*These results are supplied for informational purposes only.*
*Prescribing decisions should be made based on the approved package insert in the country of prescription.*

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| **Sponsor:** Sanofi Pasteur<br><br>**Drug substance(s):** CYD Dengue Vaccine | **Study Identifiers:** NCT00740155<br><br>**Study code:** CYD11 |

**Title of the study:** Safety and Immunogenicity of Bivalent and Tetravalent Formulations of Dengue Vaccine Candidates in Healthy Flavivirus-Naïve Adults Aged 18 to 45 Years

**Study center(s):** The trial was conducted at 2 centers in Mexico

**Study period:**

Date first subject enrolled: 11/Aug/2008

Date last subject completed: 30/Sep/2009

**Phase of development:** IIa

**Objectives:**

1) To describe the safety after each vaccination with bivalent and tetravalent formulations of dengue vaccine candidates.
2) To describe the viremia after each vaccination with bivalent and tetravalent formulations of dengue vaccine candidates.
3) To describe the humoral immune response before and after each vaccination with bivalent and tetravalent formulations of dengue vaccine candidates.
4) To describe the cellular immune response before and after each vaccination with bivalent and tetravalent formulations of dengue vaccine candidates in some groups of subjects.

**Methodology:**

This was an open, randomized, controlled, multicenter, Phase IIa study with 5 groups of subjects. The subjects were vaccinated according to the following schedule:

| | **Day 0** | **Day 105** |
|---|---|---|
| Group 1: | CYD biv (1;3) | CYD biv (2;4) |
| Group 2:* | CYD biv (1;3) + CYD biv (2;4) | CYD biv (1;3) + CYD biv (2;4) |
| Group 3: | tv [CYD(1;3;4) + VDV(2)] | tv [CYD(1;3;4) + VDV(2)] |
| Group 4: | CYD tv (1;2;3;4) | CYD tv (1;2;3;4) |
| Group 5: | JE vaccine | CYD tv (1;2;3;4) |

* separate (2 arms) and concomitant administration

CYD=ChimeriVax™ dengue; VDV=Vero dengue vaccine; biv=bivalent; tv=tetravalent JE=Japanese encephalitis. Serotypes are shown in parentheses (1;2;3;4).

For Group 5, JE vaccination was given according to a 3-dose schedule (3 doses 7 days apart) starting 14 days before Day 0 (D0). The third JE vaccine dose was given at the same time as the first vaccination of the other groups (D0).

| | |
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| **Number of subjects:** | Planned: 150<br>Randomized: 155 |
| **Evaluated:** | Immunogenicity: 152<br>Viremia: 151<br>Safety: 152 |

**Diagnosis and criteria for inclusion:**

1) Healthy as determined by medical history, clinical examination, and biological safety parameters (Screening and D-14 for all groups + V01 for Groups 1 to 4)
2) Aged 18 to 45 years on the day of inclusion (Screening)
3) Informed consent form signed (Screening)
4) Able to attend all scheduled visits and to comply with all trial procedures (Screening and D-14 for all groups + V01 for Groups 1 to 4)
5) For a woman of child-bearing potential, use of an effective method of contraception or abstinence for at least 4 weeks prior to the first vaccination and at least 4 weeks after the last vaccination (Screening and D-14 for all groups + V01 for Groups 1 to 4)

**Study treatments**

**Investigational Product 1:** CYD dengue bivalent (1,3)

Form: Powder and solvent for suspension for injection.

Composition: Each 0.5 mL dose of reconstituted vaccine contains ~5 log10 tissue culture infectious dose (CCID50) per CYD serotype (1,3).

Solvent: Sodium Chloride (NaCl) 0.4% containing human serum albumin (HSA) 2.5%.

Route of administration: Subcutaneous (SC)

**Investigational Product 2:** CYD dengue bivalent (2,4)

Form: Powder and solvent for suspension for injection.

Composition: Each 0.5 mL dose of reconstituted vaccine contains ~5 log10 CCID50 per CYD serotype (2,4).

Solvent: NaCl 0.4% containing HSA 2.5%.

Route of administration: SC

**Investigational Product 3:** Tetravalent blending VDV2/CYD-1,3,4 Dengue (Vero)

Form: Powder and solvent for suspension for injection.

