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Sponsor: Sanofi Pasteur	Study Identifiers: U1111-1116-4913, NCT01436396,2014-001714-26
substance: CYD Dengue Vaccine	Study code: CYD29
Title of the study: Immunogenicity and Safety of Yellow Fever Vaccine (Stamaril®) Administered Concomitantly with Tetravalent Dengue Vaccine in Healthy Toddlers at 12-13 Months of Age in Colombia and Peru	
Study center(s): This was a multi-center, multinational trial involving 2 investigators over 2 countries.	
Study period: Date first subject enrolled: 07/Sep/2011 Date last subject completed: 02/Sep/2013	
Phase of development: Phase III	
Objectives: Primary Objective: <i>Immunogenicity</i> To demonstrate the non-inferiority of the immune response against YF in FV non-immune subjects at baseline receiving one dose of Stamaril vaccine administered concomitantly with the first dose of CYD dengue vaccine compared to subjects receiving one dose of Stamaril vaccine concomitantly with placebo. Secondary Objective: <i>YF Immunogenicity (All Subjects)</i> <ol style="list-style-type: none">1) To assess the non-inferiority of YF immune response 28 days post-Stamaril vaccination based on seroconversion rates regardless of the FV status of subjects at baseline.2) To describe the YF immune response 28 days post-Stamaril vaccination in both groups. <i>Dengue Immunogenicity (Dengue Immunogenicity Subset)</i> <ol style="list-style-type: none">1) To describe the Ab response to each dengue virus serotype 28 days post CYD dengue vaccine (V05 and V07), following CYD dengue vaccine Dose 1 and Dose 2 from Group 2 versus following CYD dengue vaccine Dose 2 and Dose 3 for Group 1 (effect of YF vaccination). <i>Safety (All Subjects)</i> <ol style="list-style-type: none">1) To describe the safety of Stamaril vaccine administered concomitantly with the first dose of CYD dengue vaccine, or Stamaril administered concomitantly with placebo.2) To describe the safety of CYD dengue vaccine after the first dose of CYD dengue vaccine administered concomitantly with Stamaril vaccine or CYD dengue vaccine administered alone.3) To describe the safety of the CYD dengue vaccine in all subjects after each dose.	

Methodology:

Randomized, observer-blind (for the group allocation), multi-center, Phase III trial in 792 healthy toddlers in Colombia and Peru administered an injection of Stamaril vaccine concomitantly with the first dose of CYD dengue vaccine at 12-13 months of age.

At Visit (V) 01, subjects were randomized in a 1:1 ratio into 2 groups to receive a total of 9 injections throughout the whole trial:

Trial Timeline (month)	M0*	M1 [†]	M6	M7 [†]	M12	M13 [†]
Age of Subjects (months)	12-13	13-14	18-19	19-20	24-25	25-26
Visit Number	V01	V03	V04	V05	V06	V07
Group 1	Stamaril + CYD dengue vaccine Dose 1	MMR vaccine + pneumococcal conjugated vaccine + hepatitis A vaccine	CYD dengue vaccine Dose 2	DTaP-IPV//Hib vaccine	CYD dengue vaccine Dose 3	Hepatitis A vaccine
Group 2	Stamaril + Placebo	MMR vaccine + pneumococcal conjugated vaccine + hepatitis A vaccine	CYD dengue vaccine Dose 1	DTaP-IPV//Hib vaccine	CYD dengue vaccine Dose 2	Hepatitis A vaccine

MMR: measles, mumps and rubella; DTaP-IPV//Hib: diphtheria, tetanus, acellular pertussis, polio and *Haemophilus influenzae* type b (Hib).
*At M0 the vaccine(s) or placebo were administered in separate locations (the Stamaril subcutaneous [SC] vaccination was in one deltoid and the CYD dengue vaccine or placebo SC vaccination was in the other deltoid).
[†]There was not to be any assessment of safety or immunogenicity for the MMR, pneumococcal conjugated, hepatitis A or DTaP-IPV//Hib vaccines.

An interactive voice response system (IVRS) / interactive web response system (IWRS) were used for randomization of the treatment group and immunogenicity subset and side of vaccination at V01, and for the vaccine assignments and side of vaccination at V04 and V06.

All subjects were to provide one blood sample for flavivirus (FV) immune status at baseline before the first vaccination (BL1 at V01) and one blood sample (BL2 at V03) for yellow fever (YF) vaccine immunogenicity.

Vaccine immunogenicity assessments for dengue neutralizing Abs were performed in a randomized subset of 250 subjects (125 subjects from each group) on BL3 and BL4, drawn at V05 and V07, respectively.

