NCT02628444

Immunogenicity and Safety of Tetravalent Dengue Vaccine Given in 1-, 2-, or 3-Dose Schedules (STAGE I) Followed by a Single Booster Injection of the Same Vaccine (STAGE II) 1 or 2 Years after the Last Primary Dose in Healthy Subjects 9 to 50 Years of Age in Colombia and the Philippines

Two-stage, observer-blind, randomized, Phase II immunogenicity and safety study of tetravalent CYD dengue vaccine administered as a 1-, 2-, or 3-dose regimen (STAGE I) followed 1 or 2 years after the last primary dose by a single booster dose of CYD dengue vaccine (STAGE II).

Clinical Trial Protocol, Amendment 1

Health Authority File Number(s):	BB-IND #: 11219
WHO Universal Trial Number (UTN):	U1111-1161-3242
Trial Code:	CYD65
Development Phase:	Phase II
Sponsor:	Sanofi Pasteur 14, Espace Henry Vallée, F-69007 Lyon, France represented by Sanofi Pasteur Inc Discovery Drive, Swiftwater, PA 18370-0187 USA
Investigational Product(s):	CYD Dengue Vaccine
Form / Route:	Powder and solvent for suspension for injection / Subcutaneous
Indication For This Study:	Healthy subjects between 9 and 50 years of age
Manufacturer:	Same as Sponsor
Coordinating Investigators:	[redacted]

Sponsor's Responsible Medical Officer:	[redacted]
Regional Director of Clinical Development	
Product Safety Officer:	
Coordinating Clinical Trial Manager:	
Regional Clinical Trial Manager:	

Version and Date of the Protocol: Version 4.0 dated 12 December 2017

This protocol version 4.0 is the first amendment to the initial trial protocol version 3.0, dated 20 October 2015.

History of Protocol Versions

Table 1: Previous versions of the protocol

Version*	Date	Comments
1.0	9 October 2015	Internal version not submitted
2.0	20 October 2015	Internal version not submitted
3.0	20 October 2015	Original study protocol (first version used in the study)
		Amendment 1

* Versions in bold font have been approved by the Independent Ethics Committee(s) (IEC[s]) / Institutional Review Board(s) (IRB[s]) and used in the study.

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Synopsis

Company:	Sanofi Pasteur
Investigational Product:	CYD dengue vaccine
Active Substance(s):	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 Tetravalent Dengue Vaccine Given in 1- 2- or 3-Dose Schedules (STAGE I) Followed by a Single Booster Injection of the Same Vaccine (STAGE II) 1 or 2 Years after the Last Primary Dose in Healthy Subjects 9 to 50 Years of Age in Colombia and the Philippines
Development Phase:	Phase II
Coordinating Investigators:	[redacted] **Philippines:** [redacted]
Trial Centers:	This study will be conducted at 6 clinical sites in 2 countries: Colombia (3 sites), and Philippines (3 sites) Investigators and sites are listed in the "List of Investigators, Trial Centers, and Sponsor's Personnel Involved in the Trial" document.
Planned Trial Period:	May 2016 (Philippines, first visit of the first subject [FVFS] to last contact in February 2020 telephone contact [PC])
Trial Design and Methodology:	This observer-blind, randomized, Phase II non-inferiority trial will be conducted in 2 sequential stages: STAGE I (2-Dose vs 3-Dose Schedule and 1-Dose vs 3-Dose Schedule): At enrollment, for STAGE I of the study, approximately 1050 healthy subjects between 9 and 50 years of age will be randomized (1:1:1) to 1 of 3 treatments arms (Groups 1, 2, or 3) to receive 3 injections (in various schedules of CYD dengue vaccine and placebo administration) over a 12-month period (administered 6 months apart: at D0; D0 + 6 months; and D0 + 12 months). For STAGE II, subjects randomized to each of the 3 treatment arms in STAGE I will also be randomized (1:1) to 1 of 2 subgroups (eg, subgroup a or b). The subject's dengue serostatus at baseline, based on plaque reduction neutralization test (PRNT), will determine her/his eligibility to receive the booster injection. Only the subjects identified as dengue seropositive at baseline will receive a booster injection of CYD dengue vaccine at either 12 months (subgroup a, Injection 3 + 12 months) or 24 months (subgroup b, Injection 3 + 24 months) following the last primary series injection. At each injection visit in STAGE I, subjects will receive a single dose of either tetravalent

CYD dengue vaccine (CYD) or placebo (PLA): Group 1 subjects (N = 350) will receive CYD dengue vaccine at all 3 injection visits; Group 2 subjects (N = 350) will receive placebo at the first injection visit and CYD dengue vaccine at the last 2 visits; and Group 3 subjects (N = 350) will receive placebo at the first 2 visits and CYD dengue vaccine at the last injection visit.

An interactive voice response system / interactive web response system (IVRS/IWRS) will be used to assign treatment groups and subject numbers at each clinic site.

Table S1 presents the number of subjects randomized to each treatment group and the schedule for the administration of CYD / PLA injections for each group.

Table S1: STAGE I group assignments

Treatment Group	STAGE I		
	Schedule*	**N**	**Product Received**
Group 1	CYD1/CYD2/CYD3	350	CYD
Group 2	PLA1/CYD1/CYD2	350	PLA/CYD
Group 3	PLA1/PLA2/CYD1	350	PLA/CYD
Total		**1050**	

* Injections are given at D0, D0 + 6 months, and D0 + 12 months
CYD: CYD dengue vaccine; PLA: placebo.

Blood samples will be taken from each subject for assessing neutralizing antibody (Ab) titers against each of the 4 parental dengue virus strains at study baseline (immediately prior to first injection), at pre-Injection 2, 28 days post-Injection 2, pre-Injection 3, 28 days post-Injection 3, and 1 year post-injection 3. In addition to helping to maintain the blind, blood samples taken pre-Injection 2 and pre-Injection 3 will be used to assess the kinetics of and immunological response to the first dose in Group 1 and to identify possible natural infections in Groups 2 and 3 (2-dose and 1-dose regimen subjects, respectively). Blood samples taken at pre-Injection 2 will be used as baseline for Group 2 subjects receiving their first CYD dengue vaccine injection. Blood samples taken 28 days post-Injection 2 will provide information on post-Injection 2 geometric mean titers (GMTs) in Group 2 (first CYD dengue vaccine administration); and blood samples taken at pre-injection 3 will serve as baseline data for Group 3 subjects receiving their first and only CYD dengue vaccine injection and to identify possible natural infections in Group 3. Finally, the blood sample taken 1 year post-injection 3 in all subjects will be used to assess Ab persistence.

Non-inferiority testing will be performed on the immunogenicity results of subjects seropositive at baseline after the final injection has been administered: Group 2 (2-dose schedule) will be compared to Group 1 (3-dose schedule) at both 28 days and 1 year post last injection (as part of Primary Objectives).

Neutralizing antibody titers against each of the 4 parental dengue virus strains at 28 days and 1 year after the final injection in Group 3 (1-dose schedule) and Group 2 (2-dose schedule) will be compared to Group 1 (3-dose schedule).

