SANOFI PASTEUR

NCT Number: NCT02451358

Immunogenicity and Safety of a Single Dose or Two Doses Given 28 Days Apart of a Quadrivalent Influenza Vaccine Administered via the Intramuscular Route in Subjects Aged 6 Months or Older in India

Open-label, multi-center, Phase-III study in healthy subjects aged 6 months or older in India

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	QIV06
Development Phase:	Phase III
Sponsor:	Sanofi Pasteur SA 2, avenue Pont Pasteur, 69367 Lyon cedex 07, France
Investigational Product(s):	Quadrivalent Inactivated Influenza Vaccine, No Preservative
Form / Route:	Liquid/Intramuscular
Indication For This Study:	Prophylaxis of influenza in subjects aged 6 months or older
Version and Date of the SAP core body part:	Version 2.0, 04 Mar 2016

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List of Abbreviations

AE	adverse event
AESI	adverse events of special interest
AF	assent form
ALRI	influenza-associated acute lower respiratory infections
AR	adverse reaction
CI	confidence interval
CRF	electronic case report form
D	Day
DC	diary card
DCGI	Drug Controller General of India
dil	Dilution
FAS	full analysis set
FDA	Food and Drug Administration
GBS	Guillain-Barré syndrome
GCI	Global Clinical Immunology
GM	geometric mean
HA	Hemagglutinin
HAI	hemagglutination inhibition
HAU	hemagglutination units
ICF	informed consent form
ICH	International Conference on Harmonisation
LLOQ	lower limit of quantitation
LLT	lowest level term
MedDRA	Medical Dictionary for Regulatory Activities
mL	Milliliter
NDAC	New Drug Advisory Committee
NH	Northern Hemisphere
OPV	Oral Poliomyelitis Vaccine
PPAS	per-protocol analysis set
QIV	Quadrivalent influenza vaccine
RBC	red blood cell
SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SH	Southern Hemisphere
TIV	trivalent influenza vaccine
ULOQ	upper limit of quantitation

WHO	World Health Organization

1 Introduction

Influenza is a highly contagious, acute viral respiratory disease. It is typically characterized by the rapid onset of fever, myalgia, sore throat, and non-productive cough. Influenza can cause severe malaise, which lasts for several days. While influenza affects all age groups, the very young, older adults and persons with underlying health problems are at increased risk for complications.

In temperate regions of the world, influenza occurs in winter season, whereas in tropical countries like India, peak influenza activity is seen during rainy season with some activity throughout the year. North India demonstrated peak activity in winter and rainy seasons, Eastern and Western India demonstrated highest activity during rains and limited activity in winter and South India demonstrated a peak in the cooler season during rains.

Vaccination is currently the most effective protection against influenza. Current vaccines induce serum anti-hemagglutin (HA) antibody for prevention of subsequent infection and illness from natural influenza. Serum anti-HA antibody is the most consistent correlate of immunity to influenza, in that serum hemagglutination-inhibition (HAI) titer correlates inversely with frequency of influenza illnesses among vaccinated persons. Annual vaccination is recommended for the elderly population as well as for all adults at higher risk for influenza complications or in close contacts of persons at higher risk. Moreover, vaccination against influenza is recommended for any adult who wants to reduce the risk of becoming ill with influenza or of transmitting it to others. Influenza vaccination of the elderly reduces the risk of serious complication or death by 70-85%. In 2003, the World Health Assembly urged Member States with influenza vaccination policies to increase vaccination coverage of all people at high risk and to aim a vaccination coverage of elderly people of at least 50% by 2006 and 75% by 2010.

Children aged < 5 years, and particularly those < 2 years of age, have a high burden of influenza. A systematic review of the global disease burden of influenza in children, representing studies on a total of around 8 million children < 5 years of age, estimated that in 2008, there were 90 million (95%, confidence interval [CI] 49–162 million) new cases of seasonal influenza, 20 million (95% CI: 13–32 million) cases of influenza-associated acute lower respiratory infections (ALRI), and 1 to 2 million cases of influenza associated severe ALRI, including 28,000 to 111,500 deaths. The great majority of deaths from influenza occurred in developing countries.

Antigenic variation is an important feature of the influenza virus. To accommodate for this variation, since 1978, the use of trivalent influenza vaccines (TIVs) containing 1 H1N1 strain, 1 H3N2 strain and 1 B strain has been the norm. Fluzone® (Influenza Virus Vaccine), an inactivated TIV, has been used in the US since 1947, first as a whole virus preparation, and since 1980 as a split virus preparation, with a very good safety profile.

Unfortunately, the ability to predict with acceptable accuracy which B lineage will be dominant during an upcoming season has been unsatisfactory, with frequent mismatches between the lineage chosen for inclusion in the vaccine and the predominant lineage in circulation. Consequently, there seems to be growing evidence among regulators, industry, and academic advisors that the time has come to move toward a Quadrivalent influenza vaccine (QIV), which would add an additional alternate-lineage B strain to the current TIV.

In this context, Sanofi Pasteur has developed an inactivated quadrivalent influenza vaccine (QIV) containing 15 µg of HA of the two A strains (A/H1N1 and A/H3N2) and 15 µg of the two B strains (from the Victoria lineage and from the Yamagata lineage), based on the same manufacturing process as Fluzone manufactured by Sanofi Pasteur, US for active vaccination of subjects from 6 months of age (a pediatric formulation containing 7.5 µg of HA has been also developed).

Sanofi Pasteur conducted 3 studies (GRC43, QIV03, and QIV04) to evaluate the safety and immunogenicity of vaccination with QIV in adults and children (from 6 months of age). In these clinical trials, QIV demonstrated non-inferiority of antibody responses for each of the common 3 strains as compared to TIVs in each age group. QIV also demonstrated superiority of antibody response to the TIV groups which did not contain corresponding B strain in each age group. In addition, the overall safety profiles were similar in Fluzone® Quadrivalent and Fluzone groups in all age groups tested.

