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| **Sponsor:** Sanofi Pasteur<br><br>**Drug substance:** CYD Dengue Vaccine | **Study Identifiers:** U1111-1115-6579, NCT01254422<br><br>**Study code:** CYD32 |
| **Title of the study:** Safety and Immunogenicity of a Tetravalent Dengue Vaccine in Healthy Children Aged 2 to 11 Years in Malaysia | |
| **Study center(s):** This was a multi-center trial conducted at 4 sites in Malaysia. | |
| **Study period:**<br>Date first subject enrolled: 02/Dec/2010<br>Date last subject completed: 14/Aug/2012 | |
| **Phase of development:** Phase III | |

**Objectives:**

**Primary Objectives:**

Safety:

To describe the safety of the CYD dengue vaccine in all subjects after each injection.

Immunogenicity:

To describe the antibody (Ab) response to each dengue virus serotype post-Injection 2 and post-Injection 3.

**Secondary Objectives:**

Safety:

1) To describe the safety of CYD dengue vaccine after each injection according to the flavivirus (FV) immune status at baseline in all subjects
2) To describe the safety of CYD dengue vaccine after each injection in children aged 2 to 5 years and 6 to 11 years

Immunogenicity:

1) To describe the Ab response to each dengue virus serotype post-Injection 2 and post-Injection 3 according to FV status at baseline in all subjects
2) To describe the Ab response to each dengue virus serotype post-Injection 2 and post-Injection 3 in children aged 2 to 5 years and 6 to 11 years.

**Methodology:**

This was a multi-center, observer-blind, randomized, placebo-controlled, Phase III trial in 250 healthy children aged 2 to 11 years in Malaysia.

Each subject was to receive 3 injections (Day 0 [D0], D0+6 months, and D0+12 months), followed by a 6-month safety follow-up. There were 2 groups of subjects:

- **Dengue group**: 200 subjects were planned to receive CYD dengue vaccine (from Phase III lots) at 0, 6, and 12 months
- **Placebo group:** 50 subjects were planned to receive a placebo at 0, 6, and 12 months

**Number of subjects:** Planned: 250

Randomized: 250

**Evaluated:**

Immunogenicity: 246

Safety: 250

**Diagnosis and criteria for inclusion:**

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

1) Aged 2 to 11 years on the day of inclusion
2) Assent form has been signed and dated by the subject (for subjects ≥ 7 years) and informed consent form has been signed and dated by the parent(s) or another legally acceptable representative, and by an independent witness if the two parents or legally acceptable representative are illiterate)
3) Subject and parent/legally acceptable representative are able to attend all scheduled visits and to comply with all trial procedures
4) Subject in good health, based on medical history and physical examination
5) For a female subject of childbearing potential, use of an effective method of contraception or abstinence for at least 4 weeks prior to the first vaccination, until at least 4 weeks after the last vaccination

**Study treatments**

**Investigational Product:** CYD Dengue Vaccine

Formulation: Powder and solvent for suspension for injection

Composition: Each 0.5 milliliter (mL) dose of reconstituted vaccine contained:

$5 \pm 1$ $\log_{10}$ cell-culture infectious dose 50% ($CCID_{50}$) of each live, attenuated, dengue serotype 1, 2, 3, and 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%

Route of administration: Subcutaneous (SC)

**Control product**: Placebo (NaCl)

Formulation: Solution

Composition: NaCl 0.9%

Route of administration: SC

**Duration of participation:** The duration of each subject's participation in the trial was approximately 18 months

**Criteria for evaluation:**

**Primary Endpoints:**

Immunogenicity:

- Neutralizing Ab levels against each of the four parental dengue virus strains of CYD dengue vaccine constructs were measured post-Injection 2 and post-Injection 3 (dengue plaque reduction neutralization test [PRNT])

Safety:

- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, action taken, and relationship to injection of any unsolicited systemic adverse events (AEs) reported in the 30 minutes after each injection
- Occurrence, time to onset, number of days of occurrence, action taken, and intensity of solicited (i.e., pre-listed in the subject's diary card [DC] and electronic case report form [eCRF]) injection site reactions occurring up to 7 days after each injection
- Occurrence, time to onset, number of days of occurrence, action taken, and intensity of solicited systemic reactions occurring up to 14 days after each injection
- Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, and relationship to injection (for systemic AEs only) of unsolicited (spontaneously reported) AEs up to 28 days after each injection
- Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, and relationship to injection of non-serious adverse events of specific interest (AESIs) occurring up to 7 days after each injection
- Occurrence of serious adverse events (SAEs), including serious AESIs, throughout the trial period

**Secondary Endpoints:**

FV (dengue and Japanese encephalitis [JE]) serology:

Neutralizing Ab level against FV (dengue and JE) on a blood sample taken at V01 (dengue PRNT and JE PRNT)

In addition, the primary endpoints of safety and immunogenicity were used to evaluate the secondary objectives.

