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<b>Sponsor:</b> Sanofi Pasteur	<b>Study Identifiers:</b> U1111-1161-2855, IND 11219, NCT02623725
<b>Drug substance(s):</b> CYD dengue vaccine	<b>Study code:</b> CYD64
<b>Title of the study:</b> Immunogenicity and Safety of a Tetravalent Dengue Vaccine given as a Booster Injection in Adolescents and Adults who previously completed the 3-dose schedule in a study conducted in Latin America	
<b>Study center(s):</b> This was a multi-center trial conducted at 5 sites, one in each of the following countries: Brazil, Colombia, Honduras, Mexico, and Puerto Rico.	
<b>Study period:</b> Date first subject/patient enrolled: 14/Apr/2016 Date last subject/patient completed: 28/Oct/2018	
<b>Phase of development:</b> Phase 2	
<b>Objectives:</b> <b>Primary objective:</b> To demonstrate the non-inferiority, in terms of geometric mean of titer ratios (GMTRs), of a CYD Dengue Vaccine booster compared to the third CYD Dengue Vaccine injection in subjects from CYD13 and CYD30 trials (subjects from Group 1 only). <b>Secondary objectives:</b> <b>Immunogenicity</b> <ol style="list-style-type: none"><li>1) If the primary objective of non-inferiority is achieved: To demonstrate the superiority, in terms of GMTRs, of a CYD Dengue Vaccine booster compared to the third CYD Dengue Vaccine injection in subjects from CYD13 and CYD30 trials (subjects from Group 1 only).</li><li>2) To describe the immune responses elicited by a CYD Dengue Vaccine booster and placebo injection in subjects who received 3 doses of the CYD Dengue Vaccine in the CYD13 and CYD30 trials in all subjects.</li><li>3) To describe the neutralizing Ab levels of each dengue serotype PD3 (CYD13 and CYD30 subjects) and immediately prior to booster or placebo injection in all subjects.</li><li>4) To describe the neutralizing Ab persistence 6 months, 1 year, and 2 years post-booster, or placebo injection in all subjects.</li></ol> <b>Safety</b> To evaluate the safety of booster vaccination with the CYD Dengue Vaccine in all subjects.	

**Methodology:**

This was a multi-center, observer-blind, randomized, placebo-controlled, Phase II trial conducted in Latin America i) to assess the non-inferiority of the immune response induced by a booster injection of a tetravalent dengue vaccine versus that induced by the third injection of the 3-dose schedule of the same vaccine received 4 to 5 years earlier in healthy adolescents and adults from the CYD13 and CYD30 trials; ii) to evaluate the safety and antibody persistence of the booster injection up to 2 years.

Subjects were randomized into Groups 1 and 2 in a 3:1 ratio and either received the CYD dengue vaccine as a booster or a placebo.

A total of 372 subjects aged 9 to 16 years on the day of first injection of study vaccine in CYD13/CYD30 were planned to be recruited into the following 2 Groups.

Treatment Group	From CYD13-CYD30	CYD64	
		N	Product to be injected
Group 1	CYD1/CYD2/CYD3*	279	CYD booster
Group 2	CYD1/CYD2/CYD3*	93	Placebo
Total		372	

\* CYD Dengue Vaccine dose 1, dose 2, and dose 3

An interactive voice response system/interactive web response system (IVRS/IWRS) was used to assign treatment Group and subject number at each clinical site, with stratification for trial center.

At the first visit (V01), each subject received a single dose of the CYD Dengue Vaccine as booster or a placebo. Blood samples were collected at pre-booster (immediately prior to injection), at 28 days (V04), 6 months (V05), 1 year (V06), and 2 years post-injection (V07) for assessing neutralizing antibody (Ab) titers against each of the 4 parental dengue virus strains.

Reactogenicity data was collected in all subjects after the booster or placebo injection. Solicited injection site reactions were collected for Days 0–7. Solicited systemic reactions were collected for Days 0–14. Unsolicited events were collected for Days 0–28. Serious adverse events (SAEs) were reported throughout the study and serious and non-serious adverse events of special interest (AESIs) were collected in defined time windows according to the type of AESI.

In addition, hospitalized suspected dengue cases occurring at any time in the trial were documented. Hospitalized suspected dengue disease was defined as an acute febrile illness with diagnosis of dengue requiring hospitalization (with bed attribution). In such cases, 1 unplanned blood sample (acute sample) was to be collected for virological confirmation within the first 5 days after fever onset\*. A suspected hospitalized dengue case was to be considered virologically-confirmed dengue (VCD) case if there was a detection of wild-type (WT) dengue virus by non-structural protein 1 (NS1) antigen enzyme-linked immunosorbent assay (ELISA) and/or wild-type dengue reverse transcription-polymerase chain reaction (RT-PCR).

\*Note: An acute blood sample was to be collected within the pre-specified timeframe as described above for all suspected hospitalized dengue cases. If this could not be accomplished, this sample was still to be obtained as soon as possible thereafter, for Independent Data Monitoring Committee (IDMC) severity assessment.