On June 7, 2013, US Food and Drug Administration (FDA) approved Sanofi Pasteur request to supplement the biologics license application for Influenza Virus Vaccine to include a Quadrivalent influenza virus vaccine formulation (Fluzone Quadrivalent) for use in persons 6 months of age and older (1).

This Quadrivalent vaccine is currently marketed in the US, Canada, Guatemala, Mexico, Panama, Venezuela, Hong Kong, and Malaysia. This vaccine is planned to be marketed during Southern Hemisphere (SH) 2015 influenza season in some other countries in Latin America and Asia.

2 Trial Objectives

2.1 Immunogenicity Objective

To describe in each age group the immune response induced by a single injection (subjects aged 9 years or older) or 2 injections (subjects aged 6 months to 8 years) of QIV

The endpoints for the immunogenicity objective are presented in Section 4.1.1.

2.2 Safety Objective

To describe in each age group the safety profile of QIV

The endpoints for the safety objective are presented in Section 4.2.2.

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

This will be an open-label multi-center trial. A total of 400 subjects aged 6 months or older will be included in 4 age groups: 6 to 35 months, 3 to 8 years, 9 to 17 years, and 18 years or older (100 subjects per age group).

Subjects will be sequentially enrolled as follows:

Per requirement from New Drug Advisory Committee (NDAC) / Subject Expert Committee (SEC) and approval letters from the Drug Controller General of India (DCGI) dated 22 July 2014, 15 May 2015, and 17 August 2015, the following recruitment will be performed: 100 adult subjects (18 years and older) will be enrolled first to receive QIV injection.

The safety events (line listings) with an occurrence within 28 days after vaccination will be submitted to the DCGI for evaluation by SEC experts.

When the DCGI gives the Go decision, the younger age groups will be sequentially enrolled, as follows: firstly the 100 subjects from the 9 to 17 years group, secondly the 100 subjects from the 3 to 8 years group, and thirdly the 100 subjects from the 6 to 35 months group.
Safety events (line listings) with an occurrence within 28 days after vaccination for subjects aged 9 to 17 years and 28 days after the first vaccination for subjects aged 3 to 8 years will be reviewed by the Sponsor, and then submitted to the DCGI for information before moving to each of the younger age groups (3 to 8 years and 6 to 35 months).

All subjects will be vaccinated with the QIV (split-virion, inactivated) formulation recommended by the WHO Northern Hemisphere (NH) or SH according to the study timelines, by the intramuscular (IM) route.

The vaccination regimen will be as follows:

100 previously unvaccinated[a] subjects aged 6 to 35 months will receive 2 injections 4 weeks apart (28 days) of QIV 7.5µg HA/strain (0.25 milliliters [mL])

100 previously unvaccinated subjects aged 3 to 8 years will receive 2 injections at least 4 weeks apart (28 days) of QIV 15µg HA/strain (0.5 mL)

200 subjects aged 9 years or older (i.e., 100 adolescents aged 9 to 17 years and 100 adults aged 18 years or older) will receive 1 injection of QIV 15µg HA/strain (0.5 mL)

Immunogenicity of the vaccine will be assessed at baseline (Day 0 [D0]) and 28 days after the last injection. Safety data will be collected up to 28 days after each vaccination. Serious adverse events (SAEs), including adverse events of special interest (AESIs) will be collected throughout the trial (i.e., up to 28 days after the final vaccination).

[a] "previously unvaccinated": a child aged 6 months to 8 years not adequately primed, e.g., if he / she has not been vaccinated with 2 doses (with an approximate interval of 4 weeks) for at least 1 previous influenza season, then 2 doses of influenza vaccine will be administered

3.2 Trial Plan

The trial plan is summarized in the Tables of Study Procedures (see Table 3.1 and Table 3.2).

Recruitment and information of subjects / parents / legally acceptable representatives:

Prior to enrollment, the investigator or a designee will inform subjects / parents / legally acceptable representatives of potentially eligible subjects about the trial. They will explain to subjects / parents / legally acceptable representatives that they may have to first sign an assent form and/or informed consent form (ICF) for Audio-Video Recording, if required by local regulation.

Then, candidates will be given a verbal description of the trial design, including but not limited to, the potential risks and benefits, discomforts, and subject responsibilities. Subjects / parents / legally acceptable representatives must voluntarily sign an ICF prior to enrollment in the trial. AFs may be voluntarily signed by the subject in accordance with local EC requirements or local regulations.

Trial description and enrollment of subjects:

After the subjects / parents / legally acceptable representatives (if applicable) have signed the ICF and, if applicable after the subject has signed the AF, and following confirmation by the Investigator that the subject has satisfied all inclusion / exclusion criteria, eligible subjects will be included in the study. They will provide their first blood sample (BL) and will be vaccinated with QIV.

Sequential approach

Per requirement from NDAC and approval from DCGI, adult subjects (18 years and older) will be enrolled first to receive QIV injection and will be followed for safety assessment. The younger age groups will then be enrolled sequentially when DCGI gives the Go decision as follows: firstly adolescents aged 9 to 17 years, secondly children aged 3 to 8 years, and then infants and toddlers aged 6 to 35 months (see Section 3.1 for more details).

Vaccination

Depending on their age, the subjects will receive 1 or 2 doses of QIV as follows:

Subjects aged 6 to 35 months will receive 2 injections 28 days apart of QIV 7.5µg HA/strain (0.25 mL)

Subjects aged 3 to 8 years will receive 2 injections 28 days apart of QIV 15µg HA/strain (0.5 mL)

Subjects aged 9 years or older will receive 1 injection of QIV 15µg HA/strain (0.5 mL)

Blood sampling

All subjects will provide a pre-vaccination baseline blood sample (3 or 5 mL[a]) at D0 and a second blood sample (3 or 5 mL[a]) 28 days after the last vaccination.

