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Sponsor: Sanofi Pasteur **Drug substance(s):** CYD Dengue Vaccine	**Study Identifiers:** NCT00880893, 2014-001713-26 **Study code:** CYD28
Title of the study: Immunogenicity and Large-Scale Safety of Tetravalent Dengue Vaccine in Healthy Subjects Aged 2 to 45 Years in Singapore	
Study centers: This trial involved 5 centers in Singapore.	
Study period: Date first subject enrolled: 07/Apr/2009 Date last subject completed: 14/Oct/2014	
Phase of development: Phase II	
Objectives: **Primary Objectives:** ***Safety*** To evaluate in all subjects: • The safety after each dengue vaccination in terms of injection site and systemic reactogenicity. • The occurrence of SAEs throughout the trial period. ***Immunogenicity*** To evaluate the humoral immune response to each dengue serotype after each vaccination in a subset of subjects **Secondary Objectives:** To evaluate the persistence of the humoral immune response during 4 years after the last vaccination in a subset of subjects.	
Methodology: CYD28 was a multicenter, observer-blind for the first injection and single-blind for the second and third injections, randomized, controlled, Phase II trial conducted in approximately 1200 subjects. Subjects were randomized to the CYD dengue vaccine group (CYD dengue vaccine) or to the control groups (Placebo + influenza or hepatitis A vaccine) and received three vaccine injections (at 0, 6 and 12 months) as follows: • **CYD Dengue Vaccine Group** received the CYD dengue vaccine as first, second, and third injections. • **Control Group** received a placebo as first vaccination. Subjects <12 years were to receive hepatitis A as second and third vaccinations. Subjects ≥12 years were to receive influenza vaccine of Northern and Southern hemisphere formulations as second and third vaccinations, respectively. Randomization was performed according to a 3:1 ratio (3 subjects in the CYD dengue vaccine group for each subject in the control group), with stratification by age (2 to 11 years, 12 to 17 years, and 18 to 45 years) and trial center.	

There were four subsets of subjects for immunogenicity assessments:

- Two main immunogenicity subsets: humoral immune response to the four dengue virus serotypes (in all age groups, N=600), divided into Cohort 1 (N=300) and Cohort 2 (N=300) with different time points for blood sampling.
- Cell-mediated immunity (CMI) subset: in adolescents and adults (N=80) from Cohort 1.
- Cross-neutralization subset: humoral immune response to field dengue isolates (in all age groups, N=240), from Cohort 2

All subjects were followed up to 4 years after the third vaccination (i.e., 4-year follow-up).

Safety data (immediate AEs, solicited injection site and systemic reactions, unsolicited AEs, and SAEs) were assessed after each vaccination for all subjects. There was a safety follow-up up to 4 years after the third vaccination for all the subjects. An Independent Data Monitoring Committee (IDMC) reviewed safety data on a regular basis. This included any fatal outcome, related SAEs, serious virologically confirmed and suspected dengue cases.

Number of subjects:

Planned : 1200

Screened: 1199

Randomized: 1198

Evaluated:

Immunogenicity: 450

Safety: 1198

Efficacy: 585

Diagnosis and criteria for inclusion:

1. Aged from 2 to 45 years on the day of inclusion.
2. Subject in good health, based on medical history and physical examination.
3. Provision of informed consent form (and assent form for subjects aged 6 to 12 years) signed by the subject and by the parent(s) or another legally acceptable representative for subjects aged less than 21 years.
4. Subject and parent(s)/legally acceptable representative able to attend all scheduled visits and comply with all trial procedures.
5. For a woman of child-bearing potential, avoid becoming pregnant (use of an effective method of contraception or abstinence) for at least 4 weeks before the first vaccination until 4 weeks after the last vaccination.

Study treatments

Investigational product: CYD dengue vaccine

Form: Powder and solvent for suspension for injection.

Composition: Each 0.5 mL dose of reconstituted vaccine contains:

5 ± 1 log10 cell-culture infectious dose 50% (CCID50) of each live, attenuated, dengue serotype 1, 2, 3, 4 virus

Excipients: essential amino acids, non-essential amino acids, L-arginine chlorhydrate, saccharose, D-trehalose dihydrate, D-sorbitol, tris (hydroxymethyl) aminomethane, and urea

Solvent: NaCl 0.4% containing Human Serum Albumin 2.5%

Route of administration: Subcutaneous (SC).

Control Product 1: Placebo (NaCl).

Form: Liquid.

Composition : NaCl 0.9%.

Route of administration: SC

Control Product 2: Influenza vaccine (split virion, inactivated), Northern and Southern hemispheres year 2009-2010 formulations (Vaxigrip®)

Form: Suspension for injection in prefilled syringe.

Composition: Split influenza virus*, inactivated that contained Ags equivalent to the following serotypes:

- A/(H1N1)-like strain 15 µg hemagglutinin (HA)
- A/(H3N2)-like strain 15 µg HA
- B/-like strain 15 µg HA
- Buffered saline solution q.s. 0.5 mL

* propagated in fertilized hens' eggs from healthy chick flocks

Route of administration: SC

Duration of participation: The expected duration of each subject's participation in the trial is 5 years.

Criteria for evaluation:

Primary Endpoints:

Safety

- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term [PT]), duration, intensity, action taken, whether it led to discontinuation or not, and relationship to vaccination of any unsolicited systemic AEs reported in the 30 minutes after each vaccination.
- Occurrence, time to onset, number of days of occurrence, action taken, whether it led to discontinuation or not, and intensity of solicited (pre-listed in the subject's diary and electronic case report form [eCRF]) injection site reactions occurring up to 7 days after each vaccination.
- Occurrence, time to onset, number of days of occurrence, action taken, whether it led to discontinuation or not, and intensity of solicited systemic reactions occurring up to 14 days after each vaccination.
- Occurrence, nature (MedDRA PT), time to onset, duration, intensity, action taken, whether it led to discontinuation or not, and relationship to vaccination (for systemic AEs only) of unsolicited (spontaneously reported) AEs up to 28 days after each vaccination.
- Occurrence of SAEs for all subjects as follows:
- Up to 6 months after the last vaccination: all SAEs.
- From 6 months after the last vaccination until the end of trial: only related and fatal (even if unrelated) SAEs.