Finally, the blood sampling schedule will also provide data pertaining to the consistency of response between post-Injection 2 in Group 1 and post-Injection 3 in Group 2; see also Statistical Methods.

Reactogenicity data will be collected in all subjects after each injection: immediate adverse

events (AEs) observed to occur within 30 min post-injection will be collected; solicited injection site reactions will be collected for Days 0–7; solicited systemic reactions will be collected for Days 0–14; and unsolicited non-serious 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.

STAGE II (Booster Year 1 or Year 2)

Before the beginning of STAGE II, blood samples collected before the first injection of CYD dengue vaccine will have been assayed with PRNT to determine each subject's dengue serostatus at baseline. The blood sample closest to the first injection of CYD dengue vaccine will be assayed in priority (ie, BL1 for Group 1; BL2 for Group 2 [or BL1 if BL2 in unavailable]; BL4 for Group 3 [or BL2 if BL4 is unavailable, or even BL1 if BL2 is unavailable]). Only subjects identified as dengue seropositive at baseline will be eligible to receive the booster injection.

At 1 year following the last primary series injection, subjects dengue seropositive at baseline from subgroup a (1a, 2a, and 3a) will receive a single booster dose of CYD dengue vaccine. At 2 years following their last primary series vaccination, dengue seropositive subjects from subgroup b (1b, 2b, and 3b) will receive a single booster injection of CYD dengue vaccine (Table S2).

Table S2:STAGE II subgroup assignments (only in subjects identified as seropositive at baseline)

	STAGE II		
Stage I Group	**Subgroup**	**Post Inj. 3 + 1 year**	**Post Inj. 3 + 2 years**
Group 1	**1a**	CYD booster	--
	1b	--	CYD booster
Group 2	**2a**	CYD booster	--
	2b	--	CYD booster
Group 3	**3a**	CYD booster	--
	3b	--	CYD booster

Each STAGE II subject will provide blood samples for assessing neutralizing Ab titers against each of the 4 parental dengue virus strains. The first blood sample will be collected in all subjects approximately 1 year post last primary series injection (used to assess Ab persistence but also to assess pre-booster Ab levels in subgroup a). Subjects receiving the booster injection will provide additional blood samples. Subjects from subgroup a will provide a blood sample 28 days after receiving the booster injection. Subjects from subgroup b will provide blood samples immediately before and 28 days after receiving the booster injection.

After a dengue seronegative subject consented to continue in the study, the blood sample collected 1 year after the last primary series injection will be the last clinical procedure required from her/him. After this blood sample, a dengue seronegative subject will no longer be asked to come to the study site. However, the telephone contacts already planned in the study will be maintained as a safety follow-up.

Additional Immunological Tests (AIT) Subset –STAGE I and STAGE II

In addition to the blood samples described above, samples will also be drawn from 60 subjects at a specific site in Colombia who consented to participate in additional immunological testing, the additional immunological tests (AIT) subset.

For evaluations of cell-mediated immunity (CMI), Ab specificity and affinity maturation, blood samples will be taken from STAGE I subjects immediately before Injection 1 (specificity, affinity maturation and CMI) and at 28 days following injection 3 (specificity and affinity maturation only).

During STAGE II, only subjects identified as seropositive at baseline and thus eligible to booster injection will continue to be part of the AIT subset. For these subjects, samples will be drawn immediately before the booster injection, and at 7 days, 14 days, and 28 days post-booster injection for CMI, additional neutralizing Abs, Abs specificity and affinity maturation (depending on the time points).

Table S3 presents the subset of study subjects to be tested for CMI, Abs specificity and affinity maturation, following the administration of Year 1 or Year 2 boosters**.**

Table S3: Subset of subjects by age group and volume of blood samples taken for AIT

		Children and Adolescents (9-17 Years of Age)			Adults (18-50 Years of Age)		
STAGE	**Timeline**	**Neutralizing Abs**	**CMI**	**Total Volume**	**Neutralizing Abs**	**CMI**	**Total Volume / Visit**
		Volume for each assessment (mL)					
STAGE I	**D0 (pre-injection 1)**	5*	25	30	5*	35	40
	28 days Post-Injection 3	5*	--	5	5*		5
STAGE II (For booster dose at Y1 or Y2, as applicable)§	**D0 (pre-Booster)**	5*	25	30	5*	35	40
	Booster + 7 days	5†	15	20	5†	25	30
	Booster + 14 days	5†	15	20	5†	25	30
	Booster + 28 days	5*	15	20	5*	25	30

* These samples will also be assessed for Ab specificity and affinity maturation

† These sample will also be assessment for Ab specificity if required

§ Besides the pre-booster injection blood sample, STAGE II blood samples will be drawn from subjects dengue seropositive at baseline alone.

For subjects receiving a booster injection, reactogenicity data will be collected in all subjects after booster injection: immediate AEs observed to occur within 30 min post-injection will be collected; solicited injection site reactions will be collected for Days 0–7; solicited systemic reactions will be collected for Days 0–14; and unsolicited events will be collected for Days 0-28.

SAEs will be reported throughout STAGE II (and until 6 months following booster injection, at V07) and AESIs will be collected in defined time windows according to the type of AESI.

	THROUGHOUT THE TRIAL To further understand the effects of exposure to wild-type dengue on vaccination against dengue in endemic areas, 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 acute blood sample (within the first 5 days after fever onset*) will be collected for virological confirmation. A suspected hospitalized dengue case will be considered a virologically-confirmed dengue (VCD) case if there is a detection of wild-type dengue virus by NS1 antigen enzyme-linked immunosorbent assay (ELISA) and/or wild-type dengue reverse transcription polymerase chain reaction (RT-PCR). ** Note: Acute blood sample for all suspected hospitalized dengue cases should be collected within the pre-specified time frame described. If this cannot be accomplished, this sample should be obtained as soon as possible thereafter for Independent Data Monitoring Committee (IDMC) severity assessment.*
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 countries 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 / legally acceptable representative and should assure appropriate therapy and follow-up. An internal safety evaluation team (SET) will perform a blinded analysis of the safety data after each injection. 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 AE of interest will be promptly reviewed by the IDMC.
Primary Objectives:	***Immunogenicity:*** <u>STAGE I:</u> • To demonstrate the non-inferiority (NI) of the immune response elicited against each dengue serotype by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 28 days after last injection, in terms of Geometric Mean Ratio (GMR) • To demonstrate the NI of the immune response elicited against each dengue serotype by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 1 year after last injection, in terms of GMR <u>STAGE II:</u> • To demonstrate the NI of the immune response elicited against each dengue serotype, in subjects seropositive at baseline, 28 days after administration of a booster dose of CYD dengue vaccine, in terms of geometric mean of titer ratio (GMTR, within a group) or GMR (between groups): *Booster Dose at One Year* • Post-Year 1 booster Group 1/post-Dose 3 Group 1 (GMTR)