Composition: Each 0.5 mL dose of reconstituted vaccine contains ~5 log10 CCID50 per CYD serotype (1, 3, 4) and ~4 log10 CCID50 per VDV serotype (2).

Solvent: NaCl 0.4% containing HSA 2.5%.

Route of administration: SC

**Investigational Product 4:** CYD dengue tetravalent (1;2;3;4)

Form: Powder and solvent for suspension for injection.

Composition: Each 0.5 mL dose of reconstituted vaccine contains ~5 log10 CCID50 per CYD serotype (1, 2, 3, 4).

Solvent: NaCl 0.4% containing HSA 2.5%.

Route of administration: SC

**Investigational Product 5:** Japanese encephalitis virus vaccine inactivated (JE-VAX®)

Form: Powder and solvent for suspension for injection.

Composition: Inactivated JE virus, BIKEN Nakayama-NIH strain.

Solvent: Sterile water for injection.

Route of administration: SC

**Duration of participation:** The expected total duration of follow-up was 13 months per subject (including screening).

**Criteria for evaluation:**

***Safety:***

For each of the vaccinations at D0 and at D105, the following endpoints were evaluated:

- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] Version 10.0 preferred term), duration, intensity, and relationship to vaccination of any unsolicited systemic adverse events (AEs) reported in the 30 minutes after vaccination.
- Occurrence, time to onset, number of days of occurrence, and intensity of solicited (pre-listed in the subject's diary and case report form [CRF]) injection site reactions occurring up to 7 days after each vaccination.
- Occurrence, time to onset, number of days of occurrence, and intensity of solicited systemic reactions occurring up to 21 days after each vaccination (except between 0 and 30 minutes).
- Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, and relationship to vaccination (for systemic AEs only) of unsolicited (spontaneously reported) AEs up to 28 days after each vaccination.
- Occurrence, nature (MedDRA preferred term), time to onset, duration, and relationship to vaccination of serious adverse events (SAEs) throughout the trial.

***Biological Safety***

- Occurrence and timing of out-of-range biological test results up to 28 days after each vaccination.

***Vaccinal Viremia***

- Occurrence and maximum level of dengue vaccine virus in sera collected on D7, D14, D21, Visit 07 (V07)+7 days, V07+14 days, and V07+21 days: quantitative and CYD non-serotype-specific reverse transcriptase-polymerase chain reaction (YF RT-PCR) was carried out in all samples and CYD serotype specific RT-PCR (CYD RT-PCR) was performed in YF RT-PCR positive samples. In addition, dengue plaque assay (PA) was carried out in the 30 highest YF RT-PCR positive samples and in up to 30 Dengue (DEN)-2 RT-PCR positive samples.

***Immunogenicity:***

Cellular Immune response

- Specific immunity was evaluated at the Sponsor's Research Department by cytometric bead array (CBA) and intracellular cytokine staining (ICS) after antigenic stimulation of cells collected in four groups of subjects (Groups 1, 2, 3 and 4) on D0, D28, D105, and V07+28 days.

Humoral immune response

- Neutralizing antibody (Ab) levels against each parental dengue virus serotype of CYD dengue vaccine was measured in sera collected from Group 5 subjects on D-14 and from all subjects on D0, D28, D60, D105, V07+28 days, V07+60 days, and D365 (microneutralization assay). Neutralizing Ab levels against the Thai Mahidol strain for dengue virus serotype 2 were measured in sera from Group 3 subjects on D0, D28, D60, D105, V07+28 days, V07+60 days, and D365 (microneutralization assay). Derived endpoints:
- Neutralizing Ab level ≥10 1/dilution (1/dil)
- Individual titer ratio: V07+28 days/D105
- Individual titer ratio: V07+60 days/D105

**Statistical methods:**

The analysis was descriptive and presented by group with 95% confidence intervals (CIs) for the main parameters.

Three analyses were performed on data of safety, viremia, and immunogenicity. A first interim statistical analysis was performed on D28 after first vaccination and a second interim statistical analysis was performed on D28 after second vaccination.

The final statistical analysis performed after D365 and included all data.

