NCT Number: NCT02824198

Immunogenicity and Safety of a Tetravalent Dengue Vaccine given as a Booster Injection in Adolescents and Adults who previously completed the 3-dose schedule in a study conducted in Singapore

Multi-center, observer-blind, randomized, placebo-controlled, Phase II trial conducted in Singapore i) to assess the non-inferiority of the immune response induced by a booster injection of a tetravalent dengue vaccine versus that induced by the third injection of the 3-dose schedule of the same vaccine received 5 years (or more) earlier in healthy adolescents and adults from the CYD28 trial; ii) to evaluate the safety and antibody persistence of the booster injection up to 2 years

Clinical Trial Protocol, Amendment 1

Health Authority File Number(s):	BB-IND #: 11219
WHO Universal Trial Number (UTN):	U1111-1161-2813
Trial Code:	CYD63
Development Phase:	Phase II
Sponsor:	Sanofi Pasteur SA 2, avenue Pont Pasteur, 69367 Lyon cedex 07, France
Investigational Product(s):	CYD Dengue Vaccine
Form / Route:	Powder and solvent for suspension for injection / Subcutaneous
Indication For This Study:	Prevention of dengue fever in adolescents and adults
Manufacturer:	Same as Sponsor
Investigators	This is a multi-center trial with multiple investigators. Investigators and study sites are listed in the "List of Investigators and Centers Involved in the Trial" document.
Sponsor's Responsible Medical Officer:	[redacted], Sanofi Pasteur Limited [redacted]
Regional Director of Clinical Development:	**Same as Responsible Medical Officer**

Product Safety Officer: [redacted], Sanofi Pasteur SA [redacted]

Regional Clinical Trial Manager: [redacted], Sanofi Pasteur Limited [redacted]

Version and Date of the Protocol: Version 3.0 dated 26 May 2016

This protocol version 3.0 is the first amendment to the initial trial protocol version 2.0, dated 17 September 2015.

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Synopsis

Company:	Sanofi Pasteur
Investigational Product:	CYD dengue vaccine
Active Substances:	Live, attenuated, dengue serotype 1 virus Live, attenuated, dengue serotype 2 virus Live, attenuated, dengue serotype 3 virus Live, attenuated, dengue serotype 4 virus

Title of the Trial:	Immunogenicity and Safety of a Tetravalent Dengue Vaccine given as a Booster Injection in Adolescents and Adults who previously completed the 3-dose schedule in a study conducted in Singapore
Development Phase:	Phase II
Investigators:	This is a multi-center trial with multiple investigators. Investigators and study sites are listed in the "List of Investigators and Centers Involved in the Trial" document.
Trial Centers:	This will be a multi-center trial conducted at 3 sites in Singapore.
Planned Trial Period:	Quarter 2 2016 (June 2016; First visit of the first subject) to Quarter 3 2018 (September 2018, Last visit of the last subject)
Trial Design and Methodology:	This will be a multi-center, observer-blind, randomized, placebo-controlled Phase II trial conducted in Singapore to assess i) the non-inferiority of the immune response induced by a booster injection of a tetravalent dengue vaccine versus that induced by the third injection of the 3-dose schedule of the same vaccine received 5 years (or more) earlier in healthy subject from the CYD28 trial; ii) to evaluate the safety and antibody persistence of the booster injection up to 2 years. Subjects will be randomized into Groups 1 and 2 in a 3:1 ratio and will receive either the CYD dengue vaccine as a booster or a placebo. A total of 260 subjects aged 9 to 45 years on the day of first vaccination of the study vaccine in CYD28 are planned to be recruited into the following 2 groups.

	Vaccination in CYD28	CYD63	
		N	Product to be injected
Group 1	CYD1/CYD2/CYD3*	195	CYD dengue vaccine booster
Group 2	CYD1/CYD2/CYD3*	65	Placebo

* CYD dengue vaccine dose 1, dose 2, and dose 3

An interactive voice response system/interactive web response system (IVRS/IWRS) will be used to assign treatment group and subject number at each clinical site, with stratification for age (9 to 17 years, and 18 to 45 years, on the day of first injection in CYD28) and trial center.

At the first visit, each subject will receive a single dose of the CYD dengue vaccine booster or a placebo. Blood samples will be collected at baseline (immediately prior to injection), at 28 days, 6 months, 1 year and 2 years post-injection for assessing neutralizing antibody (Ab) titers against each of the four parental dengue virus strains. A subset of 60 subjects in Group 1 and Group 2 (45 and 15 subjects, respectively) will provide additional blood samples at baseline and at 7, 14, 28 days and 1 year post-injection. Depending on the time points, these blood samples will be used for

	measurement of cell mediated immunity (CMI), Ab specificity and affinity maturation, and neutralizing Ab (exploration of the Ab response's kinetics). Reactogenicity data will be collected in all subjects after the booster or placebo injection. Solicited injection site reactions will be collected for Days 0–7. Solicited systemic reactions will be collected for Days 0–14. Unsolicited events will be collected for Days 0-28. Serious adverse events (SAEs) will be reported throughout the study and serious and non-serious adverse events of special interest (AESIs) will be collected in defined time-windows according to the type of AESI. In addition, hospitalized suspected dengue cases occurring at any time in the trial will be documented. Hospitalized suspected dengue disease is defined as an acute febrile illness with diagnosis of dengue requiring hospitalization (with bed attribution). In such cases, 1 unplanned blood sample (acute sample*) will be collected for virological confirmation within the first 5 days after fever onset. A suspected hospitalized dengue case will be considered virologically-confirmed dengue (VCD) case if there is a detection of wild type (WT) dengue virus by NS1 antigen ELISA and/or wild-type dengue RT-PCR. ***Note:** Acute blood sample for all suspected hospitalized dengue cases should be collected within the pre-specified timeframe as described above. If this cannot be accomplished, this sample should still be obtained as soon as possible thereafter.
Early Safety Data Review:	This trial will not include an early review of safety data. However, it may be interrupted at any time if new data about the investigational product become available, and/or on advice of the Sponsor, the Independent Ethics Committees/ Institutional Review Boards (IECs/IRBs), or the governing regulatory authorities in the country where the trial is taking place. If the trial is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators, the IECs/IRBs, and the regulatory authorities of the reason for termination or suspension. If the trial is prematurely terminated for any reason, the Investigator will promptly inform the trial subjects / subjects' parents/guardians and should assure appropriate therapy and follow-up. An internal safety management team (SMT) will perform a blinded safety analysis on safety data after vaccination. An IDMC will be involved in the regular review of hospitalized VCD cases, including assessment of severity. Additionally, any related SAE or death or serious AESI will be promptly reviewed by the IDMC.
Primary Objective:	To demonstrate the non-inferiority, in terms of geometric mean of titer ratios (GMTRs), of a CYD dengue vaccine booster compared to the third CYD dengue vaccine injection in subjects from CYD 28 trial (subjects from Group 1 only).
Primary Endpoint:	Neutralizing Ab levels against each dengue virus serotype measured 28 days after the third CYD dengue vaccine injection and 28 days after the booster injection in Group 1 using dengue plaque reduction neutralization test (PRNT).

Secondary Objectives:	***Immunogenicity*** 1) If the primary objective of non-inferiority is achieved: To demonstrate the superiority, in terms of GMTRs, of a CYD dengue vaccine booster compared to the third CYD dengue vaccine injection in subjects from CYD28 trial (subjects from Group 1 only). 2) To describe the immune responses elicited by the CYD dengue vaccine booster or placebo injection in subjects who received three doses of the CYD dengue vaccine in the CYD28 trial in all subjects. 3) To describe the neutralizing Abs levels of each dengue serotype PD3 (CYD28 subjects) and immediately prior to booster or placebo injection in all subjects. 4) To describe the neutralizing Ab persistence 6 months, 1 year and 2 years post booster or placebo injection in all subjects. ***Safety*** To evaluate the safety of booster vaccination with CYD dengue vaccine in all subjects.
Secondary Endpoints:	***Immunogenicity:*** 1) Neutralizing Ab levels against each of the four parental dengue virus strains of CYD dengue vaccine as determined by PRNT measured 28 days after the third CYD dengue vaccine injection land 28 days post-booster injection (subjects from Group 1 only). 2) Neutralizing Ab levels against each of the four parental dengue virus strains of the CYD dengue vaccine as determined by PRNT immediately prior and 28 days post-booster or placebo injection. 3) Individual post-booster/pre-booster GMTRs for each of the four parental dengue virus strains of the CYD dengue vaccine as determined by PRNT immediately prior and 28 days post-booster or placebo injection. 4) Seroconversion rates 28 days after the booster injection for each of the four parental dengue virus strain of CYD dengue vaccine: percentages of subjects with either a pre-booster titer < 10 (1/dil) and a post-booster dose titer ≥ 40 (1/dil), or a pre-booster titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-booster dose titer as determined by PRNT immediately prior and 28 days post-injection. 5) Neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT at 28 days after the third CYD dengue vaccine injection and immediately prior to booster or placebo injection in all study subjects. 6) Neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT at 6 months, 1 year and 2 years post booster or placebo injection. ***Safety:*** 1) Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, action taken, whether it leads to discontinuation or not, and relationship to vaccination of any AEs reported in the 30 minutes after vaccination. 2) Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited (pre-listed in the subject's diary card and electronic case report form [CRF]) injection site reactions (pain, erythema, and swelling) occurring up to 7 days after vaccination.

	3) Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited systemic reactions (fever, headache, malaise, myalgia, and asthenia) occurring up to 14 days after vaccination. 4) Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, whether it leads to discontinuation or not, action taken and relationship to vaccination (for systemic AEs only) of unsolicited spontaneously reported AEs up to 28 days after vaccination. 5) Occurrence of SAEs, including serious AESIs (with specific time window according to the nature of event), throughout the trial. 6) 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 vaccination. 7) Occurrence of hospitalized virologically-confirmed dengue cases throughout the trial (i.e., from D0 through end of the study).
Additional Objectives:	**Only for the Additional Immunological Tests (AIT) subset** 1) To describe dengue neutralizing Ab levels (exploration of the Ab response's kinetics), Ab specificity and affinity maturation post-booster or placebo injection 2) To describe CMI responses post-booster or placebo injection. **In all subjects** 3) To assess post-booster neutralizing Ab levels against each dengue virus serotype while controlling for baseline neutralizing Ab levels against each dengue virus serotype
Additional Endpoints:	**Subjects from the AIT subset** (i.e., the first 60 randomized subjects from 2 specific sites (30 subjects per site; 45 subjects in Group 1, 15 subjects in Group 2). 1) Dengue PRNT Two additional time points for neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT at 7 and 14 days post-injection. 2) Ab specificity and affinity maturation a) Heterotypic and homotypic serotype-specific neutralizing Ab responses will be assessed qualitatively immediately prior to and 28 days post-injection as a priority, and at 7 and 14 days post-injection if necessary. Homotypic Abs for individual serotypes will be defined based on values above lower limits of quantitation for the neutralizing titer and % of Ab remaining following depletion. b) Serotype-specific affinity (K_D, nM) and Ab concentration (µg/mL) will be measured against the parental wild-type strains in the sera immediately prior to and at 28 days post-injection. 3) CMI responses The specific B and T immune response against the 4 dengue serotypes elicited by the CYD dengue vaccine booster will be assessed by ELISPOT or flow cytometry, using intracellular staining and phenotyping: a) T-cell response immediately prior to and 28 days, and 1 year after booster or placebo injection:

	i) Cytokine secreting CD4 and CD8 T-cells count. ii) T-cell subclasses (naïve, effector, central and terminally differentiated memory T-cells) count. iii) Cytotoxic T-cell effector markers. b) B-cell response: i) Ex vivo B-cells (plasmablast) count (measured by ELISPOT) immediately prior to and 7 and 14 days after booster or placebo injection. ii) Memory B-cells count (measured by ELISPOT) immediately prior to and 28 days and 1 year after, booster or placebo injection for a subset of subjects. **For all subjects** 4) Post booster neutralizing Ab levels against each of the 4 parental dengue virus strains as determined by PRNT
Planned Sample Size:	A total of 260 subjects from CYD28 trial are planned to be enrolled. • 195 subjects in Group 1 (CYD dengue vaccine booster) • 65 subjects in Group 2 (placebo)
Schedule of Study Procedures:	Visits/phone calls: During the trial period there will be 5 or 7 planned visits (depending whether the subject is included in the AIT subset or not) and 9 phone calls. Vaccination: All subjects (N=260) will receive 1 injection at Day 0; 195 subjects will receive the CYD dengue vaccine booster (group 1), 65 subjects will receive placebo (groups 2). Blood sampling: • All subjects will provide one pre-vaccination blood sample (BL1) at enrollment (Day 0) for determination of baseline dengue immune status; and 4 blood samples (BL4-BL7) 28 days, 6 months, 1 year and 2 years post-injection for dengue immunogenicity. • The 60 subjects from the AIT subset will provide two additional blood samples (BL2 and BL3) at 7 and 14 days post-injection for dengue immunogenicity. • The blood of the subjects from the AIT subset will be assayed for CMI, additional neutralizing Ab, Ab specificity and affinity maturation (depending on the time points). These subjects will provide larger volume blood samples (BL1 to BL4 and BL6) for determination of cellular and additional humoral immune response (see Table 1). • Additional biological samples may be collected from any subject in case of the occurrence of AESIs or SAEs (including hospitalized suspected dengue case for virological dengue confirmation) during the trial.

Table 1: Blood Sampling Schedule and Volume to be Collected

Visit Number (V)	V01	V02	V03	V04	V05	V06	V07
Trial Timelines (Days/Months)	D0	D 07	D 14	D 28	M 06	Y 01	Y 02
Time Windows (Days)		+2	+7	+7	+20	±30	±30
Blood sample (BL)	BL1	BL2	BL3	BL4	BL5	BL6	BL7
Volume for each assessment (mL)							
Neutralizing Ab ***All subjects***	5*			5*	5	5	5
Additional neutralizing Ab ***Adolescents and adults subjects in the AIT subset***		5†	5†				
CMI ***Adolescents in the AIT subset***	25	15	15	15		15	
CMI ***Adults in the AIT subset***	35	25	25	25		25	
Total Volume for each subject (mL)							
Subjects **not** in the AIT subset	5			5	5	5	5
Adolescents in the AIT subset	30	20	20	20	5	20	5
Adults in the AIT subset	40	30	30	30	5	30	5

* These blood samples will also be used to assess **Ab specificity and affinity maturation** in subjects from the AIT subset.

† These blood samples will also be used to assess **Ab specificity** in subjects from the AIT subset (if necessary).

Collection of safety data:

Clinical site personnel will record immediate AEs that occur within the 30 min after injection. Subjects or parents/legally acceptable representative will record in the Diary Card (DC) information about solicited injection site reactions from Day 0 to Day 7 post-injection, about solicited systemic reactions from Day 0 to Day 14 post-injection and unsolicited AEs from Day 0 to Day 28 post-injection. Information on non-serious AESIs will be collected within 7 days post-injection. Information on SAEs (including serious AESIs) will be collected throughout the trial.

Subjects or parents/legally acceptable representative will record safety information in memory aids (MAs) when DCs are not being used. Clinical site personnel will record information about SAEs and serious AESIs written in the MAs.

Duration of Participation in the Trial:	The duration of each subject's participation in the trial will be 24 months.

Investigational Product:	CYD Dengue Vaccine (5-dose formulation)
Form:	Powder and solvent for suspension for injection
Composition:	Each 0.5 mL dose of reconstituted tetravalent vaccine contains: Active Ingredients: 4.5 – 6 $\log_{10}$ cell-culture infectious dose 50% (CCID50) of each live, attenuated, recombinant dengue serotype 1, 2, 3, 4 virus Excipients: Essential amino acids, non-essential amino acids, L-arginine hydrochloride, sucrose, D-trehalose dihydrate, D-sorbitol, trometamol, urea, and sodium chloride. Solvent: NaCl 0.9%
Route:	Subcutaneous (SC)
Batch Number:	To be defined
Control Product:	Placebo
Form:	Solution
Composition:	NaCl 0.9%
Route:	SC
Batch Number:	To be defined
Inclusion Criteria:	An individual must fulfill all of the following criteria in order to be eligible for trial enrollment: 1) Has been identified as a potential subject by the Sponsor, and is included in the list provided to the investigator (i.e., aged 9 to 45 years on the day of first injection of CYD dengue vaccine in CYD28, has received 3 doses of CYD dengue vaccine in the CYD28 trial, and has a post-Dose 3 [PD3] serum sample available [at least 300 μL of serum]). 2) Presently in good health, based on medical history and physical examination. 3) Informed consent form (ICF) has been signed and dated by the subject (based on local regulations), and ICF has been signed and dated by the parent(s) or another legally acceptable representative (and by an independent witness if required by local regulations) 4) Subject and parent(s)/legally acceptable representative(s) able to attend all scheduled visits and to comply with all trial procedures
Exclusion Criteria:	An individual fulfilling any of the following criteria is to be excluded from trial enrollment: 1) Subject who received any other dengue vaccination that was not part of CYD28 2) Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be pre-menarche or post-menopausal for at least 1 year, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to vaccination until at least 4 weeks after vaccination)

	3) Participation at the time of study enrollment (or in the 4 weeks preceding the trial vaccination) or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure 4) Receipt of any vaccine in the 4 weeks preceding the trial vaccination or planned receipt of any vaccine in the 4 weeks following the trial vaccination 5) Receipt of immune globulins, blood or blood-derived products in the past 3 months 6) Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy, such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months) 7) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccines used in the trial or to a vaccine containing any of the same substances 8) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion 9) Receipt of blood or blood-derived products in the past 3 months, which might interfere with assessment of the immune response 10) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily 11) Current alcohol abuse or drug addiction 12) Moderate or severe acute illness/infection (according to investigator judgment) on the day of vaccination or febrile illness (temperature ≥ 38.0°C). A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided 13) Identified as an Investigator or employee of the Investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study
Statistical Methods:	The analysis will be performed under the responsibility of the Sponsor's Biostatistics platform with the SAS software, version 9.2 or higher (SAS Institute, Cary, North Carolina, USA). Three statistical analyses of safety and immunogenicity will be performed on unblinded data (one 28 days following the booster vaccination, one after the 1 year follow-up, and the other one after the end of the trial). A specific process will be implemented to maintain the blind at both subject and Investigator levels until completion of the trial. The immunogenicity populations (full and per-protocol analysis sets) will be used for the immunogenicity analyses, and the safety analysis set will be used for the safety analysis.

Hypothesis and Statistical Method for the Primary Objective (Group 1 only)

Hypotheses

Individual Hypotheses for Each Serotype to Demonstrate Non-inferiority:

A non-inferiority testing approach will be performed for each antigen specific endpoint to demonstrate the non-inferiority of a CYD dengue vaccine booster dose compared to the third CYD dengue vaccine dose in subjects from CYD28, in term of GMTRs.

Individual hypotheses for each Serotype will be as follows:

$$H_0{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) \leq 1/2$$
$$H_1{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) > 1/2$$

$Where\ i = 1,2,3\ and\ 4;\ V^i_{Booster}$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V^i_{PD3} is the immunogenicity titer 28 days after the third CYD dengue vaccine dose in CYD28 subjects.

Overall Hypothesis to Demonstrate Non-Inferiority:

The overall null hypothesis can be stated as: for at least one serotype, the post-booster dose response (28 days after the CYD dengue vaccine booster injection) is inferior to the PD3 response (28 days after the third CYD dengue vaccine dose in CYD28 subjects).

$$H_0^G : \text{at least one } H_0^i \textit{ not rejected}$$
$$H_1^G : \text{all } H_0^i \textit{ are rejected}$$

Statistical Methods

A non-inferiority test will be performed using the 95% two sided confidence interval (CI) of $GM\left(V_{Booster}/V_{PD3}\right)$ for each serotype and the 95% CI will be calculated using paired t-test. Subjects with non-missing PD3 and post-booster dose titer will be included in this analysis.