	• Post-Year 1 booster Group 2/post-Dose 3 Group 1 (GMR)* *Booster Dose at Two Years* • Post-Year 2 booster Group 1/post-Dose 3 Group 1 (GMTR) • Post-Year 2 booster Group 2/post-Dose 3 Group 1 (GMR)* * *Note: The Stage II Primary Objectives involving Group 2 will apply only if NI has been achieved in Stage I; within Group 1 testing in STAGE II will be performed regardless of the STAGE I test results* If non-inferiority is not achieved, immunogenicity-related analyses will be descriptive.
Primary Endpoints:	***Immunogenicity:*** STAGE I: • Neutralizing Ab titers against each dengue virus serotype 28 days after the last CYD dengue vaccine injection in the Group 1 and Group 2 primary series schedules • Neutralizing Ab titers against each dengue virus serotype 1 year after the last CYD dengue vaccine injection in the Group 1 and Group 2 primary series schedules STAGE II: • Neutralizing Ab titers against each dengue virus serotype 28 days post-booster dose (Year 1 and Year 2, respectively, for each group that will be tested for NI in STAGE II)

Secondary Objectives:	***Immunogenicity:*** STAGE I: • To demonstrate the superiority of the immune response elicited by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 28 days post-final injection, in terms of GMR • To demonstrate the superiority of the immune response elicited by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 1 year post-final injection, in terms of GMR *Note: NI has to be achieved before assessing for superiority* • To describe the neutralizing Ab levels of each dengue serotype at 28 days post-Injection 3 to the Ab levels immediately before receiving booster injection, by baseline serostatus, in all 3 groups • To describe the neutralizing Ab levels of each dengue serotype at 28 days post-Injection 2 and 28 days post-Injection 3 from Group 1 in a primary series schedule by baseline serostatus STAGE II: • To demonstrate the superiority of the immune response elicited against each dengue serotype, in subjects seropositive at baseline, 28 days after administration of a booster dose of CYD dengue vaccine, in terms of GMTR (within group) or GMR (between groups): *Booster Dose at One Year** • Post-Year1 booster Group 1 compared to post-Dose 3 Group 1 (GMTR) • Post-Year 1 booster Group 2 compared to post-Dose 3 Group 1 (GMR) ** Note: NI has to be achieved before assessing for superiority* *Booster Dose at Two Years** • Post-Year 2 booster Group 1 compared to post-Dose 3 Group 1 (GMTR) • Post-Year 2 booster Group 2 compared to post-Dose 3 Group 1 (GMR) **Note: NI has to be achieved before assessing for superiority* • To describe the seroconversion rate 28 days post-booster injection in all 3 groups. ***Safety:*** STAGE I and STAGE II: • To describe all hospitalized VCD cases that have occurred at any time during the trial • To evaluate the safety profile of CYD dengue vaccine after each and any injection (reactogenicity; unsolicited AEs; SAEs throughout the trial and for 6-months following any injection; and AESIs at defined time windows throughout the trial according to the type of AESI)

Secondary Endpoints:	***Immunogenicity:*** STAGE I: • Neutralizing Ab titers against each dengue virus serotype 28 days post-Injection 2 in Group 1, 28 days post-Injection 3, and 1 year post-injection 3 in all 3 groups STAGE II: • Neutralizing Ab titers against each dengue virus serotype 28 days post-Injection 3 in Group 1, immediately prior to booster injection and 28 days post-booster injection in all 3 groups • Seroconversion rates 28 days after injection for each of the 4 parental dengue virus strains of CYD dengue vaccine: percentages of subjects with either a pre-booster titer < 10 (1/dil) and a post-booster titer ≥ 40 (1/dil), or a pre-booster titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-booster titer as determined immediately prior to and 28 days post-booster in all 3 groups. ***Safety:*** STAGE I and STAGE II • 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 injection. • Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited (prelisted in the subject's diary card and electronic case report form [eCRF]) injection site reactions (pain, erythema, and swelling) occurring up to 7 days after vaccination. • 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 injection. • 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 injection. • Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, and relationship to injection of non-serious AESIs occurring up to 7 days after injection. • Occurrence of SAEs, including serious AESIs (with specific time windows according to the nature of the event) throughout the trial. • Occurrence of hospitalized VCD cases throughout the trial (ie, from D0 through end of study.
Additional Objectives:	***Immunogenicity:*** STAGE I • To describe the PRNT Ab response of flavivirus (dengue/Japanese Encephalitis [JE] in the Philippines, and dengue/Yellow Fever [YF] in Colombia) serological status by age group and country at baseline in all 3 groups • To describe the neutralizing Ab response to each dengue serotype at each available time point, by baseline serostatus, in all 3 groups • To compare the immune response elicited against each dengue serotype by the CYD

	dengue vaccine given as a 2-dose schedule (Group 2) or a 1-dose schedule (Group 3) and given as a 3-dose schedule (Group 1) at 28 days, in subjects seropositive at baseline, in terms of GMR. • To compare the immune response elicited against each dengue serotype by the CYD dengue vaccine given as a 2-dose schedule (Group 2) or a 1-dose schedule (Group 3) and given as a 3-dose schedule (Group 1) at 1 year after last injection, in subjects seropositive at baseline, in terms of GMR. STAGE II: *Booster Dose at One and Two Years* • To describe the neutralizing Ab response to each dengue serotype 28 days after a booster injection *One Year Booster* • To describe the immune response elicited against each serotype 28 days after administration of a booster dose of CYD dengue vaccine 1 year after the last primary series vaccination in a 2-dose schedule (Group 2) or 1-dose schedule (Group 3) when compared to a 3-dose schedule (Group 1) *Two Year Booster* • To describe the immune response elicited against each serotype 28 days after administration of a booster dose of CYD dengue vaccine 2 years after the last primary series vaccination in a 2-dose schedule (Group 2) or 1-dose schedule (Group 3) when compared to a 3-dose schedule (Group 1) AIT Subset Only: • To describe dengue neutralization Ab levels (exploration of Ab response kinetics), Ab specificity and affinity maturation in a subset of subjects (N=60) in all groups (Ratio between subgroups 1:1 = 10 subjects per subgroup). • To describe CMI responses at Day 0 (STAGE I) and at 0, 7, 14, 28 days post booster dose (Y1 and Y2) in this subset of subjects participating in all groups.
Additional Endpoints:	***Immunogenicity*** STAGE I: • Neutralizing Ab titers against each dengue virus serotype at all STAGE I time points • Neutralizing Ab titers against flavivirus at baseline prior to first injection of CYD dengue vaccine (JE in the Philippines; YF in Colombia) STAGE II: • Neutralizing Ab titers against each dengue virus 28 days post-booster injection (Y1 for subgroups a and Y2 for subgroups b) AIT Subset Only: The immune response against the 4 dengue serotypes elicited by the boosting dose of dengue vaccine will be assessed by the measurement of neutralizing antibodies, to include: 1) Neutralizing Ab response a. Neutralizing Ab levels against each of the 4 parental dengue virus strains immediately prior to and at 7, 14, and 28 days post-booster 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 3 (STAGE I); and immediately prior to and at 28 days post-booster injection as a priority, and at 7 and 14 days post-injection if necessary (STAGE II). Homotypic Abs for individual serotypes will be defined based on values above lower limit of quantitation (LLOQ) 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 (STAGE I) and immediately prior to and at 28 days post-booster injection (STAGE II) 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: • Cytokine secreting CD4 and CD8 T cells count immediately prior to the primary dose schedules (STAGE I) and immediately prior to and 7, 14, and 28 days after the Year 1 and Year 2 booster dose for a subset of subjects in Groups 1, 2, and 3. • T-cell subclasses (naïve, effector, central and terminally differentiated memory T cells) count immediately prior to the primary dose schedules (STAGE I), and immediately prior to and 7, 14, 28 days after the Year 1 or year 2 booster dose for a subset of subjects in Groups 1, 2, and 3. • Cytotoxic T-cell effector markers immediately prior to the primary dose schedules (STAGE I), and immediately prior to and 7, 14, and 28 days after the Year 1 or Year 2 booster dose for a subset of subjects in Groups 1, 2, and 3. b. B-cell response: • Ex vivo B-cells (plasmablast) count (measured by ELISPOT) immediately prior to the primary dose schedules and immediately prior to and 7 and 14 days after the Year 1 or Year 2 booster dose for a subset of subjects in Groups 1, 2, and 3. • Memory B-cells count (measured by ELISPOT) immediately prior to the primary dose schedules and immediately prior to and 28 days after the year 1 or year 2 booster dose for a subset of subjects in Groups 1, 2, and 3. The assays, along with the corresponding samples, will be managed by the specific Sanofi Pasteur organization (eg, Research), or with an external laboratory, whichever applies.

