

NCT02992418

Immunogenicity and Safety of a Tetravalent Dengue Vaccine Administered Concomitantly or Sequentially with Adacel® in Healthy Subjects Aged 9 to 60 Years in the Philippines

Phase IIIb, randomized, multicenter, open-label study in 688 subjects aged 9 to 60 years in the Philippines.

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	CYD66
Development Phase:	Phase IIIb
Sponsor:	Sanofi Pasteur SA 14, Espace Henry Vallée, 69007 Lyon, France
Investigational Product(s):	CYD Dengue Vaccine
Form / Route:	Powder and solvent for suspension for injection/Subcutaneous
Indication For This Study:	Prevention of dengue fever in 9- to 60-year-old subjects
Version and Date of the SAP core body part:	Version 2.0, 19 September 2019

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List of Abbreviations

Ab	antibody
AE	adverse event
AEFI	adverse event of special interest
Ag	antigen
ANOVA	analysis of variance
ap	acellular pertussis
BL	blood sample
CI	confidence interval
CRF	case report form
CSR	clinical study report
d	diphtheria
D	Day
DC	diary card
dil	dilution
FAS	full analysis set
FHA	filamentous hemagglutinin
FIM	fimbriae
GM	geometric mean
GMC	geometric mean of concentration
GMCR	geometric mean of concentration ratio
GMT	geometric mean of titer
GMTR	geometric mean of the titer ratios
IU	international units
LLOQ	lower limit of quantification
M	month
MD	missing data
MMR	Measles, Mumps and Rubella
mL	milliliter
NM	non-measurable
NR	not-reportable
PC	phone call
PPAS	per-protocol analysis set
PPC	per-protocol analysis set for CYD dengue vaccine
PPC3	per-protocol analysis set for CYD dengue vaccine on 3 rd vaccination

PPT	per-protocol analysis set for Tdap
PRN	Pertactin
PRNT ₅₀	50% plaque reduction neutralization test
PT	preferred term
RCDC	reverse cumulative distribution curve
SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SD	standard deviation
T	tetanus
V	visit

1 Introduction

The current Statistical Analysis Plan (SAP) is based on the Protocol version 1.0 (dated March 17th, 2016), and the eCRF dated on August 3rd, 2016. The study will assess the safety and immunogenicity of CYD dengue vaccine when administered concomitantly or sequentially with a single booster dose of the tetanus (T), reduced-dose diphtheria (d), and 5-component acellular pertussis vaccine (ap), consisting of pertussis toxoid (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae types 2 and 3 (FIM2+3); known by preferred product trade names ADACEL®/Adacel®/COVAXIS®/Triaxis®/TRIAXIS®, and further referred to as Tdap in this document. Preventive measures against dengue presently rely on mosquito control and personal protection. These measures are limited in efficacy, difficult to enforce, and expensive. The best method of prevention lies with the development of a safe and effective vaccine directed at the 4 serotypes of dengue virus responsible for the disease.

It is preferable that CYD dengue vaccine is administered at an existing scheduled visit as part of the routine immunization schedule. There is currently no data on concomitant administration of CYD dengue vaccine with other vaccines in the targeted population aged 9-60 years of age. Co-administration of CYD dengue vaccine with other vaccines has been assessed in clinical studies outside the age indication, in infants and toddlers 9 to 15 months of age at inclusion in dengue endemic countries. A Phase II study was conducted to evaluate the co-administration of CYD dengue vaccine together with Measles, Mumps and Rubella (MMR, Trimovax®, Live attenuated virus vaccine against Measles [Schwartz strain], Mumps [Urabe AM-9 strain] and Rubella [Wistar RA 27/3M strain; Sanofi Pasteur] (CYD08) (1), and 2 Phase III studies (CYD29 and CYD33) were conducted to evaluate the co-administration of CYD dengue vaccine together with the YF vaccine (Stamaril®, Yellow fever vaccine [live]; Sanofi Pasteur) and the DTaP-IPV-PRP-T (Hib) (Pentaxim®, Diphtheria, Tetanus, Pertussis [acellular, component], Poliomyelitis [inactivated] vaccine [adsorbed] and *Haemophilus Influenza* Type b Conjugate Vaccine; Sanofi Pasteur). These trials demonstrated that the CYD dengue vaccine could be administered concomitantly with these pediatric vaccines and no safety concerns or impact on the immune response were observed.

The present post-licensure Phase IIIb study CYD66 will investigate the immunogenicity and safety of CYD dengue vaccine and Tdap vaccine when both vaccines are administered concomitantly or sequentially to subjects aged 9 to 60 years living in the Philippines. CYD dengue vaccine may be given concomitantly with Tdap. Tdap vaccine is indicated for the prophylaxis of diphtheria caused by *Corynebacterium diphtheriae*, tetanus caused by *Clostridium tetani*, and pertussis (whooping cough) caused by *Bordetella pertussis* in individuals 4 through 64 years of age as a booster according to the national vaccination policies. Tdap vaccine is a sterile liquid suspension of adsorbed tetanus, low-dose diphtheria toxoids, and acellular pertussis components, intended for intramuscular administration. Tdap vaccine is licensed in a total of 67 countries, and is administered as a single booster dose in adolescent and adult public and private vaccination schedules in the Philippines.

The first marketing authorization for the CYD Dengue vaccine (under the commercial name Dengvaxia®) was obtained in Mexico on 08 December 2015. As of June 2019, the CYD dengue vaccine has been registered in 20 countries plus in Europe. The CYD dengue vaccine is indicated in most of the countries for the prevention of disease caused by all four dengue virus serotypes in preadolescents, adolescents and adults (9 to 45/60 years of age, depending of the country).

Dengvaxia® is currently mostly indicated in subjects 9 to 45 years of age in the majority of licensed countries including the Philippines at the beginning of the study (the vaccine is indicated in 9-60 years in Paraguay and 9-16 years in Indonesia). In order to generate data in older subjects in endemic regions, it was decided to also administer the Dengvaxia® to 46-60 year-old subjects in the present study.

As multiple antigens (Ags) are administered concurrently, it is necessary to demonstrate that there is no impact on either the immunogenicity or safety profile of CYD vaccination on Tdap or the inverse. Furthermore, the administration of CYD dengue vaccine at the same time as a booster dose of Tdap vaccine might reduce the number of vaccination visits, and thereby increase vaccine compliance and coverage of co-administered vaccines, and facilitate dengue program implementation.

During an ad-hoc meeting held on 3-4 November 2017, the IDMC involved in the safety data review of the CYD dengue vaccine clinical development program recommended that no further vaccine was to be administered to dengue seronegative subjects in ongoing or future trials, and on precautionary basis, including partially vaccinated dengue seronegative subjects in ongoing trials. Following the IDMC recommendations, Sanofi Pasteur (the Sponsor) paused all vaccinations in CYD66 study, determined baseline serostatus and communicated that information to subjects already included in the study. It was communicated to the Philippines FDA (PFDA) dated 29 Nov 2017 and documented in CLI_00024717. The study protocol was amended accordingly to reflect the IDMC recommendations. Protocol amendment 1, Version 2.0 dated 04 January 2018, was submitted and reviewed by all IRBs of the study from 19 January to 07 February 2018 and was submitted by the Sponsor to the Philippines FDA on 05 February 2018.

