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Sponsor: Sanofi Pasteur **Drug substance(s):** CYD Dengue Vaccine	**Study Identifiers:** IND 11219 **Study code:** CYD04
Title of the study: Safety of ChimeriVax™ Dengue Tetravalent Vaccine in Healthy Adults Aged 18 to 45 Years	
Study center(s): One center in the US	
Study period: Date first subject enrolled: 11/Oct/2005 Date last subject completed: 13/Feb/2007	
Phase of development: Phase I	
Objectives: 1) To describe the **safety** of each of the three injections of ChimeriVax™ Dengue Tetravalent Vaccine. 2) To describe **viremia** after each of the three injections of ChimeriVax™ Dengue Tetravalent Vaccine. 3) To describe vaccine **virus shedding** after a first injection of ChimeriVax™ Dengue Tetravalent Vaccine. 4) To describe the **humoral immune response** against dengue before injection, and after each of the three injections of ChimeriVax™ Dengue Tetravalent Vaccine. 5) To describe the **cellular immune response** against dengue before injection, and after each of the three injections of ChimeriVax™ Dengue Tetravalent Vaccine.	
Methodology: Blind observer (first injection), open (second and third injections), monocenter, randomized, controlled, Phase I study in 66 adults in the USA. Three injections were given on D0, D0 + 4 months, and D0 + 12 months. **Group 1** received the live-attenuated ChimeriVax™ Dengue Tetravalent Vaccine as first, second, and third injection. **Group 2** (Control Group) received placebo (YF-VAX® diluent: sodium chloride, NaCl) as first injection and ChimeriVax™ Dengue Tetravalent Vaccine as second and third injection. Vaccinations were staggered such that a first cohort of 18 subjects was followed up to Day 14 and their safety was evaluated by an Independent Data Monitoring Committee (IDMC). The decision on whether to proceed with the enrolment of the 48 remaining subjects was taken by the Sponsor and the Principal Investigator, based upon the IDMC's recommendation.	
Number of subjects:	Planned: 66 (33 in each group) Randomized: 66 (33 in each group) Number of subjects who received the first vaccination: 66 subjects (33 in each group). Number of subjects who received the second vaccination: 63 subjects (30 in Group 1 and 33 in Group 2). Number of subjects who received the third vaccination: 49 subjects (23 in Group 1 and 26 in Group 2).

Evaluated:

Immunogenicity: 66

Safety: 66

Diagnosis and criteria for inclusion:

Inclusion criteria were checked at Screening only (Scr.), at Screening and at the first vaccination visit (Scr.+V01), or at the first vaccination visit only (V01).

1) Aged 18 to 45 years on the day of inclusion (Scr.).
2) Informed consent form signed (Scr.).
3) For a woman, inability to bear a child or negative serum pregnancy test at screening (Scr.).
4) Able to attend all scheduled visits and to comply with all trial procedures (Scr.+V01).
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 (Scr.+V01).
6) For a woman, inability to bear a child or negative urine pregnancy test on the day of injection (V01).

Study treatments

Investigational product: ChimeriVax™ Dengue Tetravalent Vaccine

Form: Liquid/Frozen

Composition: Each 0.5 mL dose contains: ~5 $\log_{10}$ Cell Culture Infectious Dose 50% ($CCID_{50}$) of each chimeric dengue serotype (1, 2, 3, 4)

Route of administration: Subcutaneous (SC)

Contol Product: YF-VAX® diluent: NaCl

Form: Liquid

Composition: NaCl

Route of administration: SC

Duration of participation: The total duration of follow-up was approximately 14 months for each subject.

Criteria for evaluation:

Safety:

- Occurrence, time to onset, number of days of occurrence, severity, action taken, and seriousness of solicited (i.e., prelisted in the subject's diary card and Case Report Form, CRF) injection site reactions occurring up to 7 days after each injection and solicited systemic reactions occurring up to 14 days after each injection.
- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), time to onset, duration, severity, action taken, relationship to vaccination (except for injection site adverse events [AEs]), and seriousness of unsolicited (spontaneously reported) injection site and systemic AEs up to 28 days after each injection and of serious adverse events (SAEs) throughout the trial.
- Occurrence and timing of out-of-normal-range biological test results up to 28 days after each injection.