**Statistical methods:**

All the main analyses were descriptive. For the main parameters, 95% CIs of point estimates were calculated using normal approximation for quantitative data (using log transformation of immunogenicity data), and exact binomial distribution (Clopper-Pearson method) for proportions.

Exploratory analyses of immunogenicity data included the investigation of the baseline FV (dengue and JE) immune status effect.

The first statistical analysis was performed to address the safety and immunogenicity objectives post-Injection 2. This analysis was performed once the database obtained 28 days post-Injection 2 had been locked and the blind was broken at the Sponsor level for this analysis. However the observer-blind methodology was maintained for the assessment of post-Injection 3 safety. A second statistical analysis was performed to address the safety and immunogenicity objectives post-Injection 3. This analysis was performed once the database obtained 28 days post-Injection 3 has been locked.

A final statistical analysis was produced after the 6-month safety follow-up.

Immunogenicity:

Of the 200 subjects in the dengue group, 60-140 subjects were expected to be FV (JE and/or dengue) positive at baseline, including 60-65 JE virus vaccinated subjects. With a drop-out rate of 15%, there were to be 170 subjects in the dengue group and 51-119 baseline FV positive subjects. Based on a variability of 0.7 (standard deviation), the power to detect differences of at least 0.4 in $log_{10}$ geometric mean titers (GMTs) between baseline FV positive and negative subjects was > 90%. The power to detect this 0.4 difference between age groups was >

**Summary:**

**Study population**

A total of 250 subjects were enrolled: 199 eligible subjects were randomized to the dengue group and 51 to the placebo group.

From the 199 subjects randomized in the dengue group, 196 (98.5%) subjects completed the 3-injection schedule. One (0.5%) subject did not complete the 3-injection schedule due to an AE, 1 (0.5%) subject due to non-compliance with the protocol and 1 (0.5%) subject was lost to follow-up. Five (2.6%) subjects were excluded from the Per Protocol (PP) Analysis Set 2 and 7 (3.6%) subjects were excluded from the PP Analysis Set 3.

From the 51 subjects randomized in the placebo group, 50 (98.0%) subjects completed the 3-injection schedule. One (2.0%) subject did not complete the 3-injection schedule due to an SAE. One (2.0%) subject was excluded from the PP Analysis Set 2 and 1 (2.0%) subject was excluded from the PP Analysis Set 3.

The Full analysis set (FAS) consisted of 196 (98.5%) subjects in the dengue group and 50 (98.0%) subjects in the placebo group. The Safety Analysis Set consisted of 199 (100%) subjects in the dengue group and 51 (100%) subjects in the placebo group.

All subjects who had received 3 injections (196 [98.5%] subjects in the dengue group and 50 [98.0%] subjects in the placebo group) were contacted for the 6-month follow-up.

In the dengue group, the percentage of female and male subjects was similar (51.8% [103/199] versus 48.2% [96/199]). In the placebo group, there were more male than female subjects (62.7% [32/51] versus 37.3% [19/51]). The mean age in the dengue group (6.4 years old) was similar to the placebo group (6.5 years old).

**Safety**

***Primary objective: Safety data after each injection***

Overall, the percentage of subjects experiencing at least one solicited reaction (including both solicited injection site and solicited systemic reactions) after any injection tended to be similar between the dengue group and the placebo group (89.4% [178/199] and 94.1% [48/51], respectively). The percentage tended to be slightly higher in the dengue group than in the placebo group after the first and second injections and slightly higher in the placebo group than in the dengue group after the third injection.

The percentage of subjects with solicited systemic reactions decreased after each injection (in the dengue group it was 52.8%, 45.7% and 30.3 % for the first, second and third injection, respectively, and in the placebo group it was 52.9%, 30.0% and 26.0% for the first, second and third injection, respectively). The percentage of subjects reporting solicited injection site reactions in the dengue group tended to be higher after the second injection (62.9%) compared to the first injection (57.1%) and then decreased after the third injection (51.8%) compared to the first and second injections. In the placebo group, this was higher after the second and third injections compared to the first injection.

