NCT Number: NCT02979535

Immunogenicity and Safety of a Tetravalent Dengue Vaccine Administered Concomitantly or Sequentially with Cervarix® in Healthy Female Subjects Aged 9 to 14 Years in Mexico

Phase IIIb, randomized, open-label, multicenter study in 480 healthy female subjects aged 9 to 14 years in Mexico.

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	CYD71
Development Phase:	Phase IIIb
Sponsor:	Sanofi Pasteur 14, Espace Henry Vallée, F-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 healthy female subjects aged 9 to 14 years
Version and Date of the SAP core body part:	Version 1.0, 21MAY2019

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List of Abbreviations

Ab	antibody
AE	adverse event
AESI	adverse event of special interest
BL	blood sample
CI	confidence interval
CRF	case report form
CSR	clinical study report
D	day
DC	diary card
dil	dilution
FAS	full analysis set
FV	Flavivirus
GM	geometric mean
GMT	geometric mean of the titer
GMTR	geometric mean of the titer ratios
LLOQ	lower limit of quantitation
MD	missing data
MedDRA	Medical Dictionary for Regulatory Activities
mL	milliliter
NA	Not applicable
NM	non-mesurable
NR	not-reportable
PC	phone call
PPAS	per-protocol analysis set
$PRNT_{50}$	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
V	Visit
WHO	World Health Organization

1 Introduction

The study will assess the safety and immunogenicity of the CYD dengue vaccine when administered concomitantly or sequentially with Cervarix® (Human Papillomavirus Bivalent [Types 16 and 18] Vaccine, Recombinant; GlaxoSmithKline).

Previous preventive measures against dengue relied 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. The first marketing authorization for the CYD Dengue vaccine (under the commercial name Dengvaxia®) was obtained in Mexico on 08 December 2015. As of February 2019, the CYD dengue vaccine has been registered in 19 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). Since implementation of a label update, vaccination is recommended in individuals with prior infection with dengue.

There is currently no data on concomitant administration of CYD dengue vaccine with other vaccines in the indicated population in subjects 9 years and above. Co-administration of CYD dengue vaccine with other vaccines has been assessed in clinical studies outside the age indication, in 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) vaccine (CYD08) 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) vaccine (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 was observed.

The present post-licensure Phase IIIb study CYD71 plans to investigate the immunogenicity and safety of the CYD dengue vaccine when it is administered concomitantly or sequentially with Cervarix at least 28 days apart in at least 480 healthy female subjects aged 9 to 14 years.

Cervarix is a suspension for injection of HPV bivalent (types 16 and 18) vaccine (recombinant, adjuvanted, adsorbed) indicated for the prevention of cervical cancer, CIN grade 2 or worse and adenocarcinoma in situ, and CIN grade 1 caused by oncogenic HPV types 16 and 18, in females aged 9 through 26 years of age. Cervarix is currently recommended by WHO for female subjects 9 through 14 years of age in a 2 dose schedule (0, 6 months), and 15 years to 26 years in a 3 dose schedule (0, 1, 6 months). Since 2006, HPV vaccines have been licensed in over 100 countries.

During the conduct of the CYD71 trial, new clinical data based on the results of exploratory analyses showed that the efficacy and safety profile of the CYD dengue vaccine was different between subjects previously naturally exposed to the dengue virus prior to vaccination and

subjects never exposed to the dengue virus prior to vaccination. In light of these results, the Independent Data Monitoring Committee (IDMC) involved in the safety data review of the CYD dengue vaccine clinical development program concluded, in an ad hoc meeting held on 3-4 November 2017, that there is a strong evidence that the CYD dengue vaccine protects seropositive subjects from symptomatic, hospitalized and severe dengue while, in seronegative subjects at baseline, the vaccine confers limited short term benefit against symptomatic dengue and induces an increased risk of hospitalized or severe dengue in the long term (findings are based on follow-up of dengue unexposed subjects having received 3 CYD dengue vaccine doses). Following this meeting, the IDMC recommended not to vaccinate any individuals with no prior dengue infection anymore, and to only continue vaccination in subjects with prior dengue infection. As a consequence, the Sponsor has amended the CYD71 study protocol to implement the recommendation from IDMC on February 2018. The study was put on hold between IDMC recommendation and approval of protocol amendment. As per IDMC recommendations, the following changes were applied to this trial:

- All vaccinated subjects were informed about their baseline dengue serostatus, and what it means, as soon as possible based on blood samples provided by the subjects before the first vaccination.
- All subjects were asked about their willingness to continue participating in this study. Subject's consent were formalized by signing an amended Assent Form (subject's parent[s]/legally acceptable representative[s] will sign an amended Informed Consent Form).
- Subjects identified as seronegative at baseline were not to receive further CYD dengue vaccine doses. They could continue in the study for safety follow-up at 6 months post last dengue vaccine dose, if they consented to, and had timely access to appropriate care in the event of suspected dengue, for 10 years from the date of last dengue vaccination whether they remained in the study or not.

Subjects identified as subjects seropositive at baseline who were eligible to continue dengue vaccination in the study were additionally asked to consent for further CYD dengue vaccine injection. Subjects that consented to receive the third and last dose of CYD dengue vaccine completed the study as it was initially planned. Subjects that consented to remain in the study but preferred not to receive the last injection were able to continue in the study for safety follow-up at 6 months post last dengue vaccine dose.

2 Trial Objectives

2.1 Primary Objectives

Immunogenicity

Cervarix immunogenicity

- To demonstrate that the humoral immune response (in terms of geometric mean titers [GMTs]) to Cervarix after concomitant administration with the CYD dengue vaccine is non-inferior to the humoral immune response (in terms of GMTs) after sequential

administration with the CYD dengue vaccine measured 28 days after the last dose of Cervarix*

CYD dengue vaccine immunogenicity

- To demonstrate that the humoral immune response (in terms of GMTs) to the CYD dengue vaccine after concomitant administration with Cervarix is non-inferior to the humoral immune response (in terms of GMTs) to the CYD dengue vaccine after sequential administration with Cervarix measured 28 days after the third dose of the CYD dengue vaccine*

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

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

2.2 Secondary Objectives

Immunogenicity

Cervarix immunogenicity

- To demonstrate that the humoral immune response (in terms of seroconversion) to Cervarix after concomitant administration with the CYD dengue vaccine is non-inferior to the humoral immune response (in terms of seroconversion) to Cervarix sequential administration with the CYD dengue vaccine measured 28 days after the last dose of Cervarix*
- To describe the humoral immune response to Cervarix at baseline and after each dose of Cervarix in each and any group

CYD dengue vaccine immunogenicity

- To describe the humoral immune response to the CYD dengue vaccine at baseline and after each dose of the CYD dengue vaccine, in each and any group

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

Safety

Cervarix and CYD dengue vaccine safety

- To describe the safety of Cervarix and CYD dengue vaccine after each and any dose 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 480 healthy female subjects aged 9 to 14 years in Mexico.

Subjects were to be randomized in a 1:1 ratio into one of the 2 following groups to receive:

- Group 1 (N=240): 3 doses of CYD dengue vaccine and 2 doses of Cervarix (Human Papillomavirus Bivalent [Types 16 and 18] Vaccine, Recombinant; GlaxoSmithKline) concomitantly to the 2 first doses of CYD dengue vaccine
- Group 2 (N=240): 3 doses of CYD dengue vaccine and 2 doses of Cervarix sequentially to the 2 first doses of CYD dengue vaccine

For both vaccines, each dose is to be administered 6 months apart.

As per Protocol Amendment 1, only subjects identified as dengue immune (seropositive) before administration of the first CYD dengue injection (at V01 for Group 1 and at V02 for Group 2) and who consented to receive the remaining injection of dengue vaccine could remain in the study as per initial study procedures.

Subjects identified as non-immune (seronegative) at baseline could continue in the study for a 6-month safety follow-up. They did not receive the third and last injection of the CYD dengue vaccine.

3.2 Trial Plan

A summary of the schedule of study vaccination and blood samplings for eligible subjects is provided in Table 3.1.

Table 3.1: Study procedures

Phase IIIb Trial; 5 Injections; **Group 1**: 6 Visits and a 6-month follow-up Phone Call, 4 Blood Samples, 18 Months duration per subject, 6 interim phone calls; **Group 2**: 8 Visits and a 6-month follow-up Phone Call, 6 Blood Samples, 19 Months duration per subject, 8 interim phone calls. Interim phone calls will be given 7 days after each injection (PC1, PC2, PC4, PC5, and PC7) and 2 months after blood samples (PC3, PC6, and PC8) during the 6-month period after each vaccination. As per Protocol Amendment 1, only dengue-immune subjects before the first dengue vaccine injection will be eligible to continue study vaccinations with the CYD dengue vaccine.