Reactogenicity data were collected in all subjects after each vaccination. Serious adverse events (SAEs) were to be reported throughout the study and adverse events of special interest (AESIs) were to be collected in defined time-windows. There was a 6-month safety follow-up after the last CYD dengue vaccination for all subjects.

Number of subjects:

Planned: 792

Randomized: 792

Evaluated:

Immunogenicity: 758

Safety: 787

Diagnosis and criteria for inclusion:

An individual must fulfill all of the following criteria in order to be eligible for trial enrollment:

- 1) Aged 12 to 13 months on the day of inclusion.
- 2) Born at full term of pregnancy (≥ 37 weeks) and with a birth weight ≥ 2.5 kg as reported by the parent/legally acceptable representative.
- 3) Subject in good health, based on medical history and physical examination.
- 4) Subject has completed his/her vaccination schedule according to the official immunization calendar of Colombia and/or Peru, respectively.
- 5) Informed consent form has been signed and dated by the parent(s) or other legally acceptable representative (and by 2 independent witnesses if required by local regulations).
- 6) Subject and parent/legally acceptable representative/tutor able to attend all scheduled visits and to comply with all trial procedures.

Study treatments

Investigational product 1: CYD Dengue Vaccine

Form: Powder and solvent for suspension for injection

Composition: Each 0.5 mL dose of reconstituted vaccine contains:

$5 \pm 1 \log_{10}$ cell-culture infectious dose 50% (CCID₅₀) of each live, attenuated, dengue serotype 1, 2, 3, 4 virus.

Excipients: essential amino acids, non-essential amino acids, L-arginine chlorhydrate, saccharose, D-trehalose dihydrate, D-sorbitol, tris (hydroxymethyl) aminomethane, and urea.

Solvent: NaCl 0.4%

Route of administration: Subcutaneous (SC) in the deltoid region of the upper arm

Investigational product 2: YF Vaccine (Stamaril®) manufactured by Sanofi Pasteur, licensed vaccine in Colombia and Peru

Form: Powder and so5 mL lvent for suspension for injection in prefilled syringe

Composition: Each 0. dose contains:

YF virus (produced in specified pathogen-free chick embryos) 17 D-204 strain (live, attenuated).

Each dose of 0.5 mL contains not less than 1000 LD50 units of the virus (the statistically determined lethal dose in 50% of animals tested).

Powder: lactose, sorbitol, L-histidine hydrochloride, L-alanine, sodium chloride, potassium chloride, disodium phosphate, monopotassium phosphate, calcium chloride, magnesium sulphate.

Solvent: sodium chloride 2.0 mg (0.4%), water for injection (0.5 mL).

Route of administration: SC, in the deltoid region of the upper arm

Investigational Product 3: Placebo (NaCl)

Form: Solution

Composition: NaCl 0.9%

Route of administration: SC, in the deltoid region of the upper arm

Other Products: MMR, Pneumococcal Conjugate, Hepatitis A and DTaP-IPV//Hib vaccines.

One dose of Trimovax™, 2 doses of AVAXIM™, one dose of Prevenar®13 and a booster dose of PENTAXIM™ were administered to trial subjects, to comply with the vaccination calendars for second year of life in both countries or as benefit vaccines.

Duration of participation: The duration of each subject's participation is approximately 18 months.

Criteria for evaluation:

Primary Endpoint:

Immunogenicity

Level of neutralizing antibodies (Abs) 28 days post-Stamaril vaccination against YF were measured in all subjects using a YF virus plaque reduction neutralization test (YF Plaque Reduction Neutralizing Test [PRNT]₅₀) assay.

Subjects with YF seroconversion status 28 days post-Stamaril vaccination were defined as YF Ab (≥ 10 1/ dilution [dil]) in FV non-immune subjects at baseline.

The FV status at baseline was defined as the presence of Abs against YF (YF PRNT₅₀) and/or against at least one dengue serotype in the baseline sample (dengue PRNT₅₀) in the blood sample collected at V01 (BL1) from all subjects.

Secondary Endpoints:

YF Immunogenicity (All Subjects)

- 1) Subjects with YF seroconversion 28 days post-Stamaril vaccination based on neutralizing Ab titers (YF PRNT₅₀)
 - Seroconversion was defined:
 - YF Abs (≥ 10 [1/dil]) in subjects YF-seronegative at baseline
 - 4-fold increase from pre- to post-YF Abs titers in subjects YF-seropositive at baseline
- 2) Level of neutralizing Abs 28 days post-Stamaril vaccination against YF were measured in all subjects in terms of geometric mean of titers (GMTs), geometric mean of titers ratio (GMTR), and reverse cumulative distribution curves (RCDCs).