**Summary:**

For description purposes “first vaccination” refers to Visit 01 (i.e., third dose of JE vaccine in Group 5; 1st dose of dengue vaccine in Groups 1, 2, 3, and 4) and “second vaccination” refers to Visit 07 (i.e., second dose of dengue vaccine in Groups 1,2, 3, and 4; and first dose of dengue vaccine in Groups 5).

**Population characteristics:**

A total of 215 subjects were screened from 16 to 21 days before the D0 vaccination. There were 2 randomizations, V_JE1 (D-14) and V01 (D0). A total of 155 subjects were randomized to 5 groups (30 subjects in Group 1, 31 subjects in Group 2, 30 subjects in Group 3, 32 subjects in Group 4, and 32 subjects in Group 5).

All the subjects received the vaccines as randomized, except for 1 subject in Group 3, who was randomized to Group 3 (tetravalent blending vaccine), but instead received CYD biv (1;3) vaccine at the first vaccination and CYD biv (2;4) vaccine at the second vaccination.

Of the 155 subjects that were enrolled in the study, 143 (92.3%) subjects completed the study and 12 (7.7%) subjects discontinued the study, the most common reason being non-compliance with the protocol. Seven (4.5%) subjects were non-compliant with the protocol, 4 (2.6) subjects were lost to follow-up, and 1 (0.6%) subject withdrew consent. There were no discontinuations due AEs or SAEs.

There were slightly more females, (89 [58.6%] subjects) than males (63 [41.4%] subjects) in the Full Analysis Set (FAS). The number of females was higher in Group 1 (22 [71.0%]) and Group 5 (20 [66.7%]) as compared to the other groups. The age at vaccination was on average 29.3 years, and ranged from 18 through 44 years. BMI was similar across the groups.

**Safety:**

There were no deaths during the study. No SAE occurred within 28 days after the first vaccination. Three SAEs were reported after more than 28 days after first vaccination.

Three subjects reported 3 SAEs after the first vaccination (increased blood creatine phosphokinase [CPK] in Group 1, intra-uterine death of the foetus in Group 2 and abdominal hernia in Group 5). The SAEs were considered not related to the vaccination by the Investigator and the Sponsor. All subjects recovered. There were no SAEs reported after the second vaccination.

There was no safety issue in subjects reporting viremia after the first (11 subjects) or second vaccination (4 subjects) regarding SAEs, immediate AEs, Grade 3 AEs, or Grade 3 or clinically significant biological abnormalities.

*Solicited injection site reactions:*

**After the first vaccination**: The most frequently reported injection site reaction within 7 days after vaccination was injection site pain in all groups. The majority of the solicited injection site reactions were Grade 1 in intensity (none were Grade 3), appeared within 3 days of vaccination and the number of days of occurrence ranged from 1 to 3 days.

**Solicited injection site reactions within 7 days after the first vaccine injection - Safety Analysis Set**

| | Group 1 (N=31) | | Group 2 (N=31) | | Group 3 (N=29) | | Group 4 (N=31) | | Group 5 (N=30) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subjects experiencing at least one:** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** |
| **Injection site pain** | 7/31 | 22.6 | 8/31 | 25.8 | 7/29 | 24.1 | 8/31 | 25.8 | 6/29 | 20.7 |
| **Injection site erythema** | 1/31 | 3.2 | 1/31 | 3.2 | 1/29 | 3.4 | 4/31 | 12.9 | 2/29 | 6.9 |
| **Injection site edema** | 0/31 | 0.0 | 0/31 | 0.0 | 1/29 | 3.4 | 0/31 | 0.0 | 0/28 | 0.0 |

**After the second vaccination**: The most frequently reported injection site reaction was injection site pain in all groups. The majority of the solicited injection site reactions were Grade 1 in intensity, appeared within 3 days of vaccination and the number of days of occurrence ranged from 1 to 3 days. Grade 3 injection site reactions were reported by 3 subjects (1 subject each from Groups 3 and 5 reported injection site pain and 1 subject from Group 4 reported injection site erythema).

There were no relevant differences between the groups and the vaccinations. Injection site pain remained on the same level after both the vaccinations in all groups. A trend of a few additional subjects reporting injection site erythema (in Groups 1, 2 and 3) and injection site edema (in Groups 1, 2, 4 and 5) after the second vaccination was observed. However, both injection site erythema and edema were reported by a low number of subjects at both first and second vaccinations.