[a] 3 mL for subjects aged 6 months to 11 years and 5 mL for subjects aged 12 years or older

Collection of safety data

Solicited reactions from D0 to D7 and non-serious unsolicited adverse events (AEs) from D0 to D28 will be collected after each vaccination.

Information on SAEs, including AESIs, will be collected throughout the study.

Table 3.1: Table of Study Procedures for Subjects Aged 6 Months to 8 Years[a]

Phase III Trial, 3 Visits, 2 Vaccinations, 2 Blood Samples

Visit	**V01**	**Phone Call or Home Visit**	**V02**	**Phone Call or Home Visit**	**V03**
Trial timelines (days)	**D0**	**V01 + 3 days**	**V01 + 28 days**	**V02 + 3 days**	**V02 + 28 days**
Trial windows (days)	**-**	**+2**	**± 2**	**+2**	**± 2**
Indicative Months (M)			**M1**		**M2**
Informed consent / assent forms signed and dated	X				
Inclusion / exclusion criteria	X				
Demographics	X				
History of seasonal influenza vaccination / history of influenza diagnosis*	X				
Significant medical history	X				
Physical examination	X		X		X
Temperature (axillary)	X		X		
Temporary and definitive contraindications			X		
Assign subject number	X				
Blood sampling (3 mL)	BL1†				BL2
Vaccine injection	VAC1		VAC2		
30-minute surveillance period	X		X		
Diary Card (DC): **Provided** **Collected**	DC1		DC2 DC1		DC2
Contact with Subjects‡		X		X	
Recording of solicited injection site & systemic reactions			X		X
Recording of unsolicited non-serious adverse events			X		X
Reportable concomitant medication	X		X		X
Termination record					X
Serious adverse events§	Collected throughout the trial				

[a] '6 months to 8 years' means from the day of the 6th month after birth to the day before the 9th birthday

* History of seasonal influenza vaccination and influenza diagnosis will be collected within the past 3 years or since birth for subjects aged 6 to 35 months

† Collection of the first blood sample (BL1) will be before vaccination

‡ Telephone Calls or Home Visits:: trial personnel will ensure correct completion of DC, ask the subjects / subject's parent(s) / legally acceptable representative(s) whether the subject experienced any SAEs not yet reported, confirm the next visit and remind the subjects / subject's parent(s) / legally acceptable representative(s) that the DC should be brought to the trial center at the next visit

§ Including all AESIs

Table 3.2: Table of Study Procedures for Subjects Aged 9 Years or older[a]

Phase III Trial, 2 Visits, 1 Vaccination, 2 Blood Samples

Visit	**V01**	**Phone Call or Home Visit**	**V02**
Trial timelines (days)	**D0**	**Visit 1 + 3 days**	**Visit 1 + 28 days**
Trial windows (days)	**-**	**+2**	**± 2**
Indicative Months (M)			**M1**
Informed consent / assent forms signed and dated	X		
Inclusion / exclusion criteria	X		
Demographics	X		
History of seasonal influenza vaccination / history of influenza diagnosis*	X		
Significant medical history	X		
Physical examination	X		X
Temperature (axillary)	X		
Urine pregnancy test†	X		
Assign subject number	X		
Blood sampling (3 or 5 mL‡)	BL1§		BL2
Vaccine injection	VAC1		
30-minute surveillance period	X		
Diary Card (DC) **Provided** **Collected**	DC1		 DC1
Contact with Subjects**		X	
Recording of solicited injection site & systemic reactions			X
Recording of unsolicited non-serious adverse events			X
Reportable concomitant medication	X		X
Termination record			X
Serious adverse events††	Collected throughout the trial		

* History of seasonal influenza vaccination and influenza diagnosis will be collected within the past 3 years

† For women of childbearing potential, the urine pregnancy test is to be performed before vaccination.

‡ 3 mL for subjects aged 9 to 11 years and 5 mL for subjects aged 12 years or older

[a] ‘ '9 years or older’ means from the day of the 9th birthday

§ Collection of the first blood sample (BL1) will be before vaccination

** Telephone Calls or Home Visit: trial personnel will ensure correct completion of DC, ask the subjects / subject's parent(s) / legally acceptable representative(s) whether the subject experienced any SAEs not yet reported, confirm the next visit and remind the subjects / subject's parent(s) / legally acceptable representative(s) that the DC should be brought to the trial center at the next visit

†† Including all AESIs

4 Endpoints and Assessment Methods

4.1 Immunogenicity Endpoints and Assessment Methods

See Section 9.1 of the protocol.

4.1.1 Immunogenicity Endpoints

Immunogenicity will be evaluated before and 28 days after the final vaccination[a] using the HAI technique. For each vaccine strain, serum HA antibody titers will be expressed as geometric mean (GM) of HAI titers obtained in duplicates for pre- (D0) and post-vaccination (28 days after the final vaccination[a]).

The derived endpoints will be:

Individual geometric mean of duplicate titers (GM of titers) pre- and post-vaccination

Detectable HAI: titer ≥10 (1/dilution [1/dil]) pre- and post-vaccination

Individual titer ratio post-vaccination/pre-vaccination

Seroprotection status: titer ≥ 40 (1/dil) pre- and post-vaccination

Seroconversion or significant increase status:

- Seroconversion status: pre-vaccination titer < 10 (1/dil) and post-vaccination titer ≥ 40 (1/dil)
- Significant increase status: pre-vaccination titer ≥ 10 (1/dil) and ≥ 4-fold increase of post-vaccination titer

4.1.2 Immunogenicity Assessment Methods

Anti-HA antibody titers will be measured using the HAI method from the sera obtained on D0 and D28 after the last vaccination[a], according to a reference technique. For each vaccine strain, samples obtained pre- and post-vaccination from a same subject will be tested simultaneously in

[a] D56 for subjects aged 6 months to 8 years, and D28 for subjects aged 9 years and older

duplicates. The titer assigned at the time of the statistical analysis to each sample will be the GM of 2 independent determinations.