	Time period prior to Protocol Amendment 1						**Time period post Protocol Amendment 1***		
Visit (V) Number	**V01**	**V02**	**V03 (Group 2)**	**V04**	**V05**	**V06†‡ (Group 2)**	**V07†**	**V08†**	**Phone Call§**
Trial Timelines	**D0**	**M1 (V01+28D)**	**M2 (V02+28D)**	**M6 (V01 + 6M)**	**M7 (V04+28D) (Group 1)** **(V02+6M) (Group 2)**	**M8 (V05+28D)**	**M12 (V01 + 12M)** (Group 1) **M13 (V02+ 12M)** (Group 2)	**M13 (V07+28D)** (Group 1) **M14 (V07+28D)** (Group 2)	**M18 (V07+6M)** (Group 1) **M19 (V07+6M)** (Group 2)
Time Windows (days)		**+14**	**+14**	**±20**	**+14**	**+14**	**±20**	**+14**	**+30**
Informed Consent and assent form (if applicable) signed	X								
Amendment 1to AF and ICF						X (Amdt 1) (as applicable)	X (Amdt 1) (as applicable)		
Inclusion/Exclusion Criteria	X								
Significant Medical History	X								
History of Dengue Infection/Vaccination	X								
Demography/Body Stature	X								
Physical/Clinical Examination and Temperature**	X	X (Group 2)	X (Group 2)	X	X	X (Group 2)	X	X	
Concomitant Therapy††	X	X	X (Group 2)	X	X	X (Group 2)	X	X	
Urine Pregnancy Test‡‡	X	X (Group 2)		X	X (Group 2)		X		
Randomization	X								
Contraindications		X (Group 2)		X	X (Group 2)	X	X		

	Time period prior to Protocol Amendment 1						Time period post Protocol Amendment 1*		
Visit (V) Number	**V01**	**V02**	**V03 (Group 2)**	**V04**	**V05**	**V06†‡ (Group 2)**	**V07†**	**V08†**	**Phone Call§**
Trial Timelines	**D0**	**M1 (V01+28D)**	**M2 (V02+28D)**	**M6 (V01 + 6M)**	**M7 (V04+28D) (Group 1)** **(V02+6M) (Group 2)**	**M8 (V05+28D)**	**M12 (V01 + 12M)** (Group 1) **M13 (V02+ 12M)** (Group 2)	**M13 (V07+28D)** (Group 1) **M14 (V07+28D)** (Group 2)	**M18 (V07+6M)** (Group 1) **M19 (V07+6M)** (Group 2)
Blood Sampling§§									
Dengue neutralizing Abs	X (Group 1)	X	X (Group 2)		X (Group 1)	X (Group 2)		X	
HPV Abs	X	X			X				
Virological confirmation of dengue* **	**All acute febrile illness with diagnosis of dengue requiring hospitalization within the first 5 days after fever onset, occurring anytime throughout the trial period**								
Vaccine injection	**Cervarix Dose 1** (Groups 1 and 2) **CYD Dose 1** (Group 1)	**CYD Dose 1** (Group 2)		**Cervarix Dose 2** (Groups 1 and 2) **CYD Dose 2** (Group 1)	**CYD Dose 2** (Group 2)		**CYD Dose 3** (Groups 1 and 2)		
Post-injection phone call†††	**Interim phone calls will be given 7 days after each injection and 2 months after blood samples during the 6-month period after each vaccination.**								
Diary Card (DC)									
Provided	X	X (Group 1 for SAE only and Group 2)	X (Group 2 for SAE only)	X	X (Group 1 for SAE only and Group 2)	X (Group2 for SAE only)	X		
Checked		X	X (Group 2)	X	X	X (Group 2)	X	X	
Collected		X	X (Group 2)	X	X	X (Group 2)	X	X	
Memory Aid (MA)									
Provided‡‡‡						X	X	X	
Checked									X
30-Min. Observation Period	X	X (Group 2)		X	X (Group 2)		X		
Injection Site Reactions & Systemic Events Assessment§§§	X	X	X (Group 2)	X	X	X (Group 2)	X	X	
Collection of SAEs, and serious AESIs§§§	**Throughout the trial period (for SAEs) or in defined time windows according to the type of AESI**								
Termination Record****								X	