Immunogenicity

- Neutralizing Ab level against each of the four dengue virus serotypes of CYD dengue vaccine constructs were measured by dengue plaque reduction neutralizing test [PRNT] assay in sera collected from a subset of 600 subjects as follows:
- Before the first and the third injections and 28 days after the first and the third injections in a randomized subset of 300 subjects (Cohort 1)
- Before the first and the second injections, and 28 days after the second and the third injections in a randomized subset of 300 subjects (Cohort 2)

Secondary Endpoints:

Neutralizing Ab levels against each of the four dengue virus serotypes of tetravalent dengue vaccine constructs were planned to be measured in sera collected from a subset of 600 subjects each year during 4 years after the last vaccination

Statistical methods:

Statistical Methods for Primary Endpoints

No hypotheses were tested for the primary endpoints.

Safety

For each age group/all subjects combined within each treatment group, the number and percentage of subjects experiencing any injection site reaction (solicited and unsolicited) after each injection were calculated. These events and reactions were also presented according to their nature (MedDRA PT), intensity (Grade 1, 2 or 3), time of onset, number of days of occurrence (solicited reactions), duration (unsolicited events), relationship (unsolicited events), action taken, and whether they led to trial termination, by each age group/all subjects within each treatment group. Solicited reactions were systematically considered as related to vaccination.

The number and percentage of subjects with SAEs up to 6 months after the last injection by each age group/all subjects within each treatment group were calculated by outcome, seriousness, and relationship to the study vaccine. In addition, the number and percentage of subjects with related and fatal (even if unrelated) SAEs were summarized during the 4-year follow-up period.

The number and percentage of subjects with a detected viremia (i.e., ≥ Lower Limit of Detection [LLOD]) and with a quantified viremia (i.e., ≥ Lower Limit of Quantification [LLOQ]) obtained from acute blood samples for whatever the serotype (vaccinal viremia by yellow fever (YF) RT-PCR; WT viremia by dengue screen RT-PCR) and for each of the four serotypes (vaccinal viremia by CYD RT-PCRs; WT viremia by WT RT-PCRs) were calculated in subjects either with hospitalized dengue cases regardless of time of event after injection or experiencing a fever ≥ 38°C on at least 2 consecutive days within 28 days after each injection. Level of viremia was summarized for the population of subjects with quantified viremia (i.e. ≥ LLOQ) using descriptive statistics.

Likewise, the number and percentage of subjects experiencing a biological abnormality with Grade 3 intensity were calculated.

Immunogenicity

Analysis of dengue neutralizing Ab levels was done for each dengue virus serotype, by age group/all subjects within each treatment group before and 28 days after the first injection and before and 28 days after the third injection for Cohort 1, at baseline, before and 28 days after the second injection, and 28 days after the third injections for Cohort 2.

Analysis of cellular immune response from Luminex and Intracellular Cytokine Staining (ICS) tests and for each cytokines, cells and stimulations was done for adolescent (12 to 17 years old) and adult group (18 to 45 years old) in Cohort 1 using geometric means (GMs), number and percentage of subjects with value ≥ LLOD, median, Q1, Q3, Min, Max, and 95% confidence interval (CI).

The numbers and percentages of subjects with geometric mean titer (GMT) ≥ 10 [1/dil] and GMTs of both humoral and cellular immune response were presented for each of the four dengue virus serotypes, by Cohort, by age group, in all subjects of each treatment group according to baseline serological status. The 95% CIs for the single proportion of each humoral and cellular immune response were calculated using the exact binomial method (Clopper-Pearson methods).

Summary:

Trial population

A total of 1199 subjects were screened in the study and 1198 subjects were randomized (898 subjects in the CYD dengue vaccine group and 300 in the control group): 1 subject was not randomized because of pregnancy. By age group, 236 children, 141 adolescents, and 521 adults in the CYD dengue vaccine group and 80 children, 46 adolescents, and 174 adults in control group were randomized. Five centers participated in the study and 114 to 337 subjects were randomized in each center.

Among the 898 subjects randomized in the CYD dengue vaccine group, all received the first injection, 861 (95.9%) subjects received the second injection, and 838 (93.3%) subjects received the third injection. Among the 300 subjects randomized in the control group, all received the first injection, 285 (95.0%) subjects received the second injection, and 280 (93.3%) subjects received the third injection. Six randomized subjects were subsequently found to be not-eligible and a total of 895 in the CYD dengue vaccine group and 297 in the control group were eligible. The 6 subjects randomized but found to be not-eligible were withdrawn due to non-compliance.

In the CYD dengue vaccine group, 835 (93.0%) subjects completed the injection phase (from V01 to V06). The most frequent reasons for not completing were non-compliance with the protocol (12 [1.3%] subjects), lost-to-follow-up (14 [1.6%]), and voluntary withdrawal not due to an AE (31 [3.5%] subjects).

In the control group, 276 (92.0%) subjects completed the injection phase. The most frequent reasons for not completing were non-compliance with the protocol (6 [2.0%] subjects), due to lost-to-follow-up (6 [2.0%] subjects), and voluntary withdrawal not due to an AE (10 [3.3%] subjects).

A total of 585 subjects (438 in the CYD dengue vaccine group and 147 in the control group) were included in the Full Analysis Set (FAS) (290 in Cohort 1 and 295 in Cohort 2).

The Per Protocol (PP) population was defined for each injection, i.e. PP1, PP2 and PP3. A total of 430 subjects from the CYD dengue vaccine group were included in the PP1 set, 382 in the PP2 set, and 353 in the PP3 set. A total of 142 subjects from the control group were included in the PP1 analysis set, 124 subjects in the PP2 analysis set, and 109 subjects in the PP3 analysis set. The reasons for exclusion from one of the PP sets were inclusion/exclusion criteria not fulfilled, blood samples taken outside of pre-defined window after injection, not receiving the correct number of doses of vaccine, the time between injections being outside the pre-defined windows and other definitive contraindications.

