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Sponsor: Acambis **Drug substance(s):** CYD Dengue Vaccine	**Study Identifiers:** IND 11219 **Study code:** CYD02
Title of the study: Randomized, double-blind, placebo controlled Phase I study of the safety, tolerability and immunogenicity of live attenuated tetravalent ChimeriVax™-Dengue vaccine and yellow fever vaccine (YF-VAX®)	
Study center(s): One center in the US	
Study period: Date first subject enrolled: 17/Nov/2003 Date last subject completed: 13/Nov/2004	
Phase of development: Phase I	

Objectives: The specific objectives of the study, in order of priority, were to investigate:

- The safety, tolerability, and viremia levels following receipt of a subcutaneous injection of ChimeriVax™-DEN vaccine containing serotypes DEN1, DEN2, DEN3, and DEN4, in one tetravalent formulation and to compare safety and tolerability with licensed yellow fever (YF) vaccine, YF-VAX®, and placebo (YF-VAX® diluent).
- Neutralizing antibody response in terms of seroconversion rate and geometric mean titer (GMT) 30 days after SC injection of tetravalent ChimeriVax™-DEN compared with YF-VAX®. Supplementary endpoints included analysis of GMT and seroconversion rates at intermediate time points indicated in the study schedule to show the kinetics of the immune response up to 5-9 months.
- The effect of prior YF vaccination and boosting on safety and immunogenicity following administration of tetravalent ChimeriVax™-DEN at a 5-9-month interval.

Methodology:

The duration of the study for each subject was approximately 13 months. The study comprised a screening period of 3 to 21 days, a double-blind treatment period of 30 days (Stage 1), and a second screening period of 3 to 21 days followed by an open-label treatment period of 30 days (Stage 2) commencing 5 to 9 months after the double-blind treatment period. All subjects were to attend a follow-up visit at 12 months.

In the first stage of the study, 3 groups of 33 subjects received a vaccination of tetravalent ChimeriVax™-DEN (Group 1), YF-VAX® (Group 2) or placebo (YF-VAX® diluent - Group 3). Dosing was staggered such that the first cohort of 18 subjects (6 subjects received each treatment - tetravalent ChimeriVax™-DEN, YF-VAX®, and placebo) were followed up to Day 31. Safety data collected through to Day 31 from the first cohort of subjects was reviewed: 1) The Data Safety Monitoring Board (DSMB) independently reviewed all safety data collected up to Day 31 and provided a written opinion regarding dosing of the remaining subjects for Stage 1 of the study; 2) The Medical Monitor and Principal Investigator reviewed all safety data; 3) Selected Sponsor representatives independent of the clinical management of the study reviewed immunogenicity data. Dosing of additional cohorts was to be suspended if 1 or more of the following criteria was met:

- a serious adverse event (SAE) considered possibly, probably, or definitely related to vaccination, or
- any other adverse event (AE) considered possibly, probably, or definitely related to vaccination with a toxicity grade 3 in 1 subject or grade 2 in 2 subjects.

After all subjects completed the 30-day treatment period and database lock, the trial was unblinded.

In the second stage of the study, following a safety assessment and eligibility review at 5 to 9 months, eligible subjects received a vaccination of tetravalent ChimeriVax™-DEN (as documented by protocol amendment 3, 1 April 2004) as open-label treatment and were followed through 12 months after initial vaccination as shown in the following table.

	Stage 1 (Double-Blind)	Stage 2 (Open-Label)
Group 1	ChimeriVax™-DEN	ChimeriVax™-DEN
Group 2	YF-VAX®	ChimeriVax™-DEN
Group 3	Placebo	ChimeriVax™-DEN

During each treatment period (Stage 1 and Stage 2), subjects were followed-up at selected time points for up to 30 days post-vaccination for safety and immunogenicity assessments. Subjects were asked to complete a diary card for 30 days after vaccination to record signs/symptoms, changes in medication, and oral temperature. AE details were collected by questioning subjects and review of the diary card at scheduled clinic visits. A scripted questionnaire was used to prompt each subject for "expected" AEs based on the established reactogenicity of ChimeriVax™-DEN2 and YF17D vaccine. Subjects who developed an undifferentiated febrile illness were evaluated for intercurrent infection by attempting virus isolation from nasopharyngeal swab samples, and other investigations as appropriate.

