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Sponsor: Sanofi Pasteur **Drug substance(s):** CYD Dengue Vaccine	**Study Identifiers:** U1111-1161-2813, IND 11219, NCT02824198 **Study code:** CYD63
Title of the study: 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 Singapore	
Study center(s): This was a multi-center trial conducted at 3 sites in Singapore.	
Study period: Date first subject/patient enrolled: 01/Jul/2016 Date last subject/patient completed: 18/Jan/2019	
Phase of development: Phase 2	

Objectives:

Primary objective:

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 CYD 28 trial (subjects from Group 1 only).

Secondary objectives:

Immunogenicity

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 CYD28 trial (subjects from Group 1 only).

2) To describe the immune responses elicited by the CYD dengue vaccine booster or placebo injection in subjects who received three doses of the CYD dengue vaccine in the CYD28 trial in all subjects.

3) To describe the neutralizing Abs levels of each dengue serotype PD3 (CYD28 subjects) and immediately prior to booster or placebo injection in all subjects.

4) To describe the neutralizing Ab persistence 6 months, 1 year and 2 years post booster or placebo injection in all subjects.

Safety

To evaluate the safety of booster vaccination with CYD dengue vaccine in all subjects.

Methodology:

This was a multi-center, observer-blind, randomized, placebo-controlled Phase II trial conducted in Singapore to assess i) 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 5 years (or more) earlier in healthy subject from the CYD28 trial; 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 260 subjects aged 9 to 45 years on the day of first vaccination of the study vaccine in CYD28 were planned to be recruited into the following 2 groups.

	Vaccination in CYD28	CYD63	
		N	Product to be injected
Group 1	CYD1/CYD2/CYD3*	195	CYD dengue vaccine booster
Group 2	CYD1/CYD2/CYD3*	65	Placebo

* 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 age (9 to 17 years, and 18 to 45 years, on the day of first injection in CYD28) and trial center.

At the first visit, each subject received a single dose of the CYD dengue vaccine booster or a placebo. Blood samples were to be collected at baseline (immediately prior to injection), at 28 days, 6 months, 1 year and 2 years post-injection 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. Immediate adverse events (AE) observed to occur within 30 min post-injection were collected. 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 to be 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 a 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 WT dengue 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. .

Number of subjects: Planned: 260

Randomized: 118

Treated: 118

Evaluated:

Immunogenicity: 116

Safety: 118

Diagnosis and criteria for inclusion:

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

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 45 years on the day of first injection of CYD dengue vaccine in CYD28, has received 3 doses of CYD dengue vaccine in the CYD28 trial, and has a post-Dose 3 [PD3] serum sample available [at least 300 L of serum]).
2) Presently in good health, based on medical history and physical examination.
3) 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)
4) Subject and parent(s)/legally acceptable representative(s) able to attend all scheduled visits and to comply with all trial procedures

Study treatments

Investigational medicinal product: CYD Dengue Vaccine (5-dose presentation)

Form: Powder and solvent for suspension for injection

Composition: Each 0.5 mL dose of reconstituted vaccine contains:

Active ingredients:

4.5 – 6 log10 cell-culture infectious dose 50% (CCID50) of each live, attenuated, recombinant dengue serotype 1, 2, 3, 4 virus

Excipients:

Essential amino acids, non-essential amino acids, L-arginine hydrochloride, sucrose, D-trehalose dihydrate, D-sorbitol, trometamol, urea, and sodium chloride.

Solvent:

NaCl 0.9%

Route of administration: Subcutaneous (SC)

Investigational medicinal product: Placebo

Form: Solution

Composition: NaCl 0.9%

Route of administration: SC

Duration of participation: The duration of each subject's participation in the trial was approximately 24 months.

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 and 28 days post-booster or placebo injection.

3) Individual post-booster/pre-booster GMTRs for each of the 4 parental dengue virus strains of the CYD dengue vaccine as determined by PRNT immediately prior 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 dose titer ≥ 40 (1/dil), or a pre-booster titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-booster dose titer as determined by PRNT immediately prior and 28 days post- 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 in all study subjects.