All the 1198 injected subjects (898 in the CYD dengue vaccine group and 300 in the control group) were included in the Safety Analysis Set for post-injection 1; 1146 (861 in the CYD dengue vaccine group and 285 in the control group) for post-injection 2, and 1118 (838 in the CYD dengue vaccine group and 280 in the control group) for post-injection 3.

Of the 585 subjects included in the FAS, 552 (94.4% [414 in the CYD dengue vaccine group and 138 in the control group]) were present for the 1-year follow-up. The most frequent reasons for not completing the 1-year follow-up were non-compliance with the protocol (6 [1.0%] subjects), lost-to-follow-up (24 [4.1%] subjects), and voluntary withdrawal not due to an AE (3 [0.5%] subjects). A total of 513 subjects (87.7% [390 subjects in the CYD dengue vaccine group and 123 subjects in the control group]) were present for the 4-year follow-up. The most frequent reasons for not completing the 4-year follow-up were lost-to-follow-up (38 [6.5%] subjects), voluntary withdrawal not due to an AE (22 [3.8%] subjects) and non compliance with the protocol (11 [1.9%] subjects); 1 subject in the CYD dengue vaccine group did not complete the 4-year follow-up due to an SAE.

Demography

In the CYD dengue vaccine group, there were slightly more females than males in children and in adults, and there was a similar number of females and males in adolescents. In the control group, there were slightly more males than females in all age groups.

For each age group (children aged 2 to 11 years, adolescents aged 12 to 17 years, and adults aged 18 to 45 years), the height, weight, and body mass index were similar between the two treatment groups and were in accordance with the age. The observation of minimum and maximum values showed that no values were of particular concern.

Safety

In the control group, the first injection was a placebo for all subjects and the second and the third injection were active controls (hepatitis A vaccines for children and influenza vaccines for adolescents and adults); subjects in the CYD dengue vaccine group received the CYD dengue vaccine for each injection.

Solicited reactions after each injection

Table 1: Solicited reactions by age group - Safety Analysis Set

		CYD Dengue Vaccine Group (N=898)			Control Group (N=300)		
		Post-injection 1	Post-injection 2	Post-injection 3	Post-injection 1	Post-injection 2	Post-injection 3
		n/N	n/N	n/N	n/N	n/N	n/N
Children	**Solicited reaction**	53.4% (126/236)	53.6% (126/235	48.1% (112/233)	43.8% (35/80)	55.3% (42/76)	38.2% (29/76)
	Solicited injection site reaction	35.2% (83/236)	41.3% (97/235)	35.6% (83/233)	30.0% (24/80)	42.1% (32/76)	35.5% (27/76)
	Solicited systemic reaction	39.4% (93/236)	41.3% (97/235)	33.9% (79/233)	28.8% (23/80)	39.5% (30/76)	26.3% (20/76)
Adolescents	**Solicited reaction**	58.9% (83/141)	39.7% (54/136)	43.0% (58/135)	47.8% (22/46)	46.7% (21/45)	45.5% (20/44)
	Solicited injection site reaction	28.4% (40/141)	29.4% (40/136)	26.7% (36/135)	28.3% (13/46)	44.4% (20/45)	38.6% (17/44)
	Solicited systemic reaction	53.2% (75/141)	30.9% (42/136)	28.9% (39/135)	41.3% (19/46)	37.8% (17/45)	36.4% (16/44)
Adults	**Solicited reaction**	55.7% (287/515)	54.1% (264/488)	51.7% (242/468)	41.5% (71/171)	71.3% (117/164)	69.0% (109/158)
	Solicited injection site reaction	30.9% (159/515)	38.5% (188/488)	39.3% (184/468)	16.4% (28/171)	62.8% (103/164)	62.7% (99/158)
	Solicited systemic reaction	46.8% (241/515)	38.7% (189/488)	34.6% (162/468)	39.8% (68/171)	42.7% (70/164)	43.0% (68/158)

First injection

The frequency of solicited reactions (including both solicited injection site and solicited systemic reactions) was slightly higher in the CYD dengue vaccine group than in the control group that received placebo (53.4% *versus* 43.8% in children; 58.9% *versus* 47.8% in adolescents; 55.7% *versus* 41.5% in adults). The same trend was observed when solicited reactions were taken individually, except for solicited injection site reactions in adolescents where the frequencies were similar in both treatment groups (28.4% *versus* 28.3%).

Second injection

The overall frequencies of solicited reactions after the second injection of the CYD dengue vaccine were similar to those after the first injection. The frequency of solicited reactions was similar in the CYD dengue vaccine group to the control group in children (53.6% *versus* 55.3%, slightly lower in adolescents (39.7% *versus* 46.7%), and lower in adults (54.1% *versus* 71.3%). The same trend was observed when solicited reactions were taken individually.

Third injection

The overall frequencies of solicited reactions after the third injection of the CYD dengue vaccine were similar to those after the first and second injections. The frequency of solicited reactions was slightly higher in the CYD dengue vaccine group than in the control group in children (48.1% *versus* 38.2%), similar in adolescents (43.0% versus 45.5%), and slightly lower in adults (51.7% *versus* 69.0%). The same trend was observed when solicited reactions were taken individually. The systemic reactions did not increase after second and third doses of CYD dengue vaccine, but rather had a tendency to decrease especially in the adolescent group.

Solicited injection site reactions after each injection

The most frequently reported solicited site reaction in both treatment groups and in all age groups was pain. After the first injection, pain was reported by approximately 30.0% of subjects in the CYD dengue vaccine group regardless of the age group, and by approximately 30.0% of children and adolescents, and 16.4% of the adults in the control group. After the second injection, the frequency of pain in the CYD dengue vaccine group was similar to the frequency of pain after the first injection in children and adolescents and in adults (approximately 30-40%). In the control group, where children received hepatitis vaccine and adults influenza vaccine at the second injection, pain was more frequently reported than in the CYD dengue vaccine group (approximately 40.0% of children and adolescents, and by 62.8% of adults). The frequency of pain after the third injection, was similar to that reported after the second injection.

