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

Sponsor: Sanofi Pasteur **Drug substance(s):** CYD Dengue Vaccine	**Study Identifiers:** NCT00730288 **Study code:** CYD10
Title of the study: Safety of ChimeriVax™ Dengue Tetravalent Vaccine in Adult Subjects Previously Immunized with an Investigational Dengue or Yellow Fever Vaccine	
Study center(s): One center in Australia	
Study period: Date first subject enrolled: 02/Aug/2006 Date last subject completed: 13/Mar/2007	
Phase of development: Phase IIa	
Objectives: 1) To describe the safety of one injection of ChimeriVax™ dengue tetravalent vaccine. 2) To describe viremia after one injection of ChimeriVax™ dengue tetravalent vaccine. 3) To describe the cellular and humoral immune response against dengue before and after one injection of ChimeriVax™ dengue tetravalent vaccine.	
Methodology: Open, controlled, monocenter, Phase IIa study. Subjects were recruited from the pool of subjects who completed the one-year follow-up of the DIV12 trial (conducted by the Sponsor in the same study center). In DIV12, 24 subjects received one injection of the monovalent Vero dengue vaccine (VDV1 or VDV2) and 12 subjects received one injection of yellow fever (YF) vaccine. In addition, a control group of flavivirus-naive subjects (who did not participate in the DIV12 trial) were included in the present trial. Three groups of subjects received one dose of the investigational vaccine. • VDV group: subjects who previously received one injection of monovalent VDV1 or VDV2. • YFV group: subjects who previously received one injection of YF vaccine. • Control group: flavivirus-naive subjects.	
Number of subjects:	Planned sample size was 48 subjects: VDV group: 24 subjects, YFV group: 12 subjects, and Control group: 12 subjects. Actual number of subjects enrolled was 35 subjects (Full Analysis Set [FAS] and Safety Analysis Set): VDV group: 15 subjects, YFV group: 8 subjects, and Control group: 12 subjects. All subjects completed the trial. Treated: 35
Evaluated:	Immunogenicity: 35 Safety: 35

Diagnosis and criteria for inclusion:

Criteria 1 to 4 were checked at screening only:

1) Aged 18 to 40 years on the day of inclusion.
2) Informed consent form signed.
3) For a woman, inability to bear a child or negative serum pregnancy test.
4) Completed the one-year follow-up of Study DIV12 (applicable to DIV12 subjects only).

Criteria 5 and 6 were checked at screening and at the vaccination visit (V01):

5) Able to attend all scheduled visits and to comply with all trial procedures.
6) For a woman of child-bearing potential: use of an effective method of contraception or abstinence for at least 4 weeks prior to vaccination and at least 4 weeks after vaccination.

Criterion 7 was checked at the vaccination visit (V01) only:

7) For a woman, inability to bear a child or negative urine pregnancy test.

Study treatments

Investigational Product: ChimeriVax™ dengue tetravalent vaccine

Form: Liquid/frozen

Composition: Each 0.5 mL dose contains approximately 5 $\log_{10}$ cell culture infectious dose ($CCID_{50}$) of each chimeric dengue serotype (1, 2, 3, and 4).

Route of administration: Subcutaneous

Dose regimen: One injection of dengue vaccine was administered on D0.

Duration of participation: The planned total duration of participation in the trial (from V01 to last visit [V07]) was approximately 6 months per subject.

Criteria for evaluation:

Safety

- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, severity, and relationship to vaccination of any systemic adverse events (AEs) reported in the 30 minutes after vaccination.
- Occurrence, time to onset, number of days of occurrence, and severity of solicited (pre-listed in the subject diary and Case Report Form [CRF]) injection site reactions occurring up to 7 days after vaccination and solicited systemic reactions occurring up to 14 days after vaccination.
- Occurrence, nature (MedDRA preferred term), time to onset, duration, severity, and relationship to vaccination (for systemic AEs only) of unsolicited (spontaneously reported) AEs up to 28 days after vaccination.
- Occurrence, nature (MedDRA preferred term), time to onset, duration, and relationship to vaccination of serious adverse events (SAEs) throughout the trial.
- Occurrence and timing of out-of-range biological test results before vaccination and 7, 14, and 28 days after vaccination.