In both study groups, after any injection, pain was the most frequently reported solicited injection site reaction (69.3% [138/199] in the dengue group and 56.9% [29/51] in the placebo group), followed by erythema. Most of the solicited injection site reactions were of Grade 1 intensity. In both study groups, malaise (54.3% [108/199] in the dengue group and 41.2% [21/51] in the placebo group) and headache (52.3% [104/199] in the dengue group and 39.2% [20/51] in the placebo group) were the most frequently reported solicited systemic reactions followed by asthenia. Fever was the least frequently reported reaction. In both groups, most of the systemic reactions were of Grade 1 intensity. The few Grade 3 reactions were mostly reported for fever. Approximately half of the Grade 3 fever episodes were reported concomitantly with intercurrent infections.

One subject in the dengue group experienced an immediate unsolicited AE not related to vaccination. No immediate unsolicited ARs were reported.

The percentage of subjects experiencing at least one unsolicited AE was similar in both dengue and placebo groups after the first and second injections. However, it was higher in the dengue group than in the placebo group after the third injection (22.4% [44/196] and 14.0% [7/50], respectively). Infections and infestations were the most frequently reported unsolicited AEs, with upper respiratory tract infections being predominant. The percentage of subjects experiencing at least one unsolicited AR was similar between the dengue group (11.1% [22/199]) and the placebo group (9.8% [5/51]). The largest proportion of subjects reported general disorders and administration site conditions, among which injection site induration was the most frequent AR. The percentages of both unsolicited AEs and unsolicited ARs were subsequently lower after each injection for both dengue and placebo groups. Most of the unsolicited AEs and ARs were considered as non-serious in both groups and did not lead to subject discontinuation.

During the trial, a total of 23 SAEs were reported by 18 (7.2%) subjects. Fourteen SAEs were reported by 11 (5.5%) subjects in the dengue group and 9 SAEs were reported by 7 (13.7%) subjects in the placebo group. All were considered as unrelated to CYD dengue vaccine or placebo, except 1 SAE of VIIth nerve paralysis in the placebo group, reported 19 days after the administration of the placebo and considered by the Investigator as related to the placebo injection. No deaths were reported during this study.

Non-serious AESIs included hypersensitivity/allergic reactions occurring within 7 days after injection. Serious AESIs included serious hypersensitivity/allergic reactions occurring within 7 days after injection, serious neurotropic disease occurring within 30 days after injection, serious neurotropic disease occurring within 30 days after injection and serious dengue disease occurring at any time during the study.

No serious AESI was reported. A total of 5 (2.0%) subjects reported non-serious AESIs, all allergic reactions, were reported after the first and second injections (4 subjects in the dengue group and 1 subject in the placebo group). No AESI was reported after the third injection. All were considered as related to study vaccine/placebo. No anaphylactic reaction, viscerotropic or neurotropic events, or serious/severe dengue cases were reported during the study.

***Secondary objective 1*: *Safety by baseline FV immune status***

At baseline, in the FAS, 109/196 (55.6%) subjects were FV immune (i.e., neutralizing Ab titers ≥ 10[1/dil] against FV) and 87/196 (44.4%) subjects were FV non-immune (i.e. neutralizing Ab titers < 10[1/dil] in the dengue group.

In the dengue group, the percentage of subjects reporting solicited reactions after the first injection was slightly lower in the subjects who were FV immune at baseline (72.1% [80/111]) compared to the subjects who were FV non-immune (79.5% [70/88]). After the second injection, the percentages decreased to 69.1% and 74.7% for FV immune and FV non-immune subjects, respectively. After the third injection, the percentages further decreased to 50.0% and 67.8% for the FV immune and FV non-immune subjects, respectively.

The number of solicited injection site reaction was lower in the FV immune subjects compared to the FV non-immune subjects after each injection: 54.5% [60/110]) versus 60.2% [53/88] after first injection, 60.0% [66/110]) versus 66.7% [58/87]) after second injection and 44.4% [48/108]) versus 60.9% [53/87] after third injection respectively.