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 AEs 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 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 window 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 (i.e., 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 end 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 to Demonstrate Non-inferiority:

A non-inferiority testing approach was performed for each serotype specific endpoint to demonstrate the non-inferiority in terms of GMTRs for each subject, 28 days post-injection, of a CYD dengue vaccine booster dose compared to the third CYD dengue vaccine dose in subjects from CYD28.

Individual hypotheses for each serotype were as follows:

$H_0{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) \leq 1/2$

$H_1{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) > 1/2$

$Where\ i = 1,2,3\ and\ 4;\ V^i_{Booster}$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V^i_{PD3} is the immunogenicity titer 28 days after the third CYD dengue vaccine dose in CYD28 subjects.

Overall Hypothesis to Demonstrate Non-Inferiority:

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 CYD28 subjects).

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

H_1^G : all H_0^i *are rejected*

Statistical Methods

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

For each serotype, the non-inferiority was 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 rejected if the 4 individual null hypotheses were rejected simultaneously.

Hypotheses and Statistical Methods for the First Secondary Objective

Non-inferiority was not demonstrated for the primary endpoint. As a consequence, superiority hypotheses below were not performed.

Hypotheses

Individual Hypotheses for Each Serotype to Demonstrate Superiority:

A superiority hypotheses testing approach was to be performed for each serotype to demonstrate the superiority of a CYD dengue vaccine booster dose (28 days after the booster dengue vaccine injection) compared to the third CYD dose 28 days post vaccination in subjects from CYD28 trial, in terms of GMTR for each subject.

Individual hypotheses for each serotype were as follows:

$$H_0{}^i : GM\left(V^i_{Booster}/V^i_{PD3}\right) \leq 1$$
$$H_1{}^i : GM\left(V^i_{Booster}/V^i_{PD3}\right) > 1$$

$Where\ i = 1,2,3\ and\ 4;\ V^i_{Booster}$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V^i_{PD3} is the immunogenicity titer 28 days after third CYD dengue vaccine dose in CYD28.

Overall Hypothesis to Demonstrate Superiority:

The overall null hypothesis can be stated as: for at least 1 serotype, the post-booster dose response is not superior to the PD3 response.

H^G_0 : at least one H^i_0 *not rejected*
H^G_1 : all H^i_0 *are rejected*

Statistical Methods

A superiority test was to be performed using the 95% two sided confidence interval (CI) of $GM(V_{Booster}/V_{PD3})$ for each serotype; the 95% CI was to be calculated using paired t-test. Subjects with non-missing PD3 and post-booster dose titers were to be 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 analyses for other secondary objectives and additional objectives 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 GMTs (difference between GMTs on log scale). Assuming that log_{10} transformation of the titers/titers ratio follows 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 and GMTRs and their 95% CIs. The 95% CIs for percentages were calculated using the exact binomial distribution (Clopper-Pearson's method).

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 118 subjects were randomized out of the 260 eligible subjects (ie, aged 9 to 45 years on the day of first vaccination in CYD28, had received the 3-dose schedule of CYD dengue vaccine, and had a PD3 serum sample available [at least 300 µL of serum]). Following randomization, 89 subjects were allocated to the CYD Dengue Vaccine Group and 29 subjects to the Placebo Group. The overall distribution of randomized subjects by treatment group is summarized in table hereafter:

Subjects by center and randomized group – Randomized Subjects

Center	CYD Dengue Vaccine Group (N=89) n (%)	Placebo Group (N=29) n (%)	All (N=118) n (%)
All	89 (100.0)	29 (100.0)	118 (100.0)
001	20 (22.5)	6 (20.7)	26 (22.0)
002	30 (33.7)	10 (34.5)	40 (33.9)
005	39 (43.8)	13 (44.8)	52 (44.1)

n: number of subjects fulfilling the item listed

Overall, at 28 days post-booster injection (V04), 116 (98.3%) subjects were present and provided a blood sample. At 6 months post-booster injection (V05), 110 (93.2%) subjects were present and provided a blood sample. At 12 months post-booster injection (V06), 106 (89.8%) subjects were present and provided a blood sample. At 24 months post-booster injection (V07), 100 (84.7%) subjects were present and provided a blood sample.