Viremia

- Occurrence and maximum level of the four dengue vaccine serotypes in sera collected before vaccination and 7, 14, and 21 days after vaccination: quantitative and serotype-specific reverse transcriptase-polymerase chain reaction (RT-PCR) and dengue plaque assay (PA).

Immunogenicity

Humoral immune response

- Neutralizing antibody levels against the four parental dengue virus strains of ChimeriVax™ dengue constructs and

against the four Thai Mahidol dengue virus strains were measured in sera collected from all subjects before vaccination and 28, 60, and 180 days after vaccination (Plaque Reduction Neutralization Test [PRNT]).

- Neutralizing antibody levels against YF virus 17D strain were measured in sera collected from all subjects before vaccination and 28 days after vaccination (PRNT).
- Non-serotype specific dengue immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA) before vaccination, and 14 and 28 days after vaccination.

Cellular immune response

- Specific immunity was evaluated by cytometric bead array (CBA) after lymphocyte proliferation and by intracellular cytokine staining (ICS) after antigenic stimulation of cells collected before and 28 days after vaccination.

Statistical methods:

Analysis was descriptive and presented by group with 95% confidence intervals (CIs) for main parameters. An interim statistical analysis of safety, viremia, and immunogenicity was conducted on D28 data. A final statistical analysis was performed at the end of the D180 follow-up.

Summary:

Population characteristics:

A total of 35 subjects (15 subjects in the VDV group, 8 subjects in the YFV group and 12 subjects in the Control group) were vaccinated at V01 and had blood samples taken at each visit of the trial. All 35 subjects completed the trial and formed the FAS (and the Safety Analysis Set). One subject in the YFV group was excluded from the PP analysis set since his blood sample was taken outside the planned time window after injection.

The three groups were well balanced in terms of demographic characteristics. The mean age of subjects was 25.9 ± 5.9 years and the mean body mass index was 25.4 ± 3.8 kg/m^2.

Safety:

The safety profile is presented by group in the table below:

	VDV		YFV		Control	
	n/N	%	n/N	%	n/N	%
Subjects with at least one:						
- Event	11/15	73.3	7/8	87.5	12/12	100.0
- Reaction	10/15	66.7	6/8	75.0	11/12	91.7
- Immediate event	0/15	0.0	0/8	0.0	0/12	0.0
- Injection site reaction	6/15	40.0	0/8	0.0	5/12	41.7
- Systemic reaction	8/15	53.3	6/8	75.0	9/12	75.0
- Clin. sign. biological abnormality	1/15	6.7	3/8	37.5	2/12	16.7
- Severe event	1/15	6.7	3/8	37.5	2/12	16.7
- Severe reaction	1/15	6.7	1/8	12.5	0/12	0.0
- Severe injection site reaction	0/15	0.0	0/8	0.0	0/12	0.0
- Severe systemic reaction	1/15	6.7	1/8	12.5	0/12	0.0
- Severe biological abnormality	0/15	0.0	0/8	0.0	1/12	8.3
- SAE since vaccination (D0)	0/15	0.0	0/8	0.0	0/12	0.0
- Related SAE since vaccination (D0)	0/15	0.0	0/8	0.0	0/12	0.0
- SAE leading to withdrawal since vaccination (D0)	0/15	0.0	0/8	0.0	0/12	0.0

Adverse events

No SAE, withdrawal due to AE, or significant AE occurred during this trial.

Overall, the incidence of AEs was slightly lower in the VDV group (11 subjects, 73.3%) than in the other two groups (7 subjects [87.5%] and 12 subjects [100.0%] in the YFV and Control groups, respectively). The same trend was observed for systemic reactions (8 subjects [53.3%] in the VDV group, 6 subjects [75.0%] in the YFV group and 9 subjects [75.0%] in the Control group). Injection site reactions were more frequently reported in the VDV group (6 subjects [40.0%]) and in the Control group (5 subjects [41.7%]) than in the YFV group (none [0.0%]).

AEs were mainly mild or moderate. Most AEs required no action; medications were occasionally prescribed. No severe injection site reaction was reported. Severe solicited systemic reactions were observed in two subjects, one subject (6.7%) in the VDV group reported headache on D13, which resolved after one day following intake of medication, and one subject (12.5%) in the YFV group reported malaise on D10, which resolved after one day, following intake of medication.