PFDA has not provided a response nor approval for protocol amendment 1. The Sponsor has not received any indication of feedback timelines by the Philippines FDA. Consequently, dengue seropositive subjects included in CYD66, vaccinations have not resumed. At the time of study pause following IDMC recommendation and pending protocol amendment 1 implementation, out of the 688 subjects initially enrolled, 676 subjects (98%) received dengue dose 1, 643 subjects (93%) received dengue dose 2, and none received dengue dose 3. In terms of serostatus at baseline, out of 676 subjects who received the first injection, 629 (93%) were dengue seropositive and 47 (7%) were dengue seronegative. Due to the lack of answer from PFDA, the subjects became out of time window to finalize their vaccination schedule and to complete the last safety follow up call (6 months after the last dose). For this reason, the Sponsor decided to put an end to the CYD66 study with a last visit (considered as the termination visit) of the subjects in order to perform the last safety follow up as originally planned in the protocol. Therefore, no more dengue dose injections were administered to any subjects included in the study since the recommendation of IDMC to stop vaccinating seronegative individuals.

This Statistical Analysis Plan is based on protocol version 1.0 dated 17 March 2016.

2 Trial Objectives

2.1 Primary Objectives

Immunogenicity

Tdap immunogenicity

- To demonstrate the non-inferiority of the humoral immune response to the Tdap booster dose concomitantly administered with the first dose of CYD dengue vaccine as compared to sequential administration, measured 28 days after Tdap booster dose*

CYD dengue vaccine immunogenicity

- To demonstrate the non-inferiority of the humoral immune response to the first dose of CYD dengue vaccine concomitantly administrated with Tdap compared to sequential administration, measured 28 days after the first dose of CYD dengue vaccine*

The endpoints for the primary objectives are presented in Section 4.1.

* Providing that the number of evaluable subjects allows a global power of at least 80% (otherwise analyses will be descriptive).

2.2 Secondary Objectives

CYD dengue vaccine immunogenicity

- To demonstrate the non-inferiority of the humoral immune response of 3 doses of CYD dengue vaccine with the first dose concomitantly administered with Tdap as compared to sequential administration, measured 28 days after the third dose of CYD dengue vaccine*
- To describe the humoral immune response at baseline and 28 days after the first and third doses of CYD dengue vaccine in each and any group*.

* Objectives on the 3rd dose are not applicable due to the early termination of study (cf. section 5.7)

Tdap immunogenicity

- To describe the humoral immune response of Tdap vaccine at baseline and 28 days after concomitant administration with the first dose of CYD dengue vaccine as compared to the sequential administration, in each and any group

Safety

Tdap and CYD dengue vaccine safety

- To describe the safety of the CYD dengue vaccine and of the Tdap booster dose after each and any injection in each group

The endpoints for the secondary objectives are presented in Section 4.2.

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

This is a Phase IIIb, randomized, open label, multicenter study in 688 healthy subjects aged 9 to 60 years in the Philippines.

Subjects were to receive the CYD dengue vaccine according to a 3-dose schedule, given 6 months apart. A booster dose of Tdap vaccine (Adacel®) was to be administered either concomitantly to or before (sequential administration) the first dose of CYD dengue vaccine.

Subjects were to be randomized according to a 1:1 ratio into one of the 2 following groups with stratification on age (9 to 11 years, 12 to 17 years, 18 to 45 years and 46 to 60 years):

- **Group 1:** subjects were to be administered the first dose of CYD dengue vaccine concomitantly with the booster dose of Tdap vaccine at the second visit (V02; M1)
- **Group 2:** subjects were to receive Tdap vaccine at the first visit (V01; D0), and then CYD dengue vaccine 28 days later at the second visit (V02; M1)

The stratification was to be considered in order to balance the potential difference in terms of booster response by age group for the pertussis antigens (PT, FHA, PRN and FIM2+3).

Since the CYD dengue is to be administered according to a 3-dose schedule, given 6 months apart, initial trial design included administration of the second and third doses of CYD dengue vaccine at V04 (Month 7 [M7] and at V06 (M13) in all subjects.

At the time of study hold linked to the recommendation from IDMC not to vaccinate seronegative individuals anymore based on the results of the exploratory case-cohort study, all but 19 subjects (from both, Group 1 and Group 2) did receive dose 2 of the CYD dengue vaccine as planned. Following the IDMC recommendations, Sanofi Pasteur paused all vaccinations in CYD66 study, determined baseline serostatus and the Principal Investigators have communicated serostatus information to subjects already included in the study. In addition, the study protocol was amended to reflect the IDMC recommendations. The Sponsor has not received any feedback nor approval for the protocol amendment from Philippines FDA and has therefore decided to put an end to the CYD66 study with a last visit (which served as termination visit) of the subjects in order to inform them of the end of the study and to perform the last safety follow up. As a consequence, no more CYD dengue vaccine were administered to any subjects included in the study since the study hold. More details will be mentioned in the CSR

Blood samples (BL) collected before dose 1 of the vaccine at V02 were used to determine dengue serostatus at baseline. Dengue serostatus was determined using the Dengue 50% plaque reduction neutralization test (PRNT50).

3.2 Trial Plan

A schedule of assessments and study vaccinations was provided in the table of study procedures (Table 3.1).

All information collected during the study visits should be reported into the source documents. Some of the following information was to be recorded in the electronic case report form (eCRF).

Table 3.1: Study procedures as per initial study protocol

Phase IIIb Trial, 4 Vaccinations, 7 Visits and 3 or 4 Blood Samples (depending on group allocation), 19 Months Duration Per Subject. One Safety Follow-up Phone Call (PC) will be given 6 months after the last injection and 3 Interim phone calls will be given during the 6-month period after each CYD dengue vaccine injection (approximately 3 months after vaccination). .

Visit Number (V)	V01	V02	V03	V04	V05	V06†††	V07†††	Phone call***†††††
Trial Timelines* (Days/Months)	D0	M1 (V01+28D)	M2 (V02+28D)	M7 (V04+28D)	M8 (V06+28D)	M13 (V06+28D)	M14 (V06+28D)	Last vaccination+6M
Time Windows (Days)	+14	+14	+14	+20	+14	+20	+14	+30
Assent or Informed Consent	X							
Inclusion/Exclusion Criteria	X	X (Group 1)						
Contraindication	X (Group 2)	X		X			X	
Significant Medical History	X							
History of Dengue Infection/Vaccination	X							
Temperature	X (Group 2)	X		X			X	
Physical Examination†	X	X	X	X	X	X	X	X
Urine Pregnancy Test‡	X (Group 2)	X		X			X	
Demography/Body Stature	X							
Randomization	X							
Blood Sampling (BL) for Immunogenicity††								
T, D, PT, FHA, PRN, and FIM2+3	BL (Group 2)	BL	BL (Group 1)				BL	
Dengue Serotypes 1, 2, 3, and 4		BL	BL					
Tdap Vaccination §	X (Group 2)	X (Group 1)						
CYD dengue Vaccination §		Dose 1		Dose 2		Dose 3		
Post-injection phone calls (PC)**								Interim phone calls will be given during the 6-month period after each CYD dengue vaccine injection. The last phone call will be held 6 months after the last injection.
30-Min. Observation Period	X (Group 2)	X (Group 2)		X				
Injection Site and Systemic Events Collected***				X	X	X	X	X