Abs: antibodies; AE: Adverse event; AESI: AE of special interest; BL: blood sample; CYD: CYD dengue vaccine; D: Day; M: Month; SAE: Serious adverse event; V: Visit;

*	Visits to be performed by dengue immune subjects only, if they consent to receive the next CYD vaccine injection
†	Vaccinations and associated or subsequent procedures may be out of the defined time-windows in dengue-immune subjects continuing the study due to study pause
‡	All subjects will either attend an unscheduled visit (V00) or have an unscheduled phone call (PC00) during the study pause and **before** the next scheduled visit (V07 for most subjects; V08 for 2 subjects). During the unscheduled visit or phone call, the subject will be informed about the new safety data thanks to a "dear participant letter" and on their baseline serostatus result. The signature of the ICF/AF by the parents/subjects and the check of contraindications to continue in the study will be performed at the next visit planned, as applicable.
§	The 6-month phone call will be given to all subjects who consented to continue in the study whether they were identified as "dengue-immune" or "dengue non-immune"
**	A full physical and clinical examination will be performed and documented on each vaccination visit (mandatory before vaccination) and at the Investigator's discretion if necessary based on the health status of the subject for the other visits.
††	Concomitant therapy will be collected for Days 0–28 after each injection only
‡‡	In healthy female subjects of childbearing potential. Result of urine pregnancy test should be confirmed as negative before vaccination.
§§	Blood samples planned during vaccination visits will be taken before vaccination
***	In such case, 1 unplanned acute blood sample (approximately 3 mL) will be collected for virological confirmation of dengue disease, by NS1 (nonstructural protein 1) antigen test (ELISA), and/or WT dengue RT-PCR
†††	Interim phone calls will be given 7 days (+8 days of time window) after each injection (PC1: V01 + 7 days, PC2: V02 + 7 days, PC4: V04 + 7 days, PC5: V05 + 7 days, and PC7: V07 + 7 days) and approximately 2 months after blood samples (PC3: M3 or M4, PC6: M9 or M10, and PC8: M15 or M16) during the 6-month period after each vaccination.
‡‡‡	MA will be delivered to subjects who will not receive further CYD dengue vaccine injection but consent to continue their participation in the study.
§§§	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. 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 disease within 30 days after each CYD dengue vaccine injection, serious neurotropic disease within 30 days after each CYD dengue vaccine injection; serious dengue cases requiring hospitalization [ie, hospitalized suspected dengue case] will be reported during the entire study).
****	Termination record will be checked either during a planned study visit or a phone call

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

See Section 9.1 of the protocol.

4.2 Secondary Endpoints and Assessment Methods

See Section 9.2 of the protocol.

4.3 Observational Endpoints and Assessment Methods

There are no observational objectives in this study.

4.4 Derived Endpoints: Calculation Methods

4.4.1 Safety

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 case report form (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 recorded the intensity level (Grade 1, 2, or 3) in the DC. For the measurable reactions of redness and swelling, they recorded 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 >= 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 recorded the intensity level (Grade 1, 2, or 3) in the DC. For the measurable reactions of redness and swelling, they recorded the size of the reaction and the classification as Grade 1, 2, or 3 will be 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 ≥°38.0°C (≥ 100.4°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: ≥ 38.0°C to ≤ 38.4°C, **or** ≥ 100.4°F to ≤ 101.1°F	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, **or** ≥ 101.2°F to ≤ 102.0°F	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 **or** ≥ 102.1°F	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, subjects or parents/legally acceptable representatives recorded the intensity level (Grade 1, 2, or 3) in the DC. For fever, they recorded the body temperature and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis.

4.4.1.1 Solicited Reactions

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 of 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 of onset is the first day of the first occurrence.

Time of onset will be displayed by period as follows:

Table 4.4: Categories for Time of Onset

Period of Time of Onset	
Injection Site Reactions (D0-D7)	**Systemic Reactions (D0-D14)**

Period of Time of Onset	
D0 - D3 D4 - D7	D0 - D3 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 ≥ 38°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:

- (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".