**Solicited injection site reactions within 7 days after the second vaccine injection - Safety Analysis Set**

| | Group 1 (N=31) | | Group 2 (N=30) | | Group 3 (N=28) | | Group 4 (N=29) | | Group 5 (N=28) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subjects experiencing at least one:** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** |
| **Injection site pain** | 10/31 | 32.3 | 8/30 | 26.7 | 7/28 | 25.0 | 8/28 | 28.6 | 5/28 | 17.9 |
| **Injection site erythema** | 6/31 | 19.4 | 3/30 | 10.0 | 5/28 | 17.9 | 4/28 | 14.3 | 3/28 | 10.7 |
| **Injection site edema** | 4/31 | 12.9 | 2/30 | 6.7 | 1/28 | 3.6 | 3/28 | 10.7 | 2/28 | 7.1 |

*Solicited systemic reactions:*

**After the first vaccination**: The most frequently reported solicited systemic reaction within 21 days after the first vaccination was headache in all groups. The majority of the solicited systemic reactions were Grade 1 or Grade 2 in intensity, appeared within 3 days of vaccination and the number of days of occurrence was 1 to 3 days. Grade 3 systemic reactions were reported by 8 subjects across 4 groups (except Group 2), with headache (n=4) being the most frequent.

**Solicited systemic reactions within 21 days after the first vaccine injection - Safety Analysis Set**

| | Group 1 (N=31) | | Group 2 (N=31) | | Group 3 (N=29) | | Group 4 (N=31) | | Group 5 (N=30) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subjects experiencing at least one:** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** |
| **Fever** | 6/31 | 19.4 | 6/31 | 19.4 | 7/29 | 24.1 | 4/31 | 12.9 | 5/29 | 17.2 |
| **Headache** | 20/31 | 64.5 | 20/31 | 64.5 | 18/29 | 62.1 | 21/31 | 67.7 | 15/29 | 51.7 |
| **Malaise** | 13/31 | 41.9 | 8/31 | 25.8 | 11/29 | 37.9 | 7/31 | 22.6 | 13/29 | 44.8 |
| **Myalgia** | 16/31 | 51.6 | 10/31 | 32.3 | 11/29 | 37.9 | 10/31 | 32.3 | 13/29 | 44.8 |
| **Asthenia** | 16/31 | 51.6 | 12/31 | 38.7 | 10/29 | 34.5 | 13/31 | 41.9 | 15/29 | 51.7 |

**After the second vaccination:** The most frequently reported solicited systemic reaction was headache in all groups. The majority of the solicited systemic reactions were Grade 1 or Grade 2 in intensity, appeared within 3 days of vaccination and the number of days of occurrence was 1 to 3 days. Grade 3 systemic reactions were reported by 5 subjects across Groups 1, 2, and 5.

**Solicited systemic reactions within 21 days after the second vaccine injection - Safety Analysis Set**

| | Group 1 (N=31) | | Group 2 (N=30) | | Group 3 (N=28) | | Group 4 (N=29) | | Group 5 (N=28) | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Subjects experiencing at least one:** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** | **n/M** | **%** |
| **Fever** | 9/31 | 29.0 | 8/30 | 26.7 | 6/28 | 21.4 | 5/28 | 17.9 | 4/28 | 14.3 |
| **Headache** | 15/31 | 48.4 | 12/30 | 40.0 | 12/28 | 42.9 | 12/28 | 42.9 | 14/28 | 50.0 |
| **Malaise** | 8/31 | 25.8 | 9/30 | 30.0 | 10/28 | 35.7 | 3/28 | 10.7 | 7/28 | 25.0 |
| **Myalgia** | 13/31 | 41.9 | 7/30 | 23.3 | 9/28 | 32.1 | 8/28 | 28.6 | 13/28 | 46.4 |
| **Asthenia** | 10/31 | 32.3 | 8/30 | 26.7 | 8/28 | 28.6 | 7/28 | 25.0 | 11/28 | 39.3 |

***Immediate unsolicited ARs/AEs:*** *There were no immediate (i.e., within 30 minutes after vaccination) unsolicited systemic AEs or ARs after the first vaccination. Immediate unsolicited systemic ARs of abdominal cramp (Group 2), nausea (Group 3) and dizziness (Group 4) were reported by 3 subjects after the second vaccination and 1 subject from Group 3 reported an immediate unsolicited AE of drowsiness after the second vaccination.*