<p><b>Number of subjects:</b></p> <p>Planned: 372</p> <p>Randomized: 251</p> <p>Treated: 251</p> <p><b>Evaluated:</b></p> <p>Immunogenicity: 248</p> <p>Safety: 251</p>
<p><b>Diagnosis and criteria for inclusion:</b></p> <p>An individual had to fulfill <i>all</i> of the following criteria in order to be eligible for trial enrollment:</p> <ol style="list-style-type: none"> <li>1) Has been identified as a potential subject by the Sponsor and is included in the list provided to the Investigator (ie, aged 9 to 16 years on the day of first vaccination of CYD Dengue Vaccine in CYD13/CYD30 and has a PD3 serum sample available [at least 400 µL of serum])</li> <li>2) Presently in good Health, based on medical history, and physical examination</li> <li>3) Assent form (AF) or informed consent form (ICF) has been signed and dated by the subject (based on local regulations), and ICF has been signed and dated by the parent(s) or another legally acceptable representative (and by an independent witness if required by local regulations)</li> <li>4) Subject and parent(s)/legally acceptable representative(s) able to attend all scheduled visits and to comply with all trial procedures</li> </ol>
<p><b>Study treatments</b></p> <p><b>Investigational medicinal product:</b> CYD Dengue Vaccine (5-dose presentation)</p> <p>Form: Powder and solvent for suspension for injection</p> <p>Composition: Each 0.5 mL dose of reconstituted vaccine contains:</p> <p style="padding-left: 40px;"><u>Active ingredients:</u></p> <p style="padding-left: 80px;">4.5 – 6 log<sub>10</sub> cell-culture infectious dose 50% (CCID<sub>50</sub>) of each live, attenuated, recombinant dengue serotype 1, 2, 3, 4 virus</p> <p style="padding-left: 40px;"><u>Excipients:</u></p> <p style="padding-left: 80px;">Essential amino acids, non-essential amino acids, L-arginine hydrochloride, sucrose, D-trehalose dihydrate, D-sorbitol, trometamol, urea, and sodium chloride</p> <p style="padding-left: 40px;"><u>Solvent:</u> NaCl 0.9%</p> <p>Route of administration: Subcutaneous (SC)</p>
<p><b>Investigational medicinal product:</b> Placebo</p> <p>Form: Solution</p> <p>Composition: NaCl 0.9%</p> <p>Route of administration: SC</p>
<p><b>Duration of participation:</b> The duration of each subject's participation in the trial was approximately 24 months.</p>

**Criteria for evaluation:****Primary endpoint:**

Neutralizing Ab levels against each dengue virus serotype measured 28 days after the third CYD Dengue Vaccine injection and 28 days after the booster injection in Group 1 using dengue plaque reduction neutralization test (PRNT).

**Secondary endpoints:*****Immunogenicity***

- 1) Neutralizing Ab levels against each of the 4 parental dengue virus strains of CYD Dengue Vaccine as determined by PRNT measured 28 days after the third CYD Dengue Vaccine injection and 28 days post-booster injection (subjects from Group 1 only).
- 2) Neutralizing Ab levels against each of the 4 parental dengue virus strains of the CYD Dengue Vaccine as determined by PRNT immediately prior to and 28 days post-booster or placebo injection.
- 3) Individual post-booster/pre-booster GMTs for each of the 4 parental dengue virus strains of the CYD Dengue Vaccine as determined by PRNT immediately prior to and 28 days post-booster or placebo injection.
- 4) Seroconversion rates 28 days after the booster injection for each of the 4 parental dengue virus strain of CYD Dengue Vaccine: percentages of subjects with either a pre-booster titer < 10 (1/dil) and a post-booster titer  $\geq$  40 (1/dil), or a pre-booster titer  $\geq$  10 (1/dil) and a  $\geq$  4-fold increase in post-booster titer as determined by PRNT immediately prior to and 28 days post-booster or placebo injection.
- 5) Neutralizing Ab levels against each of the 4 parental dengue virus strains as determined by PRNT at 28 days after the third CYD Dengue Vaccine injection and immediately prior to booster or placebo injection.
- 6) Neutralizing Ab levels against each of the 4 parental dengue virus strains as determined by PRNT at 6 months, 1 year, and 2 years post-booster, or placebo injection.

***Safety***

- 1) Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, action taken, whether it leads to discontinuation or not, and relationship to vaccination of any Adverse Events (AE) reported in the 30 minutes after vaccination.
- 2) Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited (pre-listed in the subject's diary card [DC] and electronic case report form [CRF]) injection site reactions (pain, erythema, and swelling) occurring up to 7 days after vaccination.
- 3) Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited systemic reactions (fever, headache, malaise, myalgia, and asthenia) occurring up to 14 days after vaccination.
- 4) Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, whether it leads to discontinuation or not, action taken and relationship to vaccination (for systemic AEs only) of unsolicited spontaneously reported AEs up to 28 days after vaccination.
- 5) Occurrence of SAEs, including serious AESIs (with specific time windows according to the nature of event), throughout the trial.
- 6) Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, and relationship to vaccination of non-serious AESIs occurring up to 7 days after vaccination.
- 7) Occurrence of hospitalized virologically-confirmed dengue cases throughout the trial (ie, from D0 through end of the study)

**Statistical methods:**

The analysis was 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).

Three statistical analyses of safety and immunogenicity were performed on unblinded data (one 28 days following the booster vaccination, one after the 1 year follow-up, and the other one after the completion of the trial). A specific process was implemented to maintain the blind at both subject and Investigator levels until completion of the trial.

The immunogenicity populations (full and per-protocol analysis sets) were used for the immunogenicity analyses, and the safety analysis set was used for the safety analysis.

**Hypothesis and Statistical Method for the Primary Objective (Group 1 only)**

**Hypotheses:**

*Individual Hypotheses for Each Serotype:*

A non-inferiority testing approach was performed for each serotype to demonstrate the non-inferiority, in terms of GMTRs, 28 days post-injection, of a CYD Dengue Vaccine booster dose compared to the third CYD Dengue Vaccine dose in subjects from CYD13 and CYD30 trials.

Individual hypotheses for each serotype were as follows:

$$H_0^i : GM(V_{Booster}^i / V_{PD3}^i) \leq 1/2$$

$$H_1^i : GM(V_{Booster}^i / V_{PD3}^i) > 1/2$$

Where  $i = 1, 2, 3$  and  $4$ ;  $V_{Booster}^i$  is the immunogenicity titer 28 days after the CYD Dengue Vaccine booster dose and  $V_{PD3}^i$  is the immunogenicity titer 28 days after the third CYD Dengue Vaccine dose in CYD13 and CYD30 subjects.