Dengue Immunogenicity (Dengue Immunogenicity Subset)

Neutralizing Ab levels against each of the 4 dengue virus serotype strains contained in the CYD dengue vaccine were measured in sera collected from a randomized subset of 250 subjects at V05 and V07 (dengue PRNT₅₀ assay).

Safety (All Subjects)

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

Statistical methods:

Primary objective:

Hypothesis and Statistical Method for the Primary Objective

The difference in terms of proportion of FV- non-immune subjects at baseline reaching YF seroconversion between test Group 1 (Stamaril administered concomitantly with CYD dengue vaccine Dose 1) and reference Group 2 (Stamaril administered concomitantly with placebo), is inferior or equal to the clinically relevant limit for non-inferiority ($-\delta$).

The tested hypotheses was as follows:

$$H_0 : P_{Tested} - P_{Reference} \leq -\delta$$

$$H_1 : P_{Tested} - P_{Reference} > -\delta$$

δ non-inferiority limit is set at 10%.

A non-inferiority test was performed using the 95% two-sided confidence interval (CI)

of the difference $P_{test}-P_{reference}$ between the seroconversion rates ($\alpha=2.5\%$). The 95% CI was calculated based on the Wilson score method without continuity correction as described by Newcombe and colleagues (Newcombe, 1998). Non-inferiority was demonstrated if the lower limit of the two-sided 95% CI is greater than $-\delta$. This is equivalent to testing and rejecting hypothesis H_0 .

The primary hypotheses of non-inferiority was tested on the Per-Protocol analysis set (PPAS) and was to be confirmed by using the modified Intention To Treat (mITT) analysis set.

Hypothesis and Statistical Methods for the Secondary

The same hypotheses and methods as for the primary objective were used as a secondary objective regardless of the FV status of subjects at baseline ($\delta=10\%$, $\alpha=2.5\%$).

All other analyses were descriptive; no other hypotheses were tested.

The Full Analysis Set (FAS) was used for the immunogenicity analyses, and the Safety Analysis Set (SafAS) was used for the safety analyses.

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

Statistical Analyses

A blinded review of safety data collected within 14 days after the first vaccination of the first 40 subjects enrolled was performed by the internal SMT. A final statistical analysis was produced after the 6-month safety follow-up.

Power for the primary analysis

% of Drop-out From the Analysis Set	n Evaluable per Group	Power
17.5%	326	89.2%
15.0%	336	90.0%
12.5%	346	90.9%
10.0%	356	91.6%
7.5%	366	92.3%

Assuming a non-inferiority delta margin (clinically significant differences) of 10%, an overall drop-out from the FAS of 5%, an $\alpha=2.5\%$ (one-sided test), a seroconversion rate of 80% (Stefano, 1999), a 92.9% power was achieved with a total of 752 evaluable subjects (376 subjects per group) based on simulations using the Wilson score method (without continuity correction).

Power for the secondary analysis

% of Drop-out From the Analysis Set	n Evaluable per Group	Power
5%	376	92.9%
4%	380	93.2%
3%	384	93.4%
2%	388	93.6%
1%	392	93.9%

With 396 subjects per group, the probability to detect any uncommon AE (incidence of 0.75% or more) was to be 95%.

With 125 subjects in the dengue immunogenicity subset, the immunogenicity assessment had CI widths of less than 20% (distance between the estimate and the upper or lower bound of less than 10%) for any estimate.

95% CI for proportions (Exact method) in the dengue immunogenicity subset

% Observed	95% CI (N=125)
50%	(41.3; 59.5)
60%	(50.9; 68.7)
70%	(61.6; 78.2)
80%	(71.9; 86.6)
90%	(83.8; 94.9)

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Summary

Disposition and Demographic Data

A total of 792 subjects were enrolled and randomized: 472 subjects (59.6%) in Colombia and 320 subjects in Peru (40.4%). The overall compliance to vaccination and blood sampling was good in both groups as well as both sites. A total of 197 subjects (24.9%) reported at least one deviation to the protocol and were therefore excluded from the PPAS.

Overall in the SafAS, there were slightly more female (51.0%) than male subjects (49.0%); these proportions were similar in each treatment group. The mean age at enrolment was 12.2 ± 0.25 months in both treatment groups. There were 155 subjects (19.6%) out of all subjects randomized reporting at least one past and current significant medical history. Among those, 30 subjects (3.8%) reported at least one still ongoing at inclusion.