Frequencies of erythema and swelling were low in each treatment group and after each injection (the highest frequency was 13.3% for erythema in adults after the third injection of the control vaccine) and neither erythema nor swelling was reported in adolescents.

Most injection site reactions were of Grade 1 or 2 intensity. The only Grade 3 solicited injection site reaction was pain, and was reported by 1.0% in the CYD dengue vaccine group and 2.0% in the control group. Grade 3 erythema and swelling were not reported. Most solicited injection site reactions emerged within 3 days after injection and had a duration of 1 to 3 days. Almost no solicited site reactions required medication, health care or hospitalization.

Solicited systemic reactions after each injection

The most frequently reported solicited systemic reactions were headache, malaise, and myalgia after each injection in both treatment groups; fever and asthenia were less frequent. After the first injection, the frequencies of each solicited systemic reaction in the CYD dengue vaccine group were slightly higher than the frequencies in the control group, where the subjects received placebo. However, in all age groups, the frequencies of solicited systemic reactions after the second and third injections were similar in the CYD dengue vaccine group and the control group (which received hepatitis A or influenza vaccine). The exception to this was myalgia, where the frequencies in adolescents and adults after the second and third injections were slightly higher in the control group than in the CYD dengue vaccine group. The frequency of headache, malaise, myalgia, and asthenia was more common in adolescents or adults and less common in children. In contrast, fever was more common in children and less common in adults.

Most solicited systemic reactions were of Grade 1 or Grade 2. Most solicited systemic reactions emerged within 3 days after injection and had a duration of 1 to 3 days. Most solicited systemic reactions did not require medication, health care contact or hospitalization.

Unsolicited non-serious AEs after each injection

Table 2: Unsolicited non-serious AE and Adverse Reactions (ARs) reactions by age group - Safety Analysis Set

		CYD Dengue Vaccine Group (N=898)			Control Group (N=300)		
		Post-injection 1	Post-injection 2	Post-injection 3	Post-injection 1	Post-injection 2	Post-injection 3
		n/N	n/N	n/N	n/N	n/N	n/N
Children	**Unsolicited AE**	15.3% (36/236)	17.9% (42/235)	12.9% (30/233)	20.0% (16/80)	15.8% (12/76)	13.2% (10/76)
	Unsolicited AR	0.8% (2/236)	3% (7/235)	0.4% (1/233)	0.0% (0/80)	0.0% (0/76)	0.0% (0/76)
Adolescents	**Unsolicited AE**	14.9% (21/141)	11.0% (15/136)	8.1% (11/136)	21.7% (10/46)	8.9% (4/45)	4.5% (2/44)
	Unsolicited AR	0.7% (1/141)	0.7% (1/136)	0.0% (0.0/136)	0.0% (0/46)	0.0% (0/45)	0.0% (0/44)
Adults	**Unsolicited AE**	18.4% (96/521)	13.9% (68/490)	11.3% (53/470)	20.7% (36/174)	15.2% (25/164)	11.3% (18/160)
	Unsolicited AR	2.7% (14/521)	2.9% (14/490)	1.3% (6/470)	2.3% (4/174)	2.4% (4/164)	2.5% (4/160)

One adolescent in each treatment group reported an immediate unsolicited AE. One adolescent from the CYD dengue vaccine group reported Grade 1 neck pain after the first injection that resolved on the day of onset and one adolescent from the control group reported Grade 1 hypoesthesia after the first injection that resolved on the day of onset. None of the 2 immediate unsolicited AEs led to discontinuation.
After the first injection, unsolicited AEs were reported by 15.3% of children, 14.9% of adolescents, and 18.4% of adults in the CYD dengue vaccine group and by 20.0% of children, 21.7% of adolescents, and 20.7% of adults in the control group. These proportions slightly decreased after the second (ranging from 11.0% to 17.9% in the CYD dengue vaccine group and from 8.9% to 15.8% in the control group) and third injections (8.1% to 12.9% in the CYD dengue vaccine group; 4.5% to 13.2% in the control group).

Unsolicited non-serious AE by system organ class (SOC)
The most frequently unsolicited AEs after the first and second injections in both groups were infections (which belongs to the SOC Infections and infestations) (mostly upper respiratory tract infections), and respiratory disorders (which belongs to the SOC Respiratory, thoracic, and mediastinal disorders) (mostly cough and rhinorrhea); the majority of them were considered not to be related to injection. After the third injection in both groups, only infections were frequently reported (especially upper respiratory tract infections).

Unsolicited non-serious AE by seriousness criterion
Most unsolicited AEs reported within 28 days after each injection were non-severe. Grade 3 unsolicited AEs occurred with similar frequency in both treatment groups: 36 (4.0%) subjects reported 44 Grade 3 unsolicited AEs in the CYD dengue vaccine group and 13 (4.3%) subjects reported 16 Grade 3 unsolicited AEs in the control group. Grade 3 unsolicited AEs were more frequently reported in adults: up to 9 subjects (adults from the CYD dengue vaccine group; 1.7%) reported Grade 3 unsolicited systemic AEs after the first injection, 8 subjects (adults from the CYD dengue vaccine group; 1.6%) after the second injection, and 9 subjects (adults from the CYD dengue vaccine group; 1.9%) after the third injection. One child and 1 adolescent from the control group reported Grade 3 unsolicited AE after the first injection. Neither children nor adolescents in the control group reported Grade 3 unsolicited AEs after the second and third injections.