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

- Time of 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 of onset will be considered as "Missing". Events that occur before vaccination (negative time of 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 of onset between 0 and 28 days after vaccination or missing. An AE 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.

Time of onset will be displayed by period as follows:

- D0-D3
- D4-D7
- D8-D14
- ≥ 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 of 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 of 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 reaction 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. 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. 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 hospitalization[1] 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 GMTs, any titer reported as < the lower limit of quantitation (LLOQ) will be converted to a value of ½ LLOQ.

While a single approach will be used for GMTs, two different approaches for geometric mean of the titer ratios [GMTR] will be applied:

- For CYD dengue vaccine, < LLOQ will be converted to ½ LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator
- For Cervarix, < LLOQ will be converted to ½ LLOQ.

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 and 3rd dose of CYD Dengue vaccine, the thresholds considered are:

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

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

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 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 Cervarix Seroconversion

Seroconversion 28 days after each dose is defined as changing serostatus from seronegative at baseline to seropositive (> LLOQ of the assay) or ≥ 4-fold rise in Ab titer if seropositive at baseline.

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 serostatus 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 [$PRNT_{50}$]) in the blood sample collected at V01 for group 1 and at V02 for group 2. The baseline dengue status will be derived for each subject as follows:

- Dengue immune subjects (seropositive) at baseline 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 (seronegative) at baseline are defined as those subjects with titers <10 (1/DIL) (not quantified) for all serotypes with parental dengue virus strains with available and "valid" results (i.e. not coded "NR") in the serology database.
- Otherwise the baseline status will be classified as undetermined and subjects will have their dengue baseline status derived as non-immune.

4.4.6 Baseline Zika status

The Zika serostatus at baseline is defined as the presence of Abs against Zika in the baseline blood sample (by Zika 50% microneutralization). The baseline Zika status will be derived for each subject as follows:

- Zika immune subjects (seropositive) at baseline are defined as those subjects with titers ≥100 (1/DIL) with the parental Zika virus .
- Zika non-immune subjects (seronegative) at baseline are defined as those subjects with titers <100 (1/DIL) (not quantified) for parental Zika virus with available and "valid" results (i.e. not coded "NR") in the serology database
- Otherwise the baseline status will be classified as undetermined and subjects will have their Zika baseline status derived as non-immune.

4.4.7 Baseline flavivirus (FV) status

Flavivirus (FV) status at baseline is defined as

- Immune for subjects with quantified (≥ 10 (1/DIL)) neutralizing Abs against at least one dengue serotype and ≥100 (1/DIL) against Zika virus in the baseline sample.
- Non-immune for subjects without quantified (< 10 (1/DIL)) neutralizing Abs against any of the four dengue serotypes and <100 (1/DIL) against Zika virus in the baseline sample. To be classified in this group, all of the titers planned to be measured at baseline must be available, and "valid" results (i.e., not coded "NR") in the serology database
- Otherwise the baseline status will be classified as undetermined and subjects will have their FV baseline status derived as non-immune.

5 Statistical Methods and Determination of Sample Size

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

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

For descriptive purposes, the following statistics will be presented:

Table 5.1: Descriptive statistics produced

Baseline characteristics and follow-up description	**Categorical data**	Number of subjects. Percentage of subjects.
	Continuous data	Mean, standard deviation, quartiles, minimum, and maximum.
Clinical safety results	**Categorical data**	Solicited: Number and percentage (95% confidence interval [CI]) of subjects and number of events Unsolicited: Number and percentage (95% CIs) of subjects and number of events. Hospitalized suspected dengue case: Number and percentage (95% CIs) of subjects
Immunogenicity results	**Categorical data (cutoff)**	Number and percentage (95% CIs) of subjects.
	Continuous data (titer / titer ratio)	Log_{10}: Mean and standard deviation. Anti-Log_{10} (work on Log_{10} distribution, and anti-Log_{10} 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 (1), i.e., using the inverse of the beta integral with SAS®.

For immunogenicity, assuming that Log_{10} transformation of the titers follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log_{10} 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 geometric means (GMs) and their 95% CI.

GM is defined as follows:

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

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

5.1 Statistical Methods

In a general way, non-inferiority testing will be performed on dengue immune subjects. The tests will be done only if the number of evaluable subjects provides a global power of at least 80% for the co-primary objectives and secondary objective. In case the global power is insufficient to perform non-inferiority testing, only descriptive analyses will be performed.