Number of patients: Planned: 99

Randomized: 99

Treated: 99 (79 of the 99 Stage 1 subjects started and completed Stage 2 of the study)

Evaluated:

Efficacy/Immunogenicity: 99

Safety: 99

Diagnosis and criteria for inclusion:

Healthy males or females aged 18-40 inclusive. No history of prior vaccination or infection with flaviviruses (including Japanese encephalitis, tick-borne encephalitis, YF, St Louis encephalitis, West Nile virus, dengue fever). No history of residence in or travel for a period of 2 weeks or more to the tropics, including Mexico, Africa, India, South-east Asia, Central America, the Caribbean or South America.

Study treatments

Test product: ChimeriVax™-DEN

Formulation: tetravalent combination containing serotypes 1, 2, 3, and 4, each component at ~ 4 $\log_{10}$ 50% tissue culture infectious dose ($TCID_{50}$)/dose. ChimeriVax™-DEN was given in a volume of 0.5 mL.

Route of administration: Subcutaneous (SC)

Dose regimen: Single vaccinations given on Day 1 (Stage 1) and at 5 to 9 months (Stage 2)

Reference therapy: YF-VAX® and Placebo

Formulation: YF-VAX® at a nominal dose of 4.74 log10 plaque forming units (PFU) per dose or placebo (YF-VAX® diluent) (Stage 1 only). YF-VAX® and placebo were given in a volume of 0.5 mL.

Route of administration: SC

Dose regimen: Single vaccinations given on Day 1 (Stage 1)

Duration of participation: The duration of the study for each subject was approximately 13 months.

Criteria for evaluation:

Safety:

Safety criteria included AE incidence rates, physical and neurological examination, vital signs (blood pressure, pulse, respiratory rate and oral body temperature), routine laboratory safety assessments (hematology, clinical chemistry, and urinalysis), and viremia (using quantitative tests for viremia by plaque assay).

The primary safety analyses were evaluation of AE incidence rates and clinical laboratory changes among treatment groups up to 30 days post-vaccination (in Stages 1 and 2). For viremia, ChimeriVax™-DEN was compared against YF-VAX® up to Day 21 in Stage 1. In Stage 2, AE incidence rates, clinical laboratory evaluations, and viremia up to 30 days were compared between groups, and within the ChimeriVax™-DEN group, comparing 1 versus 2 doses of tetravalent ChimeriVax™-DEN.

Efficacy/Immunogenicity:

Immunogenicity endpoints were secondary and included:

- The seroconversion rate to wild-type (WT) dengue serotypes 1-4 (for tetravalent ChimeriVax™-DEN subjects) or to YF17D (for YF-VAX® subjects) at Day 31 and Month 5-to-9 + 30 days, using constant-virus, serum-dilution 50% plaque-reduction neutralization test ($PRNT_{50}$).

The analysis defined seroconversion rates to all 4 dengue serotypes and to each individual serotype as a 4-fold or greater rise in neutralizing antibody titer between pre- and post-immunization samples. Subjects who were seronegative at baseline (< 1:10) required a PRNT50 titer of ≥ 1:20 to meet the criteria for seroconversion.

- Neutralizing antibody response to homologous ChimeriVax™-DEN virus by $PRNT_{50}$ assay.
- Geometric mean neutralizing antibody titer (GMT) and seroconversion rate 10 and 30 days and 6 months after each vaccination to WT DEN serotypes 1-4, homologous tetravalent ChimeriVax™-DEN virus, and YF between treatment groups (both stages) and within the ChimeriVax™-DEN group (Stage 2; to show the boosting effect of 2 versus 1 doses of tetravalent ChimeriVax™-DEN).

