalization[a]
- Results in persistent or significant disability/incapacity[b]
- Is a congenital anomaly/birth defect
- Is an important medical event[c]

Additionally, the following important medical events are to be considered as SAEs and reported to the Sponsor according to the procedure described in Section 10:

- 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), 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 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 between Day 0 and Day 7 post-vaccination, or headache between Day 0 and Day 7.

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[a] 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 outpatient treatment with no hospitalization.

[b] "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[c] 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.

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 AE/AR:

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. For example, if headache between Day 0 and 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.

An unsolicited non-serious AE is an unsolicited AE excluding SAEs.

Injection Site Reaction:

An injection site reaction[a] is an AR at and around the injection site. Injection site reactions are commonly inflammatory reactions.

Systemic AE:

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:

AEs 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.

9.3.1.2 Safety Endpoints

The observational endpoints for the evaluation of safety are:

1) Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, and relationship to vaccination of any unsolicited systemic AEs reported in the 20 minutes after each 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 between Day 0 and Day 7 after each 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 between Day 0 and Day 7 after each vaccination.
4) Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, relationship to vaccination (for systemic AEs only), and whether the event led to early

[a] All injection site AEs are considered to be related to vaccination; therefore, all *injection site* AEs are *injection site reactions*.

termination from the study, of unsolicited AEs between Visit 1 and Visit 2 for subjects receiving 1 dose or between Visit 1 and Visit 3 for subjects receiving 2 doses.

5) Occurrence, nature (MedDRA preferred term), time to onset, seriousness criteria, relationship to vaccination, outcome, and whether the SAE led to early termination from the study, of SAEs between Visit 1 and Visit 2 for subjects receiving 1 dose or between Visit 1 and 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), and anaphylaxis or other hypersensitivity/allergic reactions between Visit 1 and Visit 2 for subjects receiving 1 dose, or between Visit 1 and Visit 3 for subjects receiving 2 doses.

9.3.1.3 Safety Assessment Methods

At Visit 2 and Visit 3 (for subjects receiving 2 influenza doses), the Investigator or a delegate will ask the parent/guardian about any solicited reactions and unsolicited AEs recorded in the diary card, as well as about any other AEs that may have occurred since the previous visit. All relevant data will be transcribed into the eCRF according to the instructions provided by the Sponsor.

9.3.1.3.1 Immediate Post-vaccination Surveillance Period

Subjects will be kept under observation for 20 minutes after each vaccination to ensure their safety. The post-vaccination surveillance should be documented in the source document. Any AE that occurs during this period will be noted on the source document and recorded in the eCRF, as follows:

- Any unsolicited systemic AE occurring during the first 20 minutes post-vaccination will be recorded on the eCRF as an immediate unsolicited systemic AE.
- Solicited and unsolicited injection site reactions and solicited systemic reactions will be recorded and analyzed as starting on the day of vaccination.
- Any SAE occurring during the first 20 minutes post-vaccination will be reported in the same way as any other SAE and to the Sponsor, according to the procedures described in Section 10.

9.3.1.3.2 Reactogenicity (Solicited Reactions From Day 0 to Day 7 After Each Vaccination)

After each vaccination, the subject's parent/guardian will be provided with a safety diary card, a digital thermometer, and a flexible ruler, and will be instructed how to use them. The following items will be recorded by the parent/guardian in the diary card on the day of vaccination and for the next 7 days (i.e., Day 0 to Day 7) until resolution:

- Daily temperature, with the route by which it was taken
- Daily measurement or intensity grade of all other solicited injection site and systemic reactions

- Action taken for each event, if any (e.g., medication)

The action taken by the subject's parent/guardian to treat any **solicited reactions** will be classified in the eCRF using the following scale:

0: None

1: Medication (self-medication with an existing prescription or over-the-counter medication)

2: Health care provider contact (no new medication prescribed)

3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)

4: Hospitalization (inpatient)

Parents/guardians will be contacted by telephone approximately 8 days after each vaccination to remind them to record all safety information in the diary card.

If the timing of the telephone call should fall on a weekend or a holiday, the call should be made on the next business day. If contact is not made on the designated day, study staff will continue calling until contact is made. Every telephone attempt and its outcome will be documented in the source document.

Table 9.1 and Table 9.2 present, respectively, the injection site reactions and systemic reactions that are prelisted in the diary cards and eCRF, together with the intensity scales.