*Overall Hypothesis:*

The overall null hypothesis can be stated as for at least 1 serotype, the post-booster dose-response (28 days after the CYD Dengue Vaccine booster injection) is inferior to the PD3 response (28 days after the third CYD Dengue Vaccine dose in CYD13 and CYD30 subjects)

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

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

**Statistical Methods**

A non-inferiority test was performed using the 95% two-sided CI of  $GM(V_{Booster} / V_{PD3})$  for each serotype; the 95% CI was calculated using paired t-test. Subjects with non-missing PD3 and post-booster dose titer were included in this analysis.

For each serotype, non-inferiority was to be demonstrated if the lower limit of the two-sided 95% CI was greater than 1/2. If the null hypothesis was rejected, then the alternative hypothesis of non-inferiority was supported.

The overall null hypothesis was to be rejected if the 4 individual null hypotheses were rejected simultaneously.

**Hypotheses and Statistical Methods for the First Secondary Objective**

As non-inferiority was demonstrated for the primary endpoint the superiority hypotheses were performed.

### **Hypotheses:**

#### *Individual Hypotheses for Each Serotype:*

A superiority hypothesis testing approach was performed for each serotype to demonstrate the superiority, in terms of GMTRs, 28 days post-injection, of a CYD Dengue Vaccine booster dose compared to the third CYD Dengue Vaccine dose in subjects from CYD13 and CYD30 trials.

Individual hypotheses for each serotype were as follows:

$$H_0^i : GM(V_{Booster}^i / V_{PD3}^i) \leq 1$$

$$H_1^i : GM(V_{Booster}^i / V_{PD3}^i) > 1$$

Where  $i = 1, 2, 3$  and  $4$ ;  $V_{Booster}^i$  is the immunogenicity titer 28 days after the CYD Dengue Vaccine booster dose and  $V_{PD3}^i$  is the immunogenicity titer 28 days after the third CYD Dengue Vaccine dose in CYD13 and CYD30 subjects.

#### *Overall Hypothesis:*

The overall null hypothesis can be stated as for at least 1 serotype, the post-booster dose-response (28 days after the CYD Dengue Vaccine booster injection) is not superior to the PD3 response (28 days after the third CYD Dengue Vaccine dose in CYD13 and CYD30 subjects).

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

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

### **Statistical Methods**

A superiority test was performed using the 95% two-sided CI of  $GM(V_{Booster} / V_{PD3})$  for each serotype; the 95% CI was calculated using paired t-test. Subjects with non-missing PD3 and post-booster dose titer were included in this analysis.

For each serotype, superiority was to be demonstrated if the lower limit of the two-sided 95% CI was greater than 1. If the null hypothesis was rejected, then the alternative hypothesis of superiority was supported.

The overall null hypothesis was to be rejected if the 4 individual null hypotheses were rejected simultaneously.

### **Statistical Methods for Other Secondary Objectives**

All other analyses were descriptive; no hypotheses were tested.

For immunogenicity, 2 sample t-test on the  $\log_{10}$  transformed titers were used for 95% CI for the ratio of geometric mean titers (GMTs) (difference between GMTs on log scale). The 95% CIs for percentages were calculated using the exact binomial distribution (Clopper-Pearson's method). Assuming that  $\log_{10}$  transformation of the titers/titers ratio followed a normal distribution, first, the mean and 95% CIs were calculated on  $\log_{10}$  (titers/ titers ratio) using the usual calculation for normal distribution, then antilog transformations were applied to the results of calculations, to compute GMTs/GMTRs and their 95% CIs.

For safety, the exact binomial distribution (Clopper-Pearson method) for proportions was used in calculations of the 95% CIs.

**Summary:**

***Disposition of Participants***

A total of 251 subjects were randomized out of the 372 eligible subjects (ie, aged 9 to 16 years on the day of first vaccination in CYD13/CYD30, had received the 3-dose schedule of CYD Dengue Vaccine, and had a PD3 serum sample available [at least 400 µL of serum]). Following randomization, 187 subjects were allocated to the CYD Dengue Vaccine Group and 64 subjects to the Placebo Group. The overall distribution of randomized subjects by country and treatment Group is summarized hereafter:

**Subjects by center and randomized Group - Randomized Subjects**

Center	Country	CYD Dengue Vaccine Group	Placebo Group	All
		(N=187) n (%)	(N=64) n (%)	(N=251) n (%)
All	All	187 (100.0)	64 (100.0)	251 (100.0)
001	Brazil	32 (17.1)	11 (17.2)	43 (17.1)
002	Colombia	57 (30.5)	19 (29.7)	76 (30.3)
003	Honduras	32 (17.1)	10 (15.6)	42 (16.7)
004	Mexico	49 (26.2)	18 (28.1)	67 (26.7)
005	Puerto Rico	17 (9.1)	6 (9.4)	23 (9.2)

n: number of subjects fulfilling the item listed

Overall, at 28 days post-booster injection (V04), 250 (99.6%) subjects were present (ie, 1 subject from the CYD Dengue Vaccine Group was absent) and 249 (99.2%) subjects provided a blood sample (ie, 2 subjects from the CYD Dengue Vaccine Group did not provide a sample).

At 6 months post-booster injection (V05), 244 (97.2%) subjects were present (ie, 6 subjects from the CYD Dengue Vaccine Group and 1 subject from Placebo Group were absent). At 12 months post-booster injection (V06), 239 (95.2%) subjects were present and at 24 months post-booster injection (V07) 229 (91.2%) subjects were present.

Over the duration of the study, a total of 22 subjects (5.7%) had an early termination (16 in the CYD Dengue Vaccine Group and 6 in the Placebo Group). A total of 12 subjects (4.8%) had an early termination because of non-compliance with the protocol (9 in the CYD Dengue Vaccine and 3 in the Placebo Group). In the Placebo Group, 2 subjects (3.1%) had an early termination related to a serious adverse event.