Most of the Grade 3 unsolicited AEs were not related to the CYD dengue vaccine. Only few cases of Grade 3 unsolicited AEs were considered as related to treatment and recorded as Grade 3 ARs. After the first injection, Grade 3 unsolicited ARs were reported in only 1 adult in the CYD dengue vaccine group who reported a Grade 3 upper respiratory infection. Grade 3 ARs were reported by 1 child (vomiting) and 2 adults (generalized rash and arthralgia) after the second injection in the CYD dengue vaccine group. No subjects reported a Grade 3 unsolicited AR after the third injection.

SAEs up to 4-year follow-up
All SAEs were collected throughout the study period including up to 4 years after the last injection, with a particular attention for those occurring within 28 days after injection. The frequency of subjects reporting SAEs was similar between treatment groups (see Table 3 below).

Table 3: Number of subjects who reported SAEs by age group - Safety Analysis Set

		CYD Dengue Vaccine Group (N=898)			Control group (N=300)		
		Post-injection 1	Post-injection 2	Post-injection 3	Post-injection 1	Post-injection 2	Post-injection 3
		n/N	n/N	n/N	n/N	n/N	n/N
All subjects	**Within 28 days after injection**	0.3% (3/898)	0.8% (7/861)	0.2% (2/838)	0.7% (2/300)	0.4% (1/285)	0.4% (1/280)
	Up to 6 months post-injection 3	-	-	4.2% (38/898)	-	-	4.3% (13/300)
	Up to 4 years post-injection 3			4.8% (43/898)			4.3% (13/300)
Children	**Within 28 days after injection**	0.0% (0/236)	1.3% (3/235)	0.4% (11/233)	0.0% (0/80)	1.3% (3/76)	1.3% (1/76)
	Up to 6 months post-injection 3	-	-	5.1% (12/233)	-	-	7.5% (6/76)
	Up to 4 years post-injection 3			5.5% (13/233)			7.5% (6/76)
Adolescents	**Within 28 days after injection**	0.7% (1/141)	1.5% (2/136)	0.0% (0/136)	0.0% (0/46)	0.0% (0/46)	0.0% (0/46)
	Up to 6 months post-injection 3	-	-	5.7% (8/136)	-	-	0.0% (0/160)
	Up to 4 years post-injection 3			6.4% (9/136)	-	-	0.0% (0/160)
Adults	**Within 28 days after injection**	0.4% (2/251)	0.4% (2/490)	0.2% (1/470)	0.6% (1/174)	0.0% (0/160)	0.0% (0/160)
	Up to 6 months post-injection 3	-	-	3.5% (18/470)	-	-	4.0% (7/160)
	Up to 4 years post-injection 3			4.0% (21/470)	-	-	4.0% (7/160)

Thirteen SAEs were reported by 12 subjects within 28 days after any injection. Less than 2% of the subjects in each age group and treatment group reported an SAE within 28 days after injection. All the SAEs reported within 28 days after injection required hospitalization or prolonged hospitalization and none of them were considered as related to treatment, except one case of serious tension headache that was considered as related to the treatment. A 9-year old male child in CYD dengue vaccine group reported tension headache secondary to untreated allergic rhinitis 17 days after the second dose of CYD dengue vaccine. The Investigator considered the event as possibly related to the study vaccine because the event occurred within the 28-day observational period, although the causality could not be established and the events could be entirely coincidental. The subject fully recovered.

Seventy SAEs occurred during the study up to 4 years after the third injection and were reported by 43 (4.8%) subjects in the CYD dengue vaccine group and 13 (4.3%) subjects in the control group. In the CYD dengue vaccine group, 13 (5.5%) children reported 15 SAEs, 9 (6.4%) adolescents reported 10 SAEs, and 21 (3.5%) adults reported 27 SAEs. In the control group, 6 (7.5%) children reported 7 SAEs and 7 (4.0%) adults reported 11 SAEs; no adolescents reported SAEs.

Most SAEs occurred in the SOC infections and infestations. No SAE led to persistent or significant disability/incapacity.

SAEs from 6 months and up to 4-year follow-up

Only related and fatal (even if unrelated) SAEs were collected from 6 months up to 4 years after the third vaccination.

There were 3 fatal non-related SAEs reported up to 4-year follow-up

In the CYD dengue vaccine group, one SAE of dengue hemorrhagic fever (DHF) was reported 339 days after the third injection. This case resolved and was considered by the Investigator as not related to vaccination.

In the control group, one SAE of dengue fever was reported 1515 days after the third vaccination. This case resolved and was considered by the Investigator as not related to vaccination. This case is not part of the 70 cases mentioned here above because it appeared after the 4-year follow-up visit.

Viremia in case of fever after each injection

Dengue vaccine and WT dengue viremia were assessed in the event of temperature ≥ 38°C for ≥ 2 days within 28 days after each injection. In the 28 cases of fever reported within 28 days post-any injection, only one viremia was detected due to infection by WT dengue viruses in only 1 subject in the control group.

Hospitalized dengue cases up to 4 years after third injection

In the CYD dengue vaccine group, one serologically probable case of dengue and one case of dengue hemorrhagic fever (DHF) were respectively reported 152 and 339 days after the third injection. All cases resolved and were considered by the Investigator as not related to the vaccination. No additional hospitalized dengue cases were reported during the 4-year follow -up.