Table 9.1: Solicited Injection Site Reactions: Terminology, Definitions, and Intensity Scales (Subjects 6 to < 36 Months of Age)

eCRF term (MedDRA lowest level term [LLT])	**Injection site tenderness**	**Injection site erythema**	**Injection site swelling**
Diary card term	Tenderness	Redness	Swelling
Definition		Presence of redness including the approximate point of needle entry	Swelling at or near the injection site Swelling or edema is caused by a fluid infiltration in tissue or cavity and, depending on the space available for the fluid to disperse, swelling may be either soft (typically) or firm (less typical) to touch and thus can be best described by looking at the size of the swelling
Intensity scale[a]	Grade 1: Minor reaction when injection site is touched Grade 2: Cries or protests when injection site is touched Grade 3: Cries when injected limb is moved, or the movement of the injected limb is reduced	Grade 1: > 0 to < 25 mm Grade 2: ≥ 25 to < 50 mm Grade 3: ≥ 50 mm	

[a] For the subjective reaction of tenderness, parents/guardians will record the intensity level (Grade 1, 2, or 3) in the diary card. For the measurable reactions of redness and swelling, they will record just the size of the reaction, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis.

Table 9.2: Solicited Systemic Reactions: Terminology, Definitions, and Intensity Scales (Subjects 6 to < 36 Months of Age)

eCRF term (MedDRA lowest level term [LLT])	Fever	Vomiting	Crying abnormal	Drowsiness	Appetite lost	Irritability
Diary card term	Temperature	Vomiting	Abnormal crying	Drowsiness	Loss of appetite	Irritability
Definition	Elevation of temperature to ≥ °38.0°C (≥ 100.4°F)	Vomiting does not include spitting up	Inconsolable crying without a reason	Reduced interest in surroundings, or increased sleeping	See intensity scale	An excessive response to stimuli: increased fussiness, whining, and fretfulness despite attempts to comfort the infant and despite caregiver responses that would normally be soothing
Intensity scale[a]	Grade 1: ≥ 38.0°C to ≤ 38.5°C **or** ≥ 100.4°F to ≤ 101.3°F	Grade 1: 1 episode per 24 hours	Grade 1: < 1 hour	Grade 1: Sleepier than usual or less interested in surroundings	Grade 1: Eating less than normal	Grade 1: Easily consolable
	Grade 2: > 38.5°C to ≤ 39.5°C **or** > 101.3°F to ≤ 103.1°F	Grade 2: 2–5 episodes per 24 hours	Grade 2: 1–3 hours	Grade 2: Not interested in surroundings or did not wake up for a feed/meal	Grade 2: Missed 1 or 2 feeds/meals completely	Grade 2: Requiring increased attention
	Grade 3: > 39.5°C **or** > 103.1°F	Grade 3: ≥ 6 episodes per 24 hours or requiring parenteral hydration	Grade 3: > 3 hours	Grade 3: Sleeping most of the time or difficult to wake up	Grade 3: Refuses ≥ 3 feeds/meals or refuses most feeds/meals	Grade 3: Inconsolable

[a] For all reactions but fever, parents/guardians will record the intensity level (Grade 1, 2, or 3) in the diary card. For fever, they will record the body temperature, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis.

Important notes for the accurate assessment of temperature:

Parents/guardians are to measure body temperature once per day, preferably always at the same time. The optimal time for measurement is the evening, when body temperature is the highest. Temperature is also to be measured at the time of any apparent fever. The observed daily temperature and the route of measurement are to be recorded in the diary card, and the highest temperature will be recorded by the site in the eCRF. The preferred route for temperature measurement in this trial is rectal. In cases where a rectal temperature cannot be obtained, a non-preferred route (e.g., axillary) may be used. Pre-vaccination temperature is also to be systematically collected by the Investigator in the eCRF for all subjects. Tympanic thermometers must not be used.