A total of 8 subjects (3.2%) voluntarily withdrew from the study for a reason not linked to an adverse event. The decision to terminate the participation of a subject was made either by the Investigator (for 5.6% of the subjects) or by the subject / legal representative (for 3.2% of the subjects).

All subjects that were present at V05, V06 and V07 provided blood samples.

***Analysis sets***

The primary objective of non-inferiority was assessed in the PPAS. The PPAS accounted for 96.0% of the randomized subjects. Overall, there were 241 subjects in the PPAS: 177 from the CYD Dengue Vaccine Group and 64 from the Placebo Group.

The secondary objective of superiority was assessed in the FAS. The FAS included all subjects who received an injection. A total of 248 subjects out of the 251 (98.8%) randomized subjects were included in the FAS.

The SafAS was the set of subjects who received at least one injection and were analyzed according to the treatment they actually received. As all subjects were injected, it accounted for 100.0% of the study population.

The PPAS and the SafAS were used for the evaluation of the other secondary objectives.

#### *Demographic and Baseline Characteristics*

Overall, there were 112 female (46.5%) and 129 male subjects (53.5%) included in the PPAS and the male to female sex ratio was 1.15. This imbalance in the sex ratio was more pronounced in the Placebo Group (1.37) than in the CYD Dengue Vaccine Group (1.08). The mean age at enrollment was similar in both treatment Groups (19.2 years in the CYD Dengue Vaccine Group; 19.0 years in the Placebo Group).

The dengue-immune status at pre-booster was similar between the 2 treatment Groups. There was 98.4% and 96.9% of subjects that were dengue-immune at pre-booster in the CYD Dengue Vaccine Group and in the Placebo Group, respectively.

The demographic characteristics were comparable across the different populations of analyses.

#### **Primary Objective: Non-inferiority**

The non-inferiority of the CYD Dengue Vaccine booster dose compared to the third dose of the primary series in CYD13 and CYD30 was analyzed by calculating the ratio of post-booster GMTs in CYD64 on PD3 GMTs in CYD13 or CYD30, for each serotype. The results of the non-inferiority analysis are presented in the following Table:

#### **Non-inferiority of CYD Dengue Vaccine booster dose compared to the third CYD Dengue Vaccine dose from CYD13 or CYD30 – Dengue PRNT – Per-Protocol Analysis Set**

Component	Post-dose 3 in CYD13 and CYD30 (PD3) (N=177)			Post-booster* dose in CYD64 (V04) (N=177)			Ratio (Post-booster/PD3)			Non-inferiority
	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	
<b>Serotype 1 [PRNT – 1/dil]</b>	176	316	(233; 428)	177	560	(421; 744)	176	1.66	(1.33; 2.06)	Yes
<b>Serotype 2 [PRNT – 1/dil]</b>	175	356	(275; 462)	177	657	(520; 830)	175	1.82	(1.43; 2.31)	Yes
<b>Serotype 3 [PRNT – 1/dil]</b>	175	640	(516; 794)	177	671	(535; 843)	175	1.04	(0.841; 1.27)	Yes
<b>Serotype 4 [PRNT – 1/dil]</b>	176	243	(195; 303)	177	344	(279; 424)	176	1.32	(1.01; 1.74)	Yes

M: number of subjects with available data at both time points

For each serotype, non-inferiority was to be demonstrated if the lower limit of the two-sided 95% CI for the ratio was greater than ½. Overall non-inferiority was to be demonstrated if all 4 serotypes achieve non-inferiority

\*Post-booster corresponds to 28 days post-booster.

The non-inferiority of the booster dose was demonstrated for each serotype and overall. The post-booster to PD3 GMTRs were above 1 for all serotypes and the corresponding 95% CIs were above 0.5 (from 0.841 for serotype 3 to 1.43 for serotype 2).

### **First Secondary Objective: Superiority**

As the overall non-inferiority of the CYD Dengue Vaccine booster could be demonstrated, a superiority analysis of the booster dose compared to the third dose of the CYD Dengue Vaccine in CYD13 or CYD30 was performed for each serotype using GMTRs. The results of the superiority analysis are presented in the following Table:

### **Superiority of CYD Dengue Vaccine booster dose compared to the third CYD Dengue Vaccine dose from CYD13 or CYD30 – Dengue PRNT – Full Analysis Set**

Component	Post-dose 3 in CYD13 and CYD30 (PD3) (N=185)			Post-booster* dose in CYD64 (V04) (N=185)			Ratio (Post-booster/PD3)			Superiority
	M	GM	(95% CI)	M	GM	(95% CI)	M	GM	(95% CI)	
<b>Serotype 1 [PRNT – 1/dil]</b>	184	302	(224; 406)	185	536	(404; 710)	184	1.66	(1.34; 2.06)	Yes
<b>Serotype 2 [PRNT – 1/dil]</b>	183	340	(264; 439)	185	653	(519; 823)	183	1.90	(1.49; 2.41)	Yes
<b>Serotype 3 [PRNT – 1/dil]</b>	183	611	(495; 755)	185	662	(529; 827)	183	1.07	(0.870; 1.31)	No
<b>Serotype 4 [PRNT – 1/dil]</b>	184	239	(193; 295)	185	347	(283; 426)	184	1.37	(1.05; 1.78)	Yes

M: number of subjects with available data at both time points

For each serotype, superiority was to be demonstrated if the lower limit of the two-sided 95% CI for the ratio was greater than 1. Overall superiority was to be demonstrated if all 4 serotypes achieve superiority

\*Post-booster corresponds to 28 days post-booster.

The superiority of the booster dose was demonstrated for serotype 1, serotype 2, and serotype 4. The superiority of the booster dose was not demonstrated for serotype 3 as the lower limit of the two-sided 95% CI of the GMTR was < 1 for this serotype. Therefore, the overall superiority of the CYD Dengue Vaccine booster dose could not be demonstrated.