For each serotype, the non-inferiority will be demonstrated if the lower limit of the two-sided 95% CI is greater than 1/2. If the null hypothesis is rejected, then the alternative hypothesis of non-inferiority will be supported.

The overall null hypothesis will be rejected if the four individual null hypotheses are rejected simultaneously.

Hypotheses and Statistical Methods for the First Secondary Objective

If non-inferiority is demonstrated for the primary endpoint, then superiority hypotheses will be performed.

Hypotheses

Individual Hypotheses for Each Serotype to Demonstrate Superiority:

A superiority hypotheses testing approach will be performed for each serotype to demonstrate the superiority of a CYD dengue vaccine booster dose (28 days after the booster dengue vaccine injection) compared to the third CYD dose 28 days post vaccination in subjects from CYD28 trial, in terms of GMTR for each subject.

Individual hypotheses for each serotype will be as follows:

$$H_0^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) \leq 1$$
$$H_1^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) > 1$$

$Where\ i = 1,2,3\ and\ 4;\ V^i_{Booster}$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V^i_{PD3} is the immunogenicity titer 28 days after third CYD dengue vaccine dose in CYD28.

Overall Hypothesis to Demonstrate Superiority:

The overall null hypothesis can be stated as: for at least one serotype, the post-booster dose response is not superior to the PD3 response.

$$H_0^G: \text{at least one } H_0^i \textit{ not rejected}$$
$$H_1^G: \text{all } H_0^i \textit{ are rejected}$$

Statistical Methods

A superiority test will be performed using the 95% two sided confidence interval (CI) of $GM(V_{Booster}/V_{PD3})$ for each serotype; the 95% CI will be calculated using paired t-test. Subjects with non-missing PD3 and post-booster dose titer will be included in this analysis.

For each serotype, superiority will be demonstrated if the lower limit of the two-sided 95% CI is greater than 1. If the null hypothesis is rejected, then the alternative hypothesis of superiority will be supported.

The overall null hypothesis will be rejected if the four individual null hypotheses are rejected simultaneously.

Hypotheses and Statistical Methods for other Secondary Objectives and Additional Objectives

All analyses for other secondary objectives and additional objectives will be descriptive; no hypotheses will be tested.

For immunogenicity, 2 sample t-test on the $\log_{10}$ transformed titers will be used for 95% CI for the ratio of GMTs (difference between GMTs on log scale). Assuming that $\log_{10}$ transformation of the titers/titers ratio follows a normal distribution, first, the mean and 95% CIs will be calculated on $\log_{10}$ (titers/ titers ratio) using the usual calculation for normal distribution, then antilog transformations will be applied to the results of calculations, to compute GMTs/GMTRs and their 95% CIs. The 95% CIs for percentages will be calculated using the exact binomial distribution (Clopper-Pearson's method).

For safety, the exact binomial distribution (Clopper-Pearson method) for proportions will be used in calculations of the 95% CIs.

Calculation of sample size:

There will be 195 subjects in Group 1, 65 subjects in Group 2. Assuming that approximately10% of subjects from each group will not provide valid immunogenicity results, a total of 176 and 59 evaluable subjects is anticipated for Groups 1 and Group 2, respectively. With 176 evaluable subjects, the probability of observing at least 1 AE with true incidence of 1.7% is approximately 95%.

Sample size for the primary endpoint (only for Group 1 subjects) was estimated to demonstrate non-inferiority in terms of GMTR, 28 days post-injection, of a CYD dengue vaccine booster compared to the third CYD dose among subjects from CYD28 trial.

With 176 evaluable subjects in Group 1 for each serotype, there is 80.2% overall power (see Table 1) using paired t-test to reject the 4 individual null hypotheses simultaneously; calculation assumed a non-inferiority margin (delta) =2, one-sided type I error =0.025 and correlation between the responses PD3 and post-booster dose of the same serotype in the same subject = 0.6.

Table 2: Power/Sample size calculation summary table for primary endpoint (only for Group 1 subjects)

Component (Antigen)	Standard deviation (log 10)	Non-Inferiority Definition	Power for N=176
Serotype 1	(sd1=0.77,sd2=1.54)	> 1/2	0.892
Serotype 2	(sd1=0.74,sd2=1.48)	> 1/2	0.914
Serotype 3	(sd1=0.59,sd2=1.18)	> 1/2	0.970
Serotype 4	(sd1=0.53,sd2=1.06)	> 1/2	0.996
Overall			**0.802**

The calculation of the standard deviation for PD3 (sd1) is based on the weighted average of 28-day PD3 standard deviations of titers from CYD28. The standard deviation for post-booster dose (sd2) is estimated conservatively as two folds of the sd1 for each serotype.

Since four individual null hypotheses should be rejected simultaneously to reject the overall null hypothesis, so no multiplicity adjustment for alpha is necessary.

A 3:1 randomization ratio between Group 1 and Group 2 was chosen, so there will be 195 and 65 subjects enrolled in Group 1 and Group 2, respectively.

For the assessment of CMI, additional neutralizing Ab titers (at 7 and 14 days post-injection), Ab specificity and affinity maturation, the AIT subset will include the first 60 randomized subjects from two specific sites (30 subjects per site): 45 subjects in Group 1 and 15 subjects in Group 2.

Table of Study Procedures

Phase II Trial, 5 or 7 Visits, 1 Vaccination, 5 or 7 Blood Samples, 9 Phone Calls, 24-Month Duration per Subject

Visit Number (V)	**V01**	**V02***	**V03***	**V04**	**PC1**	**PC2**	**V05†**	**PC3**	**PC4**	**V06**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**V07**
Trial Timelines (Days/Months)	**D0**	**V01 + 7d**	**V01 + 14d**	**V01 + 28d**	**V01 + 2M**	**V01 + 4M**	**V01 + 6M**	**V01 + 8M**	**V01 + 10M**	**V01 + 12M**	**V01 + 14M**	**V01 + 16M**	**V01 + 18M**	**V01 + 20M**	**V01 + 22M**	**V01 + 24M**
Time Windows (Days)		**+2**	**+7**	**+7**	**+8d**	**+8d**	**+20d**	**+8d**	**+8d**	**±30**	**+8d**	**+8d**	**+8d**	**+8d**	**+8d**	**±30**
Informed Consent	√															
Inclusion/Exclusion Criteria	√															
Demography/Body Stature	√															
Significant Medical History	√															
History of dengue infection	√															
Physical Examination and Temperature‡	√	√	√	√			√			√						√
Urine Pregnancy Test§	√															
Concomitant Therapy	√	√	√	√												
IVRS/IWRS Call	√‡															
Blood Sampling: Neutralizing Ab PRNT (all subjects)	BL1**††			BL4††			BL5			BL6						BL7
Injection	**Inj. 1**															
30-Min. Observation Period	√															
Injection Site Reactions & Systemic Events Assessment‡‡	√	√	√	√												
Diary Card (DC) Provided	DC															
Checked		DC	DC	DC												
& Collected				DC												
Memory Aid (MA) Provided				MA												
Checked					MA	MA	MA	MA	MA	MA	MA	MA	MA	MA	MA	MA

Visit Number (V)	**V01**	**V02***	**V03***	**V04**	**PC1**	**PC2**	**V05†**	**PC3**	**PC4**	**V06**	**PC5**	**PC6**	**PC7**	**PC8**	**PC9**	**V07**
Trial Timelines (Days/Months)	**D0**	**V01 + 7d**	**V01 + 14d**	**V01 + 28d**	**V01 + 2M**	**V01 + 4M**	**V01 + 6M**	**V01 + 8M**	**V01 + 10M**	**V01 + 12M**	**V01 + 14M**	**V01 + 16M**	**V01 + 18M**	**V01 + 20M**	**V01 + 22M**	**V01 + 24M**
Time Windows (Days)		**+2**	**+7**	**+7**	**+8d**	**+8d**	**+20d**	**+8d**	**+8d**	**±30**	**+8d**	**+8d**	**+8d**	**+8d**	**+8d**	**±30**
Phone Call§§					√	√		√	√		√	√	√	√	√	
Termination Record																√
SAEs and Serious AESIs***	Throughout the period															
THE FOLLOWING ADDITIONAL PROCEDURES APPLY ONLY TO THE AIT SUBSET†††																
Additional neutralizing Ab PRNT (both for adolescents and adults)		BL2‡‡‡	BL3‡‡‡													
CMI **(for adolescents and adults)**	BL1**	BL2	BL3	BL4						BL6						

* V02 and V03 are only for subjects included in the additional immunological test (AIT) subset.

† Subjects having prematurely terminated the trial will be contacted by phone for the 6-month safety follow-up.

‡ Mandatory at injection visit (before injection). For other visits: physical examination and temperature measurement will be performed if necessary, based on the health status of the subject.

§ For all female subjects, except pre-menarche girls and post-menopausal (for at least 1 year) women.

** Blood samples planned during vaccination visit will be taken before vaccination.

†† These blood samples will also be used to assess Ab specificity and affinity maturation in subjects from the AIT subset.

‡‡ Solicited injection site reactions will be collected for 7 days after injection. Solicited systemic reactions will be collected for 14 days after injection. Unsolicited AEs will be collected for 28 days after injection.

§§ Telephone call to contact the subjects and ask them about SAEs that may have occurred.

*** Serious AESIs will be reported after each injection in defined time windows as follows: serious hypersensitivity/allergic reactions occurring within 7 days, serious viscerotropic disease occurring within 30 days, serious neurotropic disease occurring within 30 days; hospitalized suspected dengue disease will be reported during the entire study. Non-serious AESIs (i.e. hypersensitivity / allergic reactions) will be reported within 7 days after each injection.

††† In the AIT subset of 60 subjects (45 subjects in Group 1 and 15 subjects in Group 2).

‡‡‡ These blood samples will also be used to assess Ab specificity in subjects from the AIT subset.

List of Abbreviations

Ab	antibody
AE	adverse event
AESI	adverse event of special interest
AF	assent form
AIT	additional immunological tests
ALT	alanine transaminase
AR	adverse reaction
AST	aspartate aminotransferase
BL	blood sample
$CCID_{50}$	cell-culture infectious dose 50%
CDM	Clinical Data Management
C&MQO	Clinical and Medical Quality Operations
CI	confidence interval
CMI	cell mediated immunity
CRA	Clinical Research Associate
CRF	electronic case report form
CTA	clinical trial agreement
CTL	Clinical Team Leader
CRO	contract research organization
D	day
DC	diary card
DF	dengue fever
DHF	dengue hemorrhagic fever
DSS	dengue shock syndrome
EC	Ethics Committee
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
FAS	full analysis set
FDA	Food and Drug Administration
FV	flavivirus
FVFS	first visit, first subject
FVLS	first visit, last subject
GCI	Global Clinical Immunology

GCP	Good Clinical Practice
GMT	geometric mean of titer
GMTR	geometric mean of titer ratio
GPV	Global PharmacoVigilance
IATA	International Air Transport Association
ICF	informed consent form
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
Ig	immunoglobulin
IND	investigational new drug (application)
IRB	Institutional Review Board
IVRS	interactive voice response system
IWRS	interactive web response system
LLT	lowest level term
LLOD	lower limit of detection
LLOQ	lower limit of quantification
M	month
MA	memory aid
MedDRA	Medical Dictionary for Regulatory Activities
mL	milliliter
NI	non-inferiority
NR	non-reportable
NS	non-structural protein
NSAID	non-steroidal anti-inflammatory drug
PC	phone call
PD	post-dose
PFU	plaque-forming unit
PPAS	per-protocol analysis set
PSO	Product Safety Officer
RCDC	reverse cumulative distribution curves
RMO	Responsible Medical Officer
RNA	ribonucleic acid
RT-PCR	reverse transcription-polymerase chain reaction
SAE	serious adverse event
SafAS	safety analysis set
SC	subcutaneous
Sd	standard deviation

SMT	safety management team
TMF	trial master file
UAR	unexpected adverse reaction
ULOQ	upper limit of quantification
VCD	virologically-confirmed dengue
VE	vaccine efficacy
WHO	World Health Organization
YF	yellow fever

1 Introduction

1.1 Background

This study is a booster study of Sanofi Pasteur's CYD Dengue vaccine. It will assess the immunogenicity and safety of the CYD dengue vaccine booster in healthy children, adolescents and adults who received 3 doses of the tetravalent dengue vaccine 5 years (or more) earlier in the CYD28 trial conducted in Singapore (1). As the CYD dengue vaccine candidate claimed indication for the prevention of dengue disease is for individuals 9 through 60 years of age, the subjects enrolled in CYD63 will be a subset of CYD28 subjects that were aged ≥ 9 years on the day of the first injection of study vaccination.

Dengue disease is caused by 4 closely related, but antigenically distinct, dengue virus serotypes (1, 2, 3, and 4) of the genus flavivirus (FV). Infection with a dengue virus is usually asymptomatic but can produce a spectrum of clinical illnesses ranging from a non-specific viral syndrome to severe, fatal hemorrhagic disease.

Dengue fever (DF) is characterized by biphasic fever, headache, pain in various parts of the body, prostration, rash, and lymphadenopathy. Recovery from DF is usually complete in 7 to 10 days, but prolonged asthenia is common. Decreases in leukocytes and platelet count are frequent. The incubation period of DF after the mosquito bite averages 4 days (range from 3 to 14 days).

Dengue hemorrhagic fever (DHF) is characterized by abnormalities of homeostasis and increased vascular permeability that can lead to hypovolemia and hypotension (dengue shock syndrome [DSS]), often complicated by severe internal bleeding. The case fatality rate of DHF can be as high as 10% without therapy, but is below 1% in most centers with modern intensive supportive therapy.

Human infection occurs by injection of the virus into the extravascular tissues during blood feeding by an infected *Aedes aegypti* mosquito or *Aedes albopictus* mosquito (2). The primary cell subset infected after inoculation is the dendritic cells, which subsequently migrate to the draining lymph nodes. After initial replication in the skin and draining lymph nodes, the virus appears in the blood during the acute febrile phase, generally for 3 to 5 days.

Mosquito vectors for dengue viruses, *Aedes aegypti* and *Aedes albopictus*, are now present in all tropical and sub-tropical areas of the world and in some temperate areas of the United States of America (USA), Europe, Africa, and the Middle East. Following its rapid spread in recent years, DF/DHF is now endemic/epidemic in Latin America, South East Asia, India, Africa, and the Caribbean and Pacific regions.

According to the World Health Organization (WHO), over 2.5 billion people are now at risk from dengue in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. The American, South East Asia and the Western Pacific regions are the most seriously affected. WHO currently estimates there may be 50–100 million dengue infections worldwide every year. An estimated 500,000 people with severe dengue require hospitalization, a large proportion of whom are children. About 2.5% of those affected die (3).

Thus, according to WHO, there is an urgent need to develop a safe and effective vaccine against the four serotypes of dengue virus to protect people in endemic countries (4).

In endemic areas, DF is suspected in patients who develop sudden fever, headache, myalgias, and adenopathy, particularly with the characteristic rash or recurrent fever. Routine laboratory diagnosis of dengue infections is based on the detection of dengue virus-specific antibodies (Abs), immunoglobulin (Ig) M and/or isolation of the virus or detection of viral ribonucleic acid (RNA) by reverse transcription-polymerase chain reaction (RT-PCR) or viral non-structural protein (NS) 1 antigen (Ag) by enzyme-linked immunosorbent assay (ELISA) (5) (6) (7). The diagnosis of dengue falls into 2 stages: Stage I, the acute fever period lasting a few days when viremia may be detected; and Stage II, the early post-febrile period lasting a few weeks when IgM and IgG Abs are increased. The confirmatory dengue diagnosis is performed through virological detection (e.g., dengue non-structural protein 1 (NS1), dengue RT-PCR).

There is no licensed vaccine to prevent dengue infection or disease and no specific treatment exists[a]. Preventive measures presently rely on mosquito control and personal protection. These measures are limited in efficacy, difficult to enforce, and expensive. The best method of prevention lies with the development of a safe and effective vaccine directed at the 4 serotypes of dengue virus responsible for the disease.

1.2 Background of the Investigational Product

Sanofi Pasteur's tetravalent CYD dengue vaccine, using recombinant technology to obtain a live-attenuated vaccine, has been extensively evaluated in subjects from 2 to 60 years.

- In previous Phase I trials, a total of 185 adult subjects, 71 adolescents (aged 12 to 17 years), and 140 children (aged 2 to 11 years), that is 396 subjects overall, in both Flavivirus (FV)-naïve and -immune populations have been exposed to at least one dose of Phase I lots of CYD dengue vaccine containing either 4 $\log_{10}$ or 5 ± 1 $\log_{10}$ cell-culture infectious dose 50% ($CCID_{50}$) per serotype.
- In Phase II trials, approximately 893 adult subjects, 472 adolescents, 3370 children aged between 2 and 11 years and 179 toddlers, that is more than 4,900 subjects overall, have received at least one dose of Phase II lots of CYD dengue vaccine (5 ± 1 $\log_{10}$ $CCID_{50}$ per serotype). The primary efficacy study (CYD23) has been completed in 2012 (8). Subjects from CYD23 are being followed for safety in a long-term follow-up study (CYD57).
- Specifically in Singapore, a Phase II study (CYD28) was completed in 2014; 1198 subjects (2 to 45 years) received 3 injections of CYD dengue vaccine or a placebo/control vaccine[b] at 0,

[a] Since protocol version 2.0 dated 17 September 2015 was reviewed and approved by ECs and Singapore Health Authorities, the CYD dengue vaccine (commercial name Dengvaxia®) has been licensed in Brazil, El Salvador, Mexico, and the Philippines for the prevention of dengue disease caused by all 4 dengue serotypes (1, 2, 3, 4) in individuals 9-45 years of age living in endemic areas.

[b] Control Group received a placebo as first vaccination. Subjects <12 years were to receive hepatitis A as second and third vaccinations. Subjects ≥12 years were to receive influenza vaccine of Northern and Southern hemisphere formulations as second and third vaccinations, respectively.

6, and 12 months with a 4 years Abs persistence and safety follow-up after the third injection. Study objectives were to describe safety and immunogenicity of CYD dengue vaccine. Overall, CYD dengue vaccine in Singapore showed satisfactory safety and immune responses against all 4 serotypes after 3 doses of CYD dengue vaccine, and the results were comparable to other Phase II trials in the CYD dengue vaccine program (1).

- In Phase III trials, approximately 694 adult subjects, 20,968 children aged between 2 and 17 years, and 1478 toddlers, that is 23,140 subjects overall, have received at least one dose of Phase III lots of CYD dengue vaccine (4.5 – 6.0 $\log_{10}$ $CCID_{50}$ per serotype). Two large efficacy studies (CYD14 and CYD15), conducted in Asia Pacific (in children from 2 to 14 years old) and Latin America (in children & adolescents from 9 to 16 years old) respectively, have completed the 3-years follow-up after the third vaccination (9) (10) (11). The two large efficacy studies were sufficiently powered to demonstrate significant efficacy of the CYD dengue vaccine in preventing the occurrence of VCD due to any serotype after 3 injections and the primary endpoint in each study was met, demonstrating efficacy against VCD cases post-dose (PD) 3 due to any serotype with the lower bound of the 95% CI >25%. In both studies, the vaccine showed a good safety profile over the Active Phase. In CYD14, results of the first year of the Hospital Phase showed a difference of incidence of hospitalized severe and non-severe virologically-confirmed dengue (VCD) cases between the Dengue and Control Groups, in particular in the 2 to 5 years old age group. Other available long-term follow-up data showed no evidence of increase in severity of dengue disease in subjects aged 9 to 16 years (11).