9.3.1.3.3 Unsolicited Non-serious Adverse Events From Visit 1 to Visit 2 or Visit 3

In addition to recording solicited reactions, the parent/guardian will be instructed to record any other medical events that may occur from Visit 1 to Visit 2 (Diary Card 1) for all subjects and from Visit 2 to Visit 3 (Diary Card 2) for subjects receiving 2 doses. Space will be provided in the diary card for this purpose. For each unsolicited non-serious AE, the following information is to be recorded:

- Start and stop dates[a]
- Intensity of the event:
    - For measurable unsolicited non-serious AEs that are part of the list of solicited reactions, the size of the AE as well as the temperature for fever will be collected and analyzed based on the corresponding scale used for solicited reactions (see Table 9.1 and Table 9.2)
    - Other unsolicited non-serious AEs will be classified according to the following intensity scale:
        - Grade 1: No interference with activity
        - Grade 2: Some interference with activity
        - Grade 3: Significant; prevents daily activity
- Action taken for each AE, if any (e.g., medication)

The action taken by the parent/guardian to treat any **unsolicited AEs** will be classified in the eCRF using the following scale:

0: None

1: Medication (self-medication with an existing prescription or over-the-counter medication)

2: Health care provider contact (no new medication prescribed)

[a] The stop date of all related AEs will be actively solicited. For other events, the Investigator will provide the stop date when it becomes available. Adverse events for which no stop date was obtained during the course of the trial will be considered as ongoing at the end of the trial.

3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)

- Whether the AE led to discontinuation
- Whether the AE was related to vaccination (for unsolicited systemic AEs)

9.3.1.3.4 Serious Adverse Events

Information on SAEs will be collected and assessed throughout the trial, from Visit 1 until Visit 2 (or from Visit 1 to Visit 3 for those subjects receiving 2 doses).

Any SAE occurring at any time during the trial will be reported by the Investigator through the EDC system and according to the completion guidelines provided by the Sponsor. All information concerning the SAE is to be reported, either as part of the initial reporting or during follow-up reporting if relevant information became available later (e.g., outcome, medical history, results of investigations, copy of hospitalization reports). The Investigator will assess the causal relationship between the SAE and the investigational product as either "Not related" or "Related", as described in Section 9.3.1.3.6.

See Section 10 for further details on SAE reporting.

9.3.1.3.5 Adverse Events of Special Interest

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), and anaphylaxis or other hypersensitivity/allergic reactions between Visit 1 and Visit 2 for subjects receiving 1 dose, or between Visit 1 and Visit 3 for subjects receiving 2 doses.

9.3.1.3.6 Assessment of Causality

At each visit, the Investigator or a delegate will perform a directed examination, if indicated, based on interim history, and will ask the parent/guardian about any solicited reactions and unsolicited AEs recorded in the diary card, as well as about any other AEs that may have occurred since the previous visit. All relevant data will be transcribed into the eCRF according to the instructions provided by the Sponsor.

The Investigator will assess the ***causal relationship*** between each unsolicited systemic AE and vaccination as either not related or related, based on the following definitions[a]:

0: Not related – The AE is clearly/most probably caused by other etiologies such as subject's underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the AE is incompatible with a causal relationship; or the AE started before the first vaccination (screening phase, if applicable)

[a] ICH Guidelines, Clinical Safety Data Management E2A

1: Related – There is a "reasonable possibility" that the AE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship

Note: By convention, all injection site AEs (solicited and unsolicited) and all solicited systemic reactions are considered to be related to vaccination and referred to as reactions, and therefore do not require the Investigator's opinion on relatedness.

Adverse events likely to be related to the product, whether serious or not, that persist at the end of the trial will be followed up by the Investigator until their complete disappearance or the stabilization of the subject's condition (unless the subject's parent/guardian specified that they do not want to be contacted for follow-up and it is documented in the source document). The Investigator will inform the Sponsor of the date of final disappearance of the event.

9.3.2 Immunogenicity

The observational immunogenicity objective is presented in Section 2.3.2.

9.3.2.1 Immunogenicity Endpoints

Immunogenicity will be evaluated in a subset of 1600 randomly selected subjects prior to vaccination on Day 0 (Visit 1) and at 28 (window, 28–35) days after the final vaccination using the HAI technique. For each influenza vaccine strain, HAI assay titers at pre-vaccination (Day 0) and at 28 (window, 28–35) days after the final vaccination will be determined in duplicate.