Only descriptive analyses will be conducted in dengue non-immune subjects and in the overall population.

5.1.1 Hypotheses and Statistical Methods for Primary Objectives

5.1.1.1 Hypotheses

Non-inferiority on Cervarix

The objective is to demonstrate that the humoral immune response to Cervarix administered concomitantly with CYD dengue vaccine is non-inferior to the immune response to Cervarix administered sequentially with CYD dengue vaccine.
Individual hypotheses on Cervarix response for each antigen:
A non-inferiority testing approach will be used to compare geometric mean of titer (GMTs) for the 2 antigens (HPV-16 and HPV-18) 28 days after the last dose of Cervarix, based on the following individual hypotheses:

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

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

with:

i, antigen (HPV-16 and HPV-18)

δ non-inferiority limit is set at 2, ie, 0.301 (=$\log_{10}$[2]), for each antigen "i"

Non-inferiority for antigen i will be demonstrated if the lower bound of the 2-sided 95% CI is greater than -δ (α=2.5% one-sided).

<u>Global hypotheses for non-inferiority on Cervarix response</u>
The global hypotheses are:

H_{0G}: Non inferiority of Cervarix co-administered with CYD dengue vaccine versus Cervarix administered sequentially with CYD dengue vaccine is not demonstrated for at least one antigen.

H_{1G}: Non-inferiority of Cervarix co-administered with CYD dengue vaccine versus Cervarix administered sequentially with CYD dengue vaccine is demonstrated for all the antigens.

$$H_0^G : \text{at least one } H_0^i \textit{ not rejected}$$

$$H_1^G : \text{all } H_0^i \textit{ are rejected}$$

Non-inferiority on CYD Dengue Vaccine

The objective is to demonstrate that the humoral immune response to the CYD dengue vaccine administered concomitantly with Cervarix is non-inferior to the immune response to CYD dengue vaccine administered sequentially with Cervarix.

Individual hypotheses on CYD response for each antigen:
A non-inferiority testing approach will be used to compare GMTs 28 days after the third injection of CYD dengue vaccine between groups for each serotype "i" based on the following individual hypotheses:

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

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

with:

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

δ non-inferiority limit is set at 2 ie, 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 -δ.

Global hypotheses for non-inferiority on CYD response

$$H_0^G: \text{at least one } H_0^i \textit{ not rejected}$$

$$H_1^G: \text{all } H_0^i \textit{ are rejected}$$

Overall, non-inferiority among the groups will be demonstrated if, for each antigen of Cervarix and each serotype of CYD dengue vaccine, the 2-sided 95% CIs lie above -δ.

5.1.1.2 Statistical Methods

The non-inferiority test will be performed using the 95% 2-sided CI of the difference of the means of the $\log_{10}$ transformed post- vaccination titers between Group 1 and Group 2 (α=2.5% one-sided). 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 Cervarix

The objective is to demonstrate that the humoral immune response (in terms of seroconversion) to Cervarix after concomitant administration with CYD dengue vaccine is non-inferior to sequential administration with CYD dengue vaccine measured 28 days after the last of dose of Cervarix.

A non-inferiority testing approach will be used to compare seroconversion rates for the 2 antigens (HPV-16 and HPV-18) 28 days after the last dose of Cervarix.

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

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

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

where the non-inferiority limit δ is set at 5% for each antigen "i".

Non-inferiority on CYD dengue vaccine

No statistical hypothesis will be tested on CYD dengue vaccine for secondary objectives.

5.1.2.2 Statistical Methods

Non-inferiority on Cervarix

Non-inferiority for antigen "i" will be demonstrated if the lower bound of the 2-sided 95% CI is greater than -δ.
The non-inferiority test will be performed using the 95% 2-sided CI of the difference between groups (α=2.5% one-sided). The 95% CI will be calculated based on the Wilson score method without continuity correction as quoted by Newcombe for seroconversion rates.

Descriptive analysis on Cervarix and CYD dengue vaccine immunogenicity

No hypotheses will be tested.
Immunogenicity point estimates and their 95% CI will be presented for each and any group, at baseline and after each dose of Cervarix and CYD dengue vaccine.
A complementary analysis on CYD dengue vaccine immunogenicity after each dose will be conducted for each and any group according to the dengue status at baseline (dengue immune, dengue non-immune, and all subjects).