***Unsolicited ARs:***

**After the first vaccination**: The percentage of subjects with unsolicited ARs ranged from 6.9% to 12.9% and was similar across all the 5 groups (Group 1: 4/31 [12.9%] subjects, Group 2: 3/31 [9.7%] subjects, Group 3: 2/29 [6.9%] subjects, Group 4: 3/31 [9.7%] subjects, Group 5: 3/30 [10.0%] subjects). The most frequently reported unsolicited AR was injection site pruritus recorded in 2/31 (6.5%) subjects from Group 2, 1/31 (3.2%) subject from Group 1 and 1/30 (3.3%) subject from Group 5. All the other unsolicited ARs were reported by not more than 1 subject each.

**After the second vaccination**: The percentage of subjects with unsolicited ARs was similar in Group 2 (4/30 [13.3%] subjects), Group 3 (4/28 [14.3%] subjects) and Group 5 (5/28 [17.9%] subjects) and was lower in Groups 1 (2/31 [6.5%] subjects) and Group 4 (1/29 [3.4%] subjects). The most frequently reported unsolicited ARs were influenza recorded in 2/28 (7.1%) subjects from Group 3; pharyngitis recorded in 2/28 (7.1%) subjects from Group 5; and burning sensation recorded in 2/28 (7.1%) subjects from Group 5.

***Unsolicited AEs:***

**After the first vaccination**: Unsolicited AEs were reported by 20/31 (64.5%) subjects from Group 1, 19/31 (61.3%) subjects from Group 2, 19/29 (65.5%) subjects from Group 3, 20/31 (64.5%) subjects from Group 4 and 19/30 (63.3%) subjects from Group 5. Pharyngitis was the most frequently reported unsolicited AE in all groups: 2/31 (6.5%) subjects from Group1, 3/31 (9.7%) subjects from Group 2, 4/29 (13.8%) subjects from Group 3, 5/31 (16.1%) subjects from Group 4 and 3/30 (10.0%) subjects from Group 5. Other unsolicited AEs reported by more than 5 subjects were influenza, nasopharyngitis, pharyngolarygeal pain, rhinitis, and headache. In general, the percentage and types of unsolicited AEs were generally similar across the groups.

**After the second vaccination**: Unsolicited AEs were reported by 18/31 (58.1%) subjects from Group 1, 20/30 (66.7%) subjects from Group 2, 17/28 (60.7%) subjects from Group 3, 15/29 (51.7%) subjects from Group 4 and 18/28 (64.3%) subjects from Group 5. Influenza was the most frequently reported unsolicited AE in all groups: 8/31 (25.8%) subjects from Group1, 6/30 (20.0%) subjects from Group 2, 7/28 (25.0%) subjects from Group 3, 1/29 (3.4%) subjects from Group 4 and 10/28 (35.7%) subjects from Group 5. Other unsolicited AEs reported by more than 5 subjects were pharyngitis, nasopharyngitis, burning sensation and upper abdominal pain.

***Pregnancies***: Five pregnancies were reported during the study. Four pregnancies had a normal outcome with live birth and the fifth pregnancy was terminated due to intrauterine death (SAE mentioned above).

***Biological abnormalities:***

Most subjects in all five groups had biological values within normal ranges both at baseline (screening) and after the first and second vaccinations.

A trend of increased mean AST, ALT and CPK levels was observed after the first dose of tetravalent blending formulation in Group 3 (compared to pre-vaccination levels and compared to the other vaccine groups who received CYD dengue vaccine formulations). After the second vaccination CPK levels tended to slightly increase in Group 3 compared to pre-vaccination levels and compared to the other vaccine groups who received CYD dengue vaccine formulations. The mean CPK levels returned to pre-vaccination level on D28 after both first and second vaccinations.

Grade 3 biological abnormalities after first vaccination were reported in 2 subjects who both presented high values of CPK (1 subject each from Group 1 [2348 U/L] and Group 3 [7602 U/L]). These 2 subjects with Grade 3 abnormalities and another subject from Group 5 with increased levels of CPK (884 U/L) and eosinophils (0.3*10^3/µL) were considered to have clinically significant biological abnormalities.