The derived endpoints are:

- Geometric means of HAI assay titers for individual subjects at pre-vaccination (Day 0) and at 28 (window, 28–35) days after the final vaccination.
- Ratios of individual post-final vaccination titers divided by individual pre-vaccination titers.
- Seroprotection: subjects with a titer ≥ 40 (l/dil) at pre-vaccination or at 28 (window, 28–35) days after the final vaccination.
- Seroconversion: subjects with either a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil), or a pre-vaccination titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-vaccination titer at 28 (window, 28–35) days after the final vaccination.

9.3.2.2 Immunogenicity Assessment Methods

The immunogenicity assessment methods for the observational endpoints are the same as those presented in Section 9.2.2.2.

9.3.3 Efficacy

No clinical efficacy data will be obtained in the trial.

9.3.4 Serum Collection

The observational objective for serum collection is presented in Section 2.3.3.

9.3.4.1 Serum Collection Endpoints and Assessment Methods

There are no observational endpoints for the serum collection objective.

10 Reporting of Serious Adverse Events

To comply with current regulations on SAE reporting to health authorities, the Investigator must document all SAEs regardless of causal relationship, and notify the Sponsor and the Clinical Research Associate (CRA) within the notification timelines stated in the following sections. The Investigator will give access and provide the Sponsor and the CRA with all necessary information to allow the Sponsor to conduct a detailed analysis of the safety of the investigational product(s). It is the responsibility of the Investigator to request all necessary documentation (e.g., medical records, discharge summary, autopsy) to provide comprehensive safety information. All relevant information must then be transcribed into the electronic SAE reporting form (eSAE Form).

10.1 Initial Reporting by the Investigator

Serious adverse events occurring during a subject's participation in the trial must be reported within 24 hours to the Sponsor's GPV Department and to the CRA. Every SAE must be reported, even if the Investigator considers that it is not related to the vaccine. The SAE form must be signed by a licensed physician (MD or DO) for whom the task is listed on the Study Task Delegation and Signature List after each update to the eSAE Form.

The Investigator must complete the eSAE Form in the EDC application. After validation, an e-mail alert will automatically be sent to the GPV mailbox, the CRA, and the RMOs. This message will include the country, the study code, the subject number, whether the report is an initial report or a follow-up report, the diagnosis and/or symptoms, the seriousness criteria, the relationship, if related, and the outcome, if fatal.

If the EDC system is unavailable, the site must notify the Sponsor using the paper version of the SAE Reporting Form, as follows:

The Investigator must complete the SAE Reporting Form, check off the "Initial Reporting Form" box, and send it to the Sponsor by 1 of the following means:

- By fax, to the following number: [redacted]
- In PDF format to the following e-mail address, using a method of transmission that includes password protection: [redacted]
- By express mail, to the following address:

  [redacted]

When the system becomes available, the Investigator must transcribe the information from the paper version of the eSAE Form into the EDC system.

If there is need for urgent consultation, the Investigator is to contact 1 of the RMOs as described in Section 5.3.

10.2 Follow-up Reporting by the Investigator

The eSAE Form completed initially must be updated within 24 hours after the Investigator has become aware of any new relevant information concerning the SAE (e.g., outcome, precise description of medical history, results of the investigation). After validation, an e-mail alert will be sent automatically to the GPV Department and to the CRA. All relevant information must be included directly in the eSAE Form. Copies of documents (e.g., medical records, discharge summary, autopsy) may be requested by the GPV Department.

The anonymity of the subject must always be respected when forwarding this information.

10.3 Reporting of SAEs Occurring After a Subject Has Completed the Study

Any SAE that occurs after a subject has completed the study but that is likely to be related to the product or to the trial must also be reported as soon as possible. In such a case, the reporting procedure to be followed is identical to that described in Section 10.1.

10.4 Assessment of Causality

The causal relationship between the SAE and the product will first be evaluated by the Investigator, using the following definitions:

> **0 - Not related**: The AE is clearly/most probably caused by other etiologies such as an underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the SAE is incompatible with a causal relationship; or the SAE started before the first vaccination (screening phase, if applicable).
>
> **1 - Related**: There is a "reasonable possibility" that the SAE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship.
>
> *(ICH Guidelines, Clinical Safety Data Management E2A)*

Following this, the Sponsor's Product Safety Officer (PSO) will also assess the causal relationship to the product, based on the available information and current medical knowledge.

The decision to modify or discontinue the trial may be made after mutual agreement between the Sponsor and the Investigator(s).