Additional analyses may be performed on the blood samples if required by the Sponsor to obtain further influenza antibody titration. In such a case, these analyses will not require additional blood samplings.

Anti-Influenza Virus Antibody Titration by Inhibition of Hemagglutination

Assays will be performed by the Sponsor's laboratory (GCI, Swiftwater, PA, USA) or at a contract research organization laboratory under GCI responsibility. The address is provided in the Operating Guidelines.

Test serum samples and quality control sera are incubated with Neuraminidase to eliminate non-specific inhibitors. Adsorption of spontaneous anti-species agglutinins is then performed by incubating the serum samples and quality control sera with a red blood cell (RBC) suspension. The mixtures are then centrifuged and the supernatants containing the treated sera are collected for testing.

Ten two-fold dilutions of the initial 1/10 dilution of the treated serum samples and quality control sera are incubated with previously titrated HA antigen (4 hemagglutination units [HAU]/25 μL). HA antigen is not added to serum control wells containing only serum and RBCs. The mixture is then incubated and a RBC suspension is added. Following incubation, the results are read. The serum titer against each influenza strain tested is determined as the reciprocal of the highest dilution that exhibits complete inhibition of hemagglutination. Each serum sample is titrated in independent duplicates and the two values, which cannot differ by more than a two-fold dilution, are reported. The lower limit of quantitation (LLOQ) is set at the reciprocal of the lowest dilution used in the assay, i.e., 10 (1/dil). Titers below this level are reported as <10 (1/dil). The upper limit of quantitation (ULOQ) is 10240 (1/dil). Titers above this level are reported as ≥ 10240 (1/dil).

4.2 Safety Endpoints and Assessment Methods

See Section 9.2 of the protocol.

4.2.1 Safety Definitions

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

Adverse Event (AE):

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 (SAE):

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]

Requires inpatient hospitalization or prolongation of existing hospitalization[b]

Results in persistent or significant disability / incapacity[c]

Is a congenital anomaly / birth defect

Is an important medical event[d]

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[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.

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

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

[d] 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

Additionally, AESIs listed in Section 4.3.1.4.6 are to be considered as SAEs.

Adverse Reaction:

All noxious and unintended responses to a medicinal product related to any dose should be considered adverse reactions (AR).

(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 adverse reaction 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 AE that is prelisted in the CRF. The assessment of these AEs post-vaccination is mandatory. A solicited reaction is defined by a combination of:

Symptom and

Onset post-vaccination

e.g., injection site swelling between D0 and D7 post-vaccination, or fever between D0 and D7.

A solicited reaction is therefore an AR observed and reported under the conditions (symptom and onset) prelisted (i.e., solicited) in the CRF 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 CRF in terms of diagnosis and / or onset post-vaccination, i.e., excluding solicited reactions, e.g., if fever between D0 and D7 is a solicited reaction (i.e., prelisted in the CRF), then a fever starting on D7 is a solicited reaction, whereas fever starting on D8 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.

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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, or the development of drug dependency or drug abuse, new onset diabetes or autoimmune disease.

[a] All injection site AEs are considered to be related to vaccination and are therefore all *injection site 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 (AESIs):

AESIs are AEs that are considered by the Sponsor to be relevant for the monitoring of the safety profile of the investigational vaccine (see Section 4.3.1.4.6).

4.2.2 Safety Endpoints

The endpoints for the evaluation of safety are:

- Occurrence of unsolicited AEs reported in the 30 minutes after each / any injection
- Occurrence of solicited (prelisted in the subject diary card (DC) and CRF) injection site reactions and systemic reactions within 7 days following each / any injection
- Occurrence of unsolicited (spontaneously reported) AEs within 28 days following each / any injection
- Occurrence of SAEs (including AESIs) throughout the trial (i.e., from D0 through end of the study)

Depending on the item, endpoints described include: nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), time of onset, duration, number of days of occurrence, Grade of severity, relationship to vaccine, whether the AE led to early termination from the study, seriousness, or outcome.

4.2.3 Safety Assessment Methods

At Visit 2 (subjects aged 9 or older) or at Visit 2 and Visit 3 (subjects aged 6 months to 8 years), the Investigator or a delegate will perform a clinical or medically-driven physical examination, and will ask the subjects / parents / legally acceptable representatives about any solicited reactions and unsolicited AEs recorded in the DC, as well as about any other AEs that may have occurred since the previous visit. All relevant data will be transcribed into the CRF according to the instructions provided by the Sponsor.

4.2.3.1 Immediate Post-vaccination Surveillance Period

Subjects will be kept under observation for 30 minutes after vaccination to ensure their safety. Any AE that occurs during this period will be noted on the source document and identified as an immediate event / reaction; and will additionally be recorded in the CRF, as follows:

- Any unsolicited systemic AE occurring during the first 30 minutes post-vaccination will be recorded on the CRF as 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 30 minutes post-vaccination will be reported in the same way as any other SAE and to the Sponsor.

4.2.3.2 Reactogenicity (Solicited Reactions From Day 0 to Day 7 After the Vaccination)

After vaccination, subjects / parents / legally acceptable representatives will be provided with a safety DC, a digital thermometer, and a flexible ruler, and will be instructed how to use them. The following items will be recorded by the subjects in the DC on the day of vaccination and for the next 7 days (i.e., D0 to D7) 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 subjects / parents or legally acceptable representatives to treat any **solicited reactions** will be classified in the CRF 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)

Table 4.1, Table 4.2, Table 4.3, Table 4.4 and Table 4.5 present, respectively, the injection site reactions and systemic reactions that are prelisted in the DCs and CRF for the different age group, together with the intensity scales.