Therefore, up to April 2015, around 28,500 subjects have received at least one dose of the final tetravalent CYD dengue vaccine formulation, regardless of the administration schedule. Results demonstrate the favorable risk/benefit profile of the dengue vaccine candidate and supports the claimed indication for the prevention of dengue disease caused by dengue virus serotypes 1, 2, 3 and 4 in individuals from 9 through 60 years of age living in endemic areas. The vaccination schedule consists of 3 injections of 0.5 mL to be administered at 6-month intervals.

1.3 Potential Benefits and Risks

Detailed risk/benefit analysis is presented in the Investigator's Brochure.

1.3.1 Potential Benefits to Subjects

The subjects participating in the present clinical trial and being injected with CYD dengue vaccine booster may enhance their current immunity and protection against dengue disease after vaccination with CYD dengue vaccine booster.

The possibility for subjects to have their immune response against dengue brought back to (or even brought above) the level following primary vaccination is certainly an important perspective in light of recently published data (12) (13).

Indeed, results from two large-scale Phase III efficacy trials showed CYD dengue vaccine's potential to reduce probability for subject to have symptomatic VCD, hospitalized VCD due to any of the 4 serotypes (vaccine efficacy [VE] estimates against symptomatic VCD during the whole Active Phase due to any of the 4 serotypes were 56.5% (95%CI: 43.8; 66.4) for CYD14

and 60.8% (95%CI: 52.0; 68.0) for CYD15. In addition, efficacy was observed against each of the 4 serotypes with high efficacy seen against severe VCD cases and hospitalized VCD cases during the Active Phase).

1.3.2 Potential Risks to Subjects

CYD Dengue Vaccine

During the clinical development of dengue vaccine as well as during the Active Phase of the Phase III efficacy studies, no safety concerns after administration of the CYD dengue vaccine emerged from the pooled safety analysis, providing sufficient evidence that the safety profile of the CYD dengue vaccine is acceptable and similar to the safety profile of licensed vaccines in similar population.

Potential unwanted effects also include injection site reactions such as erythema, swelling, induration, and pain. General disorders may also be observed such as fever, malaise, asthenia, myalgia and headache. As for any drugs, a risk of allergic reaction cannot be excluded. Vasovagal malaise linked to the injection procedure may be observed in susceptible individuals. Full list of expected adverse events (AEs) can be found in the Investigator's Brochure.

As CYD dengue vaccine has a yellow fever (YF) vaccine backbone, and YF vaccination has been rarely associated with viscerotropic and neurotropic AEs, this risk has to be considered. This theoretical risk linked to viscerotropism and neurotropism is further addressed in the "Guidelines for assessing viscerotropic and neurotropic AE" document. In the previous studies conducted with the CYD dengue vaccine, no confirmed viscerotropic or neurotropic AEs have been observed.

Although an unexplained higher incidence of hospitalization for dengue in year 3 of follow-up among children younger than 9 years was observed in CYD14 efficacy trial, in particular in children from 2 to 5 years old, the combined analysis of the efficacy trials during year 3 showed a lower risk of hospitalization for dengue among participants who were 9 years of age or older in the vaccine group than among those in the control group. All subjects who were hospitalized due to dengue fully recovered after receiving appropriate supportive treatment (11).

Considering the totality of long-term follow-up data during the Hospital Phase across the 3 efficacy studies, no evidence of increased severity of dengue disease or increase in frequency of hospitalized dengue cases has been observed in subject from 9 to 16 years old vaccinated with the dengue vaccine.

Placebo

No adverse reactions are expected from placebo, except local reactions due to the injection process (e.g., bruising, local pain).

All Subjects

Potential risks may also include the unwanted effects of blood sampling (i.e., the discomfort from having blood taken lasting only seconds to minutes) and/or bruising.

1.4 Rationale for the Trial

At the time of the protocol writing, there is currently no licensed vaccine against dengue and no specific drug treatment against the disease[a].

Dengue disease is endemo-epidemic in Singapore and is a major cause of childhood mortality. Despite nationwide *Aedes* mosquito control program set up in 1970, major epidemics still occur. The last big outbreak occurred in 2005 (14) (15). The number of reported cases has been lower during the last years: 2,800 cases in 2006, 8,300 in 2007, 6,600 in 2008 and 4,200 in 2009. There is currently no licensed vaccine to prevent dengue infection and no specific treatment exists. Preventive measures presently rely on mosquito control and personal protection. These measures are limited in efficacy, difficult to enforce, and expensive. The best method of prevention lies with the development of a safe and effective vaccine directed at the four serotypes of dengue virus responsible for the disease (16) (17).

Sanofi Pasteur's CYD dengue vaccine was shown to have an acceptable safety profile throughout a number of Phase II and Phase III studies and Vaccine efficacy (VE) was demonstrated in subjects from 2 to 16 years (12) (13). Data regarding the levels of neutralizing Abs from long-term follow-up studies have shown a predictable decrease in the level of circulating Abs (geometric mean of titers [GMTs]) against all 4 serotypes 1 year after the third injection, regardless of age group, which was followed by a trend to stabilization during the subsequent years. However, long-term GMTs for each serotype remained overall higher than GMT values before vaccination. These post-Dose 3 observations need to be considered for the development of an immunization program against dengue.

Indeed, and based on the key findings from Phase III efficacy studies (i.e., higher Abs levels decrease the probability to get dengue disease) (9) (10), and as no correlate of protection or correlate of risk has yet been established for the CYD dengue vaccine, it is assumed that higher neutralizing Ab levels are associated with higher VE. One of the implications of this assumption is that it is possible that the progressive decline of Ab levels is linked with the waning of protection against dengue infections. Before this can be confirmed or disproved with data from ongoing studies, Sanofi Pasteur is initiating the development of a booster vaccination strategy with two trials sharing a very similar design, CYD63 and CYD64.

As the CYD dengue vaccine candidate claimed indication for the prevention of dengue disease is for individuals 9 through 60 years of age, the subjects enrolled in CYD63 will be a subset of CYD28 subjects that were aged ≥9 years on the day of the first injection of study vaccination. The general purpose of CYD63 is to assess and describe the booster effect of la CYD dengue vaccine dose administered 5 years or more after the completion of a 3-dose vaccination schedule. To do so, this study will be looking at the non-inferiority of the neutralizing Ab responses 28 days after

[a] Since protocol version 2.0 dated 17 September 2015 was reviewed and approved by ECs and Singapore Health Authorities, the CYD dengue vaccine (commercial name Dengvaxia®) has been licensed in Brazil, El Salvador, Mexico, and the Philippines for the prevention of dengue disease caused by all 4 dengue serotypes (1, 2, 3, 4) in individuals 9-45 years of age living in endemic areas.

booster vaccination as compared to the Ab responses 28 days after the third dose of the primary vaccination, in a subset of subjects from CYD28. The assumption here is that booster vaccination should induce neutralizing Ab to at least the same levels as primary vaccination (i.e., PD3 GMTs).

CYD63 will also be pursuing a number of secondary objectives. Firstly, and if non-inferiority can be demonstrated, the study will assess whether the CYD dengue vaccine booster can induce superior neutralizing Ab responses 28 days after booster vaccination as compared to Ab responses 28 days after the third dose of the primary vaccination. Secondly, the persistence of immune responses at baseline and following booster vaccination, and the influence of natural boosting through dengue wild-type infection at baseline and following booster vaccination, will be both assessed during this 2 years study. Thirdly, this study will be assessing the safety of booster vaccination, and ensure that booster vaccination does not raise safety issues that were not observed during and following the primary vaccination schedule.

Lastly, CYD63 will be describing the humoral immune response's dynamics and characterizing the cellular immune response, following booster injection. To further assess the post-booster dose Ab titer, an analysis controlling for baseline antibody titer will be explored as well. These additional objectives will be pursued in order to further develop the current understanding of the mechanisms involved in CYD dengue vaccine-induced immune response.

2 Trial Objectives

2.1 Primary Objective

To demonstrate the non-inferiority[a], in terms of geometric mean of titer ratios (GMTRs), of a CYD dengue vaccine booster compared to the third CYD dengue vaccine injection in subjects from CYD 28 trial (subjects from Group 1 only).

The endpoints for the primary objective are presented in Section 9.1.1.1.

2.2 Secondary Objectives

Immunogenicity

1) If the primary objective of non-inferiority is achieved: To demonstrate the superiority, in terms of GMTRs, of a CYD dengue vaccine booster compared to the third CYD dengue vaccine injection in subjects from CYD28 trial (subjects from Group 1 only).
2) To describe the immune responses elicited by the CYD dengue vaccine booster or placebo injection in subjects who received three doses of the CYD dengue vaccine in the CYD28 trial in all subjects.

[a] If the planned sample size is not achieved, the analysis may be descriptive.

3) To describe the neutralizing Abs levels of each dengue serotype PD3 (CYD28 subjects) and immediately prior to booster or placebo injection in all subjects.

4) To describe the neutralizing Ab persistence 6 months, 1 year and 2 years post booster or placebo injection in all study subjects.

Safety

To evaluate the safety of booster vaccination with CYD dengue vaccine in all subjects.

The endpoints for the secondary objectives are presented in Section 9.2.1.1 and Section 9.2.2.2, respectively.

2.3 Additional Objectives

Only for the additional immunological tests (AIT) subset

1) To describe dengue neutralizing Ab levels (exploration of the Ab response's kinetics), Ab specificity and affinity maturation post-booster or placebo injection

2) To describe CMI responses post-booster or placebo injection.

In all subjects

3) To assess post-booster neutralizing Ab levels against each dengue virus serotype while controlling for baseline neutralizing Ab levels against each dengue virus serotype

The endpoints for the additional objectives are presented in Section 9.3.1.1.

3 Investigators and Trial Organization

This trial will be conducted in 3 centers in Singapore. Details of the trial centers and the Investigators at each center are provided in the "List of Investigators and Centers Involved in the Trial" document.

An Independent Data Monitoring Committee (IDMC) will be involved in the regular review of hospitalized virologically-confirmed dengue (VCD) cases[a], including assessment of severity. Additionally, any related serious adverse event (SAE) or death or serious adverse events of special interest (AESIs) will be promptly reviewed by the IDMC.

An internal safety management team (SMT) will perform a blinded safety analysis on safety data after vaccination.

The laboratories involved in this study will be:

[a] Hospitalized suspected dengue disease is defined as an acute febrile illness with diagnosis of dengue requiring hospitalization (with bed attribution). In such cases, one unplanned blood sample will be collected for virological confirmation: an acute sample (within the first 5 days after fever onset). A suspected hospitalized dengue case will be considered a serious VCD case if there is a detection of wild type (WT) dengue virus by NS1 antigen ELISA and/or wild-type dengue RT-PCR.

- Sanofi Pasteur Global Clinical Immunology (GCI), Swiftwater, Pennsylvania, USA or outsourced laboratory under the management of GCI: Neutralizing Ab titration and virological confirmation of dengue
- Sanofi Pasteur Research & Non Clinical Safety department, Marcy L'Etoile, France: CMI response
- Sanofi Pasteur VaxDesign, Orlando, Florida, USA: Ab specificity and affinity maturation and associated statistical analysis

Biostatistics, data management, monitoring, and medical writing will be either subcontracted to a CRO or performed in-house by the Sponsor.

The Sponsor's Responsible Medical Officer (RMO) (the person authorized to sign this protocol and any amendments on behalf of the Sponsor) is [redacted], [redacted] and Clinical Team Leader (CTL) of this trial.

4 Independent Ethics Committee / Institutional Review Board

Before the investigational product can be shipped to the investigational site and before the inclusion of the first subject, this protocol, the informed consent form(s) (ICF), subject recruitment procedures, and any other written information to be provided to subjects must be approved by, and / or receive favorable opinion from, the appropriate Independent Ethics Committee (IEC) or Institutional Review Board (IRB).

In accordance with Good Clinical Practice (GCP) and local regulations, each Investigator and / or the Sponsor are responsible for obtaining this approval and / or favorable opinion before the start of the trial. If the protocol is subsequently amended, approval must be re-obtained for each substantial amendment. Copies of these approvals, along with information on the type, version number, and date of document, and the date of approval, must be forwarded by the Investigator to the Sponsor together with the composition of the IEC / IRB (the names and qualifications of the members attending and voting at the meetings).

The Investigator or the Sponsor will submit written summaries of the status of the trial to the IEC /Health Science Authority annually, or more frequently if requested. All SAEs (related to the vaccination or not) occurring during the trial will be reported by the Investigator to the site's IEC. Death (and related SAEs if required by the IEC) will be reported within 24 hours, and other SAEs will be reported within 7 or 14 days (according to IEC's policy). Cross-reporting to the other sites' IECs will be done according to the IEC's policies.

5 Investigational Plan

5.1 Description of the Overall Trial Design and Plan

5.1.1 Trial Design

This is a multi-center, observer-blind, randomized, placebo-controlled, Phase II trial of the CYD dengue vaccine booster in 260 healthy subjects aged 9 to 45 years on the day of the first injection of study vaccination, and who received 3 doses of the CYD dengue vaccine in the CYD28 trial in Singapore.

There will be 1 vaccination at Day (D) 0 and 2 groups of subjects:

- **Group 1**: 195 subjects will receive CYD dengue vaccine booster
- **Group 2**: 65 subjects will receive placebo

A total of 60 subjects (45 subjects in Group 1 and 15 subjects in Group 2) will also be included in a specific subset (AIT subset).

Blood samples will be taken at several time points throughout the study for CMI, additional neutralizing Ab, and Ab specificity and affinity maturation assessments (depending on the time points). More details are provided in Section 5.1.3.

The duration of each subject's participation in the trial will be approximately 24 months.

5.1.2 Justification of the Trial Design

The trial design will enable a thorough evaluation of the administration of a CYD dengue vaccine booster. Indeed, by assessing booster vaccination according to three complementary perspectives this trial will provide information on *i)* booster vaccination's overall relevance; *ii)* booster vaccination's immunogenicity and safety, as compared to both the last dose of 3-dose vaccination schedule and the placebo; and *iii)* the impact of booster vaccination on humoral and cell-mediated immunity.

Firstly, in order to demonstrate the relevance of booster vaccination, the present study will be looking at the non-inferiority of the neutralizing Abs response 28 days after booster vaccination as compared to the Abs response 28 days after the third dose of the primary vaccination, in a subset of approximately 195 subjects from CYD28 trial. If non-inferiority can be demonstrated, a superiority hypothesis will also be tested. This two-step approach will allow assessing whether booster vaccination induces neutralizing Abs to at least (non-inferiority) or even above (superiority) the levels observed PD3.

Secondly, the trial will be assessing changes in neutralizing Ab levels between different time points in order to gather data on *i)* neutralizing Abs persistence 5 years or more PD3 (before booster vaccination), *ii)* the effect of booster vaccination on neutralizing Ab levels, and *iii)* neutralizing Abs persistence up to 2 years after booster vaccination. Also, the trial will be assessing the safety of booster vaccination, and ensure that booster vaccination does not raise

safety issues that were not observed during and following the primary vaccination schedule. SAEs and AESIs will be assessed throughout the trial. A blinded observer procedure will be used, meaning that the vaccines will be prepared and administered by persons different from the Investigators responsible for safety evaluation. This will enable Investigators to maintain the blinded design. The assessment of CYD dengue vaccine's immunogenicity and safety will involve all subjects enrolled in the study.

Thirdly, the trial will be assessing different features of the humoral and cellular immune responses in a subset (the AIT subset) of 60 subjects (45 subjects in Group 1 and 15 subjects in Group 2). Measuring features of the immune response, and drawing descriptive comparisons between the 2 treatments (CYD dengue vaccine and placebo), will allow assessing the impact of booster vaccination on CMI, neutralizing Abs at D7 and D14, Ab specificity and affinity maturation, and on both T cell and B-cell responses at specific time points. See the Table of Study Procedures and Section 5.1.3 for details of the sampling schedule.

It is noteworthy that hospitalized suspected dengue cases occurring at any time in the trial will be documented. Hospitalized suspected dengue disease is defined as an acute febrile illness with diagnosis of dengue requiring hospitalization (with bed attribution). In such cases, 1 unplanned blood sample (acute sample[a]) will be collected for virological confirmation (within the first 5 days after fever onset). A suspected hospitalized dengue case will be considered as a serious VCD case if there is a detection of wild type (WT) dengue virus by non-structural protein 1 (NS1) ELISA and/or wild-type dengue RT-PCR. Hospitalized VCD cases will be assessed by an IDMC for severity assessment.

5.1.3 Trial Plan

Eligible subjects will be identified by the Sponsor. Each Investigator will be provided with a list of potential subjects to recruit. Once enrolled, each subject (and subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) will sign and date consent forms (ICF). All included subjects will attend 5 study visits and will receive 9 phone calls. Subjects from the AIT subset will attend 2 additional study visits:

- Study visits at Day (D) 0, D28, Month (M) 6, M12, M24 after vaccination
- Additional study visits for subjects in AIT subset: D7 and D14
- Phone calls at M2, M4, M8, M10, M14, M16, M18, M20, and M22 after vaccination

Vaccination

All subjects will receive either CYD dengue vaccine booster or placebo on D0.

[a] Acute blood sample for all suspected hospitalized dengue cases should be collected within the pre-specified timeframe as described above. If this cannot be accomplished, this sample should still be obtained as soon as possible thereafter, for Independent Data Monitoring Committee (IDMC) severity assessment.

Blood sampling

- For all subjects

Immunogenicity will be assessed at baseline (D0), as well as 28 days, 6 months, 1 year and 2 years post-vaccination.

- For the AIT subset

A subset of 60 (45 subjects in Group 1 and 15 subjects in Group 2) will provide additional blood samples at baseline and at 7, 14, 28 days, and 1 year post-injection. Depending on the time points, these blood samples will be used for measurement of CMI, Ab specificity and affinity maturation, and neutralizing Ab (exploration of the Ab response's kinetics).

Table 5.1 presents the number of blood samples (BL) and the volume collected.

Table 5.1: Blood Sampling Schedule and Volume to be Collected

Visit Number (V)	V01	V02	V03	V04	V05	V06	V07
Trial Timelines (Days/Months)	D0	D 07	D 14	D 28	M 06	Y 01	Y 02
Time Windows (Days)		+2	+7	+7	+20	±30	±30
Blood sample (BL)	BL1	BL2	BL3	BL4	BL5	BL6	BL7
Volume for each assessment (mL)							
Neutralizing Ab ***All subjects***	5*			5*	5	5	5
Additional neutralizing Ab ***Adolescents and adults subjects in the AIT subset***		5†	5†				
CMI ***Adolescents in the AIT subset***	25	15	15	15		15	
CMI ***Adults in the AIT subset***	35	25	25	25		25	
Total Volume for each subject (mL)							
Subjects **not** in the AIT subset	5			5	5	5	5
Adolescents in the AIT subset	30	20	20	20	5	20	5
Adults in the AIT subset	40	30	30	30	5	30	5

* These blood samples will also be used to assess Ab specificity and affinity maturation in subjects from the AIT subset.

† These blood samples will also be used to assess Ab specificity in subjects from the AIT subset (if necessary).

Collection of safety data

Clinical site personnel will record immediate AEs that occur within the 30 minutes after injection. Subjects or subjects' parent(s) / legally acceptable representative(s) will record in the diary card (DC) information about solicited injection site reactions from D0 to D7 post-injection, about

solicited systemic reactions from D0 to D14 post-injection and unsolicited AEs from D0 to D28 post-injection. Information on non-serious AESIs will be collected within 7 days post-injection. Information on SAEs (including serious AESIs) will be collected throughout the trial. Subjects or subjects' parent(s) / legally acceptable representative(s) are to contact the Investigator in case of hospitalization for suspected dengue disease.

Subjects or subjects' parent(s) / legally acceptable representative(s) will record safety information in memory aids (MAs) when DCs are not being used. Clinical site personnel will record information about SAEs and serious AESIs written in the MAs.

Please refer to the Table of Study Procedures.

5.1.4 Visit Procedures

All information collected during the study visits must be reported into the source documents. Some of the following information will also be recorded in the electronic case report form (CRF).