10.5 Reporting SAEs to Health Authorities and IECs/IRBs

The Sponsor will inform the relevant health authorities of any reportable SAEs according to the local regulatory requirements. Reporting to the health authorities will be according to the Sponsor's standard operating procedures.

The Sponsor's RMOs will notify the Investigators in writing of the occurrence of any reportable SAEs. The Investigators/Sponsor will be responsible for informing the IRBs that reviewed the trial protocol.

11 Data Collection and Management

11.1 Data Collection and eCRF Completion

Individual safety diary cards, specifically designed for this trial by the Sponsor and provided to the study sites, will be given to study participants for the recording of daily safety information as described in Section 9.3.1.3. These diary cards will include prelisted terms and intensity scales (see Table 9.1 and Table 9.2) as well as areas for free text to capture additional safety information or other relevant details. Parents/guardians will also be provided with rulers for measuring the size of injection site reactions, and with standard digital thermometers for measuring daily temperatures. To ensure consistency of reporting, the study sites will instruct parents/guardians on how to correctly use these tools.

At specified intervals, the Investigator or an authorized designee will interview the parents/guardians to collect the information recorded in the diary card, and will attempt to clarify anything that is incomplete or unclear. All clinical trial information gathered by the study site will be reported electronically by the Investigator or authorized designee using a web-based eCRF. (Any information that was not documented in the diary card will first be captured in the source document and then reported electronically.) The eCRF has been designed specifically for this trial under the responsibility of the Sponsor, using a validated Electronic Records/Electronic Signature-compliant platform (21 CFR Part 11).

To ensure the correct and consistent completion of the eCRFs, the Sponsor or authorized representative will provide all necessary tools, instructions, and training to all site staff involved in data entry prior to study start. Additional instructional documents such as training manuals and completion guidelines will be provided to assist with data entry during the course of the trial.

Upon completion of training, each user requiring access to the EDC system will be issued a unique username and password. In the event of a change in trial personnel, each newly assigned individual will receive a unique username and password; the username and password of a previous user may not be reissued. If any trial personnel leave the study, the Investigator is responsible for informing the Sponsor immediately so that their access is deactivated. An audit trail will be initiated in the EDC system at the time of the first data entry in order to track all modifications and to ensure database integrity.

The Investigator is responsible for the timeliness, completeness, and accuracy of the information in the eCRFs; must provide explanations for all missing information; and must sign the eCRF using an e-signature.

11.2 Data Management

Management of Clinical Data

Data generated during the trial will be managed following 2 different processes:

- Clinical data, defined as all data reported in the eCRF, and laboratory data will be handled by the Sponsor's CDM platform or authorized representative.
- Data pertaining to SAEs, which are reported by the Investigator on the eSAE Forms or SAE Reporting Forms, will be handled by the Sponsor's GPV Department.

During the trial, clinical data reported in the eCRFs will be integrated into the clinical database under the responsibility of the Sanofi Pasteur CDM platform. Data monitoring at the sites and quality control in the form of computerized logic and/or consistency checks will be systematically applied in order to detect errors or omissions. In addition, data reviews may be performed several times by the Sponsor's staff in the course of the trial. Any questions pertaining to the reported clinical data will be submitted to the Investigator for resolution using the EDC system. Each step of this process will be monitored through the implementation of individual passwords to maintain appropriate database access and to ensure database integrity.

The validation of the immunogenicity data will be performed at the laboratory level following the laboratory's procedures. Information from the laboratory will be checked for consistency before integration into the clinical database.

After integration of all corrections in the complete set of data, and after the SAE information available from the CDM platform and the GPV Department has been reconciled, the database will be released for statistical analysis.

Serious Adverse Event Data Management

During the trial, data pertaining to SAEs reported on eSAE Forms will be integrated into the Sponsor's centralized GPV database.

Upon receipt of an eSAE Form, the data will be entered into the GPV database after a duplicate check. Each SAE case will be assigned a case identification number. Each case will be entered in the GPV database and assessed by the case management platform or its delegate before being reported to the relevant authorities as necessary. Assessment of related cases will be done in collaboration with the PSO and the RMO(s). Follow-up information concerning a completed case will be entered into the GPV database, and a new version of the case will be created.

The information pertaining to SAEs in the GPV database will be reconciled with that in the clinical database.

11.3 Data Review

A review of the data is performed on a regular basis through the data review process led by the CDM platform until the database lock.