Table 4.1: Solicited injection site reactions: terminology, definitions, and intensity scales for subjects aged 6 to 23 months

CRF term (MedDRA lowest level term [LLT])	Injection site tenderness	Injection site erythema	Injection site swelling
Diary card term	Tenderness	Redness	Swelling
Definition		Presence of a 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*	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	Grade 1: > 0 to < 25 mm Grade 2: ≥ 25 to < 50 mm Grade 3: ≥ 50 mm

* For the subjective reaction of tenderness, parents / legally acceptable representatives will record the intensity level (Grade 1, 2, or 3) in the DC. 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 4.2: Solicited injection site reactions: terminology, definitions, and intensity scales for subjects aged 2 to 11 years

CRF term (MedDRA LLT)	Injection site pain	Injection site erythema	Injection site swelling
DC term	Pain	Redness	Swelling
Definition		Presence of a 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* for subjects aged 2 to 11 years	Grade 1: Easily tolerated Grade 2: Sufficiently discomforting to interfere with normal behavior or activities Grade 3: Incapacitating, unable to perform usual activities	Grade 1: >0 to <25 mm Grade 2: ≥25 to <50 mm Grade 3: ≥50 mm	Grade 1: >0 to <25 mm Grade 2: ≥25 to <50 mm Grade 3: ≥50 mm

* For the subjective reaction of pain, subjects / parents / legally acceptable representatives will record the intensity level (Grade 1, 2, or 3) in the DC. 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 4.3: Solicited injection site reactions: terminology, definitions, and intensity scales for subjects aged 12 years or older

CRF term (MedDRA lowest level term [LLT])	**Injection site pain**	**Injection site erythema**	**Injection site swelling**
Diary card term	Pain	Redness	Swelling
Definition		Presence of a 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*	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm

* For the subjective reaction of pain, subjects / parents / legally acceptable representatives 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 4.4: Solicited systemic reactions: terminology, definitions, and intensity scales for subjects aged 6 to 23 months

CRF term (MedDRA 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	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*	Grade 1: ≥ 38.0°C to ≤ 38.5°C	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	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	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

* For all reactions but fever, parents / legally acceptable representatives will record the intensity level (Grade 1, 2, or 3) in the DC. 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.

Table 4.5: Solicited systemic reactions: terminology, definitions, and intensity scales for subjects aged 2 years or older

CRF term (MedDRA LLT)	Fever	Headache	Malaise	Myalgia	Shivering
DC term	Temperature	Headache	Feeling unwell	Muscle aches and pains	Shivering
Definition	Elevation of temperature to ≥°38.0°C	Pain or discomfort in the head or scalp. Does not include migraine.	General ill feeling. Malaise is a generalized feeling of discomfort, illness, or lack of well-being that can be associated with a disease state. It can be accompanied by a sensation of exhaustion or inadequate energy to accomplish usual activities.	Muscle aches and pains are common and can involve more than one muscle at the same time. Muscle pain can also involve the soft tissues that surround muscles. These structures, which are often referred to as connective tissues, include ligaments, tendons, and fascia (thick bands of tendons). Does not apply to muscle pain at the injection site which should be reported as injection site pain.	Cold feeling
Intensity scale*	Grade 1: ≥38.0°C to ≤38.4°C	Grade 1: No interference with activity	Grade 1: No interference with activity	Grade 1: No interference with activity	Grade 1: No interference with activity
	Grade 2: ≥38.5°C to ≤38.9°C	Grade 2: Some interference with activity	Grade 2: Some interference with activity	Grade 2: Some interference with activity	Grade 2: Some interference with activity
	Grade 3: ≥39.0°C	Grade 3: Significant; prevents daily activity	Grade 3: Significant; prevents daily activity	Grade 3: Significant; prevents daily activity	Grade 3: Significant; prevents daily activity

* For all reactions but fever, parents / legally acceptable representatives will record the intensity level (Grade 1, 2, or 3) in the DC. For fever, they will record the body temperature, and the classification as Grade 1, 2, or 3 will be assigned at the same time of the statistical analysis.

4.3 Derived Endpoints: Calculation Methods

Terms used in the clinical safety tables to describe the safety events are specified below.

AE: adverse event includes immediate, solicited, and unsolicited non-serious or serious event.

AR: adverse reaction corresponds to related AE.

Immediate AE/AR: unsolicited non-serious systemic AE ticked "immediate (within 30 minutes from the vaccination)" by the Investigator in the eCRF or electronic serious adverse event (eSAE) with time to onset within 30 minutes after vaccination with either dose 1 or dose 2.

SR: solicited reactions are events pre-listed in the eCRF, and which occurred during the solicited period (D0 through D7 after vaccination with either dose 1 or dose 2).

Unsolicited AE: AE recorded in the eCRF unsolicited form and eSAE, excluding SRs. Therefore, include immediate AEs.

Unsolicited non-serious injection site reactions are always recorded without relationship and analyzed as ARs.

Unsolicited AEs to be analyzed are AEs which occur between Visit 1 and Visit 2 (and between Visit 2 and Visit 3 for subjects requiring two doses). Unsolicited AEs occurring after the last visit - and which are not SAE - will be presented in separate listings.

SAE: unsolicited AE considered serious by the Investigator.