Grade 3 biological abnormalities after the second vaccination were observed in 2 subjects: 1 subject in Group 3 presented high values of CPK (3731 U/L) and 1 subject from Group 5 reported decreased lymphocyte values (0.2*10^3/uL). Both these subjects were considered to have clinically significant biological abnormalities. There were no clinical symptoms associated with these biological abnormalities.

**Viremia:**

**After the first vaccination:**

***Non-serotype specific CYD vaccine viremia:***

The highest percentage of subjects with detectable non-serotype specific CYD vaccine viremia was observed 7 days after vaccination, which then decreased 14 days and 21 days after vaccination. The number of subjects with detectable non-serotype specific CYD vaccine viremia on D7 after vaccination was highest in Group 3 and lowest in Group 1. Only 1 or 2 subjects in each group had quantified non-serotype specific CYD vaccine viremia at D7 after vaccination and there were no subjects with quantified non-serotype specific CYD vaccine viremia 14 and 21 days after vaccination.

***Serotype specific CYD vaccine viremia:***

Among the subjects with a positive YF RT-PCR, the most frequently detected CYD dengue vaccine virus parental serotype was serotype 4 in all groups, followed by serotypes 3, 1, and 2. The level of viremia was low and similar across the serotypes; and ranged from the lowest level of 4.93 GEq/mL in Group 4 for serotype 1 to the highest level of 5.99 GEq/mL in Group 4 for

serotype 4. In general, viremia was most frequently detected 7 days after vaccination.

Overall, there were 2 subjects in Group 4 with quantified serotype 1 viremia, no subjects with quantified serotype 2 viremia, 2 subjects in Group 1 with quantified serotype 3 viremia and 4 subjects (3 in Group 4 and 1 in Group 2) with quantified serotype 4 viremia.

***Dengue VDV2 vaccine viremia:***

There were no subjects in Group 3 with viremia on D7 after vaccination. One subject each reported detectable and quantified vaccine viremia on D14 after vaccination. Two subjects each had detectable and quantified vaccine viremia on D21 after vaccination.

***Plaque assay:***

No tested subject presented with CYD viremia or VDV2 vaccine viremia at 7, 14 or 21 days after the first vaccination as assessed using plaque assays.

**After the second vaccination:**

***Non-serotype specific CYD vaccine viremia:***

The percentage of subjects with viremia was lower after the second vaccination than after the first vaccination in all groups (except Group 5 who did not receive any dengue vaccination at the first vaccination). The highest percentage of subjects with detectable non-serotype specific CYD vaccine viremia was observed 7 days after vaccination. The highest number of subjects with detectable non-serotype specific CYD vaccine viremia 7 days after vaccination was 16/27 (59.3%) subjects in Group 5. There were 4/30 (13.3%) subjects from Group 1 and 1/28 (3.6%) subject from Group 3 with detectable viremia. No subjects from Groups 2 and 4 had detectable viremia.

There was only 1 subject (1/30 [3.3%]) from Group 1 with quantified non-serotype specific CYD vaccine viremia 7 days after vaccination.

***Serotype specific CYD vaccine viremia:***

Among the subjects with positive YF RT-PCR, the most frequently detected CYD serotype was serotype 4 in all groups, then serotype 3 in all groups. There were no subjects with serotype 1 and serotype 2 detectable viremia at 7, 14 and 21 days after vaccination. Serotype 4 detectable viremia was observed 7 days after the second vaccination, in 4/4 (100%) subjects from Group 1 and 12/16 (75.0%) subjects from Group 5. The level of viremia ranged from 5.40 GEq/ml in Group 5 for serotype 4 to 5.43 GEq/mL in Group 1 for serotype 4. When present viremia was most frequently detected at 7 days after vaccination.

Quantified viremia was only observed 7 days after the vaccination for serotype 4 in 1 subject each from Groups 1 and 5.

***Dengue VDV2 vaccine viremia:***

One subject reported detectable and quantified vaccine viremia at 7 days after vaccination in Group 3. Four subjects reported detectable vaccine viremia and 1 subject reported quantified vaccine viremia at 14 days after vaccination. There were no subjects with detectable or quantified vaccine viremia on D21 after vaccination.