Pain was the most reported solicited injection site reaction in both FV immune and non-immune groups: after the first injection, 44.5% and 37.5% for the FV immune and FV non-immune subjects, respectively; after the second injection, 48.2% and 48.3% for the FV immune and FV non-immune subjects, respectively; after the third injection, 34.3% and 47.1% for the FV immune and FV non-immune subjects, respectively.

The percentage of subjects experiencing solicited systemic reactions was similar in FV immune and non-immune groups after the first (52.3% [58/111] and 53.4% [47/88] for the FV immune and non-immune groups, respectively) and second injection (47.3% [52/110] and 43.7% [38/87] for the FV immune and non-immune groups, respectively). However, after the third injection, the percentage of subjects with solicited systemic reactions was higher in the FV non-immune group (36.8% [32/87]) than in the FV immune group (25.0% [27/108]). The percentage of solicited systemic reactions tended to decrease after the second and third injection of CYD dengue vaccine, whatever the FV status at baseline. The decrease in solicited systemic reactions with subsequent injections was slightly more pronounced in FV immune subjects.

During the trial, SAEs in the dengue group were reported more frequently in the FV immune group (9 subjects) than in the FV non-immune group (2 subjects). None of the SAEs were related to study vaccine and none of the SAEs occurred during the 6-month follow-up. AESIs were reported by 2 subjects in the FV immune group and by 2 subjects in the FV non-immune group.

***Secondary objective 2: Safety by age group***

In the dengue group, reporting of solicited reactions was comparable between the 2 to 5 year age group and the 6 to 11 year age group, and tended to decrease after the third injection.

The most frequently reported solicited injection site reaction was injection site pain in both age groups. Injection site erythema and swelling were reported in similar percentages in the two age groups.

Solicited systemic reactions tended to decrease after each injection both in the 6 to 11 year age group (from 51.0% after first injection to 32.0% after third injection) and in the 2 to 5 year age group (from 54.5% after first injection to 28.4% after third injection). The most frequently reported systemic reactions in the 2 to 5 year age group were malaise and headache, and in the 6 to 11 year age group, was headache. The percentage of subjects reporting headache and myalgia was higher in the 6 to 11 year age group than in the 2 to 5 year age group. Conversely, the percentage of subjects reporting fever was higher in the 2 to 5 year age group than in the 6 to 11 year age group. A similar trend was observed in the placebo group regarding headache, myalgia and fever.

During the trial, SAEs in the dengue group were reported more frequently in the 2 to 5 year age group (10 subjects) than in the 6 to 11 year age group (1 subject). None of the SAEs were related to study vaccine. Three AESIs were reported in the 2 to 5 year age group and 1 AESI was reported in the 6 to 11 year age group.

In the placebo group, SAEs were reported by 3 subjects in the 2 to 5 year age group and by 4 subjects in the 6 to 11 year age group. One SAE previously described (i.e., SAE of VIIth nerve paralysis) was reported in the 6 to 11 year age group and was considered by the Investigator as related (placebo group). One AESI was reported in the 2 to 5 year age group and none in the 6 to 11 year age group.

**Immunogenicity**

The immunogenicity section for the primary objective provides results from the FAS. Similar results to the FAS were observed for the PP analysis set. For the secondary objective, analyses were performed on the FAS only.

***Primary objective: Ab response against each serotype post-Injection 2 and 3***

***Seropositivity (Ab titer ≥ 10 [1/dilution [dil]) against each dengue virus serotype***

Baseline seropositivity against each of the 4 dengue virus serotypes was similar, ranging from 24.0% [47/196] for serotype 4 to 36.7% [72/196] for serotype 3).

The seropositivity rate substantially increased from baseline after the second injection for each serotype (90.3% [177/196], 94.9% [186/196], 98.5% [193/196], and 91.8% [180/196] for serotypes 1, 2, 3, and 4, respectively). The seropositivity rate further increased after the third injection (96.4% [189/196], 96.9% [190/196], 99.0% [194/196], and 98.0% [192/196] for serotypes 1, 2, 3, and 4, respectively). The largest increases between second and third injection were seen for serotypes 1 and 4.

***Seropositivity against at least 1, 2, 3 and all 4 serotypes***

After the second injection, an increase in the seropositivity rates against at least 1, 2, 3 or all 4 serotypes were observed. A total of 100.0% (196/196), 99.0% (194/196), 96.4% (189/196) and 80.1% (157/196) of subjects were seropositive against at least 1, 2, 3 or all 4 serotypes, respectively. After the third injection, the seropositivity rates against at least 1, 2, or 3 serotypes and particularly against all 4 serotypes further increased. All subjects were seropositive against at least 1 and 2 serotypes, 98.5% (193/196) of subjects were seropositive against at least 3 serotypes, and 91.8% (180/196) were seropositive against all 4 serotypes.