Viremia

- Occurrence, maximum level, number of days of presence, and Area Under the Curve (AUC) of the four dengue vaccine serotypes in sera collected every two days during the 20-day period following the first and the second injections, and 8 and 16 days after the third injection in all the subjects: quantitative and serotype-specific reverse transcriptase-polymerase chain reaction (RT-PCR) and dengue plaque assay (PA).

- Phenotypic and genetic stability of virus isolated from viremic subjects were evaluated using plaque size phenotype, growth curve, and sequencing.

Virus shedding

Presence of the four dengue vaccine serotypes in saliva and urine samples collected every two days during the 20-day period following the first injection in 16 randomized subjects: 11 in Group 1 (ChimeriVax™ Dengue) + 5 in Group 2 (Control Group).

Immunogenicity

- Humoral immune response:

  Neutralizing antibody levels against each of the four parental dengue virus serotype strains of ChimeriVax™ Dengue Tetravalent Vaccine constructs, against the Thai Mahidol four dengue virus serotype strains, and against other strains were measured on sera collected in all the subjects before injection, and 28 days after the first, second, and third injections (Plaque Reduction Neutralization Test, PRNT).
  Non-serotype specific IgM and IgG antibody levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA) before injection, 16 days, and 28 days after the first and second injections.

- Cellular immune response:

  Non-specific immunity: cytokine levels in sera were assessed at each visit during the 28-day period following the first injection. The levels of both pro-inflammatory and anti-inflammatory cytokines were measured.

  Specific immunity was evaluated by cytometric bead array (CBA) and intracellular cytokine staining (ICS) on cells collected before injection, and 28 days after the first, second, and third injections in 33 randomized subjects (22 in Group 1 and 11 in Group 2).

Statistical methods:

The analysis was descriptive and presented by group with 95% confidence intervals for main parameters. Listings of safety data were performed after the first cohort of 18 subjects had completed Day 14 visit (including viremia data in case of SAE). These data were provided by the Sponsor's Data Management department to the IDMC. The main listings included in this analysis were defined in agreement with the members of the IDMC. Two interim statistical analyses of safety, viremia, and immunogenicity were performed on unblinded Day 28 data of the first and second injections.

Summary:

Population characteristics:

A total of 66 subjects were included and received the first vaccination: 33 subjects in Group 1 were vaccinated with the dengue vaccine and 33 subjects in Group 2 received a placebo (YF-VAX® diluent). Three subjects in Group 1 discontinued the study before the following vaccination. One subject was withdrawn by the Investigator after the occurrence of an SAE (aggravated depression) and two subjects withdrew voluntarily. Therefore, 63 subjects, 30 in Group 1 and 33 in Group 2, received the second vaccination. The third vaccination was administered to 49 subjects (23 in Group 1 and 26 in Group 2); 14 subjects, seven in each group, did not receive this vaccination. Nine subjects, five in Group 1 and four in Group 2, withdrew voluntarily, three subjects in Group 2 did not comply with the protocol, one subject in Group 1 became pregnant, and one subject in Group 1 died between the second and third vaccination. This death led to the suspension of the study. After in-depth review by the IDMC, the study resumed.

Demographic characteristics were similar in the two groups. The mean age at screening was 31.5 years in Group 1 and 31.6 years in Group 2. More than half of the subjects were overweight, with a median BMI of 24.82 in Group 1 and 27.20 in Group 2.

***Safety*:**

Five SAEs occurred during the study, all in Group 1. None of these was considered to be related to the vaccine by the Investigator. Three SAEs occurred after the the first vaccination: aggravated depression, abdominal adhesions, and acute bronchitis. Two SAEs occurred after the second vaccination: fatal ischemic stroke and thyroid nodule.