Over the duration of the study, a total of 18 subjects (15.3%) had an early termination (15 [16.9%] in the CYD Dengue Vaccine Group and 3 [10.3%] in the Placebo Group).

Reasons for discontinuation during the study were mostly voluntarily withdrawals not due to an AE in 4.2% of subjects (5 [5.6%] in the CYD Dengue Vaccine Group and none in the Placebo Group), non-compliance with study protocol in 5.1% of subjects (5 [5.6%] in the CYD Dengue Vaccine Group and 1 [3.4%] in the Placebo Group), and “lost to follow-up” in 5.9% of subjects (5 [5.6%] in the CYD Dengue Vaccine Group and 2 [6.9%] in the Placebo Group). No subjects discontinued the study for an AE or SAE.

Analysis sets

The primary objective of non-inferiority was assessed in the PPAS in the CYD Dengue Vaccine Group. Overall, the PPAS accounted for 87.3% of the randomized subjects. There were 103 subjects in the PPAS: 75 from the CYD Dengue Vaccine Group and 28 from the Placebo Group.

The secondary objective of superiority was assessed in the FAS in the CYD Dengue Vaccine Group. Overall, the FAS included all subjects who received an injection. A total of 116 subjects out of the 118 (98.3%) randomized subjects were included in the FAS and 88 were in the CYD Dengue Vaccine Group.

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

Among the study population, the proportion of male (49.5%) and female subjects (50.5%) was well balanced (sex ratio was 0.98). The mean age at enrollment was similar in both treatment groups (28.6 years in the CYD Dengue Vaccine Group; 28.4 years in the Placebo Group).

The dengue immune status at pre-booster was high at pre-booster in 2 treatment groups and similar between the 2 treatment groups. There was 92.0% and 96.4% of subjects that were dengue immune at pre-booster in the CYD Dengue Vaccine Group and in the Placebo Group, respectively.

Primary Objective: Non-inferiority

The non-inferiority of the CYD dengue vaccine booster dose compared to the third dose of the primary series in CYD28 in Group 1 was analyzed by calculating the ratio of post-booster GMTs in CYD63 on PD3 GMTs in CYD28, 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 CYD28 - Dengue PRNT – Per-Protocol Analysis Set

	Post dose 3 in CYD28 (PD3) (N=75)			Post booster dose in CYD63 (V04) (N=75)			Ratio (Post booster/PD3)			
Component	**M**	**GM**	**(95% CI)**	**M**	**GM**	**(95% CI)**	**M**	**GM**	**(95% CI)**	**Non-inferiority**
Serotype 1 [PRNT – 1/dil]	74	20.3	(13.9; 29.5)	75	37.7	(26.4; 53.7)	74	1.34	(0.998; 1.79)	Yes
Serotype 2 [PRNT – 1/dil]	73	85.6	(55.7; 132)	75	56.2	(38.5; 82.1)	73	0.603	(0.439; 0.829)	No
Serotype 3 [PRNT – 1/dil]	72	102	(78.4; 133)	75	105	(77.4; 142)	72	0.979	(0.746; 1.28)	Yes
Serotype 4 [PRNT – 1/dil]	70	92.8	(72.5; 119)	75	123	(93.8; 161)	70	1.27	(0.918; 1.75)	Yes

M: number of subjects with available data at both time points
For each serotype, non-inferiority is to be demonstrated if the lower limit of the two-sided 95% CI for the ratio is greater than ½
Overall non-inferiority is to be demonstrated if all 4 serotypes achieve non-inferiority

The non-inferiority of the booster dose was demonstrated for serotypes 1, 3, and 4, even though the study was under-powered. The post-booster to PD3 GMTRs were above or close to 1 for these three serotypes and the corresponding lower limits of the two-sided 95% CIs were above 0.5 (0.998 for serotype 1, 0.746 for serotype 3, and 0.918 for serotype 4).