Statistical methods:

Incidences of all treatment-emergent AEs (i.e., those that started or became more severe after vaccination) and incidence of AEs considered related to study drug (i.e., those assessed by the Investigator as possibly, probably, or definitely related to treatment) were described for each treatment group after each vaccination. A symptom index was calculated and summary statistics presented. Vital signs, normal and abnormal clinical laboratory values (hematology, blood chemistry and urinalysis) were tabulated for each treatment group after each vaccination. Scatterplots of laboratory values were provided for each numeric laboratory parameter. Pre and post values were plotted by treatment group for each vaccination, along with the limits of the normal reference ranges.

The following summary measures of the viremia results were calculated for the 21-day period following first vaccination:

- mean peak viremia (calculated as the maximum viremia result);
- mean duration of viremia (calculated as the number of days from first to last non-zero viremia result);
- area under the viremia time curve (AUC, calculated using the linear trapezoidal method);
- proportion of subjects viremic by study day (first and second vaccinations);
- proportion of subjects viremic on 1 or more study days (first and second vaccinations).

The proportions of subjects undergoing seroconversion to any dengue (DEN) strain (defined as a PRNT50 titer of ≥ 20), as well as the proportions of subjects undergoing seroconversion to at least 1, 2, 3, or all 4 serotypes for Mahidol WT strains (DEN1 16007,

TDEN2 16681, DEN3 16562, and DEN4 1036) and homologous DEN strains, were defined. Summary statistics (mean, standard deviation, minimum, median and maximum) were calculated for GMT and described between Group 1 versus Group 2 after first vaccination, and between Group 1, Group 2 and Group 3 in stage 2. The difference between treatment groups and dose effects for each of the summary measures were tested using an appropriate analysis of variance (ANOVA) model.

GMT responses for each treatment group were plotted.

Summary:

Population characteristics:

In Stage 1, 99 subjects were randomized to the 3 treatment groups, 33 in each group. All subjects completed their Day 31 visit. In Stage 2, 79 subjects were enrolled, of whom 29 were vaccinated with a second dose of ChimeriVax™-DEN (Group 1), 26 subjects vaccinated with YF-VAX® in Stage 1 received a single dose of ChimeriVax™-DEN (Group 2), and 24 vaccinated with placebo in Stage 1 received a single dose of ChimeriVax™-DEN (Group 3).

Safety:

First vaccination: Stage 1 (double-blind treatment)

All 99 subjects were evaluable for safety.

Overview of main safety results in Stage 1:

	Group 1 ChimeriVax™-DEN	**Group 2 YF-VAX®**	**Group 3 Placebo**
N	33	33	33
Treatment-emergent AEs			
No. (%) with AEs	30 (90.9)	31 (93.9)	27 (81.8)
Total AEs, n	182	288	149
Headache	18 (54.5)	24 (72.7)	15 (45.5)
Photophobia	4 (12.1)	2 (6.1)	0 (0.0)
Lethargy	11 (33.3)	7 (21.2)	6 (18.2)
Laboratory abnormalities, n (%)			
WBC count increased	1 (3.0)	2 (6.1)	6 (18.2)
Blood CPK increased	4 (12.1)	6 (18.2)	6 (18.2)
Blood bilirubin increased	0 (0.0)	4 (12.1)	1 (3.0)
Viremia (>10 PFU), n (%)			
DEN1	1	1	0
DEN2	1	0	0
DEN3	13	0	0
DEN4	14	0	0

Adverse events:

- There were no deaths. There were 3 SAEs (1 subject each hospitalized for sunburn, septic prepatellar bursitis, and total hysterectomy [after Day 31 visit]), but none of these were related to treatment.
- The incidence of treatment-emergent AEs was similar across the groups (Group 1, 90.9%; Group 2, 93.9%; Group 3, 81.8%).
- The most commonly affected system organ classes in all 3 groups were general disorders and administration site conditions, nervous system disorders, musculoskeletal and connective tissue disorders, and investigations.
- The incidence of headache, the most commonly reported treatment-emergent AE, was higher in Group 2 (72.7% of subjects) than in Group 1 (54.5%) or Group 3 (45.5%). Most of the other commonly reported treatment-emergent AEs (including myalgia, fatigue, arthralgia, lethargy, and feeling hot and cold) were reported more frequently in Group 1 and Group 2 than in Group 3.
- Injection site erythema, pain, pruritus, and tenderness were relatively common in Group 2 (51.5% of subjects affected), but less common in Group 1 (9.1%) and Group 3 (18.2%). Overall, the reactions at the injection site in Group 1 resembled those in the placebo treated group in terms of incidence, type, severity, onset, and duration and appeared, therefore, to be related to the process of vaccination rather than the content of the vaccine.
- Slightly more subjects experienced treatment-related AEs in Group 2 than in Group 1 or Group 3 (84.8%, 72.7%, and 72.7%, respectively).

Laboratory investigations

- Mean WBC count decreased after a single vaccination with ChimeriVax™-DEN or YF VAX® to minima at Days 9-11, and thereafter increased to pre-vaccination levels. The decrease in WBC was due mainly to a decrease in neutrophil counts.
- The most commonly reported treatment-related hematological AE was a change in WBC counts (1 subject in each of Groups 1 and 2 had a decrease in WBC counts, and 2 subjects in Group 3 had an increase in WBC counts). They were considered possibly or probably related to the study treatment.
- The most commonly occurring treatment-related blood chemistry AE was elevation of CPK, which was reported in 6 subjects (2 subjects in Group 1, 1 subject in Group 2, and 3 subjects in Group 3) They wereconsidered possibly or probably related to vaccination.
- In Group 1, among subjects tested, 1 subject became viremic to both DEN1 and DEN2 strains, 13 subjects became viremic to DEN3, and 14 subjects became viremic to DEN4. In Group 2, one subject became viremic to both DEN1 and DEN4 strains, and one subject became viremic to DEN4 strain in Group 3.
- Measures of viremic load (viremia peak, duration, and AUC) were significantly higher in Group 1 than Group 2 ($p<0.0001$).

Viremia

Viremia was examined in relation to each of the homologous ChimeriVax-DEN strains. Among subjects tested in Group 1, viremia (≥10 PFU/mL) to each of DEN1 and DEN2 strains was recorded for 1 subject; 13 subjects became viremic to DEN3, and 14 subjects became viremic to DEN4. One subject in Group 2 became viremic to DEN1 and DEN4, and 1 subject in the placebo group showed viremia to DEN4.

Second vaccination: Stage 2 (open-label treatment)

All 79 subjects were evaluable for safety.

Overview of main safety results in Stage 2:

	Group 1 ChimeriVax™-DEN → ChimeriVax™-DEN	**Group 2 YF-VAX® → ChimeriVax™-DEN**	**Group 3 Placebo → ChimeriVax™-DEN**
N	29	26	24
Treatment-emergent AEs			
No. (%) with AEs	23 (79.3)	24 (92.3)	21 (87.5)
Total AEs, n	123	129	131
Headache	14 (48.3)	14 (53.8)	10 (41.7)
Photophobia	2 (6.9)	3 (11.5)	1 (4.2)
Lethargy	4 (13.8)	3 (11.5)	7 (29.2)
Laboratory abnormalities, n (%)			
WBC count increased	2 (6.9)	2 (7.7)	3 (12.5)
Blood CPK increased	5 (17.2)	4 (15.4)	4 (16.7)
Blood bilirubin increased	1 (3.4)	3 (11.5)	1 (4.2)
Viremia (>10 PFU)			
DEN1	1	1	0
DEN2	0	1	2
DEN3	5	9	10
DEN4	4	10	12