Visit Number (V)	V01	V02	V03	V04	V05	V06†††	V07†††	Phone call***†††
Diary Card (DC) DC Provided	X (Group 1 for SAE only, and Group 2)	X	X	X	X	X	X	
DC Collected		X		X		X	X	
Memory Aid (MA) MA Provided							X	
MA Checked							X	
Concomitant Therapy†‡	X	X	X	X	X	X	X	X
Telephone Contact								
Blood Sampling (BL) for virological confirmation of hospitalized suspected dengue cases§§	All acute febrile illness with diagnosis of hospitalized suspected dengue case within the first 5 days after fever onset, anytime throughout the trial period							
SAEs and AESIs collected***								
Termination Record§§§							X	

AESI: AE of special interest; BL: blood sample; D: Day; M: Month; MA: Memory Aid; SAE: Serious adverse event; V: Visit

* Timelines must be calculated based on the date of vaccination, not the date of visit.

** Interim phone calls will be given during the 6-month period after each CYD dengue vaccine injection: 3 months after Visit 2 (PC1: M4 [M1 + 3 months]), 3 months after Visit 4 (PC2: M10 [M7 + 3 months], and 3 months after Visit 6 [PC3: M16 M13 + 3 months]). The last PC will be held 6 months after the last vaccination.

† Physical examination will be performed if necessary, based on the health status of the subject.

‡ For female subjects of childbearing potential (e.g., a female subject who has reached menarche).

§ CYD and Tdap will be administered in 2 different sites, with the CYD dengue vaccine administered in one deltoid and Tdap in the other deltoid

§§ Concomitant therapy and ongoing medication will be collected for Days 0–28 after each injection only

§§§ Blood samples planned during vaccination visits will be taken before vaccination

†† Solicited injection site reactions will be collected for Days 0–7 after each injection. Solicited systemic reactions will be collected for Days 0–14 after each injection. Unsolicited events will be collected for Days 0–28 after each injection.

††† In such case, 1 unplanned acute blood sample (approximately 3 mL) will be collected for virological confirmation of hospitalized suspected dengue case SAEs will be reported throughout the study and AESIs will be collected in defined time windows according to the type of AESI (hypersensitivity/allergic reactions [serious or not] within 7 days after each CYD dengue vaccine injection, serious viscerotropic within 30 days after each CYD dengue vaccine injection, serious dengue cases requiring hospitalization (hospitalized VCD case) will be reported during the entire study).

*** Termination form will be completed

††† Due to early termination of the study, V06 and V07 were not performed. Phone call 6 month after last vaccination were replaced by a last safety visit regardless the time from last vaccination §§§ Due to early termination, of the study, V06 and V07 were not performed. Phone call 6 month after last vaccination were replaced by a last safety visit regardless the time from last vaccination

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

4.1.1 Immunogenicity

4.1.1.1 Immunogenicity Endpoints

See Section 9.1.1.1 of the protocol version 1.0 dated 17 March 2016.

4.1.1.2 Immunogenicity Assessment Methods

See Section 9.1.1.2 of the protocol version 1.0 dated 17 March 2016.

4.1.2 Safety

There are no primary objectives for safety.

4.1.3 Efficacy

There are no primary objectives for efficacy.

4.2 Secondary Endpoints and Assessment Methods

4.2.1 Immunogenicity

4.2.1.1 Immunogenicity Endpoints

See Section 9.2.1.1 of the protocol version 1.0 dated 17 March 2016.

4.2.1.2 Immunogenicity Assessment Methods

See Section 9.2.1.2 of the protocol version 1.0 dated 17 March 2016.

4.2.2 Safety

4.2.2.1 Safety Endpoints

See Section 9.2.2.2 of the protocol version 1.0 dated 17 March 2016.

4.2.2.2 Safety Assessment Methods

See Section 9.2.2.3 of the protocol version 1.0 dated 17 March 2016.

4.2.3 Efficacy

There are no secondary objectives for efficacy.

4.3 Observational Endpoints and Assessment Methods

There are no observational objectives in this study.

4.4 Derived Endpoints: Calculation Methods

4.4.1 Safety

4.4.1.1 Solicited Reactions

The solicited period for solicited injection site reactions is from Day (D) 0 to D7 and from D0 to D14 for solicited systemic reactions.

Table 4.1, Table 4.2, and Table 4.3 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 9 to 11 years

CRF term (MedDRA lowest level term [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*	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 or parents/legally acceptable representatives have recorded the intensity level (Grade 1, 2, or 3) in the DC. For the measurable reactions of redness and swelling, they have recorded just the size of the reaction, and the classification as Grade 1, 2, or 3 has been assigned at the time of the statistical analysis.

Table 4.2: Solicited injection site reactions: terminology, definitions, and intensity scales for subjects aged >= 12 years for all subjects

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*	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 have recorded the intensity level (Grade 1, 2, or 3) in the DC. For the measurable reactions of redness and swelling, they have recorded just the size of the reaction, and the classification as Grade 1, 2, or 3 has been assigned at the time of the statistical analysis

Table 4.3: Solicited systemic reactions: terminology, definitions, and intensity scales

CRF term (MedDRA LLT)	Fever	Headache	Malaise	Myalgia	Asthenia
DC term	Temperature	Headache	Feeling unwell	Muscle aches and pains	Weakness
Definition	Elevation of temperature to $\geq 38.0^{\circ}\text{C}$ ($\geq 100.4^{\circ}\text{F}$)	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.	Generalized weakness.
Intensity scale*	Grade 1: $\geq 38.0^{\circ}\text{C}$ to $\leq 38.4^{\circ}\text{C}$, or $\geq 100.4^{\circ}\text{F}$ to $\leq 101.1^{\circ}\text{F}$ Grade 2: $\geq 38.5^{\circ}\text{C}$ to $\leq 38.9^{\circ}\text{C}$, or $\geq 101.2^{\circ}\text{F}$ to $\leq 102.0^{\circ}\text{F}$ Grade 3: $\geq 39.0^{\circ}\text{C}$ or $\geq 102.1^{\circ}\text{F}$	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity

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

4.4.1.1.1 Daily Intensity

Intensity will be categorized as follows: None, Grade 1, Grade 2, Grade 3, or Missing. The daily intensity will be recorded by the Investigator for solicited reactions whose scale is not a measure. For measurable solicited reactions, the intensity will be calculated at the time of the statistical analysis based upon the intensity scales defined in the protocol.

A reaction that is too large to measure (non-measurable [NM]) will be considered as Grade 3.

4.4.1.1.2 Maximum Overall Intensity

Maximum overall intensity is derived from the daily intensities and is calculated as the maximum of the daily intensities over the period considered. The maximum intensity during the solicited period will be computed without considering the ongoing period.