Immunogenicity

Primary Objective : Humoral Immune Response During the Vaccination Period

Seropositivity against each dengue virus serotype

Table 4. Summary of the seropositivity rates against each serotype at baseline and after each injection – PP analysis sets

		CYD dengue vaccine group			
		Baseline	**Post-injection 1**	**Post-injection 2**	**Post-injection 3**
Children	**Serotype 1**	4.7% (7/148)	14.7% (11/75)	59.2% (42/71)	92.4% (122/132)
	Serotype 2	5.4% (8/147)	25.3% (19/75)	85.9% (61.71)	93.9% (124/132)
	Serotype 3	11.6% (17/147)	47.3% (35/74)	87.1% (61/70)	98.5% (130/132)
	Serotype 4	6.8% (10/148)	60.8% (45/74)	91.4% (64/70)	96.9% (127/131)
Adolescents	**Serotype 1**	5.7% (8/140)	14.9% (10/67)	48.4% (31/64)	68.3% (84/123)
	Serotype 2	10.0% (14/140)	34.3% (23/67)	73.4% (47/64)	87.0% (107/123)
	Serotype 3	11.5% (16/139)	49.3% (33/67)	87.5% (56/64)	88.5% (108/122)
	Serotype 4	6.5% (9/139)	62.1% (41/66)	81.3% (52/64)	91.1% (112/123)
Adults	**Serotype 1**	34.6% (47/136)	39.7% (27/68),	57.1% (28/49),	76.5% (75/98
	Serotype 2	34.1% (46/135)	60.3% (41.68)	81.6% (40/49),	81.6% (80/98)
	Serotype 3	37.0% (50/135)	80.6% (54/67)	87.8% (43/49)	91.8% (90/98)
	Serotype 4	26.1% (35/134)	79.4% (54/68)	83.7% (41/49)	92.8% (90/97)

Before injection, seropositivity rates (Ab titer ≥ 10 [1/dil]) against serotype 1, 2, 3, or 4 in the CYD dengue vaccine group were similar between children and adolescents (approximately 10%) and were higher in adults (approximately 30%). In the control group, the baseline seropositivity rates against each dengue serotype were similar to those of the CYD dengue vaccine group. Seropositivity rates against each of the 4 dengue virus serotypes increased in all age groups after the first injection of the CYD dengue vaccine (ranging from approximately 15-40% [serotype 1] to approximately 80% [serotype 3]) and further increased after the second injection ranging from approximately 50-60% [serotype 1] to approximately 80-90% [serotype 3]. A substantial increase of seropositivity rates against all 4 dengue virus serotypes was observed after the completion of the 3 injections of the CYD dengue vaccine. Twenty-eight days after the third dose of the CYD dengue vaccine, seropositivity rates were slightly higher in children compared to adolescents and adults and were lowest against serotype 1 (92.4% in children, 68.3% in adolescents, 76.5% in adults) and highest against serotype 4 (96.9% in children, 91.1% in adolescents, 92.8 in adults). The different seropositivity rates between dengue serotype 1 and the other serotypes were larger in adolescents and adults and have been observed in previous studies. The seropositivity rates after the third injection were highest against serotype 4 in all age groups and after each injection.

In the control group, seropositivity rates remained similar to baseline or slightly increased.

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

Table 5. Summary of the seropositivity rates against at least 1, 2, 3, or all 4 serotypes at baseline and after each injection – PP analyses set

		CYD dengue vaccine group			
		Baseline	**Post-injection 1**	**Post-injection 2**	**Post-injection 3**
Children	**At least 1 serotype**	19.6% (29/148)	77.3% (58/75)	100.0% (71/71)	100.0% (132/132)
	At least 2 serotypes	6.1% (9/148)	44.0% (33/75)	95.8% (68/71)	99.2% (131/132)
	At least 3 serotypes	2.0% (3/148)	17.3% (13/75)	74.6% (53/71)	97.0% (128/132)
	All 4 serotypes	0.7% (1/148)	8.0% (6/75)	50.7% (36/71)	84.8% (112/132)
Adolescents	**At least 1 serotype**	13.6% (19/140)	79.1% (53/67)	100.0% (64/64)	98.4% (121/123)
	At least 2 serotypes	7.9% (11/140)	44.8% (30.67)	90.6% (58/64)	95.1% (117/123)
	At least 3 serotypes	7.1% (10/140)	26.9% (18/67)	62.5% (40/64)	81.3% (100/123)
	All 4 serotypes	5.0% (7/140)	9.0% (6/67)	37.5% (24/64)	59.3% (73/123)
Adults	**At least 1 serotype**	47.1% (64/136)	97.1% (66/68)	65.9% (47/49)	100.0% (98/98)
	At least 2 serotypes	33.8% (46/136)	77.9% (53/68)	91.8% (45/49)	96.9% (95/98)
	At least 3 serotypes	27.9% (38/136)	54.4% (37/68)	71.4% (35/49)	83.7% (82/98)
	All 4 serotypes	22.1% (30/136)	29.4% (20/68)	51.0% (25/49)	61.2% (60/98)

As shown in Table 5, in the CYD dengue vaccine group, the baseline seropositivity rates against at least 1 serotype were low in children and adolescents (19.6% in children and 13.6% in adolescents) and were higher in adults (47.1%). The baseline seropositivity rate was lower for subjects seropositive to all 4 dengue serotypes than those positive to only 1, 2 or 3 serotypes but remained higher in adults (47.1%); the seropositivity rate against 4 serotypes was only 0.7% in children, 5.0% in adolescents, and up to 22.1% in adults. In the control group, the baseline seropositivity rates against at least 1, 2, 3, or all 4 serotypes were similar to those in the CYD dengue vaccine group.

In the CYD dengue vaccine group, the seropositivity rate against at least 1, 2, 3, or all 4 dengue serotypes increased 28 days after each injection in each age group and for each serotype. After the first injection, the seropositivity rates against at least 1 serotype increased compared to baseline and were similar in children and adolescents (approximately 80%) and higher in adults (97.1%). The seropositivity rate against 4 serotypes was lower than 10% in children and adolescents and was 29.4% in adults. After the second injection, the seropositivity rates against at least 1, 2, 3, or all 4 serotypes further increased. Most subjects (more than 90%) from each age group were seropositive against at least 2 serotypes. The seropositivity rate after the second injection was 50.7% in children, 37.5% in adolescents, and 51.0% in adults against all 4 serotypes. After the third injection of the CYD dengue vaccine, 84.8% of children 59.3% of adolescents, and 61.2% of adults were seropositive to all 4 dengue virus serotypes, most subjects (97.0% of children, 81.3% of adolescents, and 83.7% of adults) were seropositive to at least 3 serotypes, and almost all subjects (99.2% of children, 95.1% of adolescents, and 96.9% of adults) were seropositive against at least 2 serotypes.