12 Statistical Methods and Determination of Sample Size

12.1 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, median, and minimum and maximum), sex, race, and ethnic origin will be summarized, along with the number and description of protocol violations.

Continuous variables will be summarized by descriptive statistics (e.g., means and standard deviations for the non-immunogenicity endpoints, and geometric means and their CIs for the immunogenicity endpoints); categorical variables will be summarized by frequency distributions (frequency counts, percentages, and their CIs for the main endpoints).

12.1.1 Hypotheses and Statistical Methods for Primary Objective

12.1.1.1 Hypothesis

During the 7 days after either vaccination (Dose 1 and Dose 2 combined), the 0.5-mL dose of Fluzone Quadrivalent vaccine will be non-inferior to the 0.25-mL dose of Fluzone Quadrivalent vaccine in terms of fever reaction (temperature ≥ 100.4°F [38.0°C]) in subjects 6 to < 36 months of age.

12.1.1.2 Statistical Methods

The 0.5-mL dose of Fluzone Quadrivalent vaccine will be demonstrated as non-inferior to the 0.25-mL dose of Fluzone Quadrivalent vaccine in terms of fever reaction through an assessment of the rates of any fever (temperature ≥ 100.4°F [38.0°C]) during the 7 days following any dose (Dose 1 and Dose 2 combined). Non-inferiority will be demonstrated if the upper bound of the 2-sided 95% CI of the rate difference between Group 2 (subjects receiving 0.5 mL of vaccine) and Group 1 (subjects receiving 0.25 mL of vaccine) is < 5%. The 95% CI of the rate difference will be computed using the Wilson Score method without continuity correction. The Safety Analysis Set will be used for this objective.

12.1.2 Hypotheses and Statistical Methods for Secondary Objective

12.1.2.1 Hypotheses

- Geometric mean titers: For each of the 4 virus strains, the post-final vaccination GMTs in subjects 6 to < 36 months of age who receive the 0.5-mL dose of Fluzone Quadrivalent vaccine will be non-inferior to the post-final vaccination GMTs in those subjects who receive the 0.25-mL dose of Fluzone Quadrivalent vaccine.
- Seroconversion: For each of the 4 virus strains, the seroconversion rates in subjects 6 to < 36 months of age who receive the 0.5-mL dose of Fluzone Quadrivalent vaccine will be

non-inferior to the seroconversion rates in those subjects who receive the 0.25-mL dose of Fluzone Quadrivalent vaccine.

12.1.2.2 Statistical Methods

- Geometric mean titers: The statistical methodology will be based on a 2-sided 95% CI of the ratio of the GMTs (0.5-mL dose divided by 0.25-mL dose) at 28 (window, 28–35) days after the final vaccination. Non-inferiority for GMTs will be demonstrated if the lower limit of the 2-sided 95% CI of the GMT ratio is > 0.667 for each of the 4 virus strains. The 95% CI will be calculated using normal approximation of log-transformed titers. The Per-Protocol Analysis Set (PPAS) will be used as the primary analysis set for this objective.
- Seroconversion: The statistical methodology will be based on a 2-sided 95% CI of the difference in seroconversion rates (0.5-mL dose minus 0.25-mL dose) at 28 (window, 28–35) days after the final vaccination. Non-inferiority for seroconversion will be demonstrated if the lower limit of the 2-sided 95% CI is > -10% for the 4 strains. The 95% CI of the rate difference will be computed using the Wilson Score method without continuity correction. The PPAS will be used as the primary analysis set for this objective.

12.1.3 Statistical Methods for Observational Objectives

12.1.3.1 Safety

[redacted].

12.1.3.2 Immunogenicity

The following immunogenicity parameters will be calculated for each influenza strain with 95% CIs of point estimates calculated using the exact binomial distribution (Clopper-Pearson method) for proportions and using normal approximation for GMTs:

- Geometric mean HAI assay titers at pre-vaccination (Day 0) and at 28 (window, 28–35) days after the final vaccination.
- Geometric means of the individual titer ratios of post-final vaccination/pre-vaccination.
- Seroprotection rates: The percentages of subjects with a titer ≥ 40 (l/dil) at pre-vaccination and at 28 (window, 28–35) days after the final vaccination.
- Seroconversion rates: The percentages of subjects with either a pre-vaccination titer < 10 (1/dil) and a post-vaccination titer ≥ 40 (1/dil), or a pre-vaccination titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-vaccination titer at 28 (window, 28–35) days after the final vaccination.