4.3.1 Safety

4.3.1.1 Solicited Reactions

4.3.1.1.1 Daily Intensity

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

For measurable injection site reactions for subjects aged 6 months to 11 years:

Grade 1: > 0 to < 25 mm

Grade 2: ≥ 25 to < 50 mm

Grade 3: ≥ 50 mm

For measurable injection site reactions for subjects aged 12 years or older:

Grade 1: ≥ 25 to ≤ 50 mm

Grade 2: ≥ 51 to ≤ 100 mm

Grade 3: > 100 mm

For Fever for subjects aged 6 to 23 months:

Grade 1: ≥ 38.0°C to ≤ 38.5°C

Grade 2: > 38.5°C to ≤ 39.5°C

Grade 3: > 39.5°C

For Fever for subjects aged 2 years or older:

Grade 1: ≥ 38.0°C to ≤ 38.4°C

Grade 2: ≥ 38.5°C to ≤ 38.9°C

Grade 3: ≥ 39.0°C

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

1. Solicited reactions (except Fever) with an investigator presence recorded as "No" and with all daily records missing then all daily intensities will be derived as None.
2. For non-measurable solicited reactions, daily intensities will correspond to daily records reported in the clinical database. For measurable solicited reactions the daily measurements reported in the clinical database will be converted based upon the intensity scales defined above as in the Section 9.2.3.2 of the protocol; this assumes a reaction that is too large to measure (NM, not measurable) is Grade 3. Note: The intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., swelling measurement > 0 mm but < 25 mm for subjects aged ≥ 12 years).

Missing measurements (for temperature or length) will not be replaced.

The maximum intensity during the solicited period will be computed without considering the ongoing period. If the solicited period is D0 through D7, for an ongoing event at D7, the intensities after D7 will not be considered.

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

4.3.1.1.2 Maximum Overall Intensity

Maximum overall intensity is derived from the daily intensities computed as described above and is calculated as the maximum of the daily intensities over the period considered.

The Grade of intensity is applied following the rules described in the Section 4.3.1.1.1.

4.3.1.1.3 Presence

Presence is derived from the maximum overall intensity on the period considered:

- None: No presence
- Grade 1, Grade 2, or Grade 3: Presence
- Missing: Missing presence

Subjects with at least one non-missing presence for a specific endpoint will be included in the analysis. Conversely, those without a non-missing presence will not be included in the analysis of the endpoint.

4.3.1.1.4 Time of Onset

Time of onset is derived from the daily intensities computed as described in Section 4.3.1.1.1. It corresponds to the first day with intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (i.e., reaction occurs over two separate periods of time intervened by at least one daily intensity Missing or None) then the time of onset is the first day of the first occurrence.

Time of onset will be categorized and presented as periods of D0 to D3 and D4 to D7.

4.3.1.1.5 Number of Days of Occurrence

Number of days of occurrence over the period considered is derived from the daily intensities computed as described in Section 4.3.1.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. Number of days of occurrence on the solicited period with a specified intensity may also be derived.

For injection site and systemic reactions during the solicited period, number of days of occurrence will be displayed by periods as 1-3 days, 4-7 days, and 8 days.

4.3.1.1.6 Overall Number of Days of Occurrence

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

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

If the stop date is missing or incomplete (contains missing data [MD]), the overall number of days of occurrence will be considered as Missing.

For injection site and systemic reactions, overall number of days of occurrence will be displayed by periods as 1-3 days, 4-7 days, 8 days, and Missing.

4.3.1.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed as described in Section 4.3.1.1.1 and the maximum intensity on the ongoing period. The investigator's ongoing flag is not used because the measurement will determine the ongoing status of the reaction.

Note: a reaction could be derived as not ongoing for the analysis despite being considered as ongoing by the investigator (e.g., when the maximum measurement after D7 for subjects aged ≥ 12 years is > 0 mm but < 25 mm).

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

4.3.1.2 Unsolicited Non-serious AEs

4.3.1.2.1 Presence

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

4.3.1.2.2 Intensity

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

If the unsolicited non-serious AE is measurable and its preferred term is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule than the intensity scales defined in the in Section 4.3.1.1.1 for that measurable injection site or systemic reaction. Note the intensity could be considered "None" (not a reaction) in the analysis despite being considered a reaction by the investigator (e.g., measurement for subjects aged ≥ 12 years is > 0 mm but < 25 mm).

Intensity for the other unsolicited non-serious AEs will correspond to the value reported in the eCRF.

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

4.3.1.2.3 Last Vaccination

For subjects aged 6 months to 8 years:

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

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

If the visit number is missing, then the start date should be used to determine the last vaccination before the unsolicited non-serious AE

For subjects aged 9 years or older:

Last vaccination before any unsolicited non-serious AE is the study vaccination at V01.

4.3.1.2.4 Time of Onset

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

- start date of the unsolicited non-serious AE – date of last vaccination

The time of onset should be considered as missing only if one or both of the dates are missing or partially missing.

The unsolicited non-serious AEs analyzed "Within 28 days" correspond to AEs with a time of onset between 0 and 28 days after vaccination or missing. An AE with missing time of onset will be considered having occurred just after the last vaccination, so will be included in these tables.

Note: Unsolicited non-serious AE that occur before vaccination (negative time of onset) or with a time of onset higher than 28 days will not be included in analysis but will be listed separately.

Time of onset will be displayed as follows:

- D0-D3
- D4-D7
- ≥ D8
- Missing

4.3.1.2.5 Duration

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

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

The duration should be considered as missing only if one or both of the start and stop dates of the unsolicited non-serious AE is missing or partially missing.

Duration will be displayed by period as following:

1-3 days

4-7 days

≥ 8 days

Missing

4.3.1.3 SAEs

4.3.1.3.1 Presence

An observation will be considered an event if it has at least a verbatim term.

4.3.1.3.2 Last Vaccination

For subjects aged 6 months to 8 years:

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

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

If the visit number is missing, then the start date should be used to determine the last vaccination before the SAE

For subjects aged 9 years or older:

Last vaccination before any SAE is the study vaccination at V01.

4.3.1.3.3 Time of Onset

Time of onset will be computed using the same methodology than for unsolicited non-serious AEs described in Section 4.3.1.2.4.

SAEs will be analyzed throughout the study using the following periods:

- Within 28 days after each / any injection
- During the study (i.e., all SAEs occurred during the study)

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

Note: Any SAEs that occurred before vaccination (negative time of onset) will not be included in analysis but will be listed separately.