However, the non-inferiority of the booster dose was not demonstrated for serotype 2. The post-booster to PD3 GMTR was at 0.603 with a lower limit of the 95% CI below 0.5 (0.439). Therefore, the overall non-inferiority was not achieved and by consequence the superiority analysis was not performed. Although the non-inferiority of the CYD dengue vaccine booster was not demonstrated for serotype 2 in CYD63, the GMTs levels were not so far from the values observed at PD3. Moreover, the lack of adequate power could be a reason for failure of non-inferiority of the booster for the serotype 2 in this study.

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 treatment groups for the 4 serotypes. GMTs ranged from 13.5 (1/dil) for serotype 1 to 28 (1/dil) for serotype 4 in the CYD Dengue Vaccine Group and from 16.7 (1/dil) for serotype 1 to 44.9 (1/dil) for serotype 4 in the Placebo Group.

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

- For serotype 1, the GMT increased from 13.5 pre-booster injection to 37.7 at 28 days post-booster injection.
- For serotype 2, the GMT increased from 18.4 pre-booster injection to 56.2 at 28 days post-booster injection.
- For serotype 3, the GMT increased from 22.4 pre-booster injection to 105 at 28 days post-booster injection.
- For serotype 4, the GMT increased from 28.0 pre-booster injection to 123 at 28 days post-booster injection.

The post-booster/pre-booster GMTRs ranged from 1.74 for serotype 1 to 3.58 for serotype 4.

In the Placebo Group, the GMTs per serotype tended to remain stable after injection. The GMTRs post-booster/pre-booster ranged from 0.624 for serotype 2 to 0.822 for serotype 4.

Although the overall non-inferiority of the booster injection compared to PD3 was not achieved, there was still an effect of the booster injection on CYD Dengue Vaccine Group. First, as seen above, there was an increase of GMTs (1/dil) from pre-booster injection to 28 days post-booster injection for all 4 serotypes. Also, for all 4 serotypes, results at 28 days post-booster injection suggest that the booster dose tended to restore GMTs at the levels observed at PD3.

ii) Seropositivity against each dengue virus serotype

At pre-booster injection, seropositivity rates (neutralizing Ab titers ≥ 10 [1/dil]) against each serotypes were similar in both treatment groups in the PPAS and the FAS.

After the CYD dengue vaccine booster injection, seropositivity rates against each serotype increased compared to pre-booster injection. The seropositivity rates against each serotype were higher 28 days post booster injection as compared to pre booster injection, as there was no overlap of the 95% CIs.

After the CYD dengue vaccine booster injection, seropositivity rates against each serotype were comparable to those reached PD3 in CYD28 in all four serotypes.

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 93.3% (95% CI: 85.1; 97.8) at pre-booster injection to 100.0% (95% CI: 95.2; 100.0) 28 days post-booster injection. The seropositivity rate against all 4 serotypes increased from 24.0% (95% CI: 14.9; 35.3) to 68.0% (95% CI: 56.2; 78.3) 28 days post-booster injection. The 95% CIs did not overlap.

The percentages of subjects seropositive against at least 1, 2, 3 or all serotypes were comparable between post-booster injection and PD3 in CYD28.

Seropositivity rates against at least 1, 2, 3 or all 4 serotypes in the Placebo Group tended to remain stable pre-and post-booster injection.

iv) Seroconversion rates

The seroconversion rates for all 4 serotypes were higher in the CYD Dengue Vaccine Group 28 days post-booster injection. The seroconversion rates in the PPAS were as follows:

- For serotype 1, the seroconversion rate was 29.3% (95% CI: 19.4; 41.0) in the CYD Dengue Vaccine Group and 3.6% (95% CI: 0.1; 18.3) in the Placebo Group.
- For serotype 2, the seroconversion rate was 29.3% (95% CI: 19.4; 41.0) in the CYD Dengue Vaccine Group and 0.0% (95% CI: 0.0; 12.3) in the Placebo Group.
- For serotype 3, the seroconversion rate was 44.0% (95% CI: 32.5; 55.9) in the CYD Dengue Vaccine Group and 0.0% (95% CI: 0.0; 12.3) in the Placebo Group.
- For serotype 4, the seroconversion rate was 38.7% (95% CI: 27.6; 50.6) in the CYD Dengue Vaccine Group and 0.0% (95% CI: 0.0; 12.3) in the Placebo Group.