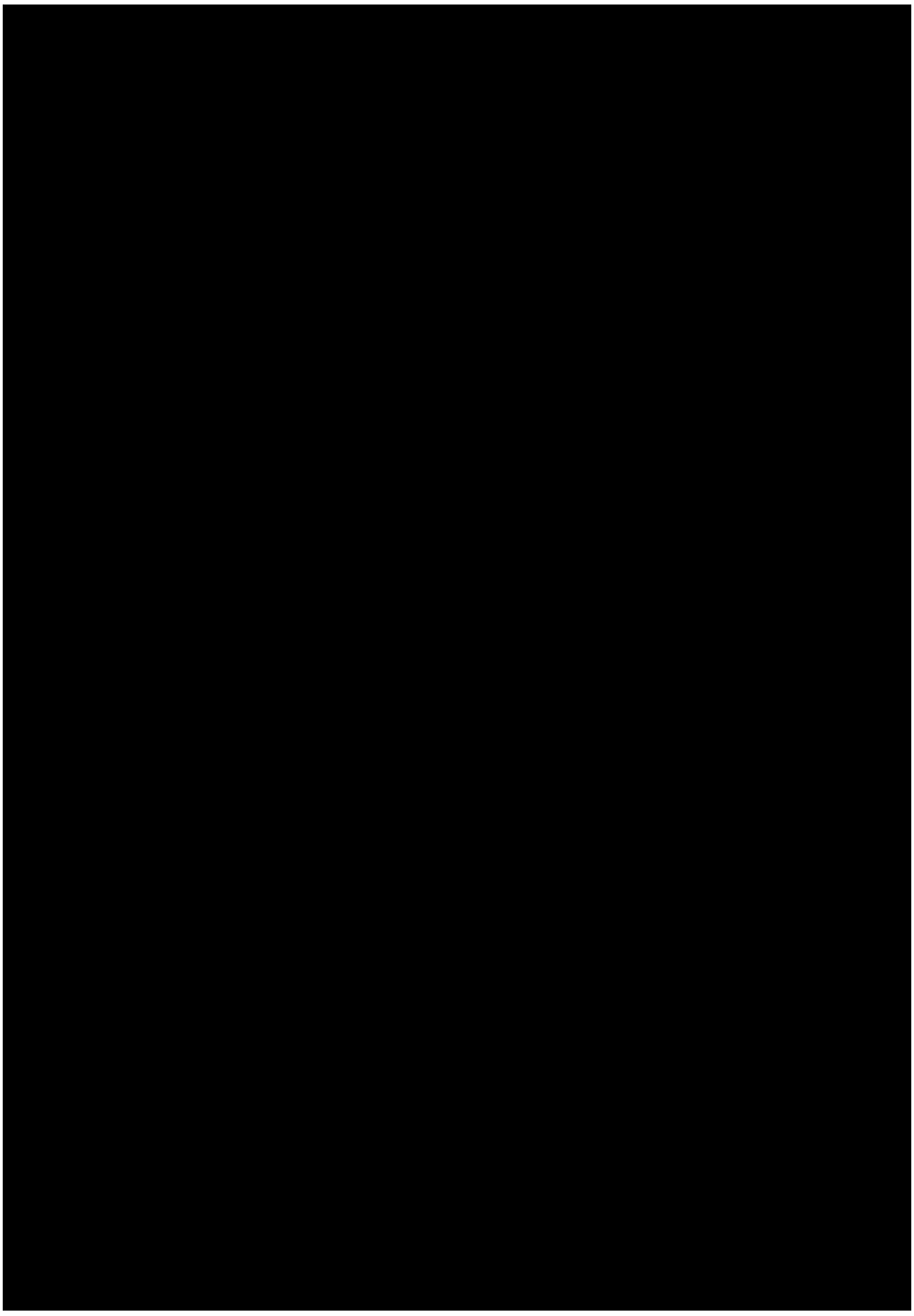
Planned Sample Size:

A total of 1050 subjects are planned to be enrolled with a 1:1:1 enrollment ratio (350 per group [or 1:1:1:1:1:1 randomization ratio, 175 per subgroup]) (Table S4). Considering a potential dropout rate of 15%, this sample size would provide 888 evaluable subjects.

Table S4: Power of the study based on the Primary Objectives - Stage I

Baseline seropositive (%)	Population per group	Objectives	Endpoints	NI Margin	Power (%)
100%	296	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	98.9
	296	Group 2 vs Group 1	1 year post-Inj 3 vs 1 year post-Inj	2	90.64
		Overall			88.6
90%	266	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	97.86
	266	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	88.52
		Overall			86.6
80%	235	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	95.56
	235	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	82.3
		Overall			78.64
70%	207	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	92.06
	207	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	74.52
		Overall			68.60
60%	178	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	86.00
	178	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	63.12
		Overall			54.28

Table S5: [redacted]



The following assumptions were considered:

- Alpha level of 2.5% (one sided) with a Bonferonni adjustment for multiplicity

- Non-inferiority margin of 2 (log10 = 0.301)
- [redacted]
- Dropout rate of 15%

The sample size will also provide a 95% probability of observing an AE that has a true incidence of >0.85% in each group (N=350).