Note: The maximum overall intensity should be considered as "Missing" only if all daily intensities over the period considered are "Missing."

4.4.1.1.3 Presence

Presence of solicited reactions will be computed based on daily records during the solicited period. For any specific period, a subject would be considered to have a reaction if the intensity is greater than or equal to Grade 1 for at least one day during that period. If no data is recorded and the presence recorded by the Investigator is different from "No", the presence will be considered as "Missing."

4.4.1.1.4 Time of Onset

Time to onset is derived from the daily intensities. 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 to onset is the first day of the first occurrence.

Time to onset will be displayed by period as follows:

Table 4.4: Categories for Time to Onset

Period of Time to Onset	
Injection Site Reactions (D0-D7)	Systemic Reactions (D0-D14)
D0 - D3	D0 - D3
D4 - D7	D4 - D7
	D8 - D14

4.4.1.1.5 Number of Days of Occurrence

The “number of days of occurrence” of a solicited reaction during the solicited period is computed as the number of days the solicited reaction is present, (intensity different from none or missing between D0 and the end of the solicited period), over the daily record period. For instance, for calculating number of days of fever occurrence, only daily temperature $\geq 38^{\circ}\text{C}$ will be considered.

Number of days of occurrence during and after the solicited period will be displayed by category (range) as follows:

Table 4.5: Categories for Number of Days of Occurrence

	Period of Number of Days of Occurrence	
	Injection Site Reactions	Systemic Reactions
During solicited period	1 - 3 days 4 - 7 days 8 days	1 - 3 days 4 - 7 days 8 - 14 days 15 days

4.4.1.1.6 Overall Number of Days of Occurrence

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

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

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

Table 4.6: Categories for Overall Number of Days of Occurrence

	Period of Overall Number of Days of Occurrence	
	Injection Site Reactions	Systemic Reactions
Ongoing period after D7 for injection site/D14 for systemic reactions	1 - 3 days 4 - 7 days ≥ 8 days Missing	1 - 3 days 4 - 7 days 8 - 14 days ≥ 15 days Missing

4.4.1.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period and the maximum intensity of the ongoing period.

If the last daily intensity of the solicited period is Grade 1, Grade 2, or Grade 3 and maximum intensity of the ongoing period is also Grade 1, Grade 2, or Grade 3, then the reaction is considered ongoing. In any other case, the reaction will not be considered as ongoing.

4.4.1.2 Unsolicited Non-serious AEs

4.4.1.2.1 Intensity

Intensity will be categorized as follows: None, Grade 1, Grade 2, Grade 3, or Missing. For unsolicited non-serious adverse events (AEs) the intensity will be recorded by the Investigator for AEs whose scale is not a measure. For measurable AEs that have the same preferred term (PT) as a solicited reaction, the intensity will be calculated at the time of the statistical analysis.

4.4.1.2.2 Last Vaccination

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

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

4.4.1.2.3 Time of Onset

Time to onset in days following the vaccination will be computed as follows:

- Time to onset = start date – date of previous vaccination

If the start date of the AE is the same as the last vaccination date, then the onset is “0”.

If one of the dates is partially missing, time to onset will be considered as “Missing”. Events that occur before vaccination (negative time to onset) will not be included in the analyses but will be listed separately. The unsolicited non-serious AEs will be analyzed within 28 days after vaccination, which corresponds to AEs with a time to onset between 0 and 28 days after vaccination or missing. An AE with missing time to onset will be considered to have occurred just after the vaccination indicated by the visit number, so will be included in these tables.

Time to onset will be displayed by period as follows:

- D0-D3
- D4-D7
- D8-D14

- $\geq D15$
- Missing

4.4.1.2.4 Duration

The duration will be computed as: Duration = stop date of event - start date of event + 1.

If start or stop dates are unfilled (“blank”) or missing (contain “MD”) or ongoing is ticked, then the duration will be considered as “Missing.”

If the start date of the AE is the same as the last vaccination date, then the duration is 1 day.

Duration will be displayed by period as following:

- 1-3 days
- 4-7 days
- 8-14 days
- 15 days or more
- Missing

4.4.1.3 SAEs

4.4.1.3.1 Last Vaccination

Last vaccination will be computed using the same methodology as for unsolicited non-serious AEs described in Section 4.4.1.2.2.

4.4.1.3.2 Time of Onset

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

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 after the vaccination indicated by the visit number, so will be included in these tables.

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

Note for SAEs: Elapsed time from last vaccination recorded will be calculated if the SAE occurred within 24 hours of vaccination.

4.4.1.3.3 Duration

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

4.4.1.4 Other Safety Endpoints

4.4.1.4.1 Action Taken

This information will be presented for solicited reactions after each injection. Listing will be also presented separately. No derivation or imputation will be done.

4.4.1.4.2 Seriousness

This information will be presented for all and related SAE within 28 days post-injection and throughout the study. Listing will be also presented separately. No derivation or imputation will be done.

4.4.1.4.3 Outcome

This information will be presented for all and related SAE within 28 days post-injection and throughout the study.. Listing will be also presented separately. No derivation or imputation will be done.

4.4.1.4.4 Causality

This information will be summarized as collected. Missing causality (relationship) will be handled as described in Section 5.3.1.2.

4.4.1.4.5 AEs Leading to Study Discontinuation

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

4.4.1.4.6 AEs of Special Interest (AESIs)

The following serious AESIs (reported as SAEs) will be considered:

- Serious hypersensitivity/allergic reactions occurring in all subjects within 7 days after vaccination
- Serious viscerotropic disease occurring in all subjects within 30 days after vaccination
- Serious neurotropic disease occurring in all subjects within 30 days after vaccination

- Serious dengue disease requiring hospitalization¹ occurring in all subjects at any time during the study

The following non-serious AESI will be considered:

Hypersensitivity/allergic reactions occurring in all subjects within 7 days after vaccination

4.4.2 Immunogenicity

4.4.2.1 Computed Values for Analysis

For the computation of GMT/GMCs, any titer/concentration reported as < the lower limit of quantification (LLOQ) will be converted to a value of $\frac{1}{2}$ LLOQ.

While a single approach was used for GMT/GMCs, two different approaches for geometric mean of the titer ratios [GMTR]/geometric mean of concentration [GMCR] will be applied:

- For CYD dengue vaccine, < LLOQ will be converted to $\frac{1}{2}$ LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator
- For Tdap Vaccine, < LLOQ will be converted to $\frac{1}{2}$ LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator when only one of either the numerator or denominator is < LLOQ. If both the numerator and denominator are < LLOQ, then both will be converted in the same way.

4.4.2.2 CYD Dengue vaccine Seropositivity and other Thresholds

Several thresholds of interest will be used to evaluate the immunogenicity of CYD Dengue vaccine.

For each threshold applied on each serotype, the derived indicator will be "Yes" for that serotype if the computed value meets the threshold, otherwise indicator will be "No".