### **Other secondary objectives: immunogenicity results**

#### **Immune response 28 days post-booster injection**

##### **i) Geometric mean titers by serotype**

At pre-booster injection, GMTs were comparable between CYD64 treatment Groups. They also tended to be within a similar range for serotype 1, serotype 2 and serotype 3. The GMTs of serotype 4 were lower in both Groups. GMTs ranged from 162 (1/dil) for serotype 4 to 360 (1/dil) for serotype 2 in the CYD Dengue Vaccine Group and from 161 (1/dil) for serotype 4 to 442 (1/dil) for serotype 3 in the Placebo Group.

After the CYD Dengue Vaccine booster injection, GMTs increased as compared to pre-booster injection level. For each serotype, the GMTs (1/dil) 28 days post-booster injection were as follows:

- For serotype 1, the GMT increased from 325 pre-booster injection to 560 28 days post-booster injection.
- For serotype 2, the GMT increased from 360 pre-booster injection to 657 28 days post-booster injection.
- For serotype 3, the GMT increased from 357 pre-booster injection to 671 28 days post-booster injection.
- For serotype 4, the GMT increased from 162 pre-booster injection to 344 28 days post-booster injection.

The post-booster/pre-booster GMTRs ranged from 1.59 for serotype 1 to 2.09 for serotype 4.

After the placebo injection, GMTs per serotype tended to remain stable. The GMTRs post-booster/pre-booster ranged from 0.798 for serotype 1 to 1.03 for serotype 2 in the Placebo Group.

#### ii) Seropositivity against each dengue virus serotype

At pre-booster injection, seropositivity rates against each serotype were high and similar in both treatment Groups.

After the CYD Dengue Vaccine booster injection, seropositivity rates (neutralizing Ab titers  $\geq 10$  [1/dil]) against each serotype tended to increase compared to pre-booster injection. Seropositivity rate against serotype 2 and serotype 3 increased with no overlap of the 95% CIs. After the placebo injection, pre-booster injection seropositivity rates per serotype tended to remain stable.

#### iii) Seropositivity against at least 1, 2, 3, or all 4 serotypes

In the CYD Dengue Vaccine Group, seropositivity rates increased compared to values at pre-booster injection. The seropositivity rate against at least 1 serotype increased from 98.9% (95% CI: 96.0; 99.9) at pre-booster injection to 100.0% (95% CI: 97.9; 100.0) 28 days post-booster injection. The seropositivity rate against all 4 serotypes increased from 85.3% (95% CI: 79.2; 90.2) to 96.0% (95% CI: 92.0; 98.4) 28 days post-booster injection. The 95% CIs did not overlap.

There was no meaningful change in the seropositivity rates against at least 1, 2, 3, or all 4 serotypes in the Placebo Group.

#### iv) Seroconversion rates

The seroconversion rates for 3 of the 4 serotypes were different between treatment Groups 28 days post-booster injection as 95% CIs did not overlap. The seroconversion rates were as follows:

- For serotype 1, the seroconversion rate was 16.9% (95% CI: 11.7; 23.3) in the CYD Dengue Vaccine Group and 3.1% (95% CI: 0.4; 10.8) in the Placebo Group.
- For serotype 2, the seroconversion rate was 19.2% (95% CI: 13.7; 25.8) in the CYD Dengue Vaccine Group and 17.2% (95% CI: 8.9; 28.7) in the Placebo Group.
- For serotype 3, the seroconversion rate was 20.3% (95% CI: 14.7; 27.0) in the CYD Dengue Vaccine Group and 4.7% (95% CI: 1.0; 13.1) in the Placebo Group.
- For serotype 4, the seroconversion rate was 19.8% (95% CI: 14.2; 26.4) in the CYD Dengue Vaccine Group and 6.3% (95% CI: 1.7; 15.2) in the Placebo Group.

One of the plausible explanations to the high seroconversion rate against serotype 2 in the Placebo Group as compared to the CYD Dengue Vaccine Group is the impact of a natural infection booster effect on GMTs.

#### Immune response PD3 and pre-booster injection

##### i) Geometric mean titers by serotype

At PD3, the GMTs tended to be comparable between serotype 1, serotype 2 and serotype 4 but were higher for serotype 3. GMTs ranged from 243 (1/dil) for serotype 4 to 640 (1/dil) for serotype 3 in the CYD Dengue Vaccine Group and from 232 (1/dil) for serotype 1 to 541 (1/dil) for serotype 3 in the Placebo Group. Overall, GMTs at PD3 in CYD13 and CYD30 were comparable between CYD64 treatment Groups.

At pre-booster injection, GMTs were comparable between CYD64 treatment Groups. They also tended to be within a similar range for serotype 1, serotype 2, and serotype 3. The GMTs for serotype 4 were lower in both Groups.

CYD Dengue Vaccine Group/Placebo Group GMTRs at PD3 and at pre-booster showed that there was no significant difference between CYD64 treatment Groups.

### **ii) Seropositivity against each dengue virus serotype**

PD3 seropositivity rates against each serotype were similar in both treatment Groups. The seropositivity rate against each serotype was above 90% in both treatment Groups at PD3.

At pre-booster injection, seropositivity rates against each serotype were similar in both treatment Groups. At pre-booster injection, seropositivity rates were still high and above 88% in the CYD Dengue Vaccine Group and above 90% in the Placebo Group.

### **iii) Seropositivity against at least 1, 2, 3, or all 4 serotypes**

The percentages of subjects seropositive against at least 1, 2, 3, and 4 serotypes were high in both treatment Groups at PD3 and at pre-booster injection.