Table S6: Number of subjects in each group (STAGE I) and subgroup (STAGE II) and schedule of vaccine/placebo injections received by all subjects

STAGE I				STAGE II		
Treatment Group	**Schedule**	**N**	**Received product**	**Treatment Group**	**Y1**	**Y2**
Group 1	CYD1 / CYD2 / CYD3	350	CYD	**Group 1a**	CYD Booster	
				Group 1b		CYD Booster
Group 2	PLA1 / CYD1 / CYD2	350	(PLA)/ CYD	**Group 2a**	CYD Booster	
				Group 2b		CYD Booster
Group 3	PLA1 / PLA2 / CYD1	350	(PLA/ CYD)	**Group 3a**	CYD Booster	
				Group 3b		CYD Booster
Total		**1050**				

For the measurement of the CMI, a convenience number consisting of 60 subjects at a specific site in Colombia who consent to participate in additional testing, the AIT subset (Table S5).

Table S7: Number of subjects in the AIT subset from each subgroup

Treatment Group	Treatment Subgroup	N
Group 1	Group 1a	10
	Group 1b	10
Group 2	Group 2a	10
	Group 2b	10
Group 3	Group 3a	10
	Group 3b	10
Total		**60**

<table>
<tr><td></td><td>It is to be noted that the 1:1 ratio per subgroup is unlikely to hold as only subjects dengue seropositive at baseline will be eligible to booster injection.</td></tr>
<tr><td>Schedule of Study Procedures:</td><td>
<u>STAGE I:</u>
Vaccination
All subjects (N=1050) will receive 1 injection at Day 0, Day180 and Day 365: a total of 350 subjects will receive 3 doses of CYD dengue vaccine (Group 1); 350 subjects will receive 1 placebo injection followed by 2 doses of CYD dengue vaccine (Group 2); and 350 subjects will receive 2 placebo injections followed by 1 dose of CYD dengue vaccine (Group 3).
Blood Sampling
All subjects will provide a blood sample at enrollment Visit 1 (V01; pre-injection 1, BL1), at V03 (pre-injection 2, BL2) at V04 (V03 + 28 days, BL3), at V05 (pre-injection 3, BL4), at V06 (V05 + 28 days, BL5), and at Year 1 (Y1) V07 (V05 + 12M, BL6) for dengue immunogenicity.
Note: AIT subset is described below.
Table S8: STAGE I injection and blood sampling schedules
<table>
<tr><th>Procedure</th><th>Product or Test</th><th>V01 (D0)</th><th>V02 (V01+ 28d)</th><th>V03 (V01+ 6M)</th><th>V04 (V03+ 28d)</th><th>V05 (V01+ 12M)</th><th>V06 (V05+ 28d)</th><th>Y1 V07 (V05+ 12M)</th></tr>
<tr><td>Injection</td><td>CYD or PLA</td><td>Inj.1</td><td>Contact visit</td><td>Inj. 2</td><td></td><td>Inj. 3</td><td></td><td></td></tr>
<tr><td>Collection of blood sample:</td><td>Neutralizing Abs (all subjects)</td><td>BL1* (pre-inj. 1) 5 mL</td><td>N/A</td><td>BL2* (pre-inj. 2) 5 ml</td><td>BL3 5 mL</td><td>BL4* (pre-inj. 3) 5 mL</td><td>BL5 5 mL</td><td>BL6† 5 mL</td></tr>
</table>
* Dengue status at baseline will be assessed for each group prior to the first CYD injection (Group 1 at V01, Group 2 at V03, and Group 3 at V05)
† In the context of STAGE I, BL6 will be used to assess neutralizing Abs persistence at 1 year post last primary series injection. In STAGE II, BL6 will be used to assess neutralizing Ab levels at pre-booster injection for subjects from subgroup <u>a</u> that were seropositive at baseline
D or d=day; M=month; N/A=not applicable
<u>STAGE II:</u>
Vaccination
It is estimated that between 60% (N=630) and 70% (N=735) of the initial number of subjects (N=1050) will be eligible to receive a CYD booster dose at Y1 or Year 2 (Y2).
• approximately 315 to 367 subjects will receive a CYD booster dose at Y1 post STAGE I last dose (Groups 1a, 2a, 3a)
• approximately 315 to 367 subjects will receive a CYD booster dose at Y2 post STAGE I last dose (Groups 1b, 2b, 3b).
Blood Sampling
All subjects will provide a blood sample 1 year post last injection (BL6; to assess Ab persistence as part of STAGE I; and pre-booster Ab levels in subgroup <u>a</u> as part of STAGE II). In addition, subgroup <u>a</u> will provide a blood sample 28 days after receiving the booster injection (BL9). Subjects from subgroup <u>b</u> identified as seropositive at baseline will provide blood samples immediately before (BL10) and 28 days after (BL13) receiving the booster injection.
</td></tr>
</table>

Table S9: STAGE II injection and blood sampling schedules (all subjects)

Procedure	Group	Y1 V07 (V05 + 12M)	Y2 V07 (V05 + 24M)	V10 (V07+28d)	6M Follow-up
Injection	**1a, 2a, 3a**	Booster Y1			PC
	1b, 2b, 3b		Booster Y2		PC
Collection of blood sample:	**1a, 2a, 3a**	BL6 (pre-Booster)*†		BL9†	PC
	1b, 2b, 3b	BL6;	BL10 (pre-Booster)†‡	BL13†‡	PC

* In the context of STAGE I, BL6 will be used to assess neutralizing Abs persistence at 1 year post last primary series injection. In STAGE II, BL6 will be used assess to neutralizing Ab levels at pre-booster injection for subjects from subgroup a that were seropositive at baseline

† This includes the volume to assess Ab specificity and affinity maturation.

‡ Only subjects identified as seropositive at baseline will provide BL10 and BL13

AIT Subset

In STAGE I, a subset of 60 subjects in groups 1a, 1b, 2a, 2b, 3a, 3b (10 each group) will provide additional blood before Injection 1 and 28 days after the injection, and again, in STAGE II, immediately before the Y1 or Y2 CYD booster injection and 7, 14, and 28 days after the booster dose for measurement of CMI response (Table S8).

Table S10: STAGE II Additional blood samples (AIT subset)

Procedure	Group	Product or Test	Y1 V07 (V05 + 12M)	Y2 V07 (V05 + 24M)	V08 (V07 + 7d)	V09 (V07 + 14d)	V10 (V07 + 28d)
Injection	**1a,2a,3a**	**CYD Booster dose**	Booster Y1				
	1b,2b,3b	**CYD Booster dose**		Booster Y2			
Blood Sampling*	**1a,2a,3a 1b,2b,3b**	**CMI response**	BL6	BL10	BL7 or BL11	BL8 or BL12	BL9 or BL13
		Additional neutralizing Abs; Abs specificity			BL7 or BL11	BL8 or BL12	

* BL6 will be taken in all subjects; BL10 will be taken in subgroup b only; BL7-BL9 will be taken in subgroups 1a, 2a, and 3a only; BL11-13 will be taken in subgroups 1b, 2b, and 3b only

Only the subjects identified as seropositive at baseline will provide BL7–BL10 or BL10-BL13, depending on the subgroup

STAGE I and II:

Additional biological samples may be collected from any subject in case of the occurrence of SAEs (including VCD cases) during the trial.