At baseline and 28 days after the 1st dose of CYD Dengue vaccine*, the thresholds considered are:

- Seropositivity: titer ≥ 10 (1/dil)
- Distribution rates of titers against each serotype according to the following predefined thresholds (1/dil): $<10, \geq 10, \geq 20, \geq 30, \geq 40, \geq 60, \geq 80, \geq 100, \geq 120, \geq 140, \geq 160, \geq 180, \geq 200, \geq 220, \geq 240, \geq 260, \geq 280, \geq 300, \geq 320, \geq 340, \geq 360, \geq 400, \geq 640$ and ≥ 1280

* Objectives on the 3rd dose are not applicable due to the early termination of study (cf. section 5.7)

¹ A hospitalized subject is any subject admitted to hospital with bed attribution or any healthcare institution and requiring in-patient care.

4.4.2.3 CYD Dengue vaccine Seropositivity against at least X serotype(s)

The criteria below will be computed for each subject and visit as soon as at least one of the four dengue serotype result is different from missing or not-reportable ("NR") (i.e. coded no result in the serology database):

- Number and percentage of subjects with antibody (Ab) titer ≥ 10 (1/dil) against at least 1, 2, 3, or the 4 serotypes with the parental dengue virus strains.

Titer(s) ≥ 10 (1/dil) for at least X serotype(s) with parental dengue virus strains is computed as a Yes/No/Missing variable (note: in the case no titer is available the variable will be missing). If at least X among the 4 serotypes titers meet the threshold then the variable is derived to "Yes", otherwise if at least one titer is available and does not meet the threshold the variable is derived to "No". For the percentage calculation, all the subjects with at least one titer available regardless of the serotype will be considered in the denominator.

4.4.2.4 Tdap Seroprotection

For each antigen, the derived indicator will be "Yes" for that test if the computed value meets the seroprotection threshold, otherwise indicator will be "No".

At baseline and 28 days after the dose of Tdap vaccine, the thresholds considered are:

- anti-D Ab concentrations ≥ 0.1 IU/mL
- anti-T Ab concentrations ≥ 0.1 IU/mL

4.4.2.5 Tdap Booster response

The booster response against pertussis components (PT, FHA, PRN and FIM2+3) will be calculated based on Ab concentration rises between pre (D0) and post-vaccination (28 days after the dose of Tdap vaccine). The booster response is defined as:

- A post-vaccination Ab concentration $\geq 4 \times$ LLOQ when pre-vaccination concentration is $<$ LLOQ
- A post-vaccination Ab concentration $\geq 4 \times$ pre-vaccination Ab concentration when pre-vaccination concentration is \geq LLOQ but $< 4 \times$ LLOQ
- A post-vaccination Ab concentration $\geq 2 \times$ pre-vaccination Ab concentration when pre-vaccination concentration is $\geq 4 \times$ LLOQ

For each pertussis components (PT, FHA, PRN and FIM2+3), the derived indicator will be "Yes" for that test if the computed value meets the booster response definition, otherwise indicator will be "No".

4.4.3 Efficacy

Not applicable.

4.4.4 Derived Other Variables

4.4.4.1 Age for Demographics

The age of a subject in the study is the calendar age.

4.4.4.2 Duration of the Study

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

Latest date of all subjects (termination date, last visit date, date of last contact) – earliest date of all subjects (date of visit V01) +1.

4.4.4.3 Subject Duration

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

Maximum (Visit dates, Termination date, Follow-up date, Last contact date) – V01 date + 1.

4.4.5 Baseline Dengue status

The dengue status at baseline is defined as the presence of Abs against at least one dengue serotype in the baseline sample (by dengue 50% plaque reduction neutralization test [PRNT₅₀] in the BL collected at V02 (BL) from all subjects. The baseline dengue status variable will be calculated for each subject as follows:

- Dengue immune subjects at baseline (seropositive) are defined as those subjects with titers ≥ 10 (1/dil) for at least one serotype with the parental dengue virus strain
- Dengue non-immune subjects at baseline (seronegative) are defined as those subjects with titers < 10 (1/dil) (not quantified) for all serotypes with parental dengue virus strains with available “valid” results (i.e. different from missing or ”NR”)
- Otherwise the baseline status will be classified as undetermined and subjects will have their dengue baseline status derived as non-immune.

5 Statistical Methods and Determination of Sample Size

The analysis will be performed under the responsibility of the Sponsor’s Biostatistics platform with the SAS software, version 9.4 or higher (SAS Institute, Cary, North Carolina, USA).

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

For descriptive purposes, the following statistics will be presented:

Table 5.1: Descriptive statistics produced

Baseline	Categorical data	Number of subjects.
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characteristics and follow-up description		Percentage of subjects.
	Continuous data	Mean, standard deviation, quartiles, minimum, and maximum.
Clinical safety results	Categorical data	Solicited: Number and percentage (95% confidence interval [CI]) of subjects. Unsolicited: Number and percentage (95% CIs) of subjects, and number of events.
Immunogenicity results	Categorical data (cutoff)	Number and percentage (95% CIs) of subjects.
	Continuous data (titer / titer ratio)	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 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, assuming that Log10 transformation of the titers / data follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log10 (titers / data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide geometric means (GMs) and their 95% CI.

GM is defined as follows:

$$GM = \left(\prod_{i=1}^n y_i \right)^{1/n} = 10^{\sum_{i=1}^n \log_{10}(y_i) / n}$$

where (y_1, y_2, \dots, y_n) are the observed titers or other data where applicable for each subject.

5.1 Statistical Methods

Non-inferiority testing will only be carried out if the number of evaluable subjects allows a global power of at least 80%. This applies to the co-primary objectives and the secondary objective. Otherwise, analyses will remain descriptive.

5.1.1 Hypotheses and Statistical Methods for Primary Objectives

5.1.1.1 Hypotheses

Non-inferiority on Tdap vaccine

The objective is to demonstrate that the humoral immune response to the Tdap booster dose administered concomitantly with the first dose of CYD dengue vaccine is non-inferior to the humoral immune response to the Tdap booster dose administered alone 28 days before the first dose of CYD dengue vaccine.

Individual hypotheses on Tdap response for each antigen:

A non-inferiority testing approach will be used to compare geometric mean concentrations (GMCs) for PT, FHA, PRN, FIM2+3, 28 days after the booster dose of Tdap, for each antigen “i” based on the following individual hypotheses:

$$H_0^i: GMC_{Group1}^i / GMC_{Group2}^i \leq \frac{1}{\delta} \Leftrightarrow \log_{10}(GMC_{Group1}^i) - \log_{10}(GMC_{Group2}^i) \leq -\log_{10}(\delta)$$

$$H_1^i: GMC_{Group1}^i / GMC_{Group2}^i > \frac{1}{\delta} \Leftrightarrow \log_{10}(GMC_{Group1}^i) - \log_{10}(GMC_{Group2}^i) > -\log_{10}(\delta)$$

with:

i, antigen {PT, FHA, PRN, FIM 2+3}.

δ , non-inferiority limit is set at 1.5, i.e., 0.176 ($=\log_{10} [1.5]$), for each antigen “i”.