In the control group, the seropositivity rate against least 1, 2, 3, or all 4 serotypes slightly increased after injections in children and adolescents, and remained similar to baseline in adults.

Geometric mean titers against each of the four serotypes

Table 6. Summary of the GMT at baseline and after each injection – PP Analysis Sets

		CYD dengue vaccine group			
		Baseline	**Post-injection 1**	**Post-injection 2**	**Post-injection 3**
Children	**Serotype 1**	5.33	6.47	16.6	60.7
	Serotype 2	5.85	9.76	39.9	95.9
	Serotype 3	6.24	16.9	62.3	138
	Serotype 4	5.64	37.5	95.1	101
Adolescents	**Serotype 1**	6.49	7.43	18.1	28.9
	Serotype 2	7.47	13.1	38.8	54.0
	Serotype 3	6.86	19.8	57.8	74.1
	Serotype 4	5.83	56.7	63.7	80.4
Adults	**Serotype 1**	16.7	21.7	45.5	59.5
	Serotype 2	17.8	41.9	98.9	72.7
	Serotype 3	14.6	70.9	111	94.9
	Serotype 4	10.4	141	132	127

Most children and adolescents had no detectable levels of Abs. GMTs were higher in adults, ranging from 10.4 (serotype 4) to 17.8 (serotype 2). In the control group, the GMTs before injection were similar to those of the CYD dengue vaccine group.

In the CYD dengue vaccine group, GMTs increased 28 days after each injection in each age group and for each serotype. After the first injection, GMTs increased compared to baseline and were similar in children and in adolescents for each serotype (ranging from 6.47 [serotype 1] to 37.5 [serotype 4] in children and from 7.43 [serotype 1] to 56.7 [serotype 4] in adolescents) and were higher in adults (ranging from 21.7 [serotype 1] to 141 [serotype 4]). After the second injection, GMTs further increased in all age groups. Similarly to what was observed after the first injection, GMTs for each serotype after the second injection were similar in children and in adolescents (ranged from 16.6 [serotype 1] to 95.1 [serotype 4] in children and from 18.1 [serotype 1] to 63.7 [serotype 4] in adolescents) and were higher in adults (ranged from 45.5 [serotype 1] to 132 [serotype 4]). After the third injection, GMT for each serotype and each age group further increased and reached high values. GMTs were higher in children (ranging from 60.7 [serotype 1] to 138 [serotype 3]) than in adolescents (ranging from 28.9 [serotype 1] to 80.4 [serotype 4]) and adults (ranging from 59.5 [serotype 1] to 127 [serotype 4]), as also observed for the seropositivity rates against each dengue serotypes. At baseline and after each injection, GMTs for serotype 1 were lower compared to the other dengue serotypes in each age group. In contrast, GMTs for serotype 4 were usually higher.

Secondary Objective : Humoral Immune Response Persistence (4-Year Follow-Up)

Seropositivity against each dengue virus serotype

1-year follow-up

Overall, at 1-year follow-up, seropositivity rates against each CYD dengue virus serotype decreased (approximately 2 fold) compared to post-injection 3 but remained generally high compared to baseline for each age group.

Seropositivity rates were higher in adults as compared to children and adolescents (with highest values for serotype 4 in each age group).

In the control group, the seropositivity rates against each parental dengue serotype 1 year after the third injection were similar to those at baseline.

2-year follow-up

Overall, at 2-year follow-up, seropositivity rates against CYD dengue virus serotype 1 and serotype 4 slightly decreased compared to 1-year follow-up but remained generally high compared to baseline for each age group. Seropositivity rates against CYD dengue virus serotype 2 and serotype 3 slightly increased compared to 1-year follow-up.

Seropositivity rates were higher in adults as compared to children and adolescents (with highest values for serotype 4 in each age group).

In the control group, the seropositivity rates against each dengue serotype 2 years after the third injection were similar to those at baseline.

3-year follow-up

Overall, at 3-year follow-up, seropositivity rates against each CYD dengue virus serotype slightly decreased compared to 2-year follow-up but remained generally high compared to baseline for each age group.

Seropositivity rates were higher in adults as compared to children and adolescents (with highest values for serotype 4 in each age group).

In the control group, the seropositivity rates against each dengue serotype 3 years after the third injection were similar to those at baseline.

4-year follow-up

Overall, at 4-year follow-up, seropositivity rates against CYD dengue virus serotype 1, serotype 2, serotype 3 and serotype 4 remained similar or slightly decreased compared to 3-year follow-up. However seropositivity rates against each CYD dengue virus serotype remained generally high compared to baseline for each age group.

Seropositivity rates were higher in adults as compared to children and adolescents (with highest values for serotype 4 in each age group).

In the control group, the seropositivity rates against each dengue serotype 4 years after the third injection were similar to those at baseline.

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

1-year follow-up

As observed for the other parameters, at 1-year follow-up, seropositivity rates against at least 1, 2, 3, or all 4 dengue virus serotypes decreased (approximately 2 to 3 fold) compared to post-injection 3 but remained generally high compared to baseline for each age group.

At 1-year follow-up, seropositivity rates against at least 1 serotype were very high compared to baseline: between 90% and 100% for all age groups compared to 19.6%, 13.5% and 46.5% at baseline for children, adolescents and adults respectively.

Seropositivity rates against all 4 serotypes were higher in adults as compared to children and adolescents.

In the control group, the seropositivity rates against each dengue serotype at 1-year follow-up were similar to those at baseline

2-year follow-up

At 2-year follow-up, seropositivity rates against at least 1 serotype were very high compared to baseline: between 90% and 100% for all age groups compared to 19.6%, 13.5% and 46.5% at baseline for children, adolescents and adults, respectively.

Seropositivity rates against all 4 serotypes were higher in adults as compared to children and adolescents.

In the control group, the seropositivity rates against each dengue serotype at 2-year follow-up were similar to those at baseline.