12.2 Analysis Sets

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

12.2.1 Safety Analysis Set

The Safety Analysis Set is defined as those subjects who have received study vaccine.[a] All subjects will have their safety analyzed after any dose according to the vaccine they actually received at the first dose. Safety data recorded for a vaccine received out of the protocol design will be excluded from the analysis (and listed separately).

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 at this dose.

12.2.2 Full Analysis Set

[redacted].

12.2.3 Per-Protocol Analysis Set

[redacted]

- Subject did not meet all protocol-specified inclusion criteria or met at least 1 of the protocol-specified exclusion criteria
- Subject did not receive vaccine or did not complete the vaccination schedule as per protocol
- Subject received a vaccine dose other than the one that he/she was randomly 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 was randomly assigned to the immunogenicity subset, but did not provide a post-dose serology sample in the proper time window or a post-dose serology sample was not drawn
- Subject received a protocol-prohibited therapy/medication/vaccine that might impact antibody response to the study vaccine
- Subject's post-vaccination serology sample did not produce a valid HAI test result for any strain

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

- Any other deviation identified during conduct of the study conduct and judged by the clinical team during data review as having a potential impact on the assessment of immunogenicity

12.2.4 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. The PPAS will be the primary analysis set for non-inferiority demonstration.

12.3 Handling of Missing Data and Outliers

12.3.1 Safety

No replacement of missing data will be done. All subjects with safety data and all safety data recorded in the eCRFs will be included in the safety analyses. No search for outliers will be performed.

12.3.2 Immunogenicity

12.4 Interim/Preliminary Analysis

No interim analyses are planned.

12.5 Determination of Sample Size and Power Calculation

13 Ethical and Legal Issues and Investigator/Sponsor Responsibilities

13.1 Ethical Conduct of the Trial/Good Clinical Practice

The conduct of this trial will be consistent with the standards established by the Declaration of Helsinki and compliant with the ICH guidelines for GCP as well as with all local and/or national regulations and directives.

13.2 Source Data and Source Documents

"Source data" are the data contained in source documents. Source documents are original documents or certified copies, and include, but are not limited to, diary cards, medical and hospital records, screening logs, informed consent/assent forms, telephone contact logs, and worksheets. The purpose of trial source documents is to document the existence of subjects and to substantiate the integrity of the trial data collected. Investigators must maintain source documents so that they are accurate, complete, legible, and up to date.

For missing or discrepant data on a diary card, the study coordinator will obtain verbal clarification from the subject, enter the response into the "Investigator's comment" page of the diary card, and transfer the information to the eCRF.

The subject pre-screening log should list all individuals contacted by the Investigators to participate in the trial, regardless of the outcome.

The Investigator must print[a] any electronic records on an ongoing basis, sign and date them immediately after creation, and keep the printouts on file as source documents that can be verified by the Sponsor or an inspector against the electronic records. Any later changes of an electronic record require the record to be re-printed, dated (with an indication of the date of change), and signed. Such records must also be kept together with the original printed copy.

13.3 Confidentiality of Data and Access to Subject Records

Prior to initiation of the trial, the Investigator will sign a fully executed confidentiality agreement with Sanofi Pasteur.

[a] Unless the electronic medical records are managed by validated computerized systems that are compliant with US 21 CFR Part 11, in which case they are acceptable on their own.

Sanofi Pasteur personnel (or designates), the IRBs, and regulatory agencies, including the FDA, require direct access to all study records, and will treat these documents in a confidential manner. In the event a subject's medical records are not at the investigational site, it is the responsibility of the Investigator to obtain those records if needed.

13.4 Monitoring, Auditing, and Archiving

13.4.1 Monitoring

Before the start of the trial (i.e., before the inclusion of the first subject in the first center), the Investigators and the Sponsor's staff or a representative will meet at the site-initiation visit to discuss the trial protocol and the detailed trial procedures. Emphasis will be placed on promoting understanding of inclusion and exclusion criteria, visit timing, safety procedures, informed consent procedures, SAE reporting procedures, eCRF completion, and the handling of samples and products. The Sponsor's staff or a representative will ensure and document that all material to be used during the trial has been received at the site; and that the study investigator team and local Sponsor/delegate staff have been properly informed about the trial, GCP and regulatory requirements, and the Sponsor's procedures. Specific training sessions for the study investigator team and the CRAs on these topics may be performed as necessary, and should be documented.