	Collection of safety data Clinical site personnel will record immediate unsolicited AEs that occur within the 30 min after any injection. Subjects or parents/legally acceptable representative will record information in the DC on: solicited injection site reactions from Day 0 to Day 7 post-injection; solicited systemic reactions from Day 0 to Day 14 post-injection; and unsolicited AEs from Day 0 to Day 28 post-injection. Information on SAEs will be collected throughout the trial, including the time period between stages, and serious and non-serious AESIs will be collected in defined time windows according to the type of AESI. 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 written in the MAs.
Duration of Participation in the Trial:	The duration of each subject's participation in the trial will be 36-42 months.
Investigational Product: ***Form:*** ***Composition:*** ***Route:*** ***Batch Number:***	CYD Dengue Vaccine Powder and solvent for suspension for injection Each individual 0.5 mL dose of reconstituted tetravalent vaccine contains: <u>Active Ingredients:</u> 4.5 to 6.0 log10 cell-culture infectious dose 50% ($CCID_{50}$) of each live, attenuated, recombinant dengue serotype 1, 2, 3, 4 virus <u>Excipients:</u> Essential amino acids, non-essential amino acids, L-arginine chlorhydrate, saccharose, D-trehalose dihydrate, D-sorbitol, tris (hydroxymethyl) aminomethane, and urea <u>Solvent:</u> NaCl 0.4% Subcutaneous (SC) To be defined
Control Product: ***Form:*** ***Composition:*** ***Route:*** ***Batch Number:***	Placebo Solution NaCl 0.9% SC To be defined
Inclusion Criteria:	An individual must fulfill *all* of the following criteria in order to be eligible for trial enrollment: 1) Aged 9 to 50 years on the day of enrollment* 2) Subject in good health, based on medical history and physical examination 3) Assent form (AF) or 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 * *"9 to 50 years" means from the day of the 9th birthday to the day before the 51st birthday*

Exclusion Criteria:	An individual fulfilling *any* of the following criteria is to be excluded from trial enrollment: 1) Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be pre-menarche, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination until at least 4 weeks after the last vaccination)* 2) Participation at the time of study enrollment (or in the 4 weeks preceding the first trial vaccination) or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device or medical procedure 3) Self-reported 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) 4) Self-reported systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccine used in the trial or to a vaccine containing any of the same substances** 5) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion*** 6) Receipt of blood or blood-derived products in the past 3 months, which might interfere with assessment of the immune response 7) Planned receipt of any vaccine in the 4 weeks following any trial vaccination 8) Previous vaccination against dengue disease with either the trial vaccine or another vaccine 9) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily 10) Current alcohol abuse or drug addiction that, based on Investigator's judgment, may interfere with the subject´s ability to comply with trial procedures. 11) Identified as a site employee of the Investigator, with direct involvement in the proposed study or other studies under the direction of that Investigator or study center, as well as family members (ie, immediate, husband, wife, and their children, adopted or natural) of the employees or the Investigator 12) A prospective subject must not be included in the study until the following conditions and/or symptoms are resolved: • Febrile illness (temperature ≥ 38.0°C) or moderate or severe acute illness/infection (according to Investigator's judgment) on the day of vaccination. • Receipt of any vaccine in the 4 weeks preceding the first trial vaccination ** For pre-menarche females, the young female subjects declare if they have not yet started menstruation; if a young female subject reaches menarche during the study, she is to be considered as a woman of childbearing potential from that time forward.* *** The components of CYD dengue vaccine and placebo are listed in Section 6.1 and in the Investigator's Brochure.* **** Chronic illness may include, but is not limited to, cardiac disorders, renal disorders, auto-immune disorders, diabetes, psychiatric disorders, or chronic infection.*
Statistical Methods:	The analysis will be performed under the responsibility of the Sponsor's Biostatistics platform with the SAS software, version 9.3 or higher (SAS Institute, Cary, North Carolina, USA). Four statistical analyses of safety and immunogenicity will be performed on unblinded data

(one 1 year post-Injection 3, one 28 days after the Y1 booster injection, one 28 days after the Y2 booster injection, and one 6 months after the Y2 booster injection).

Assuming that log_{10} transformation of the titers/ratios follows a normal distribution, first, the mean and 95% CI will be calculated on log_{10} (titers/ratios) 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.

This will be a non-inferiority study in two STAGES. If non-inferiority is not achieved, assessments of immunogenicity will be descriptive.

There will be two non-inferiority tests comparing Group 2 to Group 1 in STAGE I:

- at 28 days after last injection
- at 1 year after last injection

There will be 4 non-inferiority tests planned in STAGE II:

Within Group 1 comparisons:

- Booster Group 1a vs post dose 3 Group 1a
- Booster Group 1b vs post dose 3 Group 1b

Between Group 1 comparisons:

- Booster Group 2a vs post dose 3 Group 1
- Booster Group 2b vs post dose 3 Group 1

Regardless of the outcome in STAGE I, the within Group 1 tests will be performed. However, the between Group 1 tests will only be performed for the Group(s) which demonstrated non-inferiority to Group 1 in STAGE I.

A. <u>Hypothesis and Statistical Method for the Primary Objectives</u>

<u>STAGE I:</u>

A non-inferiority testing approach will be performed for each serotype to show the non-inferiority of 2 doses of CYD dengue vaccine (28 days after last injection) compared to 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

The non-inferiority testing approach will also be performed for each serotype to show the non-inferiority of 2 doses of CYD dengue vaccine (1 year after last injection) compared to the 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

Individual hypotheses for each serotype will be as follows:

$$H_0^i: GMT_{Group\ j}^i / GMT_{Group\ 1}^i \leq \frac{1}{\delta}$$

$$H_1^i: GMT_{Group\ j}^i / GMT_{Group\ 1}^i > \frac{1}{\delta}$$

Where:

i=1, 2, 3, and 4 (each serotype),

j=2

	δ the non-inferiority margin, is set to 2, ie, 0.301 (=log10 [2]). The non-inferiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, non-inferiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1/2 (ie, the log10 of the difference should be above -0.301). STAGE II: The non-inferiority testing for within Group 1 comparisons will be performed in Stage II regardless of whether non-inferiority is achieved or not for either test in STAGE I; however, only those group(s) that achieve non-inferiority in STAGE I will continue to be tested in STAGE II for the between Group 1 comparisons. *Paired Test* A non-inferiority testing approach will be performed for each serotype to show the non-inferiority of a CYD booster dose (28 days after booster) either 1 year and/or 2 years after last vaccination compared to the third CYD dose (28 days after third vaccination) among subjects seropositive at baseline, i.e., within Group 1 comparisons. A Bonferonni alpha adjustment will be used to control for multiplicity. $H_0^i: GM\left(V^i_{Booster\,j}/V^i_{PD3j}\right) \leq \frac{1}{\delta}$ $H_0^i: GM\left(V^i_{Booster\,j}/V^i_{PD3j}\right) > \frac{1}{\delta}$ Where: i=1, 2, 3, and 4 (each serotype), j=1a, and 1b δ the non-inferiority margin, is set to 2, ie, 0.301 (=log10 [2]). The non-inferiority tests will be performed using the paired t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Booster j and post-Injection 3 (PD3) j (α=2.5% one sided). For each serotype, non-inferiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1/2 (ie, the log10 of the difference should be above -0.301). Subjects with non-missing post dose 3 and post booster titer will be included in this analysis. ***Two Sample Test*** A non-inferiority testing approach will be performed for each serotype to show the non-inferiority of a CYD booster dose (28 days after booster) either 1 year and/or 2 years after a 2-dose schedule compared to the third CYD dose (28 days after third injection), ie, between Group 1 and Group 2 comparison, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity. Individual hypotheses for each serotype will be as follows: $H_0^i: GMT^i_{Group\,j}/GMT^i_{Group\,1} \leq \frac{1}{\delta}$ $H_1^i: GMT^i_{Group\,j}/GMT^i_{Group\,1} > \frac{1}{\delta}$ Where:

	i=1, 2, 3, and 4 (each serotype), j=2a and 2b δ the non-inferiority margin, is set to 2, ie, 0.301 (=log10 [2]). The non-inferiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, non-inferiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1/2 (ie, the log10 of the difference should be above -0.301). ***B. Hypotheses and Statistical Methods for Secondary Objectives*** If non-inferiority will be demonstrated for the primary objective, then superiority hypotheses will be performed. <u>STAGE I</u> A superiority testing approach will be performed for each serotype to show the superiority of 2 doses of CYD dengue vaccine (28 days after last injection) compared to 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity. A superiority testing approach will also be performed for each serotype to show the superiority of 2 doses of CYD dengue vaccine (1 year after last injection) compared to the 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity. Individual hypotheses for each serotype will be as follows: $H_0^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} \leq 1$ $H_1^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} > 1$ Where: i=1, 2, 3, and 4 (each serotype), j=2 The superiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, superiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1 (ie, the log10 of the difference should be above 0). <u>STAGE II:</u> *Paired Test* A superiority testing approach will be performed for each serotype to show the superiority of a CYD booster dose (28 days after booster) either 1 year and/or 2 years after last injection compared to the third CYD dose (28 days after third injection), ie, within Group 1 comparisons, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity. $H_0^i: GM\left(V^i_{Booster\ j} / V^i_{PD3\ j}\right) \leq 1$ $H_0^i: GM\left(V^i_{Booster\ j} / V^i_{PD3\ j}\right) > 1$ Where:

	i=1, 2, 3, and 4 (each serotype), j=1a, and 1b The superiority tests will be performed using the paired t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Booster j and Post Dose 3 j (α=2.5% one sided). For each serotype, superiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1 (ie, the log10 of the difference should be above 0). Subjects with non-missing post dose 3 and post booster titer will be included in this analysis. ***Two Sample Test*** A superiority testing approach will be performed for each serotype to show the superiority of a CYD dengue vaccine booster dose (28 days after booster) either 1 year and/or 2 years after a 2-dose schedule compared to the third CYD dengue vaccine dose (28 days after third injection), ie, between Group 1 and Group 2 comparison, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity. Individual hypotheses for each serotype will be as follows: $H_0^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} \leq 1$ $H_1^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} > 1$ Where: i=1, 2, 3, and 4 (each serotype), j=2a and 2b The superiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, superiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1 (ie, the log10 of the difference should be above 0). ***B.1 Immunogenicity:*** **STAGE I:** In addition, immunogenicity will be assessed using the following parameters: • Geometric means of the individual titer ratios (GMTR) for each serotype (parental strains) by baseline serostatus (1 year after dose 3/post-Dose 3) • Group 1 only, geometric mean of the individual titer ratios (GMTR) for each serotype (parental strains) by baseline serostatus (post-Dose 3/post-Dose 2) ***B.2 Safety:*** STAGES I AND II: Safety profile will be described after each injection for primary schedules and for booster dose. 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 from D0 to D07, and unsolicited immediate systemic event occurring within 30 minutes after each injection. Solicited injection site reactions or solicited systemic reactions will be described according to time to 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 injection. Unsolicited immediate systemic events will be described according to nature (MedDRA preferred term) and relationship to injection. 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 injection throughout the trial. All AEs leading to study termination will be described according to nature (MedDRA preferred term) and relationship to injection. *Detection of symptomatic dengue cases* The number and percentage of subjects with a suspected dengue cases occurring during the trial after the injection will be described using safety analysis set. ***Hypotheses and Statistical Methods for Additional Objectives*** There are no hypotheses. All of the main analyses will be descriptive. *Immunogenicity*: <u>STAGES I and II</u> The immune response to each dengue serotype at each time point overall, by baseline serostatus (only in Stage I), and by age group and country will be assessed descriptively using the following parameters: • GMT for each serotype (parental strains) at each time point • Geometric mean of the individual GMTR for each serotype (parental strains) • Number and percentage of subjects with dengue neutralizing Ab titer ≥ 10 (1/dil) (parental strains) at each time point against each serotype • Number and percentage of subjects with dengue neutralizing Ab titer ≥ 10 (1/dil) (parental strains) at each time point against at least one, two, three, or the four dengue serotypes • 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 time point • Distribution of titers against each of the 4 serotypes at each time point and corresponding reverse cumulative distribution curve • Number and percentage of subjects who converted Additionally, the number and percentage of subjects immune or non-immune to flavivirus (dengue, and JE or YF) status at baseline overall and by age group and country. GMT for each serotype at 28 days and one year after last CYD dengue vaccine injection by baseline serostatus. Between groups comparisons at 28 days and 1 year after injection 3 (ie, Group 2 vs Group 1, Group 3 vs Group 1) will be described using GMR at each time point for each serotype, in subjects seropositive at baseline. Between groups comparisons within the year of the booster (ie, Group 2a vs Group 1a, Group 3a vs Group 1a, Group 2b vs Group 1b and Group 3b vs. Group 1b) will be described using GMR for each serotype 28 days after booster, in subjects seropositive at baseline.

	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 (eg, Group 2 versus Group 1 or Group 3 versus Group 1) controlling for the baseline neutralizing Ab levels against each dengue virus serotype, in subjects seropositive at baseline. Further details regarding the ANCOVA model will be outlined in the SAP. *AIT Subset:* • 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.