In addition, severe unsolicited AEs considered as unrelated to the vaccination by the Investigator were reported by one subject in the VDV group (neck pain), three subjects in the YFV group (sinusitis, upper respiratory tract infection, and laryngitis), and two subjects in the Control group (incontinence and pollakiuria, and blood creatine phosphokinase [CPK] increased).

Solicited injection site reactions between D0 and D7 consisted in injection site pain in two subjects in the Control group, injection site erythema in 6 subjects (40.0%) in the VDV group, no subject (0.0%) in the YFV group and 2 subjects (16.7%) in the Control group, and injection site edema in one subject (6.7%) in the VDV group.

Headache was the most frequently reported solicited systemic reaction in all groups (7 subjects [46.7%] in the VDV group, 5 subjects [62.5%] in the YFV group, and 8 subjects [66.7%] in the Control group), followed by malaise and myalgia (between 2 and 5 subjects per group). Malaise was less frequent in the VDV group than in the other two groups, while myalgia occurred with comparable frequency in the three groups. Asthenia and fever were more frequent in the Control group (5 subjects [41.7%] and 3 subjects [25.0%], respectively) than in the other groups (asthenia: 13.3% in the VDV group and 12.5% in the YFV group; fever: 7.1% in the VDV group and no subject in the YFV group).

Between D0 and D14, 10 unsolicited AEs occurred in 6 subjects (40.0%) in the VDV group, 7 AEs in 4 subjects (50.0%) in the YFV group, and 8 AEs in 4 subjects (33.3%) in the Control group. Three subjects reported injection site induration (VDV group), pruritus (VDV group) and injection site hemorraghe (Control group), while the remainder of AEs consisted of systemic events reported by one or two subjects each.

Between D15 and D28, 14 unsolicited AEs occurred in 7 subjects (46.7%) in the VDV group, 7 AEs in 3 subjects (37.5%) in the YFV group, and 5 AEs in 5 subjects (41.7%) in the Control group. No injections site reactions were reported; various systemic events were reported by one or two subjects each.

Laboratory tests

Before vaccination (D0), few subjects had laboratory abnormalities: 3 subjects (20.0%) in the VDV group, 3 subjects (37.5%) in the YFV group and 5 subjects (41.7%) in the Control group. The most frequent abnormality was increased CPK levels in four subjects (from the three groups).

After D0, the total number of subjects with laboratory abnormalities increased to 9 subjects (60.0%) in the VDV group, 6 subjects (75.0%) in the YFV group and 9 subjects (75.0%) in the Control group. These were generally of limited duration and only one was severe (CPK increase in a Control subject reported as AE).

Clinically significant abnormalities after vaccination were reported in a few subjects with no trend according to group (one subject [6.7%] in the VDV group, 3 subjects [37.5%] in the YFV group, and 2 subjects [16.7%] in the Control group). The abnormalities concerned WBC, neutrophils, CPK, ALT, and AST changes, and were all reported as AEs.

Vital signs variation (systolic and diastolic blood pressure, and pulse rate) remained at similar level for most subjects before and 30 minutes after vaccination, and no variation was reported as AE.

Viremia

Viremia results are summarized by group in the table below:

			VDV	YFV	Control
Any serotype (YF RT-PCR)	**D0**	**N available**	15	8	12
		n (%) with viremia detectable	0 (0.0)	0 (0.0)	0 (0.0)
		n (%) with viremia (positive)	0 (0.0)	0 (0.0)	0 (0.0)
	D7	**N available**	15	8	12
		n (%) with viremia detectable	7 (46.7)	6 (75.0)	7 (58.3)
		n (%) with viremia (positive)	2 (13.3)	1 (12.5)	0 (0.0)
	D14	**N available**	15	8	12
		n (%) with viremia detectable	0 (0.0)	1 (12.5)	6 (50.0)
		n (%) with viremia (positive)	0 (0.0)	0 (0.0)	2 (16.7)
	D21	**N available**	15	8	12
		n (%) with viremia detectable	0 (0.0)	0 (0.0)	0 (0.0)
		n (%) with viremia (positive)	0 (0.0)	0 (0.0)	0 (0.0)
	D7, D14 and/or D21	**N available**	15	8	12
		n (%) with viremia detectable	7 (46.7)	7 (87.5)	10 (83.3)
		n (%) with viremia (positive)	2 (13.3)	1 (12.5)	2 (16.7)

Before vaccination (D0), no subject had detectable non-serotype specific dengue vaccine viremia. Viremia reached a peak on D7 in the VDV and YFV groups, in terms of both detectable and positive viremia, while in the Control group the viremia peak appeared to be present at both D7 and D14. On D21, viremia was no longer detectable in any subject.