***Plaque assay:***

No tested subject presented with CYD viremia or VDV2 vaccine viremia at 7, 14 or 21 days after the second vaccination as assessed using plaque assays.

**Immunogenicity:**

***Seropositivity:***

**After the first vaccination:**

The percentage of seropositive subjects generally increased after the first vaccination in all groups, except Group 5, for the administered serotypes. No subject from Group 5 was seropositive to dengue on 28 and 60 days after the first vaccination, as JE vaccine was administered at first vaccination.

The highest percentage of seropositive subjects was observed for serotype 4 (in Group 2 [77.4% on D28 and 86.7% on D60], Group 3 [86.2% on D28 andn 92.9% on D60] and Group 4 [83.9% on D28 and 80.0% on D60] which received vaccine formulations containing the four serotypes). In these groups, the lowest response was observed for serotype 1 (Group 2: 22.6% on D28 and 23.3% on D60, Group 3: 41.4% on D28 and 53.6% on D60, and Group 4: 29% on D28 and 33.3% on D60), and serotype 2 (Group 2: 12.9% on D28 and 20.0% on D60, Group 3: 17.2% on D28 and 46.4% on D60, and Group 4: 22.6% on D28 and 30.0% on D60). In Group 1, the first dose only elicited Ab response against serotypes 1 and 3.

The highest number of seropositive subjects after the first vaccination was observed on D60 particularly for subjects in Groups 2 and 3.

**After the second vaccination:**

The second dose generally increased the percentage of seropositive subjects in all groups that received 2 CYD dengue vaccine doses, except for the response to serotype 4, which tended to remain at a similar level as after the first vaccination

In general, the highest percentage of seropositive subjects was observed on 28 days after the second vaccination. In Group 1 there was an increase in the response at 28 days after the second vaccination to serotypes 1 (58.1%) and 3 (80.6%); however, the response to serotype 2 (6.5%) and serotype 4 (29%) was low compared to other groups. Group 5 showed percentage of seropositive subjects at 28 days after the second vaccination (85.2% for serotypes 1, 2 and 3; and 92.6% for serotype 4), comparable or even higher than the other groups receiving 2 doses of the dengue vaccine formulations containing the 4 serotypes.

In general, percentage of seropositive subjects decreased gradually at D60 and D365 (260 days after the second vaccination) compared to maximum percentages after the second vaccination in all groups.

In Group 3, the percentage of seropositive subjects for the VDV2 serotype was similar to that of the CYD dengue vaccine virus parental serotype 2 at 28 days after the second vaccination, but higher at 60 days and 260 days after the second vaccination, compared to the corresponding percentages of the CYD dengue vaccine parental serotype 2.

***Seropositivity for at Least 1, 2, 3, or 4 Dengue Serotypes:***

**After the first vaccination:**

After the first vaccination (D28), the highest percentage of subjects seropositive to at least 2 serotypes was observed in Group 3 (65.5%), compared to 29.0% in Group 1, 32.3% in Group 2 and 35.5% in Group 4. No subject in Group 5 was seropositive to at least 2 serotypes, since this group did not receive any dengue vaccine at the first vaccination.

**After the second vaccination:**

After the second vaccination (D105+28 days), the majority of subjects in all groups were seropositive to at least 2 serotypes (except in Group 1, with 48.4% of the subjects seropositive to at least 2 serotypes). At 28 days after the second vaccination, the highest percentage of subjects seropositive to at least 2 and 3 serotypes was observed in Group 3 (96.4% and 89% respectively), closely followed by Group 5 (i.e., JE vaccine primed subjects receiving only one dose of dengue vaccine) (92.6% and 77.8% against 2 and 3 serotypes respectively).

A minority of subjects in all groups maintained seropositivity to at least 3 serotypes on D365 (260 days after the second vaccination). The highest persistence of Ab response to at least 3 serotypes was observed in Group 3 (40.7%), followed by Group 2 (33.3%) and lowest in Group 5 (14.8%).