### **Neutralizing Abs persistence up to 24 months**

#### **i) Geometric mean titers by serotype**

A decrease of neutralizing Ab titers was observed within 2 years following booster injection for all serotypes. The GMTs (1/dil) at 6, 12 months and 24 months post-booster injection for the 4 serotypes were as follows:

- For serotype 1, the GMT (1/dil) at 28 days was 560. The GMTs tended to be stable during this period with 270, 270, and 241 at M6, M12, and M24, respectively.
- For serotype 2, the GMT (1/dil) at 28 days was 657. The GMTs tended to decrease during this study period with 442, 269, and 246 at M6, M12, and M24, respectively.
- For serotype 3, the GMT (1/dil) at 28 days was 671. The GMTs tended to decrease during this period with 503, 303, and 341 at M6, M12, and M24, respectively.
- For serotype 4, the GMT (1/dil) at 28 days was 344. The GMTs tended to decrease during this period with 233, 169, and 150 at M6, M12, and M24, respectively.

In the Placebo Group, a gradual decrease of GMTs was observed for all serotypes from 28 days to 24 months post-injection. Overall, at 24 months post-booster injection, GMTs were somewhat higher in the CYD Dengue Vaccine Group than in the Placebo Group although the 95% CIs overlapped.

#### **ii) Seropositivity against each dengue virus serotype**

In the CYD Dengue Vaccine Group, the seropositivity rates tended to remain stable between 28 days and 24 months post-booster injection. There was a decrease in seropositivity rate against serotype 1 between 28 days and 24 months post-booster injection (the 95% CIs did not overlap). Seropositivity rates against each of the 4 serotypes remained above 87% from 28 days to 24 months post-booster injection and were higher compared to those observed at pre-booster injection for serotype 2, 3, and 4.

In the Placebo Group seropositivity rates remained above 84% throughout the study for all 4 serotypes.

Seropositivity rates 24 months post-booster injection tended to be higher in the CYD Dengue Vaccine Group compared to the Placebo Group for serotypes 3 and 4.

#### **iii) Seropositivity against at least 1, 2, 3, or all 4 serotypes**

In the CYD Dengue Vaccine Group, the seropositivity rates against at least 1, 2, and 3 serotypes tended to remain stable and above 92% between 28 days and 24 months post-booster injection. The seropositivity rates against all 4 serotypes tended to decrease from 28 days (96.0% [95% CI: 92.0; 98.4]), to 6 months (91.4% [95% CI: 86.2; 95.1]), 12 months (91.2% [95% CI: 85.9; 95.0]) and 24 months (86.6 [95% CI: 80.4; 91.4] post-booster injection. The 95% CIs associated to seropositivity rates against all 4 serotypes at 28 days and at 24 months post-booster injection did not overlap.

In the Placebo Group, the seropositivity rates against all 4 serotypes tended to be lower than in the CYD Dengue Vaccine Group and tended to remain stable between 28 days (82.8% [95% CI: 71.3; 91.1]), 6 months (85.7% [95% CI: 74.6; 93.3]), 12 months (88.7% [95% CI: 78.1; 95.3]) and 24 months (86.2% [95% CI: 74.6; 93.9]) post-booster injection.

## **Covariates**

### **i) Country**

In each country, GMTs against each serotype tended to remain stable or to decrease between PD3 and pre-booster injection. The most notable exceptions, seen in both treatment groups, were the increase of serotype 2 titers in Colombia, and of serotype 1 and serotype 2 titers in Honduras. These increases in GMTs were most likely due to natural infections.

In the CYD Dengue Vaccine Group, Ab titers against each serotype increased between pre-booster and post-booster injection in all countries except Honduras. In Honduras, post-booster GMT for serotype 1 was lower than that at pre-booster injection.

In countries with lower GMTs at pre-booster injection (ie, Brazil, Mexico, and Puerto Rico), seroconversion rates tended to be higher:

- In Brazil, seroconversion rates ranged from 18.8% for serotype 1 to 31.3% for serotype 3.
- In Mexico, they ranged from 31.8% for serotype 1 to 36.4% for serotype 3.
- In Puerto Rico, they ranged from 26.7% for serotype 4 to 40.0% for serotype 1 and serotype 3.
- In Colombia, they ranged from 3.6% for serotype 1, serotype 2, and serotype 3 to 9.1% for serotype 4.
- In Honduras, they ranged from 6.5% for serotype 1 and serotype 3 to 12.9% for serotype 2 and serotype 4.

In the Placebo Group, there was some seroconversion against serotype 2 in Colombia (21.1%) and Honduras (20.0%), and some seroconversion against all serotypes in Mexico (from 11.1% for serotype 1 to 27.8% for serotype 2).

In the CYD Dengue Vaccine Group, a decrease in GMTs was observed between 28 days and 12 months post-booster injection in all countries, however, this decay was more pronounced in some countries when compared to others. Mexico was the country with the most important decrease between 28 days and 12 months post-booster injection. On the contrary, Honduras was the country in which the difference between D28/D0 and M12/D0 GMTRs was least pronounced.

In the Placebo Group, there were no differences between D28/D0 and M12/D0 GMTRs as 95% CI overlapped. Serotype 2 in Colombia and serotype 1 in Honduras were the only exceptions with a significant difference between D28/D0 and M12/D0 GMTRs.

In the CYD Dengue Vaccine Group, in each country, changes in GMTs between 12 months and 24 months post-booster injection were less pronounced than those observed between 28 days and 12 months post-booster injection.

At 24 months post-booster injection, in the CYD Dengue Vaccine Group, Colombia was the country with the highest GMTs for serotype 1 and 4, Honduras had the highest GMTs for serotype 2 and Brazil had the highest GMTs for serotype 3. In the Placebo Group, Honduras was the country with the highest GMTs reported for all serotypes, except for serotype 3, for which the highest GMTs were observed in Brazil (followed by Honduras). For both the CYD Dengue Vaccine Group and the Placebo Group, the lowest GMTs were reported in Mexico and Puerto Rico.