Non-inferiority for antigen “i” will be demonstrated if the lower bound of the 2-sided 95% confidence interval (CI) is greater than δ ($\alpha=2.5\%$ one-sided). For each of the 4 antigens, the statistical methodology will be based on the use of the age-stratified two-sided 95% CI of the ratio of GMCs between groups. The age-stratified CI will be calculated using an analysis of variance (ANOVA) model (type II analysis) of \log_{10} -transformed titers. The age groups (9 to 11 years, 12 to 17 years, 18 to 45 years and 46 to 60 years) will be used as the stratifying factor in the model.

Additionally, a non-inferiority testing approach will be used to compare seroprotection rates of T and D, 28 days after the booster dose of Tdap vaccine. Seroprotection is defined as anti-D and anti-T Ab concentration superior to 0.1 IU/mL.

The individual tested hypotheses for the antigen “i” will be as follows:

$$H_0^i: P_{Group1}^i - P_{Group2}^i \leq -\delta$$

$$H_1^i: P_{Group1}^i - P_{Group2}^i > -\delta$$

with:

i, antigen {T, D}

δ , non-inferiority limit is set at 10% for both T and D.

Non-inferiority for antigen “i” will be demonstrated if the lower bound of the 2-sided 95% CI is greater than $-\delta$.

Global hypotheses for non-inferiority on Tdap response:

The global hypotheses are:

H_{0G} : Non inferiority of Tdap booster dose co-administered with the first dose of CYD dengue vaccine versus Tdap booster dose administered alone is not demonstrated for at least one antigen.

H_{1G} : Non-inferiority of Tdap booster dose co-administered with the first dose of CYD dengue vaccine versus Tdap booster dose administered alone is demonstrated for all the antigens.

H_0^G : at least one H_0^i not rejected

H_1^G : all H_0^i are rejected

Non-inferiority on CYD dengue vaccine after 1 dose

The objective is to demonstrate that the humoral immune response to the first dose of CYD dengue vaccine administered concomitantly with Tdap booster dose is non-inferior to the humoral immune response to the first dose of CYD dengue vaccine administered sequentially 28 days after the Tdap booster dose.

Individual hypotheses on CYD response after one dose for each serotype:

A non-inferiority testing approach will be used to compare geometric mean of titers (GMTs), for dengue serotypes 1, 2, 3, and 4, 28 days after the first injection of CYD dengue vaccine between Group 1 and Group 2 for each serotype “i” based on the following individual hypotheses:

$$H_0^i: \text{GMT}_{Group1}^i / \text{GMT}_{Group2}^i \leq \frac{1}{\delta} \Leftrightarrow \log_{10}(\text{GMT}_{Group1}^i) - \log_{10}(\text{GMT}_{Group2}^i) \leq -\log_{10}(\delta)$$

$$H_1^i: \text{GMT}_{Group1}^i / \text{GMT}_{Group2}^i > \frac{1}{\delta} \Leftrightarrow \log_{10}(\text{GMT}_{Group1}^i) - \log_{10}(\text{GMT}_{Group2}^i) > -\log_{10}(\delta)$$

with:

i, serotypes in {1, 2, 3, 4}.

δ , non-inferiority limit is set at 2, i.e., 0.301 ($=\log_{10} [2]$), for each serotype “i”.

Non-inferiority for serotype “i” will be demonstrated if the lower bound of the 2-sided 95% CI is greater than $-\delta$.

Global hypotheses for non-inferiority on CYD response after one dose:

H_0^G : at least one H_0^i not rejected

H_1^G : all H_0^i are rejected

Overall, non-inferiority among the groups will be demonstrated if, for each antigen of Tdap and each serotype of CYD dengue vaccine, the two-sided 95% CI lies above $-\delta$.

5.1.1.2 Statistical Methods

Non-inferiority on Tdap vaccine

The non-inferiority test will be performed using the 95% 2-sided CI of the difference between Group 1 and Group 2 for seroprotection rates and of the differences of the means of the \log_{10} transformed post-vaccination concentrations/titers for GMCs/GMTs ($\alpha=2.5\%$ one-sided). The 95% CIs will be calculated based on the Wilson score method without continuity correction as quoted by Newcombe (3) for seroprotection rates and using normal approximation of log-transformed titers for GMCs/GMTs.

Non-inferiority on CYD dengue vaccine after one dose

The statistical methodology will be based on the use of the two-sided 95% CI of the differences of the means of the \log_{10} transformed post-vaccination titers between Group 1 and Group 2. The CI for differences will be calculated using normal approximation of log-transformed titers.

5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

5.1.2.1 Hypotheses

Non-inferiority on CYD dengue vaccine after three doses

The objective was to demonstrate that the humoral immune response of 3 doses of CYD dengue vaccine with the first dose administered concomitantly with the Tdap booster dose is non-inferior to the humoral immune response of 3 doses of CYD dengue vaccine administered sequentially with the Tdap booster dose administered 28 days before the first dose of CYD dengue vaccine.

Due to early termination study, this analysis will not be done (cf. section 5.7)

5.1.2.2 Statistical Methods

Non-inferiority on CYD dengue vaccine after 3 doses

Analysis on the 3rd dose are not applicable due to the early termination of the study (cf. section 5.7)

Descriptive analysis on CYD dengue vaccine and Tdap vaccine immunogenicity*

No hypotheses will be tested. Immunogenicity point estimates and their 95% CI will be presented for each and any group and by baseline dengue status, before and after the first injections for CYD dengue vaccine and before and after the booster vaccination for Tdap.

A complementary analysis on CYD dengue vaccine and Tdap vaccine immunogenicity after each dose will be conducted for each and any group according to the age groups (9 to 11 years, 12 to 17 years, 18 to 45 years and 46 to 60 years).

* Analysis on the 3rd dose are not applicable due to early termination of study (cf. section 5.7)

In addition, the booster response against pertussis components (PT, FHA, PRN, and FIM2+3) will be calculated based on Ab concentration rises between pre- and post-vaccination defined as:

- A post-vaccination Ab concentration $\geq 4 \times$ the lower limit of quantification (LLOQ) when pre-vaccination concentration is $< \text{LLOQ}$
- A post-vaccination Ab concentration $\geq 4 \times$ pre-vaccination Ab concentration when pre-vaccination concentration is $\geq \text{LLOQ}$ but $< 4 \times \text{LLOQ}$
- A post-vaccination Ab concentration $\geq 2 \times$ pre-vaccination Ab concentration when pre-vaccination concentration is $\geq 4 \times \text{LLOQ}$

Safety

All analyses will be descriptive; no hypotheses will be tested. Safety will be assessed for all subjects after the booster dose of Tdap vaccine and after each and any dose of CYD Dengue vaccine and by baseline dengue status.

A complementary analysis will be conducted for each group according to the age groups (9 to 11 years, 12 to 17 years, 18 to 45 years and 46 to 60 years).

A complementary analysis after the first CYD dengue vaccine will be conducted for each and any group in the non-immune or undetermined dengue subjects at baseline.

5.2 Analysis Sets

Four analysis sets will be used: the Per-Protocol Analysis Set (PPT and PPC for Tdap and CYD dengue vaccine respectively), the Full Analysis Set (FAS), and the Safety Analysis Set (SafAS).