3-year follow-up

At 3-year follow-up, seropositivity rates against at least 1 serotype remained high compared to baseline: between 70% and 95% for all age groups compared to 19.6%, 13.5% and 46.5% at baseline for children, adolescents and adults respectively.

Seropositivity rates against all 4 serotypes were higher in adults as compared to children and adolescents.

In the control group, the seropositivity rates against each dengue serotype at 3-year follow-up were similar to those at baseline.

4-year follow-up

At 4-year follow-up, seropositivity rates against at least 1 serotype remained high compared to baseline: between 70% and 95% for all age groups compared to 19.6%, 13.5% and 46.5% at baseline for children, adolescents and adults, respectively.

Seropositivity rates against all 4 serotypes were higher in adults as compared to children and adolescents.

In the control group, the seropositivity rates against each dengue serotype at 4-year follow-up were similar to those at baseline.

Geometric mean titers against each of the four serotypes

1-year follow-up

Overall, at 1-year follow-up, GMTs (expressed in l/dil) decreased (approximately 2 to 5 fold) compared to post-injection 3 but remained generally high compared to baseline for each age group.

GMTs were higher in adults as compared to children and adolescents (with highest values for serotype 4 in each age group).

In the control group, GMTs at 1-year follow-up were similar to those at baseline.

2-year follow-up

Overall, at 2-year follow-up, GMTs (expressed in l/dil) decreased compared to post-injection 3 but remained generally high compared to baseline for each age group, except for serotype 1 in children and adolescents where GMTs tended to be similar to baseline values.

GMTs were higher in adults as compared to children and adolescents (with highest values for serotype 4 in children and adolescents and for serotype 2 in adults).

In the control group, GMTs at 2-year follow-up were similar to those at baseline.

3-year follow-up

Overall, at 3-year follow-up, GMTs (expressed in l/dil) decreased compared to post-injection 3 but remained generally high compared to baseline for each age group, except for serotype 1 in children and adolescents where GMTs tended to be similar to baseline values.

GMTs were higher in adults as compared to children and adolescents (with highest values for serotype 4 in children and adolescents and for serotype 3 in adults).

In the control group, GMTs at 3-year follow-up were similar to those at baseline.

4-year follow-up

Overall, at 4-year follow-up, GMTs (expressed in l/dil) decreased compared to post-injection 3 but remained generally high compared to baseline for each age group, except for serotype 1 in children and adolescents where GMTs tended to be similar to baseline values.

GMTs were higher in adults as compared to children and adolescents (with highest values for serotype 4 in children and adolescents and for serotype 2 in adults).

In the control group, GMTs at 4-year follow-up were similar to those at baseline.

Seropositivity and GMT according to dengue baseline status

Baseline seropositivity status was shown to have limited effect after 3 injections on the seropositivity rates: seropositivity was slightly lower in non-immune subjects at baseline but high percentages of subjects non-immune at baseline were seropositive after 3 injections (89.6% to 96.5% in children, 62.9% to 89.7% in adolescents, 59.4% to 87.5% in adults). In terms of GMTs, baseline seropositivity status may have had an effect on the level of Ab. After the third injection of the CYD dengue vaccine, GMTs were higher in subjects who were dengue immune at baseline than in subjects who were dengue non-immune at baseline: GMTs ranged from 183 (serotype 1) to 245 (serotype 3) in group 1 and 43.0 (serotype 1) to 100 (serotype 4) in group 2 for subjects immune at baseline, and from 13.9 (serotype 1) to 19.3 (serotype 2 and 3) in group 1 and 7.75 (serotype 4) to 8.89 (serotype 3) in group 2 for subjects non-immune at baseline. GMTs in subjects immune at baseline were slightly lower in children than in the adolescents and adults.

At 1-year follow-up, GMTs decreased in non-immune and immune subjects, but remained higher than baseline levels. As observed during the vaccination phase, higher GMTs were observed in immune subjects than in non-immune subjects.

At 2-year follow-up, GMTs decreased as compared to post-injection 3 but remained higher as compared to baseline (the difference was more pronounced in adults than in adolescents or in children) for each age group and for each dengue status. GMTs remained higher in immune subjects than in non-immune subjects.

At 3-year follow-up, GMTs decreased as compared to post-injection 3 but remained higher as compared to the baseline for each age group and for each dengue status, except in children (serotype 1 and 2) and adolescents (serotypes 1 and 3) immune at baseline where GMTs were close to baseline, and in adolescents (serotype 2) immune at baseline where GMTs were lower than baseline. GMTs were higher in immune subjects than in non-immune subjects; this difference was more pronounced in adults and in adolescents.

At 4-year follow-up, GMTs decreased as compared to post-injection 3 but remained higher as compared to the baseline for each age group and for each dengue status, except in children (serotypes 1 and 3) and adolescents (serotypes 1 and 3) immune at baseline where GMTs were close to baseline. GMTs were higher in immune subjects than in non-immune subjects; this difference was more pronounced in adults and in adolescents than in children.

CMI

The administration of the CYD dengue vaccine induced the generation of serotype-specific T cell responses (most likely CD4+). In addition, DEN NS3-specific CD4 T cells secreting tumor necrosis factor (TNF) α and interferon (IFN) γ were detected before vaccination in adults, which remained constant but did not increase further after vaccination.

After the third vaccination, an immune response directed against all four serotypes was detected, being the strongest against serotype 2 and the weakest against serotype 1.

YFV 17D NS3-specific and CYD-specific responses presented a slight decrease 1 year after the last vaccination, the latter one being more pronounced with only IFNγ levels staying above quantification limits. This persistent dominance of IFNγ over TNFα and interleukin (IL)-13, and other Th2 cytokines, is considered positively regarding the long-term safety of the CYD dengue vaccine. Overall, 1 year after the third injection, the cytokine profile remained unchanged. This was still the case 2, 3 and 4 years after the third injection, but some further quantitative decrease was observed in both YFV17D and DEN-specific responses, which remained dominated by IFNγ.

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