For the CYD Vaccine Group, the GMTs (1/dil) ranged from 67.7 in Mexico (95% CI: 33.1; 138) to 636 in Colombia (95% CI: 369; 1078) for serotype 1, from 57.7 in Mexico (95% CI: 79.9; 173) to 630 in Honduras (95% CI: 297; 763) for serotype 2, from 118 in Mexico (95% CI: 41.0; 587) to 545 in Brazil (95% CI: 307; 969) for serotype 3, and from 110 in Puerto Rico (95% CI: 56.9; 214) to 190 in Colombia (95% CI: 154; 236) for serotype 4

### **ii) Dengue serostatus**

The immune response to CYD Dengue Vaccine booster injection was analyzed according to the serostatus of subjects at baseline (ie, D0 in CYD13, and CYD30).

Among the 177 subjects enrolled in the CYD Dengue Vaccine Group, 136 (approximately 77%) subjects were dengue-immune at baseline and 41 (approximately 23%) were dengue non-immune. In the Placebo Group, there were 46 (approximately 72%) subjects dengue-immune at baseline and 18 (approximately 28%) non-immune subjects.

At pre-booster injection, GMTs (1/dil) ranged from 224 (serotype 4) to 668 (serotype 1) in dengue-immune subjects and from 29.6 (serotype 1) to 54.9 (serotype 4) in dengue non-immune subjects. At 28 days post-booster injection, GMTs (1/dil) ranged from 343 (serotype 4) to 940 (serotype 1) in dengue-immune subjects and from 100 (serotype 1) to 347 (serotype 4) in dengue non-immune subjects.

Regardless of the immune status at baseline, the treatment group, and the dengue serotype, there was, in general, a decay of Ab titers between 28 days and 12 months post-booster injection. In the CYD Dengue Vaccine Group, the decay was more pronounced among dengue non-immune compared to immune subjects, with a similar trend for all serotypes. In the Placebo Group, the decay of GMTs was similar to that observed in the CYD Dengue Vaccine Group among immune subjects; however, among non-immune subjects titers were low and tended to remain stable throughout the study period.

Between 12 and 24 months post-booster injection, GMTs were stable or tended to slightly decrease in both the CYD Dengue Vaccine Group and the Placebo Group, regardless of the immune status at baseline.

At 12 months and 24 months post-booster injection, GMTs against each serotype were higher in dengue-immune subjects compared to dengue non-immune subjects in both treatment groups.

For dengue immune subjects, GMTs at 24 months post-booster injection tended to be lower than those observed at pre-booster injection. However, for dengue non-immune subjects, GMTs at 24 months post-booster injection were comparable to or tended to be higher than GMTs at pre-booster injection.

At 24 months post-booster injection, GMTs against each serotype tended to be similar between treatment groups (95% CIs overlapped), both in immune and in non-immune subjects.

### Safety

All subjects were assessed for immediate reactions, solicited reactions, unsolicited events, or reactions. SAEs were collected throughout the study and serious and non-serious AESIs were collected in defined time windows according to the type of AESI. An overview of the safety and reactogenicity up to 28 days post-booster injection is provided in the Table below:

#### Safety overview after booster injection – Safety Analysis Set

	CYD Dengue Vaccine Group (N=187)			Placebo Group (N=64)		
	n/M	%	(95% CI)	n/M	%	(95% CI)
<b>Subjects experiencing at least one:</b>						
Within 30 minutes after booster injection						
Immediate unsolicited AE	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
Immediate unsolicited Adverse Reaction (AR)	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
Within 28 days after booster injection						
Solicited reaction	114/187	61.0	(53.6; 68.0)	31/64	48.4	(35.8; 61.3)
Solicited injection site reaction	47/187	25.1	(19.1; 32.0)	12/64	18.8	(10.1; 30.5)
Solicited systemic reaction	105/187	56.1	(48.7; 63.4)	28/64	43.8	(31.4; 56.7)
Unsolicited AE	48/187	25.7	(19.6; 32.6)	13/64	20.3	(11.3; 32.2)
Unsolicited AR	2/187	1.1	(0.1; 3.8)	0/64	0.0	(0.0; 5.6)
Unsolicited non-serious AE	48/187	25.7	(19.6; 32.6)	13/64	20.3	(11.3; 32.2)
Unsolicited non-serious AR	2/187	1.1	(0.1; 3.8)	0/64	0.0	(0.0; 5.6)
Unsolicited non-serious injection site AR	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
Unsolicited non-serious systemic AE	48/187	25.7	(19.6; 32.6)	13/64	20.3	(11.3; 32.2)
Unsolicited non-serious systemic AR	1/187	0.5	(0.0; 2.9)	0/64	0.0	(0.0; 5.6)
AE leading to study discontinuation*	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
SAE	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
Death	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
Serious AESI	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
Non-serious AESI	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)

During 6-month follow-up period						
SAE	8/187	4.3	(1.9; 8.3)	2/64	3.1	(0.4; 10.8)
Death	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)
During 1-year follow-up period						
SAE	11/187	5.9	(3.0; 10.3)	4/64	6.3	(1.7; 15.2)
Death	0/187	0.0	(0.0; 2.0)	1/64	1.6	(0.0; 8.4)
During 2-year follow-up period						
SAE	19/187	10.2	(6.2; 15.4)	5/64	7.8	(2.6; 17.3)
Death	0/187	0.0	(0.0; 2.0)	2/64	3.1	(0.4; 10.8)
During the study						
SAE	19/187	10.2	(6.2; 15.4)	5/64	7.8	(2.6; 17.3)
Death†	0/187	0.0	(0.0; 2.0)	2/64	3.1	(0.4; 10.8)
Serious AESI	0/187	0.0	(0.0; 2.0)	0/64	0.0	(0.0; 5.6)

n: number of subjects experiencing the endpoint listed in the first column; M: number of subjects with available data for the relevant endpoint

\* Identified in the termination form as SAE or other AE or in an AE form that was at least Grade 1 and was within the time period indicated

† One additional death was reported over the duration of the study; it was the newborn baby of a subject in the CYD Dengue Vaccine Group.