***Dengue neutralizing antibody geometric mean titers (GMTs):***

**After the first vaccination:**

The GMTs of neutralizing Abs to CYD dengue vaccine virus parental serotypes 1, 2, and 3 showed a relatively low increase after the first vaccination in Group 2, (9.23, 6.07, and 11.1 1/dil for serotypes 1, 2, and 3 respectively on D28), Group 3 (10.1, 7.90, and 19.4 1/dil for serotypes 1, 2, and 3 respectively on D28), and Group 4 (10.7, 8.61, and 9.39 1/dil for serotypes 1, 2, and 3 respectively on D28) that received the 4 serotypes. However, titers for serotype 4 showed a substantial increase (160, 167, and 159 1/dil for groups 1, 2 and 3 respectively on D28). In Group 5, there was no increase since these subjects did not receive any dengue vaccine, and in Group 1 the subjects did not respond to the serotypes 2 and 4 since they received a formulation containing only serotypes 1 and 3.

**After the second vaccination:**

The second dose generally increased the GMTs for all serotypes (in the groups receiving 2 CYD vaccine doses), except for serotype 4. In all groups except Group 1, the highest GMTs were observed for serotype 4, while the lowest response was generally observed for serotypes 1 and 2. After the second vaccination, subjects in Group 1 had the lowest GMTs for serotypes 2 and 4 at all timepoints. Subjects in Group 5 showed GMTs at D28 comparable or even higher than other groups receiving two does of dengue vaccine formulations containing the 4 serotypes (37.4, 27.4, 42.2, and 219 1/dil for serotypes 1, 2, and 3 respectively on D28).

GMTs gradually decreased at 60 and 260 days after the second vaccination. The GMTs for serotypes 1 and 2 were below 10 1/dil (the assay threshold for seropositivity) in all groups.

In Group 3, the GMT to the VDV2 serotype was similar to that of the CYD dengue vaccine parental serotype 2 at 28 days, but higher at 60 and 260 days after the second vaccination for the VDV2 serotype compared to corresponding GMTs of the CYD dengue vaccine parental serotype 2.

***Cellular immune response:***

Due to poor viability and cell yield of the PBMCs prepared at the trial sites, many samples could not be analyzed or had to be rejected, therefore the results obtained for the different visits did not derive exactly from the same set of subjects.

The administration of different bivalent and tetravalent CYD vaccine candidates induced the generation of specific cellular responses, involving envelope-specific cluster of differentiation (CD) 4+ cells which secreted mainly interferon-gamma (IFN-γ) in response to in vitro monovalent CYD restimulation, as measured by CBA. This response was serotype-specific and dominated by serotype 4 in Groups 2, 3 and 4. Subjects who received the CYD biv (1;3) formulation (Group 1) for the first vaccination showed a response dominated by serotypes 1 and 3 even after a heterologous second vaccination with CYD biv (2;4) formulation.

The ICS results showed that the administration of different CYD vaccine formulations induced the generation of YF 17D-specific CD8+ cells. The response was dominated by IFN-γ secretion over tumor necrosis factor–alpha (TNF-α) secretion, and mainly CD8+ driven. Responses against the dengue (DEN) backbone were almost exclusively detected in Group 3 who received VDV2 serotype containing the DEN non-structural 3 (NS3) protein instead of CYD serotype 2, showing the absence of cross-reactivity between NS3 from dengue and YF.

IFN-γ cytokine results obtained after serotype-specific stimulation (serotypes CYD-1 to CYD-4) were in agreement with PRNT results: a predominance of serotype 4 was observed when 4 serotypes were injected concomitantly, regardless of the type of formulation: tetravalent, two bivalent, or blending formulation. The highest percentages of seropositive subjects to the 4 serotypes after the first vaccination were found in Group 3, in which CYD-2 was replaced by VDV2. The second vaccination allowed a broadening of the response against all serotypes but not an increase in the level of Ab titer or IFN-γ secretion. Finally, the administration of CYD biv (1;3) led to a response dominated by serotypes 1 and 3, even after the administration of the complementary serotypes CYD-2-and 4.

Adaptive responses in recipients of different dengue vaccine candidates presented a Th1/Tc1 profile as judged by a predominance of IFN-γ over TNF-α secretion measured in cell supernatants upon CYD restimulation by cytometric bead array (CBA), or after dengue- and/or YF 17D-NS3 peptide stimulation in CD8 cells by ICS.

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