Visit 1 (D0): Inclusion, Blood sample and Vaccination

The Investigator or designated study personnel will:

1) Present the trial to the subject (and subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) in more detail, answer any of his/her/their questions, and ensure that he/she/they has/have been informed of all aspects of the trial that are relevant to their decision to participate.
2) Obtain the consent forms signed and dated by the subject (and subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years), date and sign the ICF (only the investigator or sub-investigator). The Investigator will keep the original document and give one copy to the subject.
3) Review the inclusion/exclusion criteria.
4) Collect demographic data (date of birth and gender).
5) Check and collect the subject's significant medical history.
6) Check and collect the subject's history of dengue infection
7) Perform a physical examination and record the subject's axillary temperature.
8) Perform a urine pregnancy test (for all female subjects, except pre-menarche girls and post-menopausal [for at least 1 year] women).
9) Check concomitant medications and record every reportable medication ongoing at the time of vaccination.
10) Call the Interactive Voice / Web Response System (IVRS / IWRS) for randomization.
11) **For all subjects:** Obtain the first blood sample (BL1; 5 mL) for neutralizing Abs; record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples).

For AIT subset only (60 subjects): an additional volume of blood will be drawn (25 mL for adolescents and 35 mL for adults) to assess CMI (see Section 7.1.2 for detailed instructions regarding the handling of blood samples).

It is important to note that if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt, even on another day. If ultimately a blood sample cannot be obtained, the reason will be recorded in the CRF. In this case, and if the subject wants to participate in the trial, he/she will be vaccinated.

12) Inject the appropriate study vaccine.
13) Record the date of injection, the site and side of injection and the route of administration, as well as the batch number of the vaccine.
14) Affix the vaccine labels in the subject's source documents.
15) Keep the subject under observation for 30 minutes, and record any adverse reaction in the source documents.
16) Give the subject / subject's parent (s) / legally acceptable representative(s) the DC to record any injection site reactions and systemic AEs, together with instructions for its completion, including explanations on the definition and use of intensity scales for collection of AEs.
17) Give the subject / subject's parent (s) / legally acceptable representative(s) a ruler to measure the size of any injection site reaction and a thermometer for temperature measurement, and instructions on how to use them.
18) Remind the subject / subject's parent (s) / legally acceptable representative(s) to call the study center if a serious medical event occurs.
19) Arrange an appointment for the second visit (7 days[+ 2 days]) (for AIT subset's subjects only).
20) Complete the relevant CRF pages for this visit.

The visit may be postponed once if the subject is temporarily not eligible at Visit 1.

Visit 2 (7 [+2] days after Visit 1; for AIT subset's subjects only): Collection of Safety Information and Blood Sample

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature (if necessary).
2) Check the information entered into the DC by interviewing the subject / subject's parent (s) / legally acceptable representative(s) and request information concerning any medical event, serious or not, that may have occurred since Visit 1.
3) Collect information regarding the subject's medication status since the previous visit.
4) Record any injection site reactions or systemic events and any reportable concomitant medications.

5) Collect the second blood sample (BL2[a]) for additional neutralizing Abs, Ab specificity (if assessment required), and CMI assessment and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples).

Note: *If the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity to return to the study site for another attempt within the visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.*

Visit 3 (14 [+7] days after Visit 1; for AIT subset's subjects only): Collection of Safety Information and Blood Sample

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature (if necessary).
2) Check the information entered into the DC by interviewing the subject / subject's parent (s) / legally acceptable representative(s) and request information concerning any medical event, serious or not, that may have occurred since Visit 2.
3) Collect information regarding the subject's medication status since the previous visit.
4) Record any injection site reactions or systemic events and any reportable concomitant medications.
5) Collect the third blood sample (BL3[b]) for additional neutralizing Ab, Ab specificity (if assessment required), and CMI assessment and record the date of collection (see Section 7.1.2 for detailed instructions regarding the handling of blood samples).

Note: *If the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity to return to the study site for another attempt within the visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.*

Visit 4 (28 [+7] days after Visit 1): Collection of Safety Information and Blood Sample

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature (if necessary).
2) Check and collect the information entered into the DC by interviewing the subject and request information concerning any medical event, serious or not, that may have occurred since Visit 3.
3) Give the subject / subject's parent (s) / legally acceptable representative(s) a MA.
4) Collect information regarding the subject's medication status since the previous visit.
5) Record any injection site reactions or systemic events and reportable concomitant medications.

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[a] BL2: 20 mL will be drawn in adolescent subjects and 30 mL in adult subjects

[b] BL2: 20 mL will be drawn in adolescent subjects and 30 mL in adult subjects

6) **For all subjects:** collect the fourth blood sample (BL4; 5 mL) for neutralizing Abs and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples).

   For AIT subset only (60 subjects): an additional volume of blood will be drawn (15 mL for adolescents and 25 mL for adults) to assess CMI (see Section 7.1.2 for detailed instructions regarding the handling of blood samples).

Note: *If the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity to return to the study site for another attempt within the visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.*

Telephone Calls 1 and 2 (2 and 4 months [+8 days] and after Visit 1)

The Investigator or an authorized designee will:

1) Ask if the subject has experienced any SAEs not yet reported. If an SAE occurred, follow the instructions in Section 9.3.1.2 for reporting it.
2) Remind the subject / subject's parent / legally acceptable representative to do the following:
   - Complete the relevant pages of the MA and bring it to the next visit
   - Notify the site in case of an SAE

Visit 5 (6 months [+20 days] after Visit 1): Collection of Safety Information and Blood Sample

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature (if necessary).
2) Check the information entered into the MA by interviewing the subject / subject's parent (s) / legally acceptable representative(s) and request information concerning any medical event, serious or not, that may have occurred since Visit 4.
3) Collect the fifth blood sample (BL5; 5 mL) for all subjects for neutralizing Abs and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples).

Note: *If the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity to return to the study site for another attempt within the visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.*

Telephone Calls 3 and 4 (8 and 10 months [+8 days] and after Visit 1)

Same procedures as for previous PC1 and PC2.

Visit 6 (1 year [±30 days] after Visit 1): Collection of Safety Information and Blood Sample

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature (if necessary).

2) Check and collect the information entered into the MA by interviewing the subject / subject's parent (s) / legally acceptable representative(s) and request information concerning any medical event, serious or not, that may have occurred since Visit 5.

3) **For all subjects:** collect the sixth blood sample (BL6; 5 mL) for neutralizing Abs and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples).

   For AIT subset only (60 subjects): an additional volume of blood will be drawn (15 mL for adolescents and 25 mL for adults) to assess CMI (see Section 7.1.2 for detailed instructions regarding the handling of blood samples).

Note: *If the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity to return to the study site for another attempt within the visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.*

Telephone Calls 5, 6, 7, 8, and 9 (14, 16, 18, 20, and 22 months [+8 days] and after Visit 1)

Same procedures as for previous PC1 and PC2.

Visit 7 (2 years [±30 days] after Visit 1): End of Trial Visit, Collection of Safety Information and Blood Sample

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature (if necessary).

2) Check the information entered into the MA by interviewing the subject / subject's parent (s) / legally acceptable representative(s) and request information concerning any medical event, serious or not, that may have occurred since Visit 6.

3) Collect the last blood sample (BL7; 5 mL) for all subjects for neutralizing Abs and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples).

Note: *If the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity to return to the study site for another attempt within the visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.*

Complete the termination record and sign the casebook in the CRF and enter the subject's termination information in the IVRS / IWRS.

SAEs and AEs That Are Related to Vaccination or That Led to Discontinuation:

At any time during the study, a subject who experiences an SAE or an AE must be followed if *either* of the following is true:

- The SAE or AE is considered by the Investigator to be related to vaccination, and is not resolved by the end of the subject's participation in the trial
- The subject has been discontinued from the trial because of the SAE or AE

Any such subject must be followed until the condition resolves, becomes stable, or becomes chronic.

5.1.5 Planned Trial Calendar

The following dates are approximate. The actual dates may differ as, for example, the trial will not start until all the appropriate regulatory and ethical approvals have been obtained.

Planned trial period - FVFS to LVLS[a]: June 2016 to September 2018

Planned inclusion period - FVFS to FVLS[b]: June 2016 to September 2016

Planned vaccination period: June 2016 to September 2016

Planned end of trial: LVLS: September 2018

Planned date of final clinical study report: CSR Sign off: December 2019

5.1.6 Early Safety Data Review

This trial will not include an early review of safety data. However, it may be interrupted at any time if new data about the investigational product become available, and/or on advice of the Sponsor, the IECs/IRBs, or the governing regulatory authorities in the country where the trial is taking place.

If the trial is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators, the IECs/IRBs, and the regulatory authorities of the reason for termination or suspension. If the trial is prematurely terminated for any reason, the Investigator will promptly inform the trial subjects / subjects' parents/guardians and should assure appropriate therapy and follow-up.

An internal SMT will perform a blinded safety analysis on safety data after vaccination.

An IDMC will be involved in the regular review of hospitalized VCD cases, including assessment of severity. Additionally, any related SAE or death or serious AESI will be promptly reviewed by the IDMC.

5.2 Enrollment and Retention of Trial Population

5.2.1 Recruitment Procedures

The Sponsor will provide a list of potential subjects to recruit to each Investigator, and subjects who received 3 doses of CYD dengue vaccine in CYD28 trial, who were aged 9 years or older at the time of first vaccination in CYD28, and for which a PD3 serum sample is available (at least 300 μL) will be contacted to enroll 260 subjects in the trial. Upon approval from IECs, subjects

[a] FVFS: first visit of first subject; LVLS: last visit of last subject

[b] FVLS: first visit, last subject

will be contacted via letter or by phone. Recruitment procedures and materials will be submitted to the IECs for approval before implementation.

5.2.2 Informed Consent Procedures

Informed consent is the process by which a subject (and an appropriate and legally acceptable representative for subjects aged < 21 years) voluntarily confirms his or her willingness to participate (or to let his/her child participate) in a particular trial. Informed consent must be obtained before any study procedures are performed. The process is documented by means of a written, signed, and dated ICF.

In accordance with GCP, prior to signing and dating the consent form, the subject (and subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) must be informed by appropriate study personnel about all aspects of the trial that are relevant to making the decision to participate, and must have sufficient time and opportunity to ask any questions.

If the subject and / or appropriate and legally acceptable representative is / are not able to read and sign the ICF, then it must be signed and dated by an impartial witness who is independent of the Investigator. A witness who signs and dates the consent form is certifying that the information in this form and any other written information had been accurately explained to and understood by the subject or his / her representative.

The actual ICF used at each center may differ, depending on local regulations and IEC / IRB requirements. However, all versions must contain the standard information found in the sample ICF provided by the Sponsor. Any change to the content of the ICF must be approved by the Sponsor and the IEC / IRB prior to the form being used.

If new information becomes available that may be relevant to the subject's (or subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) willingness to continue participation in the trial, this will be communicated to him / her in a timely manner. Such information will be provided via a revised ICF or an addendum to the original ICF.

ICFs will be provided in duplicate, or a photocopy of the signed consent will be made. One of them will be kept by the Investigator, and the other one will be kept by the subject (or subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years).

Documentation of the consent process should be recorded in the source documents.

5.2.3 Screening Criteria

There are no screening criteria other than the inclusion and exclusion criteria.

5.2.4 Inclusion Criteria

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

1) Has been identified as a potential subject by the Sponsor, and is included in the list provided to the Investigator (i.e., aged 9 to 45 years on the day of first injection of CYD dengue vaccine in CYD28, has received 3 doses of CYD dengue vaccine in the CYD28 trial, and has a PD3 serum sample available [at least 300 μL of serum]).

2) Presently in good health, based on medical history and physical examination.
3) Informed consent form (ICF) has been signed and dated by the subject (based on local regulations), and ICF has been signed and dated by the parent(s) or another legally acceptable representative (and by an independent witness if required by local regulations)
4) Subject and parent(s)/legally acceptable representative(s) able to attend all scheduled visits and to comply with all trial procedures

5.2.5 Exclusion Criteria

An individual fulfilling *any* of the following criteria is to be excluded from trial enrollment:

1) Subject who received any other dengue vaccination that was not part of CYD28
2) Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be pre-menarche or post-menopausal for at least 1 year, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the vaccination until at least 4 weeks after vaccination)
3) Participation at the time of study enrollment (or in the 4 weeks preceding the trial vaccination) or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure
4) Receipt of any vaccine in the 4 weeks preceding the trial vaccination or planned receipt of any vaccine in the 4 weeks following the trial vaccination
5) Receipt of immune globulins, blood or blood-derived products in the past 3 months
6) Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy, such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months)
7) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccines used in the trial or to a vaccine containing any of the same substances
8) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion
9) Receipt of blood or blood-derived products in the past 3 months, which might interfere with assessment of the immune response
10) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily
11) Current alcohol abuse or drug addiction
12) Moderate or severe acute illness/infection (according to investigator judgment) on the day of vaccination or febrile illness (temperature $\geq$ 38.0°C). A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided
13) Identified as an Investigator or employee of the Investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study

If the subject has a primary physician who is not the Investigator, the site should contact this physician with the subject's consent to inform him / her of the subject's participation in the study. In addition, the site should ask this primary physician to verify exclusion criteria relating to previous therapies, such as receipt of blood products or previous vaccines.

5.2.6 Medical History

Prior to enrollment, subjects will be assessed for pre-existing conditions and illnesses, both past and ongoing. Any such conditions will be documented in the source document. Significant medical history (reported as diagnosis) including conditions for which the subject is or has been followed by a physician or conditions that could resume during the course of the study or lead to an SAE or to a repetitive outpatient care will be collected in the CRF. The significant medical history section of the CRF contains a core list of body systems and disorders that could be used to prompt comprehensive reporting, as well as space for the reporting of specific conditions and illnesses.

For each condition, the data collected will be limited to:

- Diagnosis (this is preferable to reporting signs and symptoms)
- Presence or absence of the condition at enrollment

The reporting of signs and symptoms is strongly discouraged.

Dates, medications, and body systems are not to be recorded, and the information collected will not be coded. Its purpose is to assist in the later interpretation of safety data collected during the trial.

5.2.7 Contraindications for Subsequent Vaccinations

5.2.7.1 Temporary Contraindications

Should a subject experience one of the conditions listed below, the Investigator will postpone the vaccination until the condition is resolved.

- Febrile illness (temperature ≥ 38.0°C) or moderate or severe acute illness / infection on the day of vaccination, according to Investigator judgment

5.2.7.2 Definitive Contraindications

Not applicable since only one dose of vaccine will be administered in this trial.

5.2.8 Conditions for Withdrawal

Subjects (and subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) will be informed that they have the right to withdraw (or to withdraw their child) from the trial at any time. A subject may be withdrawn from the study:

- At the discretion of the Investigator or Sponsor due to safety concerns (withdrawal) without the subject's permission
- At the request of the subject (dropout)

The following will result in automatic withdrawal or exclusion of a subject from the study:

- Significant non-compliance with the protocol, based on the Investigator's judgment

The reason for a withdrawal or dropout should be clearly documented in the source documents and on the CRF.

The Investigator must determine whether voluntary withdrawal is due to safety concerns (in which case, the reason for discontinuation will be noted as "SAE" or "other AE" as appropriate) or for another reason.

Withdrawn subjects will not be replaced.

5.2.9 Lost to Follow-up Procedures

In the case of subjects who fail to return for a next visit, documented reasonable effort (i.e., documented telephone calls and certified mail) should be undertaken to locate or recall them, or at least to determine their health status while fully respecting their rights. These efforts should be documented in the CRF and in the source documents.

5.2.10 Classification of Subjects Who Discontinue the Trial

For any subject who discontinues the trial prior to completion, the most significant reason for early termination will be checked in the CRF. Reasons are listed below from the most significant to the least significant (refer to the CRF completion guidelines for additional details and examples):

- **SAE:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an SAE, as defined in Section 9.2.2.1.
- **Other AE:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an AE other than an SAE, as defined in Section 9.2.2.1.
- **Non-compliance with protocol:** To be used when the Investigator withdraws a subject from the study because of failure to follow the protocol, including when it is retrospectively discovered that a subject did not fulfill the eligibility criteria. The Investigator will provide a comment as to the specific cause of non-compliance.
- **Lost to follow-up:** To be used when the Investigator withdraws a subject from the study because of failure to establish contact, as outlined in Section 5.2.9. The Investigator will provide documentation that contact was attempted (i.e., return of unsigned certified letter receipt).
- **Voluntary withdrawal not due to an AE:** To be used when a subject drops out of the study for any reason other than those listed above.

5.2.11 Follow-up of Discontinuations

The site should complete all scheduled safety visits and contact any subject who has prematurely terminated the trial because of an SAE, other type of AE, non-compliance with the protocol, or loss of eligibility, including definite contraindications.

For subjects where the reason for early termination was lost to follow-up or if the subject withdrew informed consent and specified that they do not want to be contacted again and it is documented in the source document, the site will not attempt to obtain further safety information.

For subjects where the reason for early termination is voluntary withdrawal, the site will attempt to contact them in order to obtain further safety information, except if the subject specifies that he does not want to be contacted anymore and this will be documented in the source document.

5.2.12 Follow-up and Reporting of Pregnancies

Pregnancy is an exclusion criterion for enrollment in this study, but a subject could potentially become pregnant during her participation. In case of pregnancy after the CYD dengue booster or placebo injection, the subject will not be discontinued from the trial and will be followed for safety and immunogenicity assessment.

All pregnancy cases should be reported if they occurred during the study. To report the pregnancy case, the Investigator must fill out a Pregnancy Reporting Form in the electronic data capture (EDC) system and send it to the Sponsor within 1 month of identifying a pregnancy case.

Study staff must then maintain contact with the subject to obtain information about the outcome—i.e., details about the delivery and the newborn, or about pregnancy termination—and must update the electronic Pregnancy Reporting Form. This information should be provided to the Sponsor within 1 month of delivery. Additional follow-up visits may be performed according to the local regulations.

Pregnancy itself is not considered an AE, but any complications during pregnancy are to be considered as AEs, and in some cases could be considered SAEs. Spontaneous abortions, fetal death, stillbirth, and congenital anomalies reported in the baby are always considered as SAEs, and the information should be provided to the Global PharmacoVigilance (GPV) Department regardless of when the SAE occurs (e.g., even after the end of the trial).

5.3 Safety Emergency Call

If, as per the Investigator's judgment, a subject experiences a medical emergency, the Investigator may contact the Sponsor's RMO for advice on trial related medical question or problem. If the RMO is not available, then the Investigator may contact the Call Center - available 24 hours a day, 7 days a week - that will forward all safety emergency calls to the appropriate primary or back-up Sanofi Pasteur contact, as needed. The toll-free contact information for the Call Center is provided in the Operating Guidelines.

This process does not replace the need to report an SAE. The investigator is still required to follow the protocol defined process for reporting SAEs to GPV (Please refer to Section 10).

In case of emergency code-breaking, the Investigator is required to follow the code-breaking procedures described in Section 6.4.

5.4 Modification of the Trial and Protocol

Any amendments to this trial plan and protocol must be discussed with and approved by the Sponsor. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Sponsor, and the amended version of the protocol will replace the earlier version. All substantial amendments e.g., that affect the conduct of the trial or the safety of subjects, require IEC / IRB approval, and must also be forwarded to regulatory authorities.

An administrative / non-substantial amendment to a protocol is one that modifies some administrative or logistical aspect of the trial but does not affect its design or objectives or have an impact on the subjects' safety. The IECs / IRBs and regulatory authorities must be notified of administrative changes and will provide approval according to local regulations.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which IEC / IRB approval has already been given, are not initiated without IEC / IRB review and approval, except to eliminate apparent immediate hazards to subjects.