Few injection site reactions were recorded. They were mild or moderate and most of these reactions were present for a maximum of 3 days. There were no severe injection site reactions. Pain was the most frequent injection site reaction after one, two, or three vaccinations. The number of subjects who reported at least one episode of pain in the 7 days after vaccination was as follows: six subjects (18.2%) in Group 1 and two (6.1%) in Group 2 after the first vaccination, ten subjects (33.3%) in Group 1 and eight (24.2%) in Group 2 after the second vaccination, and two subjects (8.7%) in Group 1 and six (23.1%) in Group 2 after the third vaccination.

After a first administration of dengue vaccine, the most frequent solicited systemic reactions were (by decreasing frequency in Group 1):

- Headache: 17 subjects (51.5%) in Group 1 and 8 (24.2%) in Group 2,
- Malaise: 11 subjects (33.3%) in Group 1 and 3 (9.1%) in Group 2,
- Myalgia: 10 subjects (30.3%) in Group 1 and 6 (18.2%) in Group 2,
- Asthenia: 9 subjects (27.3%)in Group 1 and 1 (3.0%) in Group 2,
- Fever: 6 subjects (18.2%) in Group 1 and 8 (24.2%) in Group 2.

After the first administration of dengue vaccine, most solicited reactions were mild to moderate and present on a few days. In Group 1, two subjects reported severe malaise, one subject had severe myalgia, and one subject reported severe malaise and myalgia. Fever did not exceed 39°C in Group 1, while in Group 2, pyrexia, i.e. oral temperature ≥39.1°C, was reported in two subjects.

After the second administration of dengue vaccine, the proportion of subjects with at least one solicited systemic reaction did not increase compared to the first administration. In Group 1, the most frequent solicited reactions were as follows: headache (16 subjects, 53.3%), malaise (9 subjects, 30.0%), myalgia (7 subjects, 23.3%), asthenia (5 subjects, 16.7%) and fever (4 subjects, 13.3%). One subject reported severe headache, malaise, myalgia, and asthenia. In Group 2, headache and fever were the most frequent solicited systemic reactions (8 subjects, 30.8% each), followed by malaise (4 subjects, 15.4%) and asthenia (3 subjects, 11.5%).

After the third administration of dengue vaccine, the proportion of subjects with at least one solicited reaction decreased. The most frequent solicited reactions in Group 1 were headache (5 subjects, 21.7%), fever (4 subjects, 17.4%), myalgia (3 subjects, 13.0%), malaise (1 subject, 4.3%), and asthenia (1 subject, 4.3%). No severe reactions were reported.

Unsolicited AEs were reported in 57.5% of subjects in Group 1 and 36.3% in Group 2 after the first administration of dengue vaccine. These proportions were stable after the second administration of dengue vaccine with 43.3% in Group 1 and 23% in Group 2, and decreased markedly after the third vaccination with 26.0% in Group 1.

Biological abnormalities were the most frequent unsolicited AEs (3.3% to 27.3% of subjects, depending on the vaccination) and included mainly decreases in WBC and neutrophil counts after the first administration of dengue vaccine. Severe biological abnormalities were reported after the first administration of dengue vaccine and consisted in neutrophil count decrease in one subject and CPK increase in two subjects. No severe biological abnormalities were reported after two or three administrations of dengue vaccine.

Viremia

"Detection" should be understood as a value above the lower limit of quantitation or as the detection of amplicons.

After the first administration of dengue vaccine, non-serotype specific ChimeriVax™ dengue vaccine (CYD) viremia, as detected by YF RT-PCR, was detected but could not be quantified in 18.5% to 20.7% of samples with a peak at V05 (D8) and V08 (D14). Non-serotype specific CYD viremia was detected in 2.8% to 5.1% of samples after the second administration of dengue vaccine, and in 4.0% of samples after the third administration. Serotype-specific CYD viremia for dengue serotypes 1, 2, and 3 was not detected or negligible after the first, second, or third administration of dengue vaccine, and was very limited for dengue serotype 4 after the first or third administration, and absent after the second administration.