The 95% CIs will be calculated using:

- The normal approximate method for GMTsand GMTRs
  Assuming that Log_{10} transformation of the titers / data follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log_{10} (titers / data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide geometric means (GMs) and their 95% CI.
- The exact binomial distribution for single proportions (Clopper-Pearson's method, quoted by Newcombe)

Safety

All analyses will be descriptive; no hypotheses will be tested. Safety will be assessed for all subjects and for immune subjects after each and any dose of Cervarix and CYD Dengue vaccine. Moreover, a complementary analysis will be performed for each dose of CYD dengue vaccine, for each and any group, on non-immune subjects.

As there are differences in the intensity considered for measurable reactions depending on ages of subjects, a complementary analysis will be performed according to the following age groups: 9-11 years and 12 to 14 years.

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

5.1.3 Complementary output

Additional exploratory descriptive analyses based on baseline Zika and FV statuses will be provided in Appendix 15.

- Zika and FV baseline status
- Zika and FV baseline status by baseline dengue status
- Dengue GMTs by Zika and FV baseline status
- Description of immune response to each dengue serotype according to Zika status at baseline

5.2 Analysis Sets

Four analysis sets will be used: the Per-Protocol analyses sets (PPX and PPC), the Full Analysis Set (FAS) and the Safety Analysis Set (SafAS).

5.2.1 Full Analysis Set

The Full Analysis Set (FAS) is defined as the subset of subjects who received at least one dose of the study vaccine.
Subjects will be analyzed by baseline dengue status and the vaccine treatment group to which they were randomized.

5.2.2 Per-Protocol Analysis Set

Two per-protocol analysis sets will be defined: one for Cervarix (PPX) and one for CYD dengue vaccine (PPC). The per-protocol analysis sets are subsets of the FAS.

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

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subjects identified as dengue non-immune subjects (seronegative) at baseline
- Subject is seropositive at baseline for Cervarix (ie, at least one Ab levels against Cervarix HPV antigens > LLOQ at baseline)
- Subject did not complete the Cervarix vaccination schedule (until V05 for Group 1, V06 for Group 2)
- Subject received a vaccine other than the one that she was randomized to receive (until V05 for Group 1, V06 for Group 2)
- Administration of vaccine was not done as per-protocol (site and route of administration)

- Subject did not receive vaccine in the proper time window (until V05 for Group 1, V06 for Group 2)
- Subject did not provide after the 2nd dose of Cervarix a post-dose serology sample in the proper time window or a post-dose serology sample was not drawn (V05 for Group 1, V06 for Group 2)
- Subject received a protocol-prohibited medication (prohibited therapies/medications/vaccines are indicated in the Protocol Section 6.7) (until V05 for Group 1, V06 for Group 2)
- Subject's serology sample did not produce a valid test result, ie, no Cervarix antigen titer available

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
- Subjects identified as dengue non-immune subjects (seronegative) at baseline
- Subject did not complete the vaccination schedule
- Subject received a vaccine other than the one that she was randomized to receive
- Administration of vaccines was not done as per-protocol (site and route of administration)
- Subject did not receive vaccines in the proper time window
- Subject did not provide after the 3rd dose of CYD dengue vaccine a post-dose serology sample in the proper time window or a post-dose serology sample was not drawn (V08, M13 for Group 1 and M14 for Group 2)
- Subject received a protocol-prohibited medication (prohibited therapies/medications/vaccines are indicated in Section 6.7) (until V07)
- Subject's serology sample did not produce a valid test result, ie, no Neutralizing Ab titers against any of the four parental dengue virus serotypes of CYD dengue vaccine available (at V08, M13 for Group 1 and M14 for Group 2)

5.2.3 Safety Analysis Set

The SafAS is defined as those 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 first dose. The safety data will also be presented separately for dengue immune, non-immune / undetermined subjects.

Safety data recorded for a vaccine received out of the protocol design will be excluded from the analysis (and listed separately).

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 PPX and PPC respectively for Cervarix and CYD dengue vaccine comparisons. The subjects will be analyzed by the vaccine group to which they were randomized.

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

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

Complementary analyses on Zika and FV baseline statuses (and by baseline dengue status) will be performed on randomized subjects and on the FAS.