***GMTs***

In the dengue group, the GMTs before injection against each of the 4 serotypes ranged from 9.92 (1/dil) for serotype 4 to 15.9 (1/dil) for serotype 2.

After the second injection, GMTs increased for all serotypes, compared to baseline: 119 (1/dil) for serotype 1, 160 (1/dil) for serotype 2, 196 (1/dil) for serotype 3, and 110 (1/dil) for serotype 4.

After the third injection, no additional increases in GMTs compared to post-Injection 2, were observed except for serotype 1. After the third injection, GMTs were 151 (1/dil) for serotype 1, 180 (1/dil) for serotype 2, 193 (1/dil) for serotype 3, and 114 (1/dil) for serotype 4. The geometric mean of individual fold rise of Ab titers ranged from 4.8 for dengue serotype 1 to 8.07 for dengue serotype 3 from baseline to post-Injection 2 and ranged from 6.11 for dengue serotype 1 to 7.96 for dengue serotype 3 from baseline to post-Injection 3.

***Secondary objective 1: Ab response according to FV immune status at baseline***

Immune responses were induced whatever the FV immune status at baseline.

After the third injection, all FV immune and non-immune subjects (100.0%) were seropositive against at least 2 serotypes; 99.1% (108/109) of FV immune subjects and 97.7% (85/87) of FV non-immune subjects were seropositive against at least 3 serotypes; 94.5 % (103/109) of FV immune subjects and 88.5% (77/87) of FV non-immune subjects were seropositive against all 4 serotypes. GMTs were higher in the FV immune subjects compared to the FV non-immune subjects after the second and third injections. After the third injection, the GMTs ranged from 155(1/dil) for serotype 4 to 292 (1/dil) for serotype 2 for the FV immune subjects and from 78.0 (1/dil) for serotype 4 to 117 (1/dil) for serotype 3 for the FV non-immune subjects. The GMTR from baseline to post- Injection 2 ranged from 4.87 to 6.03 fold and from 4.71 to 11.6 fold for the FV immune and FV non-immune subjects, respectively. The GMTR from baseline to post-Injection 3 ranged from 4.84 to 6.08 fold and from 7.80 to 11.7 fold for the FV immune and FV non-immune subjects, respectively.

***Secondary objective 2: Ab response according to age group***

Immune responses were induced in both age groups in terms of seropositivity rates against at least 1, 2, 3 or all 4 serotypes.

After the third injection, all subjects in the 2 to 5 year age group and the 6 to 11 year age group were seropositive against at least 2 serotypes; 99.0% (95/96) of subjects in the 2 to 5 year age group and 98.0% (98/100) of subjects in the 6 to 11 year age group were seropositive against at least 3 serotypes; 92.7 % (89/96) of subjects in the 2 to 5 year age group and 91.0% (91/100) of subjects in the 6 to 11 year age group were seropositive against all 4 serotypes.

GMTs were higher in the 6 to 11 year age group compared to the 2 to 5 year age group after the second and third injections (at baseline, GMT values were also higher in the 6 to 11 year age group compared to the 2 to 5 year age group). After the third injection, GMT values ranged from 123 (1/dil) for serotype 4 to 220 (1/dil) for serotype 3 for the 6 to 11 year age group and from 105 (1/dil) for serotype 4 to 168 (1/dil) for serotype 3 for the 2 to 5 year age group. The GMTR from baseline to post-Injection 2 ranged from 5.46 to 8.61 fold and from 4.24 to 7.59 fold for the 2 to 5 year age group and 6 to 11 year age group, respectively. The GMTR from baseline to post-Injection 3 ranged from 6.69 to 8.70 fold and from 5.59 to 7.30 fold for the 2 to 5 year age group and 6 to 11 year age group, respectively.

GMTs reached higher levels for the 6 to 11 year age group (from 123 [1/dil] for serotype 4 to 220 [1/dil] for serotype 3) than the 2 to 5 year age group (from 105 [1/dil] for serotype 4 to 168 [1/dil] for serotype 3) after the third injection.

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