Immune response PD3 and pre-booster injection

i) Geometric mean titers by serotype

At PD3, the GMTs were comparable between serotype 2, serotype 3 and serotype 4. GMTs ranged from 20.3 (1/dil) for serotype 1 to 102 (1/dil) for serotype 3 in the CYD Dengue Vaccine Group and from 26.8 (1/dil) for serotype 1 to 107 (1/dil) for serotype 3 in the Placebo Group. Overall, GMTs at PD3 in CYD28 were comparable between CYD63 treatment groups compared to GMTs of other serotypes.

At pre-booster injection, GMTs were comparable between treatment groups for all serotypes. The GMTs for serotype 1 tended to be 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 CYD63 treatment groups.

ii) Seropositivity against each dengue virus serotype

PD3 seropositivity rates against serotypes 1, 3, and 4 were similar in both treatment groups in the PPAS. The PD3 seropositivity rate against serotype 2 tended to be higher in the CYD Dengue Vaccine Group (89.0%) compared to the Placebo Group (74.1%) but their 95% CIs overlapped.

At pre-booster injection, seropositivity rates against serotype 1, 2, and 3 were similar in both treatment groups. The pre-booster seropositivity rate against serotype 4 tended to be lower in the CYD Dengue Vaccine Group (70.7%) compared to Placebo Group (89.3%) but their 95% CIs overlapped.

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

At PD3, the seropositivity rate against all 4 serotypes was 43.2% in the CYD Dengue Vaccine Group and 42.9% in the Placebo Group. The percentages of subjects seropositive against all 4 serotypes were lower at pre-booster injection with 24.0% in the CYD Dengue Vaccine Group and 32.1% in the Placebo Group.

The percentages of subjects seropositive against at least 1, 2, 3 or all serotypes were comparable between post-booster injection and PD3 in CYD28.

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 and 24 months post-booster injection for the 4 serotypes were as follows:

Between 6 months and 24 months:

- For serotype 1, the GMT (1/dil) at 28 days was 37.7. The GMTs tended to decrease during this period with 25.1, 20.9, and 14.4 at M6, M12, and M24 post-injection, respectively.
- For serotype 2, the GMT (1/dil) at 28 days was 56.2. The GMTs tended to decrease during this period with 44.3, 39.3, and 41.6 at M6, M12, and M24 post-injection, respectively.
- For serotype 3, the GMT (1/dil) at 28 days was 105. The GMTs tended to decrease during this period with 65.1, 54.8, and 36.5 at M6, M12, and M24 post-injection, respectively.
- For serotype 4, the GMT (1/dil) at 28 days was 123. The GMTs tended to decrease during this period with 75.6, 61.9, and 50.1 at M6, M12, and M24 post-injection, respectively.

The GMTs (1/dil) for each serotype at 24 months post-booster injection remained above the level of GMTs pre-booster injection but were lower as compared to GMT PD3. In the Placebo Group, GMTs were stable for all serotypes from pre-booster injection to 24 months post-injection.

When considering the ratio Dengue/Placebo, there was a booster effect (lower bound of 95% CI > 1) up to 24 months post booster injection only for serotypes 3 and 4, respectively with a ratio of 2.17 (95%CI: 1.12; 4.19) and 2.05 (95%CI: 1.19; 3.51).

ii) Seropositivity against each dengue virus serotype

In the CYD Dengue Vaccine Group, the seropositivity rates tended to decrease between 28 days and 24 months post-booster injection for serotypes 1, 2, and 3. Seropositivity rates against serotype 4 tended to remain stable and were above 89.4% from 28 days to 24 months post-booster injection.