Virus shedding

PCR-based methods failed to quantify any CYD virus in saliva or urine samples from the subjects vaccinated with the dengue vaccine.

Four and five subjects vaccinated with the dengue vaccine in Group 1 had a detection signal with YF RT-PCR in saliva and urine samples respectively, but this signal was not confirmed by subsequent testing with the CYD RT-PCRs, except in one case with the CYD-4 PCR. There was no link between either presence of viremia versus detection of a signal in saliva or urine, or between detection signal in saliva versus urine samples (with regards to either subjects or samples).

In the five subjects of the control group, a signal was detected by YF-PCR (from saliva but not from urine samples).

As a result, the signal detected was deemed to be non-specific. These findings do not support the conclusion of the shedding of CYD vaccinal viruses in saliva or urine.

Immunogenicity:

- Humoral immune response

  ChimeriVax™ Dengue Tetravalent Vaccine induced neutralizing antibodies, and IgM and IgG antibodies.

  Percentages are presented as ranges since results varied depending on the laboratory and strain.

  After the first administration of dengue vaccine in Group 1, seropositivity rates for neutralizing antibodies were high against serotype 2 (30.3% to 66.7% depending on the laboratory and strain) and serotype 4 (57.6% to 72.7%), but lower against serotype 1 (12.1% to 51.5%) and serotype 3 (18.2% to 39.4%). After the first administration of dengue vaccine in Group 2, the humoral response was similar to the response observed after the first administration in Group 1. An IgM response was observed mainly after the first injection in the two groups (75.8% and 90.9% seropositivity 28 days after the first administration of dengue vaccine in Group 1 and Group 2, respectively). A high seropositivity rate to IgG, i.e. 84.8%, was observed 28 days after the first administration of dengue vaccine in Group 1 (versus 57.6% in Group 2).

  After the second administration of dengue vaccine in Group 1, the percentage of seropositive subjects increased for serotype 1 (50.0% to 86.7%) and serotype 2 (58.6% to 93.3%), and to a lesser extent for serotype 4 (73.3% to 90.0%). The change for serotype 3 was not so clear (10.0% to 73.3%). The percentage of seropositivity ranged from 63.3% to 86.7% for at least three serotypes and from 30.0% to 60.0% for four serotypes, depending on the laboratory and strain. All subjects had IgG antibodies after the second vaccination in this group. After the second administration of dengue vaccine in Group 2, the percentage of seropositive subjects and the GMTs increased for all serotypes. The percentage of seropositivity ranged from 61.5% to 100% for at least three serotypes, and from 26.9% to 100.0% for four serotypes, depending on the laboratory and strain. All subjects had IgG antibodies after the second dose of dengue vaccine in this group.

  After the third administration of dengue vaccine in Group 1, the percentage of seropositive subjects further increased for all serotypes. The percentage of seropositivity ranged from 82.6% to 100.0% for at least three serotypes, and from 39.1% to 100.0% for four serotypes, depending on the laboratory and strain.

- Cellular immune response

  For non-specific immunity, serum cytokines were measured at each visit during the 28-day period after the first vaccination in all subjects. No variations were observed in the cytokines secreted in the sera of all 66 subjects, after the first vaccination (dengue vaccine or placebo).

  Specific immunity was evaluated by CBA and ICS on cells collected before and 28 days after each vaccination in 33 randomized subjects. ChimeriVax™ Dengue Tetravalent Vaccine was able to trigger significant, although modest, Th1 and

  17D-NS3-specific CD8+ immune responses in vaccinees. After one vaccination, serotypes 2 and 4 induced Th1 responses while after the second vaccination, a broader serotype response was noted with all 4 serotypes inducing Th1 responses. No increase in responses were detected after the third vaccination in each group, regardless of the stimulation. This absence of response can probably be explained by the unusual aspect of PBMC after thawing at V27 and V30, which probably reflects a poor quality and reactivity of these cells. Responses were dominated by IFN over TNF . IL2 was not induced, suggesting that lymphoproliferation was not optimal.

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