5.2.1 Full Analysis Set

The FAS is defined as the subset of subjects who received at least one dose of the study vaccines.

Subjects will be analyzed by baseline dengue status and by the group to which they were randomized.

5.2.2 Per-Protocol Analysis Set

Two PPASs will be defined: one for Tdap (PPT) and one for CYD dengue vaccine after first dose (PPC).

The list of deviations leading to exclusion from the PP populations was adapted to exclude dengue seronegative subjects at baseline from the populations. Further details can be found in Section 5.7

PPT

The subjects presenting with at least one of the following relevant protocol deviations will be excluded from the PPT:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject had dengue seronegative status at baseline
- Subject did not complete the vaccination schedule (at V02 for Group 1, V01 for Group 2)
- Subject received a vaccine other than the one that he/she was randomized to receive (at V02 for Group 1, V01 for Group 2)
- Administration of vaccine was not done as per-protocol (site and route of administration) (at V02 for Group 1, V01 for Group 2)
- Subject did not receive vaccine in the proper time window (at V02 for Group 1)
- Subject did not provide after Tdap injection a post-dose serology sample in the proper time window or a post-dose serology sample was not drawn (V03 for Group 1, V02 for Group 2)
- Subject received a protocol-prohibited medication (prohibited therapies/medications/vaccines are indicated in the Protocol Section 6.7) (until V03 for Group 1, V01 to V02 for Group 2)
- Subject's serology sample did not produce a valid test result, i.e., no Tdap Ab concentration available (at V03 for Group 1, V02 for Group 2)

PPC

The subjects presenting with at least one of the following relevant protocol deviations will be excluded from the PPC:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject had dengue seronegative status at baseline
- Subject did not complete the vaccination schedule (at V02 for Group 1, at V01 and V02 for Group 2)
- Subject received a vaccine other than the one that he/she was randomized to receive (at V02 for Group 1, at V01 or V02 for Group 2)

- Administration of vaccine was not done as per-protocol (site and route of administration) (at V02 for Group 1, at V01 and V02 for Group 2)
- Subject did not receive vaccine in the proper time window (at V02 for Group 1 and Group 2)
- Subject did not provide after the first CYD dengue vaccine injection a post-dose serology sample in the proper time window or a post-dose serology sample was not drawn (at V03 for Group 1 and Group 2)
- Subject received a protocol-prohibited medication (prohibited therapies/medications/vaccines are indicated in the Protocol Section 6.7) (until V03 for Group 1 and Group 2)
- Subject's serology sample did not produce a valid test result, i.e., no Neutralizing Ab titers against any of the four parental dengue virus serotypes of CYD dengue vaccine available (at V03 for Group 1 and Group 2)

5.2.3 Safety Analysis Set

The SafAS is defined as the subjects who have received at least one dose of the study vaccines. All subjects will have their safety analyzed after each dose according to the vaccine they actually received, and after any dose according to the vaccine 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).

Note: Subject will be included in the “any dose” analysis according to the 1st dose received that corresponds to a protocol group.

5.2.4 Other Analysis Set

Randomized subjects

A randomized subject is a subject for whom an injection group has been allocated.

5.2.5 Populations Used in Analyses

The main immunogenicity analyses (non-inferiority tests) will be performed on the per protocol analyses sets PPT and PPC respectively for Tdap and CYD dengue vaccine comparisons For Tdap and will be confirmed on the FAS (only Dengue Immune subjects for CYD dengue vaccine). The subjects will be analyzed by the group to which they were randomized.

All other immunogenicity analyses will be performed on the FAS by baseline dengue status. Subjects will be analyzed by the vaccine group to which they were randomized.

The SafAS will be performed on the safety analysis set by baseline dengue status. Subjects will be analyzed according to the vaccine they actually received.

5.3 Handling of Missing Data and Outliers

5.3.1 Safety

No replacement will be done.

In all subject listings, partial and missing data will be clearly indicated as missing.

5.3.1.1 Immediate

For unsolicited non-serious systemic AEs, a missing response to the “Immediate” field 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.

5.3.1.2 Causality

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

5.3.1.3 Measurements

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

5.3.1.4 Intensity

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

5.3.1.5 Start Date and Stop Date

Missing or partially missing start dates for unsolicited AEs will remain missing and not be imputed. If either the start or stop date is missing or partially missing, the time 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.

5.3.1.6 Action Taken

Missing actions taken will remain missing and not be imputed.

5.3.2 Immunogenicity

No imputation of missing values and no statistical search for outliers will be performed (immunogenicity data will be considered as validated). LLOQ management will be performed as described in Section 4.3.2.1.

5.3.3 Efficacy

Not applicable.

5.4 Interim/Preliminary Analysis

The final analysis will be performed on data collected at the end of the study.

5.5 Determination of Sample Size and Power Calculation

Power calculation is based on initial sample size of the trial. 688 subjects were to be enrolled regardless of dengue baseline status: 344 subjects per treatment group (86 subjects per age group).

Considering a potential attrition rate of 10%, such sample size would provide 618 evaluable subjects in the PPAS of Tdap and CYD dengue vaccine (PPT and PPC). This will give, for the co-primary objectives, a global power over 90.0% for the testing of non-inferiority (based on SAS proc Power).

The following assumptions were considered for the non-inferiority on Tdap: an alpha level of 2.5% (one-sided hypotheses), a maximum acceptable difference of 0.176 for GMCs, and of 10% for seroprotection rates, and assuming the following rates and standard deviations (SDs):

- $T \geq 0.1$ IU/mL – 99% of subjects
- $D \geq 0.1$ IU/mL – 99% of subjects
- PT SD – 0.4
- FHA SD – 0.4
- PRN SD – 0.5
- FIM2+3 SD – 0.6

(based on TD519, TD506 and TD526 studies and on Keith S. Reisinger (4)).

The following assumptions were considered for the non-inferiority after the first dose of CYD dengue vaccine: an alpha level of 2.5% (one-sided hypotheses), a maximum acceptable difference of 0.301 for GMTs, and assuming a standard deviation of 1.0 for serotype 1, 0.8 for serotypes 2 and 3, 0.7 for serotype 4 (based on CYD22 and CYD47 studies conducted in Asia Pacific).

In addition, this sample size would have given for the non-inferiority after the third dose* of CYD dengue vaccine (on PPC3), considered as a secondary objective, a global power over 99.0% for the testing of non-inferiority (based on SAS proc Power).

The following assumptions were considered for the non-inferiority after the third dose* of CYD dengue vaccine: an alpha level of 2.5% (one-sided hypotheses), a maximum acceptable difference of 0.301 for GMTs, and assuming a standard deviation of 0.8 for serotypes 1 and 2, 0.7 for serotypes 3 and 4 (based on studies conducted in Asia Pacific).