Adverse events

- There were no deaths. One subject reported an SAE (pregnancy) that was reported as definitely not related to the study treatment. Gestation was uncomplicated, and a healthy female infant was delivered.
- The incidence of treatment-emergent AEs was slightly higher in Groups 2 and 3 (92.3% and 87.5%, respectively) than in Group 1 (79.3%). The incidence of AEs was slightly lower after the second dose of ChimeriVax™-DEN than after the first in Stage 1 of the study.
- Consistent with Stage 1 of the study, the most commonly affected system organ classes in each group were nervous system disorders, investigations, and general disorders and administration site conditions.
- As in Stage 1, the incidences of headache, the most commonly reported treatment-emergent AE, were of 48.3%, 53.8%, and 41.7% in Groups 1, 2, and 3, respectively. These incidences were similar to those recorded following vaccination with ChimeriVax™-DEN and placebo in Groups 1 and 3 in Stage 1, but lower than following vaccination with YF-VAX® in Group 2 subjects in Stage 1 of the study (72.7%).
- The incidence of commonly reported treatment-emergent AEs in Group 1 subjects following the second vaccination with ChimeriVax™-DEN was similar of less to the Stage 1 with the exception of diarrhea and increased blood CPK which were increased. The incidence of injection-site AEs was higher in Group 2 than in Group 1 or group 3, as observed in Stage 1.

Laboratory investigations

- As in Stage 1 of the study, mean WBC counts decreased after single vaccinations with ChimeriVax™-DEN in Group 2 and Group 3, with minima at Days 9-11, and thereafter increased to pre-vaccination levels. The decrease in WBC count was mainly due to a decrease in neutrophil count.
- The most common hematology AE was an increase in WBC counts, which was considered possibly or probably related to vaccination for 2 subjects in Group 1, 2 subjects in Group 2, and 3 subjects in Group 3.
- The most commonly reported blood chemistry AE was elevation of CPK. This was reported for a similar number of subjects in each treatment group: 5 subjects in Group 1, 4 subjects in Group 2, and 4 subjects in Group 3. Almost all elevations in CPK were considered possibly or probably related to the study treatment.
- Two subjects, 1 subject in each of Group 1 and Group 2, became viremic to DEN1, 1 subject in Group 2 and 2 subjects in Group 3 became viremic to DEN2, 26 subjects (5 subjects in Group 1, 9 subjects in Group 2, and 12 subjects in Group 3) became viremic to DEN3, and 26 subjects (4 subjects in Group 1, 10 subjects in Group 2, and 12 subjects in Group 3) became viremic to DEN4.
- A significantly higher proportion of subjects in Groups 2 and 3 than in Group 1 became viremic after the second vaccination (61.5% and 58.3%, vs 20.7%, p=0.0033).
- The earliest subjects became viremic was on Day 4, in Groups 1 and 2 (2 subjects in each groups) and on Day 6 in Group 3 (19 subjects).

Viremia

Viremia was examined for each of the homologous ChimeriVax-DEN strains. Among subjects tested, 2 subjects, 1 in each of Group 1 and Group 2 became viremic to DEN1, 1 subject in Group 2 and 2 subjects in Group 3 became viremic to DEN2, 26 subjects (5 in Group 1, 9 in Group 2 and 12 in Group 3) became viremic to DEN3, and 26 subjects (4 in Group 1, 10 in Group 2, and 12 in Group 3) became viremic to DEN4.

Immunogenicity:

First vaccination (Day 30)

A total of 93 subjects, 31 in each group, were evaluable for immunogenicity:

ChimeriVax™-DEN:

- Seroconversion rates and GMTs were higher for all the WT DEN strains (notably, the protocol specified WT DEN2, DEN3, and DEN4 strains) than than against the corresponding homologous strains. The highest seroconversion rate was reported for DEN3 WT 16562 (77.4%), which also had the highest GMT (GMT 118.95% CI 47.6, 291.2)
- WT DEN strains (DEN1 16007, DEN2 16681, DEN3 16562, DEN4 1036): 90.3%, 67.7%, 19.4%, and 9.7% of subjects seroconverted against at least 1, 2, 3, and all 4 serotypes, respectively.
- Homologous DEN strains: 80.6%, 45.2%, 3.2%, and 3.2% of subjects seroconverted against at least 1, 2, 3, and all 4 serotypes, respectively.