The seropositivity rates at 24 months post-booster injection remained higher compared to those observed at pre-booster injection for serotypes 2, 3, and 4.

In the Placebo Group, pre-booster injection seropositivity rates per serotype tended to remain unchanged after placebo injection.

Seropositivity rates 24 months post-booster injection tended to be higher in the CYD Dengue Vaccine Group compared to the Placebo Group for all serotypes except for serotype 1.

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

In the CYD Dengue Vaccine Group, seropositivity rates against at least 1 serotype tended to remain stable and above 93.9% between 28 days and 24 months post-booster injection. The seropositivity rates against at least 2, 3, or all 4 serotypes tended to decrease between 28 days and 24 months post-booster injection, but remained higher than the seropositivity rates pre-booster injection. The seropositivity rates against all 4 serotypes were 68.0% (95% CI: 56.2; 78.3) at 28 days and 37.9% (95% CI: 26.2; 50.7) at 24 months post-booster injection. The 95% CIs associated to seropositivity rates 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 (28.6% [95% CI: 13.2; 48.7]) and 24 months (26.9% [95% CI: 11.6; 47.8]) post-booster injection.

Covariates

i) Dengue Serostatus

The immune response to CYD dengue vaccine booster injection was analyzed according to the serostatus of subjects at baseline (i.e., D0 in CYD28).

Among the 75 subjects enrolled in the CYD Dengue Vaccine Group, 19 (approximately 25%) subjects were dengue-immune at baseline and 56 (approximately 75%) were non-immune. In the Placebo Group, there were 10 (approximately 36%) subjects dengue-immune at baseline and 18 (approximately 64%) non-immune subjects.

At pre-booster injection in CYD dengue vaccine group, GMTs (1/dil) ranged from 94.6 (serotype 4) to 230 (serotype 2) in dengue-immune subjects and from 6.79 (serotype 1) to 18.6 (serotype 4) in dengue non-immune subjects. At 28 days post-booster injection, GMTs (1/dil) ranged from 150 (serotype 4) to 278 (serotype 2) in dengue-immune subjects and from 23.1 (serotype 1) to 115 (serotype 4) in dengue non-immune subjects. GMTs at pre-booster injection were higher in dengue-immune subjects at baseline. At 6 months post-booster injection, GMTs (1/dil) ranged from 110 (serotype 1) to 301 (serotype 2) in dengue-immune subjects and from 15.4 (serotype 1) to 61.8 (serotype 4) in dengue non-immune subjects. At 12 months post-booster injection, GMTs (1/dil) ranged from 91.8 (serotype 4) to 295 (serotype 2) in dengue-immune subjects and from 11.3 (serotype 1) to 53.8 (serotype 4) in dengue non-immune subjects. At 24 months post-booster injection, GMTs (1/dil) ranged from 71.7 (serotype 1) to 354 (serotype 2) in dengue-immune subjects and from 7.88 (serotype 1) to 39.1 (serotype 4) in dengue non-immune subjects.

In the CYD Dengue Vaccine Group, dengue serostatus at baseline were different in the seroconversion rate against serotypes 2, 3, and 4. The seroconversion rates were higher in the dengue non-immune group of subjects.

ii) By age

Among the 75 subjects included in PPAS in the CYD Dengue Vaccine Group, 47 (approximately 63%) subjects were aged from 9-17 years and 28 (approximately 37%) were aged from 18-45 years when they received first dengue vaccine injection in CYD 28. In the Placebo Group, there were 17 (approximately 61%) subjects from 9-17 years and 11 (approximately 39%) from 18-45 years.