### **Solicited reactions**

Overall, 61.0% of subjects in the CYD Dengue Vaccine Group and 48.4% in the Placebo Group experienced at least 1 solicited reaction after the booster injection. Among these subjects, 8.0% of subjects in the CYD Dengue Vaccine Group and 6.3% in the Placebo Group reported at least 1 Grade 3 solicited reaction. The Grade 3 solicited reactions that were reported in each Group were mostly systemic reactions.

Following the booster injection, the 2 treatment Groups were comparable in terms of number, intensity, time to onset, and duration of solicited reactions.

#### **i) Injection site reactions**

The most frequently reported solicited injection site reaction in both Groups was injection site pain (24.6% in the CYD Dengue Vaccine Group and 18.8% in the Placebo Group). One (0.5%) subject experienced an injection site erythema (in the CYD Dengue Vaccine Group), and no injection site swelling was reported in either Group. Most solicited injection site reactions reported were of Grade 1 intensity, occurred within 3 days, and resolved spontaneously within 3 days.

One (0.5%) subject in the CYD Dengue Vaccine Group reported a Grade 3 reaction (injection site pain).

#### **ii) Systemic reactions**

The most frequently reported solicited systemic reaction in both Groups was headache. At least 1 episode of headache was reported in 46.5% of subjects in the CYD Dengue Vaccine Group and in 34.4% of subjects in the Placebo Group after injection. The proportions of subjects who reported at least 1 episode of myalgia, malaise, and asthenia were within the same range and were similar across treatment Groups (between 21% and 32%). Some 7.3% of subjects in the CYD Dengue Vaccine Group and 9.5% of subjects in the Placebo Group experienced at least 1 episode of fever.

A total of 15 (8.0%) subjects in the CYD Dengue Vaccine Group and 4 (6.3%) subjects in the Placebo Group experienced at least 1 Grade 3 solicited systemic reaction. Headache was the most frequent Grade 3 systemic reaction. It was reported by 11 (5.9%) subjects from the CYD Dengue Vaccine Group and by 2 (3.1%) subjects in the Placebo Group.

### **Unsolicited non-serious AEs and ARs**

#### **i) Immediate unsolicited non-serious AEs and ARs**

In the CYD Dengue Vaccine Group, one (0.5%) subject experienced at least 1 immediate unsolicited non-serious AR. The subject experienced a Grade 2 lump in the right axilla. This systemic event spontaneously resolved after 5 days and was assessed as related to the booster injection by the Investigator.

## ii) Unsolicited non-serious AEs

Overall, within the 28 days post-booster injection, the proportion of subjects having experienced at least 1 unsolicited non-serious AE was 25.7% in the CYD Dengue Vaccine Group (48 subjects reported 64 AEs) and 20.3% in the Placebo Group (13 subjects reported 13 AEs). Most unsolicited non-serious AEs reported belonged to the following system organ class (SOCs): "Infections and infestations" (11.8% in the CYD Dengue Vaccine Group and 9.4% in the Placebo Group), "Gastrointestinal disorders" (4.8% in the CYD Dengue Vaccine Group and 3.1% in the Placebo Group), "Respiratory, thoracic and mediastinal disorders" (4.8% in the CYD Dengue Vaccine Group and 3.1% in the Placebo Group), "General disorders and administration site conditions" (2.7% in the CYD Dengue Vaccine Group and none in the Placebo Group), and "Nervous system disorders" (2.7% in the CYD Dengue Vaccine Group and 1.6% in the Placebo Group).

Unsolicited non-serious AEs occurred mainly within 3 days post-booster injection (5.9% in the CYD Dengue Vaccine Group and 3.1% in the Placebo Group) or after 15 days post-booster injection (11.2% in the CYD Dengue Vaccine Group and 7.8% in the Placebo Group). Most unsolicited non-serious AEs were of Grade 1 and Grade 2 severity and most resolved within 7 days or less.

A total of 10 (4.3%) Grade 3 unsolicited non-serious AEs were reported in 8 subjects in the CYD Dengue Vaccine Group. One (1.6%) Grade 3 unsolicited non-serious AE was reported in 1 subject in the Placebo Group.

## iii) Unsolicited non-serious ARs

One subject in the CYD Dengue Vaccine Group experienced an immediate unsolicited systemic AR (Grade 2 lump in the right axilla). A second subject in the CYD Dengue Vaccine Group experienced 1 unsolicited non-serious AR (Grade 1 muscular weakness). For both subjects, the ARs occurred within 1 day after booster injection, spontaneously resolved within 4-5 days, and belonged to the SOC "Musculoskeletal and connective tissue disorders".

## Deaths, other SAEs, and other significant AEs

### i) Deaths

Two subjects died 307 days and 743 days after placebo injection. In addition, a newborn baby died on the 15 December 2016. The neonate's mother was exposed to the CYD Dengue Vaccine. This death was reported as a SAE in the pharmacovigilance database. It was not counted as such in the clinical database as the baby was not a subject of the study.

These deaths were considered as unrelated to vaccination by the Investigator and the Sponsor.

### ii) SAEs

No SAEs were reported within 28 days after booster injection. A total of 24 SAEs were reported in 24 subjects between 28 days (V04) and 24 months (V07) after injection of either CYD dengue vaccine (19 subjects, 10.2%) or placebo (5 subjects, 7.8%). The SAEs were considered as not related to booster or placebo injection by the Investigator and the Sponsor.

### iii) AEs considered as significant

There were no AEs considered as significant.

## Pregnancies

A total of 19 pregnancies were reported, 15 in the CYD Dengue Vaccine Group and 4 in the Placebo Group. In the CYD Dengue Vaccine Group, 9 cases of pregnancy were linked to an AE while in the Placebo Group 1 pregnancy was linked to an AE.

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