Among subjects with viremia of any serotype (as assessed by YF RT-PCR), none had serotype 1 or serotype 2 viremia. Serotype 4 was the most frequently observed serotype in all groups, followed by serotype 3.

When PA was used for subjects with viremia of any serotype, no subject presented serotype 1, 2 or 3 viremia. For serotype 4, positive viremia was observed in 2 of 6 subjects (33.3%) in the YFV group on D7 and 1 of 6 subjects (16.7%) in the Control group on D14.

Immunogenicity:

Humoral immune response

Results of neutralizing antibody levels against parental and Mahidol strains measured by $PRNT_{50}$ obtained before vaccination (D0) and 28 days after vaccination are summarized in the table below.

		VDV		YFV		Control	
Parental strain		**D0**	**D28**	**D0**	**D28**	**D0**	**D28**
Serotype 1	**N available**	15	15	8	8	12	12
	GMT (95% CI)	7.02 (4.70;10.5)	45.8 (28.5;73.8)	5.00 (5.00;5.00)	8.40 (3.76;18.8)	5.00 (5.00;5.00)	5.00 (5.00;5.00)
	n (%) >= 10 1/dil (95% CI)	3 (20.0%) (4.3;48.1)	14 (93.3%) (68.1;99.8)	0 (0%) (0;36.9)	2 (25.0%) (3.2;65.1)	0 (0%) (0;26.5)	0 (0%) (0;26.5)
Serotype 2	**N available**	15	15	8	8	12	12
	GMT (95% CI)	13.4 (7.04;25.7)	52.5 (21.8;126)	5.74 (4.15;7.94)	28.1 (10.8;73.1)	5.00 (5.00;5.00)	7.67 (4.62;12.7)
	n (%) >= 10 1/dil (95% CI)	7 (46.7%) (21.3;73.4)	12 (80.0%) (51.9;95.7)	1 (12.5%) (0.3;52.7)	6 (75.0%) (34.9;96.8)	0 (0%) (0;26.5)	3 (25.0%) (5.5;57.2)
Serotype 3	**N available**	15	15	8	8	12	12
	GMT (95% CI)	5.36 (4.62;6.20)	41.4 (20.6;83.1)	5.00 (5.00;5.00)	33.1 (13.9;78.7)	5.00 (5.00;5.00)	28.0 (8.95;87.2)
	n (%) >= 10 1/dil (95% CI)	1 (6.7%) (0.2;31.9)	13 (86.7%) (59.5;98.3)	0 (0%) (0;36.9)	7 (87.5%) (47.3;99.7)	0 (0%) (0;26.5)	7 (58.3%) (27.7;84.8)
Serotype 4	**N available**	15	14	8	8	12	12
	GMT (95% CI)	5.00 (5.00;5.00)	91.1 (37.5;222)	5.00 (5.00;5.00)	221 (64.4;762)	5.00 (5.00;5.00)	103 (20.0;527)
	n (%) >= 10 1/dil (95% CI)	0 (0%) (0;21.8)	12 (85.7%) (57.2;98.2)	0 (0%) (0;36.9)	8 (100%) (63.1;100)	0 (0%) (0;26.5)	8 (66.7%) (34.9;90.1)

Before vaccination (D0), low and comparable geometric mean titer (GMT) values were recorded in subjects from the three groups for the parental dengue strain of serotype 1. As regards the serotype 2, 7 of 15 subjects (46.7%) from the VDV group were seropositive compared with 1 of 8 subjects (12.5%) in the YFV group and no subject in the Control group. For Serotype 3, 1 of 15 subjects (6.7%) from the VDV group was seropositive, compared with no subject in both the YFV and Control groups. No subject was seropositive for serotype 4.