5.5 Interruption of the Trial

The trial may be discontinued if new data about the investigational product resulting from this or any other trials become available; or for administrative reasons; or on advice of the Sponsor, the Investigators, and / or the IECs / IRBs. If the trial is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the regulatory authorities, and the IECs / IRBs of the reason for termination or suspension, as specified by the applicable regulatory requirements.

The Investigator shall promptly inform the trial subjects and assure appropriate therapy and / or follow-up for them.

6 Vaccines Administered

6.1 Identity of the Investigational Product

6.1.1 Identity of Trial Product

Subjects included in the study will either receive one injection of the CYD dengue vaccine (booster dose after a 3-dose schedule) or a placebo. This trial will be using the 5-dose presentation of the CYD dengue vaccine. Only one dose will be used per multi-dose vial. The remaining 4 doses will be discarded as per Sponsor's Operating Guidelines.

Product:	CYD Dengue Vaccine
Vaccine:	Live, attenuated, tetravalent dengue virus vaccine
Presentation:	multi-dose (5 doses vial)
Form:	Powder and solvent for suspension for injection
Dose volume:	0.5 milliliters (mL) of the reconstituted vaccine

Route: Subcutaneous (SC) injection

Batch number: To be defined

6.1.1.1 Composition

Each 0.5 mL dose of reconstituted vaccine contains the following components:

- **Active Ingredients:** 4.5 – 6 $\log_{10}$ cell-culture infectious dose 50% ($CCID_{50}$) of each live, attenuated, recombinant dengue serotype 1, 2, 3, 4 virus
- **Excipients:** Essential amino acids, non-essential amino acids, L-arginine hydrochloride, sucrose, D-trehalose dihydrate, D-sorbitol, trometamol, urea, and sodium chloride.
- **Solvent:** NaCl 0.9%

6.1.1.2 Preparation and Administration

Sanofi Pasteur's CYD dengue vaccine consists of a powder and solvent for suspension for injection and must be stored between +2°C and +8°C.

The vaccine must be removed from the refrigerator, reconstituted with the solvent supplied for this purpose, and used immediately after reconstitution.

The vaccine is to be administered subcutaneously in the deltoid region of the upper arm in a volume of 0.5 mL. The remaining doses of the multi-dose presentation must be discarded according to Operating Guidelines.

Prior to administration, all study products must be inspected visually for cracks, broken seals, correct label content (see Section 6.3.1), and extraneous particulate matter and / or discoloration, whenever solution and container permit. If any of these conditions exists, the vaccine must not be administered. A replacement dose is to be used, and the event is to be reported to the Sponsor.

Subjects must be kept under observation for 30 minutes after vaccination to ensure their safety, and any reactions during this period will be documented in the CRF. Appropriate medical equipment and emergency medications, including epinephrine (1:1000), must be available on site in the event of an anaphylactic or other immediate allergic reaction.

If a vial or syringe is accidentally broken and the product spilled out, appropriate disinfection procedures must be used (please refer to the Operating Guidelines and/or trial center's procedures).

6.1.1.3 Dose Selection and Timing

Sanofi Pasteur's CYD dengue vaccine candidate has been evaluated for the prevention of dengue disease is for individuals 9 through 60 years of age, following a 0, 6 and 12 months vaccination schedule. Its 5 ± 1 $\log_{10}$ $CCID_{50}$ per serotype (5555) formulation reliably provided an immune response against all 4 serotypes after 3 injections in various populations, regardless of age, region, FV status at baseline, and was selected for further Phase II and Phase III studies.

The boosting of the humoral immune response with a CYD dengue vaccine dose, 5 years or more after the completion of a 3-dose vaccination schedule, is going to be assessed in this trial.

6.1.2 Identity of Control Product

6.1.2.1 Composition

Product:	Placebo
Form:	Solution
Vaccine:	NaCl 0.9%
Route:	Subcutaneous (SC) injection
Batch number:	To be defined

6.1.2.2 Preparation and Administration

The product must be stored between +2°C and +8°C.

The placebo should be allowed to reach room temperature before use. The placebo should not be used if particles are present in the solution. The placebo will be administered subcutaneously in the deltoid region of the upper arm in a volume of 0.5 mL (for more details please refer to the Operating Guidelines and/or trial center's procedures).

The procedures for preparing and administering the control product are the same as those described for the trial product in Section 6.1.1.2.

6.1.2.3 Dose Selection and Timing

Not applicable.

6.2 Identity of Other Product

Not applicable.

6.3 Product Logistics

6.3.1 Labeling and Packaging

CYD dengue vaccine and placebo will be supplied in vials/syringes and will be labeled and packaged according to national regulations. The information on the label will include at least:

- Study code
- Name of product and group assignment
- Dosage form and route of injection
- Investigational use only statement: For Clinical Trial Use Only
- Storage conditions

- Batch #
- Name of Sponsor
- Expiry date

All the products will be identified for group assignment by a dose number

6.3.2 Product Shipment, Storage, and Accountability

6.3.2.1 Product Shipment

The Sponsor's representative will contact the Investigator or a designee in order to determine the dates and times of delivery of products.

Each vaccine shipment will include a temperature-monitoring device to verify maintenance of the cold chain during transit. On delivery of the product to the site, the person in charge of product receipt will follow the instructions given in the Operating Guidelines, including checking that the cold chain was maintained during shipment (i.e., verification of the temperature recorders). If there is an indication that the cold chain was broken, this person should immediately quarantine the product, alert the Sanofi Pasteur representative, and request authorization from Sanofi Pasteur to use the product.

6.3.2.2 Product Storage

The Investigator will be personally responsible for product management or will designate a staff member to assume this responsibility.

At the site, products must be kept in a secure place with restricted access. Vaccines will be stored in a refrigerator at a temperature ranging from +2°C to +8°C. The vaccines must not be frozen and should be protected from light. The temperature must be monitored and documented (see the Operating Guidelines) for the entire time that the vaccine is at the trial site. In case of accidental freezing or disruption of the cold chain, vaccines must not be administered and must be quarantined, and the Investigator or authorized designee should contact the Sanofi Pasteur representative for further instructions.

6.3.2.3 Product Accountability

The person in charge (unblinded vaccination study staff) of product management at the site will maintain records of product delivery to the trial site, product inventory at the site, the dose given to each subject, and the disposal of or return to the Sponsor of unused doses. The necessary information on the product labels is to be entered into the source document and the CRF. If applicable, information may also be entered into the subject's vaccination card.

The Sponsor's monitoring staff will verify the trial site's product accountability records against the record of administered doses in the CRFs and the communication from the IVRS / IWRS (if applicable).

In case of any expected or potential shortage of product during the trial, the Investigator or an authorized designee should alert the Sanofi Pasteur representative as soon as possible, so that a shipment of extra doses can be arranged.

6.3.3 Replacement Doses

If a replacement dose is required (e.g., because the syringe broke or particulate matter was observed in the syringe), the site personnel must either contact the IVRS / IWRS to receive the new dose allocation, or follow the instructions given in the Operating Guidelines.

6.3.4 Disposal of Unused Products

Unused or wasted products will be returned at room temperature to the Sponsor's warehouse in Singapore in accordance with the instructions in the Operating Guidelines. Product accountability will be verified throughout the trial period.

6.3.5 Recall of Products

If the Sponsor makes a decision to launch a retrieval procedure, the Investigator(s) will be informed of what needs to be done.

6.4 Blinding and Code-breaking Procedures

An observer-blind procedure will be followed for the injection of CYD dengue vaccine or placebo. Neither the blind-observer Investigator nor the subjects (and/or subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) will know which product will be administered. The "vaccinator" will be in charge of preparing and administering the products and will not be authorized to collect any safety data. In addition, the "vaccinator" or authorized designee will have to ensure that the documents on randomization are stored in a secure place where only he/she has access.

The code may be broken by the Investigator only in the event of an SAE and if identification of the vaccine received could influence the treatment of the SAE. Code-breaking should be limited, as far as possible, to the subject(s) experiencing the SAE.

The blind can be broken by the Investigator or a sub-investigator (medical doctor only[a]), by calling the IVRS / IWRS system as explained in the code-breaking procedures described in the Operating Guidelines. Once the emergency has been addressed by the site, the Investigator must notify the Sanofi Pasteur Responsible Medical Officer if a subject's code was broken. All contact attempts with the Sponsor prior to unblinding are to be documented in the source documents.

A request for the code to be broken may be made:

- by GPV department for reporting to Health authorities in the case of an SAE as described in ICH E2A. In this case, the code will be broken only for the subject(s) in question. The

[a] according to local regulations

information resulting from code-breaking (i.e., the subject's vaccine or group assignment) will not be communicated to either the Investigator or the immediate team working on the study, except for the GPV representative.

- by the IDMC if needed to facilitate their assessment of safety of VCD.

The IEC / IRB must be notified of the code-breaking. All documentation pertaining to the event must be retained in the site's study records and in the Sanofi Pasteur files. Any intentional or unintentional code-breaking must be reported, documented, and explained, and the name of the person who requested it must be provided to the Sponsor.

Two planned analyses will be performed on data collected up to 28 days post-injection and up to 1 year post-injection, respectively. These analyses will require the unblinding of data. A specific process will be implemented to maintain the blind at both subject and Investigator levels.

A third and final analysis will be performed at the end of the trial.

Testing performed within GCI and GCI outsourced laboratories are blinded with respect to study treatment group assignment. The code(s) linking information on sample vials to study treatment group assignment are retained by the Clinical Department and cannot be accessed by GCI or contract laboratory testing personnel.

6.5 Randomization and Allocation Procedures

Each subject who meets the inclusion/exclusion criteria and signs (along with the subject's parent(s) / legally acceptable representative(s) for subjects aged < 21 years) the ICF will be randomly assigned to one of the 2 groups via an IVRS/IWRS, according to a 3:1 ratio (3 subjects included in the CYD dengue vaccine group for 1 subject included in the control group).

Site staff will call or connect to the IVRS/IWRS, enter identification and security information, and confirm a minimal amount of data in response to IVRS/IWRS prompts. The IVRS/IWRS will then state the vaccine assignment (code number). Subject numbers will be recorded on the CRFs and will not be reassigned for any reason. The full detailed procedures for randomization are described in the Operating Guidelines.

The first 60 randomized subjects from two specific sites (30 subjects per site) will be selected for the AIT subset.

Subject numbers will be 8 digits long, with a 3-digit center identifier and a 5-digit subject identifier. The first digit of the subject identifier will be a pre-defined figure (0 or 1); "1" will be used for subjects assigned to AIT subset and "0" for all other subjects. The second digit of the subject identifier will be a pre-defined figure (0 or 1); "1" will be used for subjects aged 18 to 45 years and "0" for subjects aged 9 to 17 years. For example, subject 001-11001 will be the first randomized to the AIT subject with age of 18 to 45 years from center 1.

Subject numbers used in CYD28 will be captured on the CRFs.

Randomization will be performed with permuted block method with stratification by site and age group.

A double randomization system will be used, this implies that the subject treatment allocation will be separated from doses dispensing. Each dose will have both a code number and a dose number. The code number will be used by the IVRS/IWRS while the dose number will be entered in the CRF. The unique dose numbers will be defined according to a random list to ensure that dose numbers cannot be used to distinguish between treatment groups.

Subject numbers should not be reassigned for any reason. The Clinical and Medical Quality Operations department at Sanofi Pasteur will hold the randomization codes in a secured location.

6.6 Treatment Compliance

The following measures will ensure that the vaccine doses administered comply with those planned, and that any non-compliance is documented so that it can be accounted for in the data analyses:

- All vaccinations will be administered by qualified trial personnel
- The person in charge of product management at the site will maintain accountability records of product delivery to the trial site, product inventory at the site, dose given to each subject, and the disposal of unused or wasted doses

6.7 Concomitant Medications and Other Therapies

At the time of enrollment, ongoing medications including other therapies e.g., blood products, should be recorded in the source document as well as new medications prescribed for new medical conditions / AEs during trial participation.

Documentation in the CRF of concomitant medication will be limited to specific categories of medication of interest beginning on the day of vaccination. This may include medications of interest that were started prior to the day of vaccination.

Reportable medications will be collected in the CRF from the day of vaccination to the end of the solicited and unsolicited follow-up period (e.g., 28 day safety follow-up) as they may impact the response to the vaccination and impact the consistency of the information collected on concomitant medications at any vaccination.

The “reportable” medications are distributed according to two categories. These are:

- Category 1 antipyretics, analgesics, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and other immune modulators.

Note: inhaled and topical steroids should not be captured.

- Category 2:
  - Any vaccine other than the trial vaccine in the 4 weeks before and after trial vaccination.
  - Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy or long-term systemic corticosteroids (for more than 2 consecutive weeks) in the 4 weeks after trial vaccination. Inhaled and topical steroids should not be captured.
  - Blood or blood-derived products in the 4 weeks before and after trial vaccination.

The information reported in the CRF for each reported medication will be limited to:

- Trade name
- Given as treatment or as prophylaxis
- Medication category
- Start and stop dates

Dosage and administration route will not be recorded. Homeopathic medication will not be recorded. Topical treatment will not be recorded.

The fact that a medication was given in response to an AE will be captured in the "Action Taken" column of the AE only. No details will be recorded in the concomitant medication module of the CRF unless the medication received belongs to one of the prelisted categories. Medications will not be coded.

7 Management of Samples

7.1 Sample Collection

Blood samples for the assessment of neutralizing Ab responses and / or Ab specificity and affinity maturation as well as CMI will be collected at several time points throughout the study. Subjects not included in the AIT subset will have 5 blood sampling and subjects included in the AIT subset will have 7 blood samples. See the Table of Study Procedures and Section 5.1.3 for details of the sampling schedule.

Immediately prior to drawing blood, the person in charge of the procedure will verify the subject's identity. Each tube of blood will be clearly labeled with subject identification number and sampling stage using a self-adhesive label that will be stuck onto the tube immediately before blood sampling.

7.1.1 Blood Sample for Neutralizing Antibodies, Antibody Specificity and Affinity Maturation Assessment

At Visit 01, Visit 04, Visit 05, Visit 06 and Visit 07 for all subjects (and Visit 02 and 03 for subjects in the AIT subset), 5 mL of blood will be collected in tubes provided by or recommended by the Sponsor. Immediately prior to the blood draw, the staff member performing the procedure will verify the subject's identity; will write the assigned subject's number on the pre-printed label that contains that subject's number and the sampling stage; and will attach the label to the tube. Blood is to be taken from the limb opposite to the one that will be used for vaccination. For subjects belonging in the AIT subset, these blood samples will also be used to assess Ab specificity and affinity maturation (Table 7.1).

Table 7.1: Blood sampling volume (mL) per visit for neutralizing Ab, Ab specificity and affinity maturation assays

Visit Number (V)	V01	V02	V03	V04	V05	V06	V07
Trial Timelines (Days/Months/Years)	D0	D 07	D 14	D 28	M 06	Y 01	Y 02
Time Windows (Days)		+2	+7	+7	+20	±30	±30
Volume for each assessment (mL) – subjects in AIT subset only							
Dengue Neutralizing Abs	2	2	2	2	2	2	2
Ab specificity and affinity maturation	1*	1†	1†	1*	0	0	0
Serum bank	2	2	2	2	3	3	3
Total	**5**	**5**	**5**	**5**	**5**	**5**	**5**
Volume for each assessment (mL) – all other subjects							
Dengue Neutralizing Abs	2	0	0	2	2	2	2
Serum bank	3	0	0	3	3	3	3
Total	**5**	**0**	**0**	**5**	**5**	**5**	**5**

* Samples will be assessed for both Ab specificity and affinity maturation at D0 and D28.

† Samples will be used only for assessment of Ab specificity at D7 and D14 if necessary; no assessment of affinity maturation will be performed at D7 or D14.

7.1.2 Blood for Cellular Immunity Assessment

In addition to the above, blood samples for cellular immunity assessment will be collected at Visit 01, Visit 02, Visit 03, Visit 04 and Visit 06. Between 15 and 35 mL (depending on both the study visit, and the subject's age; see Table 5.1) of blood will be collected in heparinized tubes and will then be processed for cell isolation and freezing. Labeling procedures will be done the same way as for serum samples.

7.1.3 Blood for Virological Confirmation of Suspected Hospitalized Dengue Disease and Assessment of Disease Severity

In case of hospitalized suspected dengue disease, one 3 mL acute blood sample will be collected (within the 5 days after the fever onset). The acute blood sample for all suspected hospitalized dengue cases should be collected within the pre-specified timeframe as described above. If this cannot be accomplished, this sample should still be obtained as soon as possible thereafter, for IDMC severity assessment. This blood sample will be used to confirm dengue disease, and upon confirmation of infection to identify dengue virus serotype.

Additionally, and for all hospitalized suspected dengue cases, the Investigator must ensure that key biological parameters (hematocrit, platelet count, aspartate aminotransferase [AST], and alanine transaminase [ALT]) have been checked or are planned to be checked as part of local standard of care at the hospital (ideally within the 5 days after the fever onset). If these parameters

have not been measured, additional blood specimens will be taken[a]. The aim of these tests is the assessment of severity according to the WHO/IDMC classification.

Table 7.2 presents the additional serum aliquots in the event of a suspected hospitalized dengue disease at any time during the trial.

Table 7.2: Blood Sampling Volume (mL) for Suspected Hospitalized Dengue Case

	Blood volume (mL)
GCI (USA) or GCI outsourced laboratory	
Dengue Screen RT-PCR & Simplexa™ dengue RT-PCR	1
Serum bank	1
Dengue NS1 Ag ELISA	1
Local laboratory (if needed)	*x*
TOTAL	**3** + *x*

More detailed instructions are provided in the Operating Guidelines.

7.2 Sample Preparation

7.2.1 Blood Sample for Neutralizing Antibodies, Antibody Specificity and Affinity Maturation, and Virological Confirmation of Suspected Hospitalized Dengue Disease

Detailed instructions on how to prepare blood samples for assessment of Ab response are contained in the Operating Guidelines provided to the site. An overview of the procedures is provided here.

Following the blood draw, the sampling tube should be stored at room temperature for a minimum of 60 minutes and a maximum of 2 hours to allow the blood to clot before centrifugation. The tube must be stored vertically and will not be shaken.

Beyond 2 hours, the sampling tube must be refrigerated at a temperature of 2°C to 8°C and must be centrifuged within a maximum of 24 hours.

After being allowed to clot for a minimum of 60 minutes to a maximum of 2 hours at room temperature, blood samples for serum Ab response and viremia assessment will be centrifuged before being divided into appropriate aliquots of serum. Samples will then be handled one subject at a time to avoid a mix-up of subjects' blood tubes. Serum will be transferred to the appropriate number of tubes, pre-labeled with adhesive labels that clearly identify the subject's number and sampling stage or visit number.

[a] The volume of blood samples will depend on local laboratory needs.

The subject's identification number, the date of sampling, the number of aliquots obtained, and the date and time of preparation are to be specified on a sample identification list and recorded in the source document. Space is provided on this list for comments on the quality of samples.

Serum will be aliquoted and frozen as specified in the Operating Guidelines.

7.2.2 Blood Sample for Cellular Immunity Assessment

Details on cellular immunity assessment purification will be provided in the Operating Guidelines. An overview of the procedures is provided here:

Heparinized blood will be added to LeucoSep tubes readied with a lymphocytes separation medium. Tubes will then be centrifugated at room temperature. Mononuclear cells will be collected, washed at room temperature, and resuspended in complete medium.

Cell pellets will then be resuspended in Fetal Bovine Serum at an appropriate concentration and distributed into 500 µL aliquots in Cryostat Nunc tubes on ice. Freezing medium will be added slowly down the side of the tube to avoid shocking cells and the solution will be mixed slowly with a pipette. Tubes will then be transferred in a cold freezing container and placed at -80°C for at least 16 hours before being moved to liquid nitrogen tanks.