4.3.1.3.4 Duration

Duration will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.3.1.2.5.

4.3.1.4 Other Safety Endpoints

4.3.1.4.1 Action Taken

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

4.3.1.4.2 Seriousness

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

4.3.1.4.3 Outcome

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

4.3.1.4.4 Causality

This information will be summarized as collected. Missing causality (relationship) will be handled as described in Section 5.3.2.2.

4.3.1.4.5 AEs Leading to Study Discontinuation

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

In general, the items that are counted are:

- Disposition table: A subject who has, on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" is checked.
- Safety overview table: A subject who has either on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" is checked or lists an AE on an AE page (solicited, unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated. Note: If the Grade is below 1, the AE will be excluded from the list of AEs leading to study discontinuation.
- System organ class/Preferred term (SOC/PT) table: An event (solicited, unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated.

4.3.1.4.6 AEs of Special Interest (AESIs)

AESIs will be collected throughout the trial (i.e., up to 28 days after the final vaccination). AESIs are to be reported as SAEs. These include new onset of Guillain-Barré syndrome (GBS), Bell's palsy, encephalitis/myelitis, optic neuritis, Stevens-Johnson syndrome, toxic epidermal necrolysis, and febrile seizures.

4.3.1.4.7 Pregnancy

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

4.3.1.4.8 Medical history

Medical history will be coded using MedDRA most updated version.

4.3.2 Immunogenicity

4.3.2.1 Computed Values for Analysis

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

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

If a value is between ≥ LLOQ and < ULOQ, then use the value.

If a value is ≥ ULOQ, then use the computed value ULOQ.

4.3.2.2 Seroprotection

If the computed value is ≥ 1:40 1/dil, then the derived seroprotection indicator will be "Yes" for that test, otherwise seroprotection will be "No". If the computed value is missing, seroprotection will be missing.

4.3.2.3 Fold-rise

The derived endpoint fold-rise is driven by both baseline and post-baseline computed values (assuming replicate values have been reduced to one value as described in Section 4.3.2.1) and is computed as follows. Generally, for extreme values, this algorithm minimizes the numerator and maximizes the denominator.

- If the baseline computed value is < LLOQ and the post-baseline computed value is < LLOQ then the fold-rise is 1.
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is ≥ LLOQ then the fold-rise is post-baseline computed value / baseline computed value.
- If the baseline computed value is ≥ LLOQ and the post-baseline computed value is < LLOQ then the fold-rise is (LLOQ/2) / baseline computed value.

If the baseline computed value is < LLOQ and the post-baseline computed value is ≥ LLOQ then the fold-rise is post-baseline computed value /LLOQ.

4.3.2.4 Seroconversion

Seroconversion is defined as either

- a computed value <10 1/dil at D0 and post-injection computed value ≥40 1/dil at D28 or
- a computed value ≥10 1/dil at D0 and a ≥ 4-fold increase in computed titer values 1/dil at D28 as described in Section 4.3.2.3.

4.3.3 Efficacy

Not applicable.

4.3.4 Derived Other Variables

4.3.4.1 Age for Demographics

The age of a subject in the study is the calendar age as defined in the protocol.

4.3.4.2 Duration of a Subject in the Trial

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

Maximum (visit dates, termination date, last contact date) – V01 date +1.

4.3.4.3 Duration of the Study

The durations are computed in days as follows: Latest period date - earliest period date + 1.

Maximum (visit dates, termination date, last contact date) – minimum (V01 dates) +1

5 Statistical Methods and Determination of Sample Size

The statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics platform using SAS® Version 9.4 software or later.

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

For descriptive purposes, the following statistics will be presented:

Table 5.1: Descriptive statistics produced

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

The confidence interval (CI) for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (2)), i.e., using the inverse of the beta integral with SAS.

For immunogenicity results, assuming that Log10 transformation of the titers follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log10 (titers) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide GMs and their 95% CI.

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Immunogenicity Objective

5.1.1.1 Hypotheses

No statistical hypotheses will be tested.

5.1.1.2 Statistical Methods

The following parameters will be presented:

GM of titers on pre-vaccination (D0) and post-vaccination (D28/D56[a])

GM of titers ratio D28/D0 or D56/D0

Rate of subjects with titer ≥ 10 (1/dil) on D0 and D28 or D56

Seroprotection rate (titer ≥ 40 [1/dil]) on D0 and D28 or D56

Seroconversion or significant increase rate from D0 to D28 or D56

Immunogenicity analyses will be performed according to the following age sub-groups following the schedule applied:

- 6 to 35 months
- 3 to 8 years
- 9 to 17 years
- 18 years or older: additionally a subgroup analysis will be done on 18 to 64 years, and 65 years and older

5.1.2 Hypotheses and Statistical Methods for Safety Objective

5.1.2.1 Hypotheses

No statistical hypotheses will be tested.

5.1.2.2 Statistical Methods

The analysis of safety will address the number and percentage of subjects experiencing injection site or systemic ARs or events until 28 days after each injection (solicited reactions from 0 to 7 days and unsolicited AEs/reactions from 0 to 28 days).

The number and percentage of subjects experiencing the following items will be described:

[a] D56 for subjects aged 6 months to 8 years and D28 for subjects aged 9 years or older

- solicited reactions (from 0 to 7 days) after each injection according to intensity, time of onset, and number of days of occurrence
- immediate and delayed unsolicited non-serious AEs (MedDRA System Organ Class and Preferred Term) (from 0 to 28 days) after each injection according to relationship, intensity,
- SAEs, including AESIs, (MedDRA Preferred Term) after each/any injection and throughout the study, related or not, according to seriousness and outcome.