Overall, Ab titers against each serotype at PD3, at pre-booster injection, at 28 days post-booster injection, as well as up to 24 months post-booster injection, were higher in subjects from 18-45 years group. The age at pre-booster injection seems to have no impact on the seroconversion rates against all serotypes up to 28 days post-booster injection. There was no meaningful difference in the seroconversion rates between age groups

Safety

After the CYD dengue vaccine or the placebo injection, all subjects were assessed for immediate reaction, 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 24 months post-booster injection is provided in the table below:

Safety overview after booster injection - Safety Analysis Set

Subjects experiencing at least one:	CYD Dengue Vaccine Group (N=89) n/M	%	(95% CI)	Placebo Group (N=29) n/M	%	(95% CI)
Within 30 minutes after booster injection						
Immediate unsolicited AE	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Immediate unsolicited AR	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Within 28 days after booster injection						
Solicited reaction	45/88	51.1	(40.2; 61.9)	9/28	32.1	(15.9; 52.4)
Solicited injection site reaction	29/88	33.0	(23.3; 43.8)	7/28	25.0	(10.7; 44.9)
Solicited systemic reaction	34/88	38.6	(28.4; 49.6)	8/28	28.6	(13.2; 48.7)
Unsolicited AE	18/89	20.2	(12.4; 30.1)	3/29	10.3	(2.2; 27.4)
Unsolicited AR	1/89	1.1	(0.0; 6.1)	0/29	0.0	(0.0; 11.9)
Unsolicited non-serious AE	17/89	19.1	(11.5; 28.8)	3/29	10.3	(2.2; 27.4)
Unsolicited non-serious AR	1/89	1.1	(0.0; 6.1)	0/29	0.0	(0.0; 11.9)
Unsolicited non-serious injection site AR	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Unsolicited non-serious systemic AE	17/89	19.1	(11.5; 28.8)	3/29	10.3	(2.2; 27.4)
Unsolicited non-serious systemic AR	1/89	1.1	(0.0; 6.1)	0/29	0.0	(0.0; 11.9)
AE leading to study discontinuation†	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
SAE	1/89	1.1	(0.0; 6.1)	0/29	0.0	(0.0; 11.9)
Death	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Serious AESI	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Non-serious AESI	3/89	3.4	(0.7; 9.5)	0/29	0.0	(0.0; 11.9)
During 6-month follow-up period						
SAE	3/89	3.4	(0.7; 9.5)	0/29	0.0	(0.0; 11.9)
Death	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Serious AESI	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
During 1-year follow-up period						
SAE	3/89	3.4	(0.7; 9.5)	0/29	0.0	(0.0; 11.9)
Death	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Serious AESI	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
During 2-year follow-up period						
SAE	4/89	4.5	(1.2; 11.1)	0/29	0.0	(0.0; 11.9)
Death	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Serious AESI	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
During the study						
SAE	5/89	5.6	(1.8; 12.6)	0/29	0.0	(0.0; 11.9)
Death	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)
Serious AESI	0/89	0.0	(0.0; 4.1)	0/29	0.0	(0.0; 11.9)

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

Solicited reactions

Overall, 51.1% of subjects in the CYD Dengue Vaccine Group and 32.1% in the Placebo Group experienced at least 1 solicited reaction after the booster injection. Among these subjects, 1 subject in each group reported at least 1 Grade 3 solicited reaction. The Grade 3 solicited reaction that was reported in CYD Dengue vaccine group was an injection site reaction and the Grade 3 solicited reaction that was reported in the Placebo Group was a systemic reaction.

i) Injection site reactions

The most frequently reported solicited injection site reaction in both groups was injection site pain (33.0% in the CYD Dengue Vaccine Group and 25.0% in the Placebo Group). One (1.1%) 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.

One (1.1%) subject in the CYD Dengue Vaccine Group reported a Grade 3 injection site pain.

ii) Systemic reactions

The most frequently reported solicited systemic reactions in both groups were headache and myalgia. At least 1 episode of headache was reported in 26.1% of subjects in the CYD Dengue Vaccine Group and in 10.7% of subjects in the Placebo Group after injection. At least 1 episode of myalgia was reported in 23.9% of subjects in the CYD Dengue Vaccine Group and in 25.0% of subjects in the Placebo Group after injection. The proportions of subjects who reported at least 1 episode of malaise and asthenia were within the same range and were similar across treatment groups (between 10.7% and 17.0%). Two subjects in the CYD Dengue Vaccine Group (2.3%) experienced at least 1 episode of fever.