7.3 Sample Storage and Shipment

7.3.1 Blood Sample for Neutralizing Antibodies, Antibody Specificity and Affinity Maturation, and Virological Confirmation of Suspected Hospitalized Dengue Disease Assessment

During storage, serum tubes are to be kept in a freezer whose temperature is set and maintained at either -70°C or below (for wild-type dengue viremia samples) or at -20°C or below (for neutralizing Abs, antibody specificity and affinity maturation samples). The temperature will be monitored and documented on the appropriate form during the entire trial. If it rises above -10°C (for -20°C freezers) or -40°C (for -70°C freezers) for any period of time, the Clinical Logistics Coordinator must be notified. See the Operating Guidelines for further details.

Shipments to the laboratories will be made only after appropriate monitoring, and following notification of the Clinical Logistics Coordinator. Sera will be shipped frozen, using dry ice to maintain them in a frozen state, in the packaging container provided by the carrier. Again, temperatures will be monitored. Shipments must be compliant with the International Air Transport Association (IATA) 602 regulations.

Samples for PRNT testing and virological confirmation of hospitalized dengue disease will first be shipped to GCI at Sanofi Pasteur. The address is provided in the Operating Guidelines.

From GCI, samples to be assessed for Ab specificity and affinity maturation will be sent to Sanofi Pasteur VaxDesign. Serum tubes will be stored, packed and shipped according to the processes outlined above. Samples will be shipped to the following address:

Sanofi Pasteur
VaxDesign Campus
2501 Discovery Dr., Suite 300
Orlando, FL 32826 – USA

7.3.2 Sample for Cellular Immunity Assessment

Tubes will be kept in liquid nitrogen tanks. The tanks will be filled at least once per week, and the temperature and liquid nitrogen level will be monitored and documented on the appropriate form during the entire trial. If it rises above a certain temperature defined in the Operating guidelines for any period of time, the Clinical Logistics Coordinator must be notified. See the Operating Guidelines for further details. The samples will be shipped in special nitrogen containers provided by the Sponsor to the following address:

Sanofi Pasteur
[redacted].

The shipment will be organized in accordance with the requirements applicable for the air transport of infectious substances (IATA 6.2 regulations).

7.4 Future Use of Stored Serum Samples for Research

Any unused part of the serum samples will be securely stored at the Sanofi Pasteur serology laboratory (GCI) for at least 5 years after the last license approval in the relevant market areas has been obtained for the vaccine being tested.

Subjects (and subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) will be asked to indicate in the ICF whether they will permit the future use of any unused stored serum samples for other tests. If they refuse permission, the samples will not be used for any testing other than that directly related to this study. If they agree to this use, they will not be paid for giving permission (Anonymity of samples will be ensured). The aim of any possible future research is unknown today, and may not be related to this particular study. It may be to improve the knowledge of vaccines or infectious diseases, or to improve laboratory methods. Genetic tests will never be performed on these samples without individual informed consent.

8 Clinical Supplies

Sanofi Pasteur will supply the trial sites with protocols, ICFs, CRFs, SAE reporting forms, diary cards and pregnancy forms, DCs, MAs, and other trial documents, as well as with the following trial materials: all study vaccines and injection materials, blood collection tubes, cryotubes, cryotube storage boxes, cryotube labels, temperature recorders, shipping containers, rulers, and digital thermometers.

The means for performing EDC will be defined by Sanofi Pasteur. If a computer is provided by Sanofi Pasteur, it will be retrieved at the end of the trial.

The Investigator will supply biohazard and/or safety supplies, including examination gloves, laboratory coats, sharps disposal containers, and absorbent countertop paper. The site will ensure that all biohazard wastes are autoclaved and disposed of in accordance with local practices. The Investigator will also supply appropriate space in a temperature-monitored refrigerator for the storage of the products and for the blood samples, and appropriate space in a temperature-monitored freezer for serum aliquots.

In the event that additional supplies are required, study staff must contact Sanofi Pasteur, indicating the quantity required. Contact information is provided in the Operating Guidelines. They must allow approximately one week for an order to be filled and to have the supplies sent to their site.

9 Endpoints and Assessment Methods

9.1 Primary Endpoints and Assessment Methods

9.1.1 Immunogenicity

9.1.1.1 Immunogenicity Endpoints

The primary endpoint for the evaluation of immunogenicity is:

Neutralizing Ab levels against each dengue virus serotype measured 28 days after the third CYD dengue vaccine injection and 28 days after the booster injection in Group 1 using dengue PRNT.

9.1.1.2 Immunogenicity Assessment Methods

Dengue Neutralizing Abs

Dengue neutralizing Ab levels will be measured by PRNT (using parental dengue virus strains of CYD dengue vaccine constructs) by Sanofi Pasteur GCI, Swiftwater, USA (or outsourced with a GCI selected external laboratory).

Serial, 2-fold dilutions of serum to be tested (previously heat-inactivated) are mixed with a constant challenge-dose of each dengue virus serotype 1, 2, 3 or 4 (expressed as plaque-forming unit [PFU]/mL). The mixtures are inoculated into wells of a microplate with confluent Vero cell monolayers. After adsorption, cell monolayers are incubated for a few days. The presence of dengue virus infected cells is indicated by formation of plaques. A reduction in virus infectivity due to neutralization by Ab present in serum samples is detected. The reported value (end point neutralization titer) represents the highest dilution of serum at which ≥ 50% of dengue challenge virus (in plaque counts) is neutralized when compared to the mean viral plaque count in the negative control wells which represents the 100% virus load. The end point neutralization titers are presented as discontinuous values. The lower limit of quantitation (LLOQ) of the assay is 10 (1/ dil).

This assay will be performed on blood samples that had been taken at V06 in CYD28 trial (i.e., 28 days PD3), and on samples taken in CYD63 at V01, and V04 to V07 for all subjects, and additionally at V02 and V03 for subjects from the AIT subset.

In addition to the PRNT, additional assays and other analyses might be used to assess and characterize the dengue immune response.

9.1.2 Safety

There are no primary objectives for safety.

9.1.3 Efficacy

No clinical efficacy data will be obtained in the trial.

9.2 Secondary Endpoints and Assessment Methods

9.2.1 Immunogenicity

9.2.1.1 Immunogenicity Endpoints

The secondary endpoints for the evaluation of immunogenicity are:

1) Neutralizing Ab levels against each of the four parental dengue virus strains of CYD dengue vaccine as determined by PRNT measured 28 days after the third CYD dengue vaccine injection received in CYD28 trial and 28 days post-booster injection (subjects from Group 1 only).
2) Neutralizing Ab levels against each of the four parental dengue virus strains of the CYD dengue vaccine as determined by PRNT immediately prior and 28 days post-booster or placebo injection.
3) Individual post-booster/pre-Booster GMTRs for each of the four parental dengue virus strains of the CYD dengue vaccine as determined by PRNT immediately prior and 28 days post-booster or placebo injection.
4) Seroconversion rates 28 days after the booster injection for each of the four parental dengue virus strain of CYD dengue vaccine; percentages of subjects with either a pre-booster titer $<$ 10 (1/dil) and a post-booster dose titer $\geq$ 40 (1/dil), or a pre-booster titer $\geq$ 10 (1/dil) and a $\geq$ 4-fold increase in post-booster dose titer as determined by PRNT immediately prior and 28 days post- injection.
5) Neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT at 28 days after the third CYD dengue vaccine injection received in CYD28 trial and immediately prior to booster or placebo injection in all study subjects.
6) Neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT at 6 months, 1 year and 2 years post booster or placebo injection in all study subjects.

9.2.1.2 Immunogenicity Assessment Methods

The immunogenicity assessment methods for the secondary endpoints are the same as those presented in Section 9.1.1.2.

For each particular immunogenicity endpoints, the essay will be performed on the blood samples taken at:

Endpoint 1	28 days post-Dose 3 in CYD28 and 28 days post-booster dose in CYD63 (V06 in CYD28 and V04 in CYD63)
Endpoints 2 to 4	Immediately prior to booster injection and 28 days post-injection in CYD63 (V01 and V04 in CYD63 study)
Endpoint 5	28 days post-Dose 3 in CYD28 and immediately prior to injection in CYD63 (V06 in CYD28 and V01 in CYD63)
Endpoint 6	6 months, 1 and 2 years post-injection in CYD63 (V05, V06 and V07 in CYD63)

9.2.2 Safety

9.2.2.1 Safety Definitions

The following definitions are taken from the ICH E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

Adverse Event (AE):

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Therefore an AE may be:

- A new illness
- The worsening of a concomitant illness
- An effect of the vaccination, including the comparator
- A combination of the above

All AEs include serious and non-serious AEs.

Surgical procedures are not AEs; they are the action taken to treat a medical condition. It is the condition leading to the action taken that is the AE (if it occurs during the trial period).

Pre-existing medical conditions are not to be reported as AEs. However, if a pre-existing condition worsens in frequency or intensity, or if in the assessment of the treating physician there is a change in its clinical significance, this change should be reported as an AE (exacerbation). This applies equally to recurring episodes of pre-existing conditions (e.g., asthma) if the frequency or intensity increases post-vaccination.

Serious Adverse Event (SAE):

Serious and *severe* are not synonymous. The term *severe* is often used to describe the intensity of a specific event as corresponding to Grade 3. This is not the same as *serious* which is based on patient / event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations.

An SAE is any untoward medical occurrence that at any dose

- Results in death
- Is life-threatening[a]
- Requires inpatient hospitalization or prolongation of existing hospitalization[b]
- Results in persistent or significant disability / incapacity[c]
- Is a congenital anomaly / birth defect
- Is an important medical event[d]

Additionally, the following important medical events are to be considered as SAEs and reported to the Sponsor according to the procedure described in Section 10.1.

- (Serious and Non-Serious) Adverse Events of Special Interest (AESIs)

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[a] The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

[b] All medical events leading to hospitalizations will be recorded and reported as Serious Adverse Events, with the exception of: hospitalization planned before inclusion into the study or out-patient treatment with no hospitalization.

[c] "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[d] Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the health of the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse, new onset diabetes, or autoimmune disease.

Adverse Reaction (AR):

All noxious and unintended responses to a medicinal product related to any dose should be considered AR.

(The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility)

Unexpected Adverse Reaction (UAR):

An UAR is an AR, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved investigational medicinal product).

The following additional definitions are used by Sanofi Pasteur:

Solicited Reaction:

A solicited reaction is an event that is prelisted in the CRF**.** The assessment of these AEs post-vaccination is mandatory. A solicited reaction is defined by a combination of:

- Symptom and
- Onset post-vaccination

e.g., injection site pain between D0 and D7 post-vaccination, or headache between D0 and D7.

A solicited reaction is therefore an AR observed and reported under the conditions (symptom and onset) prelisted (i.e., solicited) in the CRF and considered as related to vaccination.

Unsolicited AE / AR:

An unsolicited AE is an observed AE that does not fulfill the conditions prelisted in the CRF in terms of diagnosis and / or onset post-vaccination, i.e., excluding solicited reactions, e.g., if headache between D0 and D14 is a solicited reaction (i.e., prelisted in the CRF), then a headache starting on D14 is a solicited reaction, whereas headache starting on D15 post-vaccination is an unsolicited AE.

An unsolicited non-serious AE is an unsolicited AE excluding SAEs.

Injection Site Reaction:

An injection site reaction[a] is an AR at and around the injection site. Injection site reactions are commonly inflammatory reactions.

Systemic AE:

Systemic AEs are all AEs that are not injection site reactions. They therefore include systemic manifestations such as headache, fever, as well as localized or topical manifestations that are not

[a] All injection site AEs are considered to be related to vaccination and are therefore all *injection site reactions*.

associated with the vaccination site, e.g., erythema that is localized but that is not at the injection site.

Adverse Events of Special Interest (AESIs):

AEs of special interest are AEs that are considered by the Sponsor to be relevant for the monitoring of the safety profile of the investigational vaccine.

9.2.2.2 Safety Endpoints

The secondary endpoints for the evaluation of safety:

1) Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, action taken, whether it leads to discontinuation or not, and relationship to vaccination of any AEs reported in the 30 minutes after vaccination.
2) Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited (pre-listed in the subject's DC and CRF) injection site reactions (pain, erythema, and swelling) occurring up to 7 days after vaccination.
3) Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited systemic (DC and CRF) reactions (fever, headache, malaise, myalgia, and asthenia) occurring up to 14 days after vaccination.
4) Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, whether it leads to discontinuation or not, action taken and relationship to vaccination (for systemic AEs only) of unsolicited spontaneously reported AEs up to 28 days after vaccination.
5) Occurrence of SAEs, including serious AESIs (with specific time window according to the nature of event), throughout the trial.
6) 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 vaccination.
7) Occurrence of hospitalized virologically-confirmed dengue cases throughout the trial (i.e., from D0 through end of the study).

9.2.2.3 Safety Assessment Methods

At Visit 01, Visit 04, Visit 05, Visit 06 and Visit 07 (and also Visit 02 and Visit 03 for subjects from the AIT subset), the Investigator or an observer-blind delegate will perform a clinical or medically-driven physical examination, and will ask the subjects (or subjects' parent(s) / legally acceptable representative(s)) about any solicited reactions and unsolicited AEs recorded in the DC or MA, as well as about any other AEs that may have occurred since the previous visit. All relevant data will be transcribed into the CRF according to the instructions provided by the Sponsor.

9.2.2.3.1 Immediate Post-vaccination Surveillance Period

Subjects will be kept under observation for 30 minutes after vaccination to ensure their safety. The post-vaccination surveillance should be documented in the source document. Any AE that occurs during this period will be noted on the source document and recorded in the CRF, as follows:

- Any unsolicited systemic AE occurring during the first 30 minutes post-vaccination will be recorded on the CRF as immediate unsolicited systemic AE.
- Solicited and unsolicited injection site reactions and solicited systemic reactions will be recorded and analyzed as starting on the day of vaccination.
- Any SAE occurred during the first 30 minutes post-vaccination will be reported in the same way as any other SAE and to the Sponsor, according to the procedures described in Section 10.1.

9.2.2.3.2 Reactogenicity (Solicited Reactions From Day 0 to Day 7/Day 14 After Vaccination)

After vaccination, subjects (or subjects' parent / legally acceptable representative for subjects aged < 21 years) will be provided with a safety DC, a MA, a digital thermometer, and a flexible ruler, and will be instructed how to use them. The following items will be recorded by the subjects (or subjects' parent / legally acceptable representative for subjects aged < 21 years) in the DC on the day of vaccination and for the next 14 days (i.e., D0 to D14) until resolution:

- Daily temperature, with the route by which it was taken
- Daily measurement or intensity grade of all other solicited injection site and systemic reactions
- Action taken for each event, if any (e.g., medication)

The action taken by the subject (or subjects' parent / legally acceptable representative for subjects aged < 21 years) to treat any solicited reactions will be classified in the CRF using the following scale:

0: None

1: Medication (self-medication with an existing prescription or over-the-counter medication)

2: Health care provider contact (no new medication prescribed)

3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)

4: Hospitalization (inpatient)

Subjects (or subjects' parent(s) / legally acceptable representative(s) for subjects aged < 21 years) will be contacted by telephone 2, 4, 8, 10, 14, 16, 18, 20 and 22 months after the vaccination to remind them to record all safety information in the DC.

If the timing of the telephone call should fall on a weekend or a holiday, the call should be made on the next business day. If contact is not made on the designated day, study staff will continue

calling until contact is made. Every telephone attempt and its outcome will be documented in the source document.

Table 9.1 and Table 9.2 present, respectively, the injection site reactions and systemic reactions that are prelisted in the diary cards and CRF, together with the intensity scales.

Table 9.1: Solicited injection site reactions: terminology, definitions, and intensity scales for adolescents and adults (aged 12 >= years)

CRF term (MedDRA lowest level term [LLT])	Injection site pain	Injection site erythema	Injection site swelling
Diary card term	Pain	Redness	Swelling
Definition		Presence of a redness including the approximate point of needle entry	Swelling at or near the injection site Swelling or edema is caused by a fluid infiltration in tissue or cavity and, depending on the space available for the fluid to disperse, swelling may be either soft (typically) or firm (less typical) to touch and thus can be best described by looking at the size of the swelling
Intensity scale*	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm

* For the subjective reaction of pain, subjects (or subjects' parent(s) / legally acceptable representative(s)) will record the intensity level (Grade 1, 2, or 3) in the DC. For the measurable reactions of redness and swelling, they will record just the size of the reaction, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis

Table 9.2: Solicited systemic reactions: terminology, definitions, and intensity scales for adolescents and adults (aged 12 >= years)

CRF term (MedDRA lowest level term [LLT])	Fever	Headache	Malaise	Myalgia	Asthenia
Diary card term	Temperature	Headache	Feeling unwell	Muscle aches and pains	Weakness
Definition	Elevation of temperature to ≥°38.0°C (≥ 100.4°F)	Pain or discomfort in the head or scalp. Does not include migraine.	General ill feeling. Malaise is a generalized feeling of discomfort, illness, or lack of well-being that can be associated with a disease state. It can be accompanied by a sensation of exhaustion or inadequate energy to accomplish usual activities.	Muscle aches and pains are common and can involve more than one muscle at the same time. Muscle pain can also involve the soft tissues that surround muscles. These structures, which are often referred to as connective tissues, include ligaments, tendons, and fascia (thick bands of tendons). Does not apply to muscle pain at the injection site which should be reported as injection site pain.	Generalized weakness
Intensity scale*	Grade 1: ≥ 38.0°C to ≤ 38.4°C, Grade 2: ≥ 38.5°C to ≤ 38.9°C, Grade 3: ≥ 39.0°C	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: No interference with activity Grade 2: Some interference with activity Grade 3: Significant; prevents daily activity	Grade 1: No interference with activity. Grade 2: Some interference with activity. Grade 3: Significant; prevents daily activity

* For all reactions but fever, subjects (or subjects' parent(s) / legally acceptable representative(s)) will record the intensity level (Grade 1, 2, or 3) in the DC. For fever, they will record the body temperature, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis.

Important notes for the accurate assessment of temperature:

Subjects (or subjects' parent(s) / legally acceptable representative(s)) are to measure body temperature once per day, preferably always at the same time. The optimal time for measurement is the evening, when body temperature is the highest. Temperature is also to be measured at the time of any apparent fever. The observed daily temperature and the route of measurement are to be recorded in the DC, and the highest temperature will be recorded by the site in the CRF. The preferred route for this trial is axillary. Pre-vaccination temperature is also systematically collected by the investigator on the source document. Tympanic thermometers must not be used.

9.2.2.3.3 Unsolicited Non-serious Adverse Events From Day 0 to Day 28 After Vaccination

In addition to recording solicited reactions, subjects (or subjects' parent(s) / legally acceptable representative(s)) will be instructed to record any other medical events that may occur during the 28-day period after vaccination. Space will be provided in the DC for this purpose.

For each unsolicited non-serious AE, the following information is to be recorded:

- Start and stop dates[a]
- Intensity of the event:
  - For measurable unsolicited non-serious AEs that are part of the list of solicited reactions, the size of the AE as well as the temperature for fever will be collected and analyzed based on the corresponding scale used for solicited reactions (see Table 9.1 and Table 9.2)
  - Other unsolicited non-serious AEs will be classified according to the following intensity scale:
    - Grade 1: No interference with activity
    - Grade 2: Some interference with activity
    - Grade 3: Significant; prevents daily activity
- Action taken for each AE, if any (e.g., medication)

The action taken by the subjects (or subjects' parent(s) / legally acceptable representative(s)) to treat any **unsolicited AEs** will be classified in the CRF using the following scale:

0: None

1: Medication (self-medication with an existing prescription or over-the-counter medication)

2: Health care provider contact (no new medication prescribed)

[a] The stop date of all related AEs will be actively solicited. For other events, the investigator will provide the stop date when it becomes available. AEs for which no stop date was obtained during the course of the trial will be considered as ongoing at the end of the trial.