Safety analyses will be performed according to the following age sub-groups following the schedule and the reactogenicity scales applied:

- 6 to 23 months
- 24 to 35 months
- 3 to 8 years
- 9 to 17 years: additionally a subgroup analysis will be done on 9 to 11 years, and 12 to 17 years
- 18 to 64 years
- 65 years or older

5.2 Analysis Sets

Three main analysis sets will be used: the full analysis set (FAS), the per-protocol analysis set (PPAS) and the safety analysis set (SafAS).

5.2.1 Full Analysis Set

The FAS will include all subjects who provided at least some data that will be used in the analysis of the secondary endpoints of the study. In this trial, the FAS will consist of all subjects who received at least one dose of vaccine and had at least one valid post-vaccination serology result.

5.2.2 Per-Protocol Analysis Set

For each strain, subjects will be excluded from the PPAS for the following reasons:

- Subject did not meet all protocol-specified inclusion/exclusion criteria
- Subject received a vaccine other than the one that he/she was supposed to receive at Visit 1
- Preparation and/or administration of vaccine was not done as per protocol at Visit 1
- Subject did not provide a post-vaccination serology sample in the proper time window at Visit 2 for subjects aged 9 years or older or at Visit 3 for subjects aged 6 months to 8 years
- Subject received a protocol-restricted therapy
- Subject's serology sample at Visit 1 and after vaccination (on Visit 2 or Visit 3) did not produce a valid test result

For subjects from 6 months to 8 years old, the following deviations will also conduct to the exclusion of subjects:

- Subject received a vaccine other than the one that he/she was supposed to receive at Visit 2

- Preparation and/or administration of vaccine was not done as per protocol at Visit 2
- Subject did not receive vaccine in the proper time window at Visit 2

In the event of national immunization days for OPV, subjects who receive one or more doses of OPV at any time during the trial will not be withdrawn from the trial.
A subject will also be excluded if a deviation was assessed as having interfered with the vaccine response was reported. Such deviations will be identified through the data review process and be confirmed based on the clinical team decision.

5.2.3 Safety Analysis Set

The SafAS is defined as those subjects who have received at least one dose of the study vaccine.

5.2.4 Populations Used in Analyses

All subjects with data in the CRF will be taken into account in the description of the population. The immunogenicity analysis will be done on the FAS and confirmed on the PPAS. Finally, the SafAS will be used for the analysis of safety.

5.3 Handling of Missing Data and Outliers

5.3.1 Immunogenicity

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

5.3.2 Safety

Missing safety data will not be replaced. No search for outliers will be performed.

5.3.2.1 Immediate

For unsolicited non-serious systemic AEs, a missing response to the “Immediate” field will be assumed to have occurred after the 30-minute surveillance period and will not be imputed.

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

5.3.2.2 Causality

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

5.3.2.3 Measurements

Partially missing temperatures will be handled as described in Section 4.3.1.1.1.

5.3.2.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.3.1.1.1. For unsolicited non-serious AEs, missing intensities will remain missing and will not be imputed.

5.3.2.5 Start Date and Stop Date

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

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

5.3.2.6 Action Taken

Missing actions taken will remain missing and not be imputed.

5.4 Interim / Preliminary Analysis

Preliminary data line listings on subjects aged 18 years or older, 9 to 17 years, and 3 to 8 years will be prepared in the scope of the 3 early safety reviews.

No formal interim analyses are planned. However, the statistical analysis may be performed in 2 steps:

A first analysis of the immunogenicity and safety data from the 100 adult subjects will be performed once data are available and after first data base lock.

A final analysis at the end of the study when immunogenicity and safety data from all subjects are available and the final database lock.

5.5 Determination of Sample Size and Power Calculation

The sample size was set to 100 subjects per age group.

A 5% drop-out rate can be anticipated; therefore, 95 subjects per age group are expected to be evaluable for immunogenicity. With this sample size, the immunogenicity assessment in terms of percentages of subjects will have 95% CI widths of less than 21% (see Table 5.2).

Table 5.2: 95% Confidence intervals for proportions (Exact method)

n/N	% subjects observed	95% CI
45/95	47.40%	(37.0;57.9)
50/95	52.60%	(42.1;63.0)
55/95	57.90%	(47.3;68.0)

70/95	73.70%	(63.6;82.2)
90/95	94.70%	(88.1;98.3)
95/95	100.00%	(96.2;100.0)

For the safety assessment, a sample size of 100 subjects vaccinated will allow detecting with 0.95 probability an AE with a frequency of 3%.

5.6 Data Review for Statistical Purposes

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

Three early safety data reviews for this trial are planned as described in Section 3.1.

- When the 100 adult subjects have been vaccinated and have provided safety data from D0 to D28 post-vaccination, using the data collection methods described in the protocol. The safety data collected will be entered into the electronic case report forms (CRFs), and will be reviewed by SEC experts. It is understood that this review is based on preliminary data that have not been subject to database lock. (The usual and ongoing process of monitoring safety signals outside of those specified in the protocol-defined early interim safety analysis will continue unchanged.)

  The early safety review conducted by SEC experts will focus on the following adverse events (AEs) occurring within 28 days post-vaccination:

  - Immediate reactions
  - Solicited injection site and systemic reactions
  - Unsolicited non-serious AEs
  - SAEs
- When the 100 adolescent subjects aged 9 to 17 years have been vaccinated and have provided safety data from D0 to D28 post-vaccination, using the data collection methods described in the protocol. The safety data collected will be entered into the CRFs, will be reviewed by the Sponsor, and then submitted to the NDAC. It is understood that this review is based on preliminary data that have not been subject to database lock
- Similarly, when the 100 children subjects aged 3 to 8 years have been vaccinated and have provided safety data from D0 to D28 post-first vaccination. The safety data collected will be reviewed by the Sponsor, and then submitted to the NDAC.

6 References List

1 http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm356095.htm

2 Newcombe R.G., Two-sided confidence intervals for the single proportion: comparison of seven methods. Statistics in Medicine. 1998;17:857-72.