One (3.6%) subject in the Placebo Group experienced at least 1 Grade 3 solicited systemic reaction. This subject reported Grade 3 myalgia and malaise. Myalgia started on D4 and malaise started on D12. Both reactions resolved within 3 days with medication.

Unsolicited non-serious AEs and ARs

i) Immediate unsolicited non-serious AEs and ARs

Within 30 minutes following booster injection, none of the subjects experienced any immediate unsolicited AE.

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 19.1% in the CYD Dengue Vaccine Group (17 subjects reported 23 AEs) and 10.3% in the Placebo Group (3 subjects reported 3 AEs).

The SOCs with the highest incidence of AEs (more than 1.0% of subjects) were:

- “Infections and infestations” with 10 AEs reported by 9 subjects (10.1%) in the CYD Dengue Vaccine Group and 1 AE experienced by 1 subject (3.4%) in the Placebo Group.
- “Respiratory, thoracic and mediastinal disorders” with 3 AEs reported by 3 subjects (3.4%) in the CYD Dengue Vaccine Group and 1 AE experienced by 1 subject (3.4%) in the Placebo Group.
- “Skin and subcutaneous tissue disorder” with 6 AEs reported by 3 subjects (3.4%) in the CYD Dengue Vaccine Group and no occurrences in the Placebo Group.
- “Gastrointestinal disorders” with 1 AE reported by 1 subject (1.1%) in the CYD Dengue Vaccine Group and no occurrences in the Placebo Group.
- “Nervous system disorders” with no occurrences in the CYD Dengue Vaccine Group and 1 AE experienced by 1 subject (3.4%) in the Placebo Group.
- “Cardiac disorders” with 2 AEs reported by 1 subject (1.1%) in the CYD Dengue Vaccine Group and no occurrences in the Placebo Group.
- “Musculoskeletal and connective tissue disorders” with 1 AE reported by 1 subject (1.1%) in the CYD Dengue Vaccine Group and no occurrences in the Placebo Group.

Unsolicited non-serious AEs occurred mainly within 3 days post-booster injection (6.7% in the CYD Dengue Vaccine Group and 0.0% in the Placebo Group) or after 15 days post-booster injection (3.4%, in the CYD Dengue Vaccine Group and 6.9% 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.

Only 1 (3.4%) Grade 3 unsolicited non-serious AE was reported in 1 subject in the Placebo Group. None were reported in the CYD Dengue Vaccine Group. The Grade 3 unsolicited non-serious AE reported belonged to the SOC "Respiratory, thoracic and mediastinal disorders". The subject reported cough which started on D12 and resolved spontaneously within 3 days.

iii) Unsolicited non-serious ARs

One subject in the CYD Dengue Vaccine Group experienced 1 unsolicited non-serious AR (Grade 1 pruritus). The AR occurred within 1 day after booster injection, spontaneously resolved within 4-5 days, and belonged to the SOC "Skin and subcutaneous tissue disorders".

iv) SAEs

One subject experienced an SAE that began within 28 days after booster injection.

Three subjects experienced an SAE that occurred between 28 days and 6 months post-booster injection and one subject experienced an SAE that occurred between 12 and 24 months post-booster injection.

v) AESIs

A total of 4 non-serious AESIs were reported in 3 subjects within 28 days after booster injection. These 4 cases belonged to the SOC "Skin and subcutaneous disorder":

- One subject experienced Grade 1 pruritus which started within 1 day after injection and resolved spontaneously within 4 days.
- One subject experienced 2 episodes of Grade 2 urticaria which started 2 days and 5 days after injection and both were resolved within 1 day with medication.
- One subject experienced Grade 2 generalized rash which started within 1 day after injection and resolved spontaneously after 82 days.

vi) Deaths

There were no deaths reported within 24 months after booster injection.

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