3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)

- Whether the AE led to discontinuation
- Whether the AE was related to vaccination (for unsolicited systemic AEs). See Section 9.2.2.3.6 for the assessment of causality.

9.2.2.3.4 Serious Adverse Events

Information on SAEs will be collected and assessed throughout the trial, from inclusion until 2 years after vaccination.

Any SAE occurring at any time during the trial will be reported by the Investigator through the EDC system and according to the completion guidelines provided by the Sponsor. All information concerning the SAE is to be reported, either as part of the initial reporting or during follow-up reporting if relevant information became available later (e.g., outcome, medical history, results of investigations, copy of hospitalization reports). The Investigator will assess the causal relationship between the SAE and the investigational product as either "Not related" or "Related", as described in Section 10.4.

See for further details on SAE reporting.

9.2.2.3.5 Adverse Events of Special Interest

Serious AESIs

The following serious AESIs will be considered:

- Serious hypersensitivity/allergic reactions occurring in all subjects within 7 days after vaccination
- Serious viscerotropic disease occurring in all subjects within 30 days after vaccination
- Serious neurotropic disease occurring in all subjects within 30 days after vaccination
- Serious dengue disease requiring hospitalization[a] occurring in all subjects at any time during the study

Specific guidelines are provided to the Investigator to help in the assessment of AEs that may be indicative of viscerotropic or neurotropic disease (see Guidelines for Assessing Viscerotropic and Neurotropic AE).

Non-Serious AESIs

The following non-serious AESI will be considered:

[a] A hospitalized subject is any subject admitted to hospital with bed attribution or any healthcare institution and requiring in-patient care.

- Hypersensitivity/allergic reactions occurring in all subjects within 7 days after vaccination.

9.2.2.3.6 Assessment of Causality

The Investigator will assess the ***causal relationship*** between each unsolicited systemic AE and vaccination as either not related or related, based on the following definitions[a]:

0: Not related – The AE is clearly / most probably caused by other etiologies such as subject's underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the AE is incompatible with a causal relationship; or the AE started before the vaccination (screening phase, if applicable)

1: Related – There is a "reasonable possibility" that the AE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship

Note: By convention, all injection site AEs (solicited and unsolicited) and all solicited systemic reactions are considered to be related to vaccination and referred to as reactions, and therefore do not require the Investigator's opinion on relatedness.

AEs likely to be related to the product, whether serious or not, that persist at the end of the trial will be followed up by the Investigator until their complete disappearance or the stabilization of the subject's condition. The Investigator will inform the Sponsor of the date of final disappearance of the event.

9.2.2.4 Methods for Virological Confirmation of Suspected Dengue Disease and Assessment of Disease Severity

In the event of a suspected hospitalized dengue case, the following tests will be performed based on the process described in below.

Dengue Screen RT-PCR

Dengue screen RT-PCR test will be performed by Sanofi Pasteur GCI, Swiftwater, USA or GCI designated laboratory.

Assessment and quantitation of dengue viremia is determined by testing serum samples with a nucleic-acid based assay. RNA is extracted from the serum to discard potential Taq polymerase inhibitors or interfering factors, using a commercial kit. Then, a RT-PCR is carried out with primers from a gene sequence conserved among dengue viruses. Due to a virus standard included in each run, results can be expressed as a concentration of log10 plaque forming unit (PFU)/mL.

Simplexa™ Dengue RT-PCR

Serotype identification of post-infectious dengue viremia is determined by testing serum samples with a nucleic-acid based assay. Briefly, RNA is extracted from the serum to discard potential polymerase inhibitors or interfering factors, using a commercial kit. Then the Simplexa™ dengue RT-PCR assay is carried out which incorporates serotype-specific primers from dengue

[a] ICH Guidelines, Clinical Safety Data Management E2A

sequences. The results are expressed qualitatively and reported for each dengue serotype as detected or not detected.

This assay will be used on all DS RT-PCR positive or Dengue NS1 Ag ELISA positive samples for serotype identification. In addition sequencing analysis may be attempted on isolates from the serotyped samples.

Dengue NS1 Ag ELISA

The NS1 Ag ELISA will be performed using a commercially available kit: "Platelia™ Dengue NS1 Ag" from Bio-Rad (Marnes-la-Coquette, France). The manufacturer's instructions are followed. The Dengue NS1 Ag test is a one-step sandwich-ELISA based assay that enables detection of NS1 Ag in serum. The test uses murine monoclonal antibodies (MAbs) for capture and revelation. Samples and controls are directly and simultaneously incubated with the conjugate within the microplate wells coated with MAb. If NS1 Ag is present in the sample, an immune-complex MAb-NS1-MAb/peroxidase will be formed. The presence of immune-complex is demonstrated by addition of a chromogenic solution that initiates a color development reaction. After 30 minutes of incubation at room temperature, the enzymatic reaction is stopped by addition of an acid solution. The optical density (OD) reading obtained with a spectrophotometer set at 450/620 nm is proportional to the amount of NS1 Ag present in the sample. The presence of NS1 Ag in an individual sample is determined by comparing the OD reading of the sample to the OD of the cutoff control serum.

Sample ratios of < 0.5, ≥ 0.5 to ≤ 1.0, and > 1 will be indicative of negative, equivocal, and positive results, respectively.

Hematology – Biochemistry

Hematology and biochemistry parameters (hematocrit, platelet count, AST, and ALT) will be measured by local laboratories using standard methods as per routine standard of care in Singapore. However, the measurement of any of these biological parameters may be undertaken (or repeated), based on the Investigator's judgment, to ensure the adequate evaluation of dengue disease severity. It is noteworthy that hematocrit and platelet counts are required parameters in the WHO/IDMC severity assessment protocol. The results will be collected in the CRF.

The assessment of biological parameters will be: within normal range or outside normal range. Normal ranges for each biological parameter will be provided by the local laboratory and collected in the CRF.

Interpretation of Results

If a sample is positive for the dengue screen RT-PCR (i.e., >=LLOQ) and/or the NS1 assay is positive and/or the Simplexa™ dengue RT-PCR is positive, this will be classified as a virologically-confirmed dengue infection.

9.2.3 Efficacy

No clinical efficacy data will be obtained in the trial.

9.3 Additional Endpoints and Assessment Methods

9.3.1 Immunogenicity

9.3.1.1 Immunogenicity Endpoints

Subjects from the AIT subset (i.e., the first 60 randomized subjects from 2 specific sites (30 subjects per sites; 45 subjects in Group 1, 15 subjects in Group 2).

1) Dengue PRNT

   Two additional time points for neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT at 7 and 14 days post-injection.

2) Ab specificity and affinity maturation

a) Heterotypic and homotypic serotype-specific neutralizing Ab responses will be assessed qualitatively immediately prior to and 28 days post-injection as a priority, and at 7 and 14 days post-injection if necessary. Homotypic Abs for individual serotypes will be defined based on values above lower limits of quantitation for the neutralizing titer and % of Ab remaining following depletion.

b) Serotype-specific affinity (K_D, nM) and Ab concentration (µg/mL) will be measured against the parental wild-type strains in the sera immediately prior to and at 28 days post-injection.

3) CMI responses

The specific B and T immune response against the 4 dengue serotypes elicited by the CYD dengue vaccine booster will be assessed by ELISPOT or flow cytometry, using intracellular staining and phenotyping:

a) T-cell response immediately prior to and 28 days, and 1 year after booster or placebo injection::
   i) Cytokine secreting CD4 and CD8 T-cells count.
   ii) T-cell subclasses (naïve, effector, central and terminally differentiated memory T-cells) count.
   iii) Cytotoxic T-cell effector markers.

b) B-cell response:
   i) Ex vivo B-cells (plasmablast) count (measured by ELISPOT) immediately prior to and 7 and 14 days after booster or placebo injection.
   ii) Memory B-cells count (measured by ELISPOT) immediately prior to and 28 days and 1 year after, booster or placebo injection for a subset of subjects.

For all subjects

4) Post booster neutralizing Ab levels against each of the 4 parental dengue virus strains as determined by PRNT

The assays, along with the corresponding samples, will be managed by the specific Sanofi Pasteur organization (e.g., GCI, Research), or with an external laboratory, as it applies.

9.3.1.2 Immunogenicity Assessment Methods

9.3.1.2.1 Ab specificity and affinity maturation

Ab specificity

Sera will undergo a bead-based depletion method in a coordinated manner with the wild-type dengue parental strains (DENV1/3/4 and DENV2) and control BSA to qualitatively assess whether the neutralizing Abs elicited by the vaccine are primarily homotypic or cross-reactive heterotypic. Depletion will be confirmed by a Luminex-bead fluorescent based read-out that is similar to an ELISA but can assess responses to all 4 serotypes in a single sample run.

The neutralizing Abs in the depleted and BSA-control depleted sera will be analyzed by a flow cytometry-based neutralization assay using the U937 DC-SIGN cell line. Samples undergo a 3-fold serial dilution from 20-fold to 131,220 fold and run independently against all 4 parental wild-type viruses. The percent neutralization will be calculated and the values fitted to a sigmoidal curve in GraphPad Prism to determine the 50% neutralization titer. Neutralization titers of the DENV-depleted samples will be compared to the neutralization titers of the BSA-depleted samples to determine whether the neutralizing Ab response in homotypic or heterotypic.

Ab affinity maturation

Affinity measurements and Ab concentration will be read on the ForteBio Octet RED384. lAb binding curves will be generated by binding a serotype cross-reactive mAb to the optical fiber sensors followed by the capture of the individual parental wild-type dengue viruses. For kinetic analysis, the virus-coated sensors will be exposed to serially-diluted sera samples. The initial binding rates will be used to calculate the serum Ab concentration (µg/mL) based on established calibration curves. A global fit to a 1:1 binding model (Data analysis software, ForteBio) will be used to generate the equilibrium binding constants and the affinity constants (K_D, nM).

9.3.1.2.2 Dengue PRNT

The immunogenicity assessment methods for the secondary endpoints are the same as those presented in Section 9.1.1.2.

9.3.1.2.3 CMI responses

The Intracellular Cytokine Staining and B-cell ELISPOT tests will be performed by the Sponsor's Research Department in Marcy l'Etoile, France.

T-cell response (Intracellular Cytokine Staining)

Intracellular Cytokine Staining can measure the T-cell immune response to a variety of antigens after short-term activation of PBMCs. Cells are incubated with antigens (e.g., pools of peptides from YF 17D or dengue viruses), an antibody directed against the degranulation marker CD107a, secretion inhibitors such as brefeldin A and monensin, allowing the intracellular accumulation of cytokines in activated cells. The cells will be stained with the surface markers CD3, CD4, CD8, CD45RA, CCR7 and, after fixation and permeabilization, the cells are stained for intracellular markers CD154, IFN-γ, TNF-α, IL2, and MIP-1beta. Cells are then analyzed by flow cytometry.

B-cell response (ELISPOT B)

Antibody secreting cell (ASC) frequency will be measured by an ELISPOT assay adapted to measure dengue-specific responses. Briefly, nitrocellulose-bottomed 96-well plates will be coated with Dengue vaccine and incubated overnight at 4°C. After washing and blocking, PBMCs will be plated and incubated for 5 h at 37°C. Plates will be washed and incubated with biotinylated mouse anti-human pan-IgG Fc antibody overnight at 4°C. Plates will be washed, incubated with peroxidase-conjugated streptavidin, and developed with substrate. Spots will be enumerated with an automated spot reader.

Dengue-specific B-cells memory responses will be evaluated after 5 days of PBMC polyclonal stimulation in vitro. After 5 days in culture, the cells will be recovered, washed, and used in the ELISPOT assay described above.

9.3.2 Safety

There are no additional objectives for safety.

9.3.3 Efficacy

No clinical efficacy data will be obtained in the trial.

10 Reporting of Serious Adverse Events

In order to comply with current regulations on SAE reporting to health authorities, the Investigator must document all SAEs regardless of causal relationship, and notify the Sponsor and the Clinical Research Associate (CRA) and/or the Regional Clinical Trial Manager (RCTM) within the notification timelines stated in the following sections. The Investigator will give access and provide the Sponsor and the CRA and/or the RCTM with all necessary information to allow the Sponsor to conduct a detailed analysis of the safety of the investigational product. It is the responsibility of the Investigator to request all necessary documentation (e.g., medical records, discharge summary, autopsy) in order to provide comprehensive safety information. All relevant information must then be transcribed into the eSAE Form.

10.1 Initial Reporting by the Investigator

SAEs occurring during a subject's participation in the trial or experiment must be reported within 24 hours to the Sponsor's GPV Department and to the CRA. Every SAE must be reported, even if the Investigator considers that it is not related to the vaccine. The SAE form must be signed by a licensed physician (M.D. or D.O.) for whom the task is listed on the Study Task Delegation and Signature List after each update to the Form.

The Investigator must complete the "eSAE Form" in the EDC application. After validation, an e-mail alert will automatically be sent to the GPV mailbox, the CRA and/or the RCTM and the RDCD/RMO. This message will include the country, the study code, the subject number, whether the report is initial or a follow-up, the diagnosis and / or symptoms, the seriousness criteria, the relationship, if related and the outcome, if fatal.

If the EDC system is unavailable, the site must notify the Sponsor using the paper version of the SAE Reporting Form, as follows:

- By fax, to the following number: [redacted]
- In PDF format to the following e-mail address, using a method of transmission that includes password protection: [redacted]
- By express mail, to the following address:

  Sanofi Pasteur SA
  Global PharmacoVigilance Department
  2, Avenue Pont Pasteur
  69367 Lyon Cedex 07
  France

When the system becomes available, the Investigator must transcribe the information from the paper version of the eSAE Form into the EDC system.

If there is need for urgent consultation, the Investigator is to contact a designated Sponsor representative. The contact information is provided in the "List of Investigators and Centers Involved in the Trial" document.

10.2 Follow-up Reporting by the Investigator

The eSAE Form completed initially must be updated within 24 hours after the Investigator has become aware of any new relevant information concerning the SAE (e.g., outcome, precise description of medical history, results of the investigation). After validation, an e-mail alert will be sent automatically to the GPV Department and to the CRA and/or the RCTM. All relevant information must be included directly in the eSAE form. Copies of documents (e.g., medical records, discharge summary, autopsy) may be requested by the GPV Department.

The anonymity of the subject must always be respected when forwarding this information.

10.3 Reporting of SAEs Occurring After a Subject Has Completed the Study

Any SAE that occurs after a subject has completed the study but that is likely to be related to the product or to the experiment must also be reported as soon as possible. In such a case, the reporting procedure to be followed is identical to that described in Section 10.1.

10.4 Assessment of Causality

The causal relationship between the SAE and the product will first be evaluated by the Investigator, using the following definitions:

0 - Not related: The AE is clearly / most probably caused by other etiologies such as an underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the SAE is incompatible with a causal relationship; or the SAE started before the vaccination (screening phase, if applicable).

> **1 - Related**: There is a "reasonable possibility" that the SAE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship.
>
> *(ICH Guidelines, Clinical Safety Data Management E2A)*

Following this, the Sponsor's Product Safety Officer (PSO) will also assess the causal relationship to the product, based on the available information and current medical knowledge.

The decision to modify or discontinue the trial may be made after mutual agreement between the Sponsor and the Investigator(s).

10.5 Reporting SAEs to Health Authorities and IECs / IRBs

The Sponsor will inform the relevant health authorities of any reportable SAEs according to the local regulatory requirements. Reporting to the health authorities will be according to the Sponsor's standard operating procedures.

The Sponsor's RMO will notify the Investigators in writing of the occurrence of any reportable SAEs. The Investigators / Sponsor will be responsible for informing the IECs or IRBs that reviewed the trial protocol.

11 Data Collection and Management

11.1 Data Collection and CRF Completion

Individual safety DCs, specifically designed for this trial by the Sponsor and provided to the study sites, will be given to study participants for the recording of daily safety information as described in Section 9.2.2.3. These diary cards will include prelisted terms and intensity scales (see Table 9.1 and Table 9.2) as well as areas for free text to capture additional safety information or other relevant details. Subjects (or subjects' parent / legally acceptable representative for subjects aged < 21 years) will also be provided with rulers for measuring the size of injection site reactions, and with standard digital thermometers for measuring daily temperatures. To ensure consistency of reporting, the study sites will instruct subjects (and subjects' parent / legally acceptable representative for subjects aged < 21 years) on how to correctly use these tools.

At specified intervals, the Investigator or an authorized designee will interview the subjects (or subjects' parent / legally acceptable representative for subjects aged < 21 years) to collect the information recorded in the DC, and will attempt to clarify anything that is incomplete or unclear. All clinical trial information gathered by the study site will be reported electronically by the Investigator or authorized designee using a web-based CRF. (Any information that was not documented in the DC will first be captured in the source document and then reported electronically.) The CRF has been designed specifically for this trial under the responsibility of the Sponsor, using a validated Electronic Records / Electronic Signature-compliant platform (21 CFR Part 11).

To ensure the correct and consistent completion of the CRFs, the Sponsor or authorized representative will provide all necessary tools, instructions, and training to all site staff involved

in data entry prior to study start. Additional instructional documents such as training manuals and completion guidelines will be provided to assist with data entry during the course of the trial.

Upon completion of training, each user requiring access to the EDC system will be issued a unique username and password. In the event of a change in trial personnel, each newly assigned individual will receive a unique username and password; the username and password of a previous user may not be reissued. If any trial personnel leave the study, the Investigator is responsible for informing the Sponsor immediately so that their access is deactivated. An audit trail will be initiated in the EDC system at the time of the first data entry in order to track all modifications and to ensure database integrity.

The Investigator is responsible for the timeliness, completeness, and accuracy of the information in the CRFs; must provide explanations for all missing information; and must sign the CRF using an e-signature.

11.2 Data Management

Management of Clinical Data

Data generated during the trial will be managed following two different processes:

- Clinical data, defined as all data reported in the CRF, and laboratory data will be handled by the Sponsor's Clinical Data Management (CDM) platform or authorized representative.
- Data pertaining to SAEs, which are reported by the Investigator on the eSAE Forms or SAE Reporting Forms, will be handled by the Sponsor's GPV Department.

During the trial, clinical data reported in the CRFs will be integrated into the clinical database under the responsibility of the Sanofi Pasteur CDM platform. Data monitoring at the sites and quality control in the form of computerized logic and / or consistency checks will be systematically applied in order to detect errors or omissions. In addition, data reviews may be performed several times by the Sponsor's staff in the course of the trial. Any questions pertaining to the reported clinical data will be submitted to the investigator for resolution using the EDC system. Each step of this process will be monitored through the implementation of individual passwords to maintain appropriate database access and to ensure database integrity.

The validation of the immunogenicity data will be performed at the laboratory level following the laboratory's procedures. Information from the laboratory will be checked for consistency before integration into the clinical database.

After integration of all corrections in the complete set of data, and after the SAE information available from CDM and the GPV Department has been reconciled, the database will be released for statistical analysis.

SAE Data Management

During the trial, data pertaining to SAEs reported on eSAE Forms will be integrated into the Sponsor's centralized GPV database.

Upon receipt of an eSAE Form, the data will be entered into the GPV database after a duplicate check. Each SAE case will be assigned a case identification number. Each case will be entered in the GPV database and assessed by the case management platform or its delegate before being reported to the relevant authorities as necessary. Assessment of related cases will be done in collaboration with the PSO and the RMO. Follow-up information concerning a completed case will be entered into the GPV database, and a new version of the case will be created.

The information pertaining to SAEs in the GPV database will be reconciled with that in the clinical database.

11.3 Data Review

A blind review of the data is anticipated through the data review process led by Data Management before database lock.

12 Statistical Methods and Determination of Sample Size

12.1 Statistical Methods

The analysis will be performed under the responsibility of the Sponsor's Biostatistics platform with the SAS software, version 9.2 or higher (SAS Institute, Cary, North Carolina, USA).