As expected in this age group, most of the subjects (80.6%) were FV non-immune at baseline: 304 subjects (79.8%) in Group 1 and 307 subjects (81.4%) in Group 2 based on the FAS. Only 9% and 5.9% of subjects were YF and dengue immune at baseline, respectively.

Immunogenicity Results

Primary Objective: YF Non-Inferiority in FV Non-Immune Subjects

Non-inferiority of YF seroconversion rate at 28 days post the first injections in baseline FV non-immune subjects – YF PRNT – PPAS

	Group 1 (N=296)		Group 2 (N=299)		Difference % (Group 1-Group 2)	95% CI for difference	Non-inferiority
	n/M	%	n/M	%			
Seroconversion	296/296	100.0	298/299	99.7	0.334	(-0.976; 1.87)	Yes

Twenty-eight days after the first injections, the immune response against YF of FV non-immune subjects receiving one dose of Stamaril vaccine administered concomitantly with the first dose of CYD dengue vaccine was non inferior to the immune response against YF of FV non-immune subjects receiving one dose of Stamaril vaccine concomitantly with placebo (0.334 difference with [-0.976;1.87] CI).

Secondary Objectives: Immune Response on YF and Dengue

YF Non-Inferiority Regardless of FV Status of Subjects

Twenty-eight days after the first injections, the immune response against YF of all subjects receiving one dose of Stamaril vaccine administered concomitantly with the first dose of CYD dengue vaccine was non inferior to the immune response against YF of all subjects receiving one dose of Stamaril vaccine concomitantly with placebo: 98.7% versus 99.7% of subjects seroconverted, respectively (-1.06 difference with [-2.81;0.383] CI).

YF Immune Response in Terms of GMT and Seropositivity Rate

The YF seropositivity rates 28 days post Stamaril with CYD dengue vaccine or Stamaril with Placebo injection was high, regardless the FV status at baseline, 99.5% in Group 1 and 99.7% in Group 2. A trend to higher GMT levels was observed in subjects having received Stamaril with Placebo, 423 (1/dil) versus 369 (1/dil), respectively.

Dengue Immune Response

For serotype 4, two doses of CYD dengue vaccine in subjects previously primed with Stamaril (Group 2) elicited comparable immune response in terms of GMT levels compared to three doses of CYD dengue vaccine (Group 1) where the first dose was co-administered with YF vaccine: 74.6 (1/dil) versus 74.0 (1/dil).

For serotype 1, 2 and 3, subjects who received 3 dengue doses (Group 1) tended to have a higher immune response in terms of GMT levels than those who received only 2 doses (Group 2): 89.0 (1/dil) versus 61.3 (1/dil) for serotype 1, 173 (1/dil) versus 150 (1/dil) for serotype 2, 181 (1/dil) versus 155 (1/dil) for serotype 3.

Overall, the neutralizing Ab response to 3 doses of CYD dengue vaccine given at 12 and 18 months when the first dose was co-administered with Stamaril (Group 1) tended to be higher than 2 doses of CYD dengue vaccine given at 18 and 24 months in YF primed toddlers (Group 2), however:

- The true impact on YF priming versus YF vaccine-CYD dengue vaccine co-administration is not assessable in absence of a third group receiving 3 doses of CYD dengue vaccine and then Stamaril alone.
- The impact of administration age was not controlled, since the subjects of Group 1 received the first injection of CYD dengue vaccine at 12 months and subjects of Group 2 received it at 18 months of age.

Safety Results

Immediate AEs

There were no immediate unsolicited AEs (i.e., within the 30-minute observation period) reported in either of the groups following any injection.

Solicited Reactions

The percentage of subjects experiencing at least 1 solicited reaction (including both solicited injection site and solicited systemic reactions) was slightly higher in Group 1 (84.5%) compared to Group 2 (78.8%). After any injections, i.e. CYD dengue vaccine, Stamaril and Placebo, approximately 44% of subjects in both groups experienced at least 1 solicited injection site reaction, whereas 80.6% of subjects in Group 1 compared to 73.8% in Group 2 experienced at least 1 solicited systemic reaction. Most of these reactions were Grade 1 reactions occurring within 3 days after any injection.

Grade 3 solicited reactions were not frequent: 2 subjects (0.5%) in Group 1 and 1 subject (0.3%) in Group 2 had at least one Grade 3 solicited injection site reaction and 7.3% of subjects in Group 1 and 7.0% of subjects in Group 2 had at least one Grade 3 solicited systemic reaction.