The following instruction manuals will be provided: the eCRF Completion Guidelines for entering data into the eCRF, and the Operating Guidelines for detailing trial procedures such as those related to product management and the handling of samples.

After the start of the trial, the Sponsor's staff or a representative will be in regular contact with the investigational team through telephone calls and regular follow-up visits. The Investigator or delegate must be available for these visits and must allow the Sponsor/delegate staff direct access to subject medical files and eCRFs. During these visits, the Sponsor/delegate staff will:

- Evaluate the quality of the trial progress (adherence to protocol and any study-specific guidelines, quality of data collection and document completion, presence of appropriate signatures on consent forms, occurrence of SAEs, sample and product management, cold-chain monitoring, archiving)
- Source-verify completed eCRFs and any corresponding answered queries
- Determine the number of complete or ongoing issues identified at monitoring visits (e.g., protocol deviations, SAEs). Any identified problems will be discussed with the Investigator, and corrective or preventive actions will be determined, as appropriate.
- After all protocol procedures have been completed and the data have been entered into the eCRF, the Investigator must still be available to answer any queries forwarded by the Sponsor. All data-related queries must be completed prior to database lock.

At the end of the trial, a close-out visit will be performed to ensure that:

- The center has all the documents necessary for archiving
- All samples have been shipped to the appropriate laboratory (GCI)
- All unused materials and products have been either destroyed or returned to the Sponsor

13.4.2 Audits and Inspections

A quality assurance audit may be performed at any time by the Sponsor's Clinical and Medical Quality Operations Department or by independent auditors to verify that the trial has been conducted according to the protocol, GCP and ICH requirements, and other applicable regulations. An inspection may be conducted by regulatory authorities. The Investigator must allow direct access to trial documents during these inspections and audits.

13.4.3 Archiving

The Investigator must keep all trial documents after the completion or discontinuation of the trial, whatever the nature of the investigational center (private practice, hospital, or institution), for as long as required by applicable laws and regulations. In the absence of any applicable laws or regulations, trial documents will be kept at a minimum for the duration indicated on the Clinical Trial Agreement (CTA). In no event, should study personnel destroy or permit the destruction of any trial documents upon less than 90 days advance written notification to the Sponsor. In addition, trial documents should continue to be stored, at Sponsor's sole expense, in the event that the Sponsor requests in writing that such storage continues for a period of time that exceeds that required by any applicable law or regulation or the CTA. The Investigator will inform Sanofi Pasteur of any address change or if they will no longer be able to house the trial documents.

Archived data may be held on electronic records, provided that a back-up exists and that a hard copy can be obtained if required. The protocol, documentation, approvals, and all other documents related to the trial, including certificates attesting that satisfactory audit and inspection procedures have been carried out, will be kept by the Sponsor in the Trial Master File (TMF). Data on AEs are included in the TMF. All data and documents will be made available if requested by relevant authorities.

13.5 Financial Contract and Insurance Coverage

A CTA will be signed by all the parties involved in the trial's performance, if relevant. The Sponsor has an insurance policy to cover any liabilities that may arise from use of the product and/or the study protocol.

13.6 Stipends for Participation

Subjects may be provided with a stipend according to local practice to compensate for the time and travel required for study visits and procedures.

13.7 Publication Policy

Data derived from this trial are the exclusive property of Sanofi Pasteur. Any publication or presentation related to the trial must be submitted to Sanofi Pasteur for review before submission of the manuscript. After publication of the results of the trial, any participating center may publish or otherwise use its own data provided that any publication of data from the trial gives recognition

to the trial group. In addition, Sanofi Pasteur shall be offered an association with all such publications, it being understood that Sanofi Pasteur is entitled to refuse the association.

Sanofi Pasteur must have the opportunity to review all proposed abstracts, manuscripts, or presentations regarding this trial at least 90 days prior to submission for publication/presentation. Any information identified by Sanofi Pasteur as confidential must be deleted prior to submission, it being understood that the results of this trial are not to be considered confidential.

Sanofi Pasteur's review can be expedited to meet publication guidelines.

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