Dengue GMTs by Zika and by FV baseline statuses by Zika baseline status will be performed on all subjects and on FAS dengue immune subjects.

Description of immune response to each dengue serotype according to Zika status at baseline will be performed on FAS dengue immune subjects.

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 of onset will be considered to be missing. Nevertheless unsolicited AEs with missing time of onset will be included in analyses according to the visit 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

For the computation of GMTs, any titer reported as < LLOQ will be converted to a value of 1/2 LLOQ.

While a single approach was used for GMTs, two different approaches for GMT ratios were applied:

- For Cervarix, < LLOQ will be converted to 1/2 LLOQ
- For CYD dengue vaccine, < LLOQ will be converted to 1/2 LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator

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

5.3.3 Efficacy

Not applicable.

5.4 Interim / Preliminary Analysis

No planned interim / preliminary analyses were performed.

5.5 Determination of Sample Size and Power Calculation

A total of 480 subjects will be enrolled: 240 subjects in each group.

The reference standard deviations (SD) considered for Cervarix are 0.4 for both HPV-16 and HPV-18.

The reference seroconversion rates for Cervarix were set to 99% for both antigens.

The reference SD considered are the following for CYD dengue vaccine: 0.9 for serotype 1, 0.7 for serotypes 2 and 3 and 0.5 for serotype 4 (based on CYD13 and CYD15 efficacy studies conducted in subjects aged 9 to 16 years in Latin American countries).

Considering a potential attrition rate of 15%, such sample size would provide 204 evaluable subjects in the per protocol (PP) population per group. This will give, for the co-primary objectives, a global power of 90.2% and for secondary objectives (in terms of Cervarix seroconversion) a power of 91.8%.

Following Protocol Amendment 1, the number of evaluable subjects may be difficult to achieve (considering only dengue immune (seropositive) subjects at baseline to be included in the Per-Protocol populations). Thus the non-inferiority testing will be done only if the number of evaluable subjects provides a global power of at least 80% for the co-primary objectives and secondary objective ie, if the number of evaluable subjects is at least 163 per group for the co-primary objectives and 168 per group for the secondary objective. The power for non-inferiority in terms of GMTs per antigen and serotype is detailed in Table 5.1 and Table 5.3.

This initial study sample size will also provide a 95% probability of observing an AE that has a true incidence of 1.2% in each group (regardless of baseline serostatus).

Table 5.2: Powers for Non-inferiority between GMT for each antigen and serotype

Antigen/Serotype	Alpha	δ	References SD(s)	Power (%) for 204 evaluable subjects per group	Power (%) for 163 evaluable subjects per group
HPV-16	0.025	0.301	0.4	>99.9%	>99.9%
HPV-18	0.025	0.301	0.4	>99.9%	>99.9%
Dengue serotype 1	0.025	0.301	0.9	92.1%	85.3%
Dengue serotype 2	0.025	0.301	0.7	99.1%	97.2%
Dengue serotype 3	0.025	0.301	0.7	99.1%	97.2%
Dengue serotype 4	0.025	0.301	0.5	>99.9%	>99.9%
Power global				90.2%	80.3%

Table 5.3: Powers for Cervarix seroconversion for each antigen

Antigen	Alpha	Δ	Power (%) for 204 evaluable subjects per group	Power (%) for 168 evaluable subjects per group
HPV-16	0.025	5%	95.8%	89.6%
HPV-18	0.025	5%	95.8%	89.6%
Power global				80.4%

5.6 Data Review for Statistical Purposes

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

5.7 Changes in the Conduct of the Trial or Planned Analyses

According to Protocol amendment 1, the study population for the non-inferiority is reduced to the dengue immune subjects (seropositive) compared to initial sample size.
The full analysis set for dengue immune (seropositive) subjects population described in the protocol is no more useful because results for immunogenicity and safety will be presented by baseline dengue status.

To further assess the potential impact of prior infection by zika on the immune response induced by the CYD dengue vaccine and on dengue injection, an exploratory analysis will be performed on subjects who have accepted further testing on their BS in the ICF. Description will be done according to Zika and FV baseline status and on dengue characteristic according to the Zika and FV baseline status.

6 References List

1 Newcombe R.G., Two-sided confidence intervals for the single proportion: comparison of seven methods, Statistics in Medicine, (1998) 17, 857-872

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