* Analysis on the 3rd dose will not be done due to the early termination of study (cf. section 5.7)

Table 5.2: Powers for Non-inferiority between GMT/seroprotection for each antigen and serotype - Primary objective

Antigen/Serotype	Alpha	δ	Reference rate/SD	Power (%) for 309 evaluable subjects per group
D	0.025	10%	99%	> 99.9
T	0.025	10%	99%	> 99.9
PT	0.025	0.176	0.4	> 99.9
FHA	0.025	0.176	0.4	> 99.9
PRN	0.025	0.176	0.5	99.2
FIM2+3	0.025	0.176	0.6	95.4
Dengue serotype 1	0.025	0.301	1.0	96.2
Dengue serotype 2	0.025	0.301	0.8	99.7
Dengue serotype 3	0.025	0.301	0.8	99.7
Dengue serotype 4	0.025	0.301	0.7	> 99.9

Table 5.3: Powers for Non-inferiority between GMT for each serotype - Secondary objective

Serotype	Alpha	δ	Reference SD	Power (%) for 309 evaluable subjects per group
Dengue serotype 1	0.025	0.301	0.8	99.7
Dengue serotype 2	0.025	0.301	0.8	99.7
Dengue serotype 3	0.025	0.301	0.7	> 99.9
Dengue serotype 4	0.025	0.301	0.7	> 99.9

This sample size will also provide a 95% probability of observing an AE that has a true incidence > 0.87% in each group (N=344).

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.

5.7 Changes in the Conduct of the Trial or Planned Analyses

As protocol amendment 1 submitted following the IDMC recommendations to stop vaccinating seronegative individuals could not be implemented because of absence of response from the Philippines FDA, the Sponsor has decided to stop the trial following the study hold.

Therefore, the third dose of CYD dengue vaccine at V06 and the BS at V07 were not performed in any subject. The last phone call was replaced by a last safety follow-up visit before the end of the study (changes from study procedures table).

This early termination had an impact on the conduct of the trial and the planned analyses, as summarized below:

- 2.2 Secondary objectives: As no subjects received the third injection of the CYD Dengue vaccine, objectives associated to the third injection will not be assessed
- 4.2.1 Immunogenicity: As no subjects received the third injection of the CYD Dengue vaccine, endpoints and immunogenicity assessment methods associated to the third CYD vaccination are not applicable
- 4.2.2.2 CYD Dengue vaccine Seropositivity and other threshold: Seropositivity and threshold for the third CYD vaccination are not applicable
- 5.1.2 Hypotheses and Statistical Methods for secondary objectives:
 - Hypotheses and statistical methods on the Non-inferiority on CYD dengue vaccine after three doses are not applicable.
 - Descriptive analysis on CYD dengue vaccine will not be assessed after the third dose of CYD dengue vaccine
- 5.2.2 Per-protocol Analysis Set: As no subjects received the third injection of the CYD Dengue vaccination the Per-Protocol population on the 3rd CYD vaccination (PPC3) defined in protocol V1.0 will be removed and the Per-Protocol population on the 1st CYD vaccination (PPC1) will be renamed by PPC
- 5.2.2: Definition of the PPT and PPC were revised in order to integrate an additional deviation after IDMC recommendation and based on new label indication. Subjects seronegative at baseline will be excluded from the 2 populations.
- 5.2.5 Population Used in Analysis:
 - According to the new definition of the Per-Protocol Analysis Set, the main immunogenicity analyses (non-inferiority tests) will be reduced to the dengue immune subjects (seropositive) and analyzed by the group which they were randomized.
 - All the other immunogenicity and safety analysis will be performed according to the baseline dengue status
- 5.4 Interim /Preliminary Analysis:

- The first interim analysis scheduled on results obtained after a partial database lock of data collected up to the 28 days post first dose of CYD dengue vaccine at V03, M2) was not done due to the hold of the study.
 - The potentially second interim analyses scheduled on results collected up to the 28 days after the third dose CYD dengue vaccine at V06, M13 was not performed because no subjects received the third CYD dose.
 - Only one final statistical analysis will be performed.
- 5.5 Determination of the sample size and power calculation:

Considering a potential attrition rate of 10%, it was initially planned that such sample size would provide 618 evaluable subjects in the PPAS of Tdap and CYD dengue vaccine (PPT and PPC). This sample size (based on SAS proc Power) targeted a global power over 90.0% for the non-inferiority testing corresponding to the co-primary objectives (i.e., after the booster dose of Tdap and after the first dose of CYD), and over 99.0% for the non-inferiority corresponding to the secondary objective (ie, testing after the third dose of CYD dengue vaccine).

However, with early termination of the study, since the number of evaluable subjects will be reduced (ie, number of dengue seropositive subjects in PPT and PPC), reducing global power to 80% may be considered. Thus, the non-inferiority testing will be carried out only if a global power of at least 80% for the co-primary objectives can be attained (i.e., if the number of evaluable subjects is at least 510 for the co-primary objectives and 324 for the secondary objective). (table 5.4 and 5.5)

Table 5.4: Powers for Non-inferiority between GMT/seroprotection for each antigen and serotype - Primary objective

Antigen/Serotype	Alpha	δ	Reference rate/SD	Power (%) for 309 evaluable subjects per group	Power (%) for 255 evaluable subjects per group
D	0.025	10%	99%	> 99.9	> 99.9
T	0.025	10%	99%	> 99.9	> 99.9
PT	0.025	0.176	0.4	> 99.9	> 99.9
FHA	0.025	0.176	0.4	> 99.9	> 99.9
PRN	0.025	0.176	0.5	99.2	97.8
FIM2+3	0.025	0.176	0.6	95.4	91.1
Dengue serotype 1	0.025	0.301	1.0	96.2	92.4
Dengue serotype 2	0.025	0.301	0.8	99.7	98.9
Dengue serotype 3	0.025	0.301	0.8	99.7	98.9
Dengue serotype 4	0.025	0.301	0.7	> 99.9	99.8
Global power				90%	80%

Table 5.5: Powers for Non-inferiority between GMT for each serotype - Secondary objective

Serotype	Alpha	δ	Reference SD	Power (%) for 309 evaluable subjects per group	Power (%) for 162 evaluable subjects per group
Dengue serotype 1	0.025	0.301	0.8	99.7	96.4
Dengue serotype 2	0.025	0.301	0.8	99.7	96.4
Dengue serotype 3	0.025	0.301	0.7	> 99.9	99.0
Dengue serotype 4	0.025	0.301	0.7	> 99.9	99.0
Global power				99%	80%

This sample size will also provide a 95% probability of observing an AE that has a true incidence > 0.87% in each group (N=344).

TLFs document will be done on collected data.

6 References List

- 1 Crevat D, Brion JD, Gailhardou S, Laot TM, Capeding MR. First Experience of Concomitant Vaccination Against Dengue and MMR in Toddlers. *Pediatr Infect Dis J*. 2015;34(8):884-92.
- 2 Newcombe RG. Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in Medicine*. 1998; 17:857-72
- 3 Newcombe R. G. Interval estimation for the difference between independent proportions: comparison of eleven methods. *Statistics in Medicine* 17: 873-890, 1998.
- 4 Reisinger KD. Safety, Tolerability, and Immunogenicity of Gardasil Given Concomitantly with Menactra and Adacel. *Pediatrics* 2010;125;1142