YF-VAX®:

- Overall, 9.7% of subjects seroconverted against a WT DEN strain and 6.5% of subjects seroconverted against a homologous DEN strain. None of the subjects seroconverted against more than 1 serotype.
- A total of 96.8% of subjects seroconverted against the homologous YF strain.

Month 6, before second vaccination

- Between 40.7% and 74.1% of subjects in Group 1 were seropositive (PRNT50 ≥1:10) to WT DEN strains before the second vaccination.
- Between 11.1% and 22.2% of subjects in Group 1 were seropositive to homologous DEN strains before the second vaccination.

Second vaccination (Day 30)

Group 2 versus pooled single dose ChimeriVax™-DEN

- Seroconversion rates against the protocol-defined WT and homologous DEN2 and homologous DEN3 strains were higher in Group 2 (92.3%, 65.4%, and 65.4%, respectively) than in the pooled single dose ChimeriVax™-DEN group (25.9%, 18.5%, and 33.3%, respectively). The corresponding GMTs were also significantly higher in Group 2 compared to the pooled single dose ChimeriVax™-DEN group. The highest seroconversion rate in Group 2 at 30 days was 92.3% against WT DEN2 (16681) and the GMT was 149 (95% CI 87.9, 253.0).
- When seroconversion was defined as a $PRNT_{50}$ titer ≥ 1:20, there were similar seroconversion rates in both groups against at least 1 WT DEN serotype (92.3% vs 92.6%, respectively) or homologous serotype (84.6% vs 79.6%, respectively). Seroconversion against multiple DEN serotypes was higher in Group 2 than the pooled single dose ChimeriVax™-DEN group for both WT and homologous DEN strains. At Day 30, 38.5% of subjects in Group 2 had seroconverted against all 4 WT DEN serotypes (vs 11.1% in the pooled dose group), and 15.4% had seroconverted against all 4 homologous DEN serotypes (vs 5.6%, respectively). When seroconversion was defined as a $PRNT_{50}$ titer ≥ 1:10, seroconversion against multiple serotypes were also higher in Group 2 than in the pooled single dose ChimeriVax™-DEN group.

Booster vaccination with ChimeriVax™-DEN (Group 1) versus Group 2 or pooled single dose ChimeriVax™-DEN

- Seropositivity rates against the protocol-defined WT and homologous DEN2 and homologous DEN3 strains were higher in Group 2 than in Group 1 or the pooled single dose ChimeriVax™-DEN group. The highest seropositivity rate in Group 2 was 96.2% against WT DEN2 16681 and the GMT was 149 (95% CI 87.9, 253.0). Booster vaccination with ChimeriVax™-DEN had a limited effect on seropositivity, particularly in respect of DEN2 strains, and WT DEN1 and DEN2 strains for GMTs.
- For Group 1, Group 2, and the pooled single dose ChimeriVax™-DEN group, almost all subjects were seropositive to at least 1 WT DEN serotype. Seropositivity to all 4 WT DEN serotypes was higher in Group 2 than in Group 1 or the pooled single dose ChimeriVax™-DEN group (65.4% vs 40.7% and 16.7%, respectively).
- Seropositivity against 1 or more homologous DEN serotypes was higher in Group 2 and in the pooled single dose ChimeriVax™-DEN group than in Group 1 (88.5% and 81.5% vs 63.0%, respectively). Seropositivity to at least 3 homologous serotypes was higher in Group 2 than Group 1 and in the pooled single dose ChimeriVax™-DEN group (34.6% vs 18.5% and 7.4%, respectively).

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