Injection Site Reactions After Each Dose

The frequencies of erythema and swelling injection site reactions following Stamaril whether administered with CYD dengue vaccine concomitantly or not were similar, reported by approximately 10% and 5% of subjects, respectively. The frequency of tenderness tended to be higher when Stamaril was co-administered with CYD dengue vaccine (25.4% versus 17.6% of subjects).

The frequency of injection site erythema and swelling for CYD dengue vaccine post-dose (PD) 1 in Group 1 were similar to the frequency of each injection site reaction for CYD dengue vaccine PD1 in Group 2, while injection site tenderness was slightly higher in Group 1 (24.9% of subjects) compared to Group 2 (20.4% of subjects). Then, the frequency of each injection site reaction for CYD dengue vaccine PD2 in Group 1 were similar to the frequency of each injection site reaction for CYD dengue vaccine PD2 in Group 2.

In both groups, the frequency of injection site reactions tended to decrease after subsequent CYD dengue vaccine injections.

Systemic Reactions After Each Dose

The frequency of fever was higher when Stamaril was co-administered with CYD dengue vaccine (26.7% in Group 1 and 16.5% in Group 2). The frequency of all the other systemic reactions was similar when Stamaril was administered with CYD dengue vaccine concomitantly or not.

The frequency of each systemic reaction post-injection 2 in Group 2, i.e. dengue dose 1, was overall lower than the frequency of each systemic reaction post-injection 1 in Group 1, i.e. dengue dose 1 co-administered with Stamaril.

In both groups, the frequency of systemic reactions tended to decrease after subsequent CYD dengue vaccine injections, except fever which was sustainably reported after each injection. The frequency of each systemic reaction post-injection 3 in Group 2, i.e. dengue dose 2, tended to be lower than the frequency of each systemic reaction post-injection 2 in Group 1, i.e. dengue dose 2.

Unsolicited Non-Serious AEs

Approximately 70% of subjects in both groups experienced at least 1 unsolicited AE after any injection. The large majority of the unsolicited AEs was common medical conditions and considered non-serious in both treatment groups. In Group 1, the most common unsolicited AEs were Infections and infestations, Gastrointestinal disorders and Respiratory, thoracic and mediastinal disorders (reported by 59.4%, 21.3% and 15.0% of subjects, respectively). In Group 2, the most common unsolicited AEs were in the same SOCs (reported by 54.7%, 18.8% and 12.2% of subjects, respectively). These AEs were mainly reported as Grade 1 or Grade 2 AEs that spontaneously resolved within a few days. Unsolicited non-serious AR, i.e., AEs related to vaccination, were reported by 3 subjects (0.8%) in Group 1 and 2 subjects (0.5%) in Group 2.

Grade 3 unsolicited AEs were not frequent: 1.5% of subjects in Group 1 and 1.0% of subjects in Group 2 had at least one Grade 3 unsolicited reaction. Unsolicited non-serious adverse reactions (AR), i.e., AEs related to vaccination, were reported by 3 subjects (0.8%) in Group 1 and 2 subjects (0.5%) in Group 2. The most common AR reported was injection site hematoma, reported by 1 subject (0.3%) in each group.

The frequency of unsolicited events was similar when Stamaril was administered with CYD dengue vaccine concomitantly or not. In both groups, the frequency and severity of unsolicited events tended to decrease after the second injection compared to the first one.

AEs leading to Discontinuation

Approximately 1% of subjects in both groups reported at least one AE leading to discontinuation.

SAEs

A total of 73 subjects experienced at least one SAE until 6 months after the last CYD dengue vaccine injection: 35 subjects (8.9%) in Group 1 and 38 subjects (9.7%) in Group 2. There were no SAEs related to the trial vaccines as per Investigator. One SAE was considered as related to the trial vaccines as per the Sponsor: subject in Group 1 with medical history of febrile convulsions, who reported febrile seizure 1 day after Stamaril and CYD dengue vaccine injection. In Group 2, one non-related death was reported (severe traumatic brain injury). There were no safety concerns raised following the report of 11 cases of febrile convulsions and 1 case of seizure (secondary to trauma) during the entire clinical trial. No serious immediate allergic reactions and no serious

neurotropic or viscerotropic events were reported during the trial.

AESIs

One subject reported at least one serious AESI (dengue requiring hospitalization, virologically-confirmed but non-severe). Few subjects reported non-serious AESIs: 11 subjects (2.8%) in Group 1 and 7 subjects (1.8%) in Group 2, mainly bronchospasms. These AESIs, except one episode of urticaria, were not considered as related to the vaccines.

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