12.1.1 Hypotheses and Statistical Methods for Primary Objective

12.1.1.1 Hypotheses

Individual Hypotheses for Each Serotype to Demonstrate Non-inferiority:

A non-inferiority testing approach will be performed for each serotype specific endpoint to demonstrate the non-inferiority in terms of GMTRs for each subject, 28 days post injection, of a CYD dengue vaccine booster dose compared to the third CYD dengue vaccine dose in subjects from CYD28 trial.

Individual hypotheses for each serotype will be as follows:

$H_0{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) \leq 1/2$

$H_1{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) > 1/2$

$Where\ i = 1,2,3\ and\ 4;\ V^i_{Booster}$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V^i_{PD3} is the immunogenicity titer 28 days after the third CYD dose in CYD28 subjects.

Overall Hypothesis to Demonstrate Non-inferiority:

The overall null hypothesis can be stated as: for at least 1 serotype, the post-booster dose response (28 days after the CYD dengue vaccine booster dose) is inferior to the PD3 response (28 days after the third CYD dengue vaccine dose in CYD28 subjects).

H_0^G : at least one H_0^i *not rejected*

H_1^G : all H_0^i *are rejected*

12.1.1.2 Statistical Methods

A non-inferiority test will be performed using the 95% two sided confidence interval (CI) of $GM(V_{Booster}/V_{PD3})$ for each serotype and the 95% CI will be calculated using paired t-test (18). Subjects with non-missing PD3 and post-booster dose titers will be included in this analysis.

For each serotype, the non-inferiority will be demonstrated if the lower limit of the two-sided 95% CI is greater than 1/2. If the null hypothesis is rejected, then the alternative hypothesis of non-inferiority will be supported. The overall null hypothesis will be rejected if the four individual null hypotheses are rejected simultaneously.

12.1.2 Hypotheses and Statistical Methods for Secondary Objectives

12.1.2.1 Hypotheses and Statistical Methods for the First Secondary Objective

If non-inferiority is demonstrated for the primary endpoint, then superiority hypotheses will be performed.

Individual Hypothesis for Each Serotype to Demonstrate Superiority:

A superiority hypotheses testing approach will be performed for each serotype to demonstrate the superiority, 28 days post-injection, of a CYD booster dose compared to the third CYD dose in subjects from CYD28 trial, in terms of GMTR for each subject.

Individual hypotheses for each serotype will be as follows:

$$H_0{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) \leq 1$$

$$H_1{}^i: GM\left(V^i_{Booster}/V^i_{PD3}\right) > 1$$

$Where\ i = 1,2,3\ and\ 4$; $V^i_{Booster}$ is the immunogenicity titer 28 days after the CYD dengue vaccine booster dose and V^i_{PD3} is the immunogenicity titer 28 days after the third CYD dose in CYD28 subjects.

Overall Hypothesis to Demonstrate Superiority:

The overall null hypothesis can be stated as: for at least 1 serotype, the post-booster dose response (28 days after the CYD dengue vaccine booster injection) is not superior to the PD3 response (28 days after the third CYD dengue vaccine dose in CYD28 subjects)

H_0^G : at least one H_0^i *not rejected*

H_1^G : all H_0^i *are rejected*

Statistical Methods:

A superiority test will be performed using the 95% two sided CI of $GM(V_{Booster}/V_{PD3})$ for each serotype; the 95% CI will be calculated using paired t-test. Subjects with non-missing PD3 and post-booster dose titer will be included in this analysis.

For each serotype, superiority will be demonstrated if the lower limit of the two-sided 95% CI is greater than 1. If the null hypothesis is rejected, then the alternative hypothesis of superiority will be supported.

The overall null hypothesis will be rejected if the 4 individual null hypotheses are rejected simultaneously.

12.1.2.2 Hypotheses and Statistical Methods for the Other Secondary Objectives

There are no hypotheses. All of the analyses will be descriptive.

12.1.2.2.1 Immunogenicity Objectives

Response elicited by booster dose

The time point to be used for the descriptive comparisons to describe the immune response elicited by CYD dengue vaccine booster (Group 1) as compared to placebo among subjects who received 3 doses of CYD dengue vaccine in CYD28 trial will be immediately prior to and 28 days post-injection. Immunogenicity will be assessed descriptively using the following parameters:

- Geometric mean of titers (GMTs) against each serotype with the parental dengue virus strains at each available time point
- Geometric mean of the individual titers ratio (GMTRs) against each serotype with the parental dengue virus strains
- Number and percentage of subjects ≥ 10 (1/dil) against each dengue serotype with the parental dengue virus strains at each available time point
- Number and percentage of subjects ≥ 10 (1/dil) against at least 1, 2, 3, or 4 dengue serotypes with the parental dengue virus strains at each available time point
- Number and percentage of subjects ≥ various titer thresholds (1/dil) for at least 1, 2, 3, or 4 serotypes with parental dengue virus strains at each available time point
- Distribution of titers against each of the 4 serotypes with parental dengue virus strains at each available time point and corresponding RCDC.
- Seroconversion rates 28 days after the injection for each of the four parental dengue virus strain of CYD dengue vaccine.

Two sample t-test on the log10 transformed titers will be used for 95% CI for the ratio of GMTs (difference between GMTs on log scale).

Assuming that log10 transformation of the titers/titers ratio follows a normal distribution, first, the mean and 95% CIs will be calculated on log10 (titers/ titers ratio) using the usual calculation for

normal distribution, then antilog transformations will be applied to the results of calculations, to compute GMTs/GMTRs and their 95% CIs.

The 95% CIs for percentages will be calculated using the exact binomial distribution (Clopper-Pearson's method).

Ab persistence

- at baseline: the time points to be used for the descriptive comparisons to describe neutralizing Ab persistence will be D0 (immediately prior to booster or placebo injection) and 28 days after the third CYD dengue vaccine dose in CYD28 subjects.
- at 6 months, 1 year and 2 years: the time points to be used to describe neutralizing Ab persistence will be 6 months, 1 year, and 2 years after the booster or placebo injection

Immunogenicity will be assessed descriptively using the same statistical methods and parameters as above.

12.1.2.2.2 Safety Objectives

Safety profile will be described after booster vaccination with CYD dengue vaccine.

The safety analysis will address the number and percentage of subjects with injection site reactions (pain, erythema, and swelling) from D0 and D07, solicited systemic reactions (fever, headache, malaise, myalgia, and asthenia) from D0 to D14, unsolicited AEs until D28, non-serious AESIs (hypersensitivity/allergic reactions) from D0 to D07, and unsolicited immediate systemic event occurring within 30 minutes after a booster CYD dengue vaccine dose. Solicited injection site reactions or solicited systemic reactions will be described according to time of onset, number of days of occurrence, action taken, and intensity.

Unsolicited AEs or non-serious AESIs will be described according to nature (MedDRA preferred term), time to onset, duration, intensity, action taken, and relationship to vaccination.

Unsolicited immediate systemic events will be described according to nature (MedDRA preferred term) and relationship to vaccination.

The number and percentage of subjects with SAEs, including serious AESIs will be described according to nature (MedDRA preferred term), seriousness criteria, outcome, and relationship to vaccination throughout the trial.

All AEs leading to study termination will be described according to nature (MedDRA preferred term) and relationship to vaccination.

The exact binomial distribution (Clopper-Pearson method) for proportions will be used in calculations of the 95% CIs.

Detection of hospitalized virologically-confirmed dengue cases:

The number and percentage of subjects with a hospitalized virologically-confirmed dengue cases, occurring at any time throughout the trial after the injection will be described using safety analysis set.

The 95% CIs for percentages will be calculated using the exact binomial distribution (Clopper-Pearson's method).

12.1.3 Statistical Methods for Additional Objectives

There are no hypotheses. All of the analyses in the AIT subset will be descriptive.

- For categorical data, the number and percentage of subjects above or equal to the lower limit of detection (LLOD), and the 95% CI of the percentage of the subjects.
- For continuous data, Log10: mean and standard deviation; geometric mean, 95% CI of the geometric mean and quartiles, minimum and maximum value.

For the last additional objective, analysis of covariance (ANCOVA) will be used to compare the post booster mean response of neutralizing Ab levels against each dengue virus serotype of two groups controlling for the baseline neutralizing Ab levels against each dengue virus serotype. Further details regarding the ANCOVA model will be outlined in the SAP.

12.2 Analysis Sets

12.2.1 Per-Protocol Analysis Set

The per-protocol analysis set (PPAS) is a subset of the full analysis set (FAS). It will include all subjects who had no protocol deviations. Subjects will be excluded from the PPAS for the following reasons:

- Subject did not meet all protocol-specified inclusion/exclusion criteria
- Subject did not receive study injection
- Subject received a vaccine other than the one that he / she was randomized to receive
- Preparation and / or administration of vaccine was not done as per-protocol
- Subject did not provide the post-dose serology sample at Visit 4 (D28) in the proper time window
- Subject received a protocol-restricted medications (see Section 6.7)
- Subject's post-injection serology sample did not produce a valid test result (i.e. a result different from "not-reportable" ["NR"] or missing, for at least one dengue serotype)

Subjects will remain in this population as long as they do not meet one of the above criteria, except for blood sampling timing and validity of the serology test result.

12.2.2 Full Analysis Set

The FAS is defined as the subjects who received either CYD dengue vaccine or placebo and had blood sample drawn and valid post-injection serology results (i.e. a result different from "NR" or missing, for at least one dengue serotype). Subjects will be analyzed by the vaccine group to which they were randomized.

12.2.3 Safety Analysis Set

The safety analysis set (SafAS) is defined as those subjects who have received either CYD dengue vaccine or placebo[a]. All subjects will have their safety analyzed according to the vaccine they actually received.

Safety data recorded for a vaccine received out of the protocol design will be excluded from the analysis (and listed separately).

12.2.4 Populations Used in Analyses

The immunogenicity analyses will be performed on the PPAS, and will be confirmed on the FAS.

The SafAS will be used for the description of clinical safety. Subjects will be analyzed according to the product they actually received.

12.3 Handling of Missing Data and Outliers

12.3.1 Immunogenicity

For the computation of geometric mean titers (GMTs), a titer reported as < LLOQ will be converted to a value of 0.5 LLOQ.

For calculating GMTR, < LLOQ will be converted to 0.5 LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator

Any titer reported as > upper limit of quantification (ULOQ) will be converted to ULOQ.

Missing data will not be imputed. No test or search for outliers will be performed.

12.3.2 Safety

No replacement will be done. Nevertheless, missing relationship will be considered as related at the time of stat analysis. Details will be described in the SAP.

12.4 Interim / Preliminary Analysis

No interim analyses are planned.

Two planned statistical analyses will be performed on data collected up to 28 days post-injection and up to 1 year post-injection, respectively. These analyses will require the unblinding of data. A specific process will be implemented to maintain the blind at both subject and Investigator levels.

A third and final analysis will be conducted once the study has ended (2 years post-booster injection) and the final database lock has occurred.

[a] For which safety data are scheduled to be collected

12.5 Determination of Sample Size and Power Calculation

There will be 195 subjects in Group 1, 65 subjects in Group 2. Assuming that 10% of subjects from each group will not provide valid immunogenicity results, a total of 176 and 59 evaluable subjects is anticipated for Groups 1 and Group 2, respectively. With 176 evaluable subjects, the probability of observing at least 1 AE with true incidence of 1.7% is approximately 95%.

Sample size for the primary endpoint (only for Group 1 subjects) was estimated to demonstrate non-inferiority of a CYD dengue vaccine booster compared to the third CYD dengue vaccine dose in subjects from CYD28 trial, in terms of GMTR.

With 176 evaluable subjects in Group 1, for each serotype, there is 80.2% overall power (see Table 12.1) using paired t-test to reject the 4 individual null hypotheses simultaneously; calculation assumed a non-inferiority margin (delta) =2, one-sided type I error =0.025 and correlation between the responses PD3 and post-booster dose of the same serotype in the same subject = 0.6.

Table 12.1: Power/Sample size calculation summary table for primary endpoint (only for Group 1 subjects)

Component (Antigen)	Standard deviation (log 10)	Non-Inferiority Definition	Power for N=176
Serotype 1	(sd1=0.77,sd2=1.54)	> 1/2	0.892
Serotype 2	(sd1=0.74,sd2=1.48)	> 1/2	0.914
Serotype 3	(sd1=0.59,sd2=1.18)	> 1/2	0.970
Serotype 4	(sd1=0.53,sd2=1.06)	> 1/2	0.996
Overall			0.802

The standard deviation for PD3 (sd1) is based on 28-day PD3 standard deviations of titers from CYD28 trial. The standard deviation for post-booster (sd2) is estimated conservatively as two folds of the sd1 for each serotype.

Since four individual null hypotheses should be rejected simultaneously to reject the overall null hypothesis, no multiplicity adjustment for alpha is necessary.

A 3:1 randomization ratio between Group 1 and Group 2 was chosen, so there will be 195 and 65 subjects enrolled in Group 1 and Group 2, respectively.

For the assessment of CMI, additional neutralizing Ab titers (for exploration of the Ab response's kinetics), Ab specificity and affinity maturation, the AIT subset will include the first 60 randomized subjects from two specific sites (30 subjects per site): 45 subjects in Group 1 and 15 subjects in Group 2.

13 Ethical and Legal Issues and Investigator / Sponsor Responsibilities

13.1 Ethical Conduct of the Trial / Good Clinical Practice

The conduct of this trial will be consistent with the standards established by the Declaration of Helsinki and compliant with the ICH guidelines for good clinical practice (GCP) as well as with all local and / or national regulations and directives.

13.2 Source Data and Source Documents

“Source data” are the data contained in source documents. Source documents are original documents or certified copies, and include, but are not limited to, DCs, MAs, medical and hospital records, screening logs, informed consent, telephone contact logs, and worksheets. The purpose of trial source documents is to document the existence of subjects and to substantiate the integrity of the trial data collected. Investigators must maintain source documents so that they are accurate, complete, legible, and up to date.

For missing or discrepant data on a diary card, the study coordinator will obtain verbal clarification from the subject, enter the response into the "investigator’s comment" page of the diary card, and transfer the information to the CRF.

The subject pre-screening log should list all individuals contacted by the Investigators to participate in the trial, regardless of the outcome.

The Investigator must print[a] any electronic records on an ongoing basis, sign and date them immediately after creation, and keep the printouts on file as source documents that can be verified by the Sponsor or an inspector against the electronic records. Any later changes of an electronic record require the record to be re-printed, dated (with an indication of the date of change), and signed. Such records must also be kept together with the original printed copy.

13.3 Confidentiality of Data and Access to Subject Records

Prior to initiation of the trial, the Investigator will sign a fully executed confidentiality agreement with Sanofi Pasteur.

Sanofi Pasteur personnel (or designates), the IECs / IRBs, and regulatory agencies, including the Food and Drug Administration (FDA), require direct access to all study records, and will treat these documents in a confidential manner.

In the event a subject’s medical records are not at the investigational site, it is the responsibility of the investigator to obtain those records if needed.

[a] Unless the electronic medical records are managed by validated computerized systems that are compliant with US 21 CFR Part 11, in which case they are acceptable on their own.

13.4 Monitoring, Auditing, and Archiving

13.4.1 Monitoring

Before the start of the trial (i.e., before the inclusion of the first subject in the first center), the Investigators and the Sponsor's staff or a representative will meet at the site-initiation visit to discuss the trial protocol and the detailed trial procedures. Emphasis will be placed on inclusion and exclusion criteria, visit timing, safety procedures, informed consent procedures, SAE reporting procedures, CRF completion, and the handling of samples and products. The Sponsor's staff or a representative will ensure and document that all material to be used during the trial has been received at the site; and that the study investigator team and local Sponsor/delegate staff have been properly informed about the trial, GCP and regulatory requirements, and the Sponsor's procedures. Specific training sessions for the study investigator team and the CRAs on these topics may be performed as necessary, and should be documented.

The following instruction manuals will be provided: the CRF Completion Guidelines for entering data into the CRF, and the Operating Guidelines for detailed trial procedures such as the product management and sample-handling procedures.

After the start of the trial, the Sponsor's staff or a representative will be in regular contact with the investigational team through telephone calls and regular follow-up visits. The Investigator or delegate must be available for these visits, and must allow the Sponsor/delegate staff direct access to subject medical files and CRFs. During these visits, the Sponsor/delegate staff will:

- Evaluate the quality of the trial progress (adherence to protocol and any study-specific guidelines, quality of data collection and document completion, signature of consent forms, occurrence of SAEs, sample and product management, cold-chain monitoring, archiving)
- Source-verify completed CRFs and any corresponding answered queries
- Determine the number of complete or ongoing issues identified at monitoring visits (e.g., protocol deviations, SAEs). Any identified problems will be discussed with the Investigator, and corrective or preventive actions will be determined, as appropriate.
- After all protocol procedures have been completed and the data have been entered into the CRF, the Investigator must still be available to answer any queries forwarded by the Sponsor. All data-related queries must be completed prior to database lock.

At the end of the trial, a close-out visit will be performed to ensure that:

- The center has all the documents necessary for archiving
- All samples have been shipped to the appropriate laboratories
- All unused materials and products have been either destroyed or returned to the Sponsor

13.4.2 Audits and Inspections

A quality assurance audit may be performed at any time by the Sponsor's Clinical and Medical Quality Operations department (C&MQO) or by independent auditors to verify that the trial has been conducted according to the protocol, GCP and ICH requirements, and other applicable

regulations. An inspection may be conducted by regulatory authorities. The Investigator must allow direct access to trial documents during these inspections and audits.

13.4.3 Archiving

The Investigator must keep all trial documents after the completion or discontinuation of the trial, whatever the nature of the investigational center (private practice, hospital, or institution), for as long as required by applicable laws and regulations. In the absence of any applicable laws or regulations, trial documents will be kept at a minimum for the duration indicated on the Clinical Trial Agreement (CTA). In no event, should study personnel destroy or permit the destruction of any trial documents upon less than 90 days advance written notification to the Sponsor. In addition, trial documents should continue to be stored, at Sponsor's sole expense, in the event that the Sponsor requests in writing that such storage continues for a period of time that exceeds that required by any applicable law or regulation or the CTA. The Investigator will inform Sanofi Pasteur of any address change or if they will no longer be able to house the trial documents.

Archived data may be held on electronic records, provided that a back-up exists and that a hard copy can be obtained if required. The protocol, documentation, approvals, and all other documents related to the trial, including certificates attesting that satisfactory audit and inspection procedures have been carried out, will be kept by the Sponsor in the Trial Master File (TMF). Data on AEs are included in the TMF. All data and documents will be made available if requested by relevant authorities.

13.5 Financial Contract and Insurance Coverage

A Clinical Trial Agreement will be signed by all the parties involved in the trial's performance, if relevant. The Sponsor has an insurance policy to cover any liabilities that may arise from use of the product and / or the study protocol.

13.6 Stipends for Participation

Expenses that are directly related to the subject's participation in the trial (for example cost of transportation for attending visits) and the time given will be compensated. Subjects/subjects' parent(s)/legally acceptable representative(s) will not receive any remuneration for participation in the trial.

13.7 Publication Policy

Data derived from this trial are the exclusive property of Sanofi Pasteur. Any publication or presentation related to the trial must be submitted to Sanofi Pasteur for review before submission of the manuscript. After publication of the results of the trial, any participating center may publish or otherwise use its own data provided that any publication of data from the trial gives recognition to the trial group. In addition, Sanofi Pasteur shall be offered an association with all such publications, it being understood that Sanofi Pasteur is entitled to refuse the association.

Sanofi Pasteur must have the opportunity to review all proposed abstracts, manuscripts, or presentations regarding this trial at least 90 days prior to submission for publication / presentation.

Any information identified by Sanofi Pasteur as confidential must be deleted prior to submission, it being understood that the results of this trial are not to be considered confidential.

Sanofi Pasteur's review can be expedited to meet publication guidelines.

14 References List

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