On D28 after vaccination, at least 12 of 15 subjects (80.0%) in the VDV group were seropositive for each serotype of the parental dengue strain. For serotype 1, the proportion of seropositive subject was significantly higher in the VDV group than in the YFV and Control groups. For serotypes 2, 3, and 4, the proportion of seropositive subjects was comparable in the VDV and YFV groups. In the Control group, the number of seropositive subjects was lower than in the other groups for the four serotypes, although for serotypes 3 and 4 more than half of the control subjects were seropositive.

On D60, a delayed response was observed for serotype 1 in the YFV group: on D28, 2 of 8 subjects were seropositive, which increased to 5 of 8 subjects on D60 and further to 6 of 8 subjects on D180.

On D180, the number of seropositive subjects in the VDV group slightly decreased for serotype 1 (from 14 subjects [93.3%] on D28 to 13 subject [86.7%] on D180), serotype 2 (from 12 subjects [80.0%] on D28 to 10 subjects [66.7%] on D180), and serotype 4 (from 12 subjects [85.7%] on D28 to 11 subjects [73.3%] on D180), and decreased noticeably for the serotype 3 (from 13 subjects [86.7%] on D28 to 8 subjects [53.3%] on D180).

In the YFV group, the number of seropositive subjects increased at each visit for serotype 1, while it remained stable for serotypes 2, 3, and 4.

Results obtained for the Mahidol strain showed similar trends in proportion of seropositive subjects in the three groups (in general a lower proportion but similar kinetics) with a few exceptions.

Positive response (≥10 1/dil.) to at least three serotypes of the parental strain on D28 was observed in 11 of 15 subjects (73.3%) in the VDV group, compared to 6 of 8 subjects (75.0%) in the YFV group and 2 of 12 subjects (16.7%) in the Control group.

No effect of dengue vaccine on YF antibody titer in subjects previously immunized with YF vaccine was observed.

Non-serotype specific IgM and IgG dengue antibody levels measured using ELISA showed IgM levels that increased with time after vaccination in the VDV and Control groups. On D28, 12 of 15 subjects (80.0%) from the VDV group had IgM antibody levels of ≥1, compared with 1 of 8 subjects (12.5%) in the YFV group and 10 of 12 subjects (83.3%) in the Control group.

All subjects in the VDV and YFV groups, and 10, 11 and 11 of 12 subjects, respectively, in the Control group, had IgG levels of ≥1 on D0, D14, and D28. No subjects from any group had an IgM/IgG ratio ≥1.8 before and 14 and 28 days after vaccination.

Cellular immune **response**

Quantification of cellular cytokines in supernatants after antigenic stimulation showed that as regards T helper type 1 (Th1), enhanced responses were detected against each serotype (ChimeriVax™ dengue vaccine [CYD] 1 to 4) for interferon (IFN) γ in the VDV (monovalent ChimeriVax™ dengue [DEN]) pre-immune subjects, which presented a broader response than seen in naïve and 17D-primed subjects. In these latter groups, IFNγ secretion was mostly induced by CYD4. No marked changes were observed for tumor necrosis factor (TNF) α and interleukin (IL) 2.

No Th2 response (IL4, IL5 or IL10) was detected regardless of the stimulation used.

ICS assays showed that T cytotoxic type (Tc) 1 CD8+ T cell response was developed in the majority of the 17D-pre-immune or naïve subjects after restimulation with YF non-structural (NS)3 peptides, and in the VDV group after stimulation with DEN NS3 peptides pools. Importantly, VDV-primed subjects did not react with 17D NS3 pools before, and in most cases even after, CYD1 to 4 vaccination, and vice versa: 17D-primed or naïve subjects immunized with CYD1 to 4 did not react with DEN3 NS3 pools. This shows that no significant cross-reactivity exists between 17D and DEN NS3 at the CD8 epitope level. Moreover, it seems that pre-existing responses against DEN peptides as seen in VDV-primed subjects can subdue subsequent responses against the 17D backbone.

Moderate CD4+ T cell responses were detected in four subjects in the three groups after restimulation with YF NS3 peptides pool.

Issue date: 15-May-2020