Table of Procedures – STAGE I and STAGE II

	STAGE I								STAGE II*					
Visit Number (V)	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**6M FU (PC7)†**	**V06_Inf**	**V07 (Y1)**	**V07 (Y2)**	**V08 (Y1/Y2) (AIT only)**	**V09 (Y1/Y2) (AIT only)**	**V10 (Y1/Y2)**	**6M FU (PC)‡**
Trial timelines (Days/Months)	**V01**	**V01+ 28d**	**V01+ 180d**	**V03+ 28d**	**V01+ 365d**	**V05+ 28d**	**last inj + 6M**		**V05+ 12M**	**V05+ 24M**	**V07+ 7d**	**V07+ 14d**	**V07+ 28d**	**V07+ 6M**
Time Windows (Days)		**+14d**	**±20d**	**+14d**	**±20d**	**+14d**	**+20d**		**+60**	**+60**	**+3d**	**+7d**	**+14d (+7d for AIT)**	**+20d**
Informed Consent/Assent	√								√					
Information letter on baseline serostatus								√						
Inclusion/Exclusion Criteria	√													
Demography	√													
Contraindications	√		√		√				Subgroup a: V07 Y1 Subgroup b: V07 Y2					
Significant Medical History	√													
History of YF or JE Infection/Vaccination and/or Dengue Infection	√													
Physical Examination and Temperature§	√	√	√	√	√	√			subgroup a: V07 Y1 subgroup b: V07 Y2		√	√	√	
Urine Pregnancy Test (pre-injection)**	√		√		√									
Concomitant Therapy	√	√	√	√	√	√					√	√	√	
IVRS/IWRS Contact	√		√		√									
Injection	Inj. 1		Inj. 2		Inj. 3				Booster Inj. subgroup a: V07 Y1 subgroup b: V07 Y2					

	STAGE I								STAGE II*					
Visit Number (V)	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**6M FU (PC7)†**	**V06_Inf**	**V07 (Y1)**	**V07 (Y2)**	**V08 (Y1/Y2) (AIT only)**	**V09 (Y1/Y2) (AIT only)**	**V10 (Y1/Y2)**	**6M FU (PC)‡**
Trial timelines (Days/Months)	**V01**	**V01+ 28d**	**V01+ 180d**	**V03+ 28d**	**V01+ 365d**	**V05+ 28d**	**last inj + 6M**		**V05+ 12M**	**V05+ 24M**	**V07+ 7d**	**V07+ 14d**	**V07+ 28d**	**V07+ 6M**
Time Windows (Days)		**+14d**	**±20d**	**+14d**	**±20d**	**+14d**	**+20d**		**+60**	**+60**	**+3d**	**+7d**	**+14d (+7d for AIT)**	**+20d**
Blood Sampling††	BL1		BL2	BL3	BL4	BL5			BL6 (all subjects)	BL10 (subgroup b only)			BL9 or BL13‡‡	
30-min observation period	√		√		√				subgroup a: V07 Y1 subgroup b: V07 Y2		√	√	√	
Injection site reactions & systemic events§§	√	√	√	√	√	√								
Diary Card (DC): Provided:	DC1		DC2		DC3				DC4 or DC4 Group b or MA2	DC5				
Checked and/or collected:		DC1		DC2		DC3			***	DC4 Group b ***	DC4/DC5	DC4/DC5	DC4/DC5	
Memory Aid (MA): Provided:						MA1							MA2	
Checked:							MA1		MA1					MA2
Termination Record													√	
SAEs and Serious AESIs†††	To be reported throughout the study period													
	THE FOLLOWING ADDITIONAL PROCEDURES APPLY ONLY TO THE AIT SUBSET													
Additional neutralizing Ab											BL7 or BL11‡‡	BL8 or BL12‡‡		
Affinity maturation and specificity assays	BL1					BL5			BL6‡‡	BL10‡‡	BL7 or BL11‡‡ ‡‡‡	BL8 or BL12‡‡ ‡‡‡	BL9 or BL13‡‡	
CMI	BL1								BL6‡‡	BL10‡‡	BL7 or BL11‡‡	BL8 or BL12‡‡	BL9 or BL13‡‡	

STAGE I									STAGE II*					
Visit Number (V)	**V01**	**V02**	**V03**	**V04**	**V05**	**V06**	**6M FU (PC7)†**	**V06_Inf**	**V07 (Y1)**	**V07 (Y2)**	**V08 (Y1/Y2) (AIT only)**	**V09 (Y1/Y2) (AIT only)**	**V10 (Y1/Y2)**	**6M FU (PC)‡**
Trial timelines (Days/Months)	**V01**	**V01+ 28d**	**V01+ 180d**	**V03+ 28d**	**V01+ 365d**	**V05+ 28d**	**last inj + 6M**		**V05+ 12M**	**V05+ 24M**	**V07+ 7d**	**V07+ 14d**	**V07+ 28d**	**V07+ 6M**
Time Windows (Days)		**+14d**	**±20d**	**+14d**	**±20d**	**+14d**	**+20d**		**+60**	**+60**	**+3d**	**+7d**	**+14d (+7d for AIT)**	**+20d**

NAb: neutralizing antibodies; CMI: cellular mediated immune response; Aff: affinity; AIT: Additional Immunological Testing subset.

* As described in the synopsis, dengue seronegative subjects at baseline will attend Y1 V07 and have the possibility to provide BL6 once having consented to continue in the trial. After the Y1 V07, they will no longer have to come back to the site.

† See Table of procedures (Phone Contacts). Subjects having prematurely terminated the trial will be contacted by phone for the 6-month safety follow-up.

‡ See Table of procedures (Phone Contacts). Subjects eligible to booster injection will be contacted by phone for the post-booster injection 6-month safety follow-up (PC12 for subjects from subgroup a and PC15 for subjects from subgroup b). Subjects non-eligible to booster injection will be contacted by phone as part of the safety follow-up.

§ Axillary temperature will be obtained for vaccination visits; also, note that febrile illness on the day of vaccination is a temporary contraindication.

** Urine pregnancy test for females of child-bearing potential; to be considered of non-child-bearing potential, a female must be 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 and until at least 3 weeks after vaccination

†† Blood samples planned during vaccination visits will be taken before injection. Baseline YF and JE status will be assessed using PRNT.

‡‡ BL6 is to be taken in all subjects 1 year after last injection (at V05 + 12M). BL10 is to be taken in subjects from subgroup b 2 years after last injection (at V05 + 24M). BL7 through BL9 are taken in subgroup a only; and BL11 through BL13 are taken in subgroup b only.

§§ 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.

*** DC4 will be provided to subgroup a to collect the full safety information after booster injection at Y1. DC4 Group b will be provided to subgroup b to collect SAEs from V07 Y1 to V07 Y2. DC5 will be provided to subgroup b to collect full safety information after booster injection at Y2. Dengue seronegative subjects at baseline from both subgroups will be provided with MA2.

††† 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 (ie, hypersensitivity / allergic reactions) will be reported within 7 days after each injection.

‡‡‡ At these time points, Ab specificity may be assessed, if necessary; Ab affinity maturation will not be assessed.

Table of Procedures – Phone Contacts

	STAGE I									STAGE II					
Phone Contact (PC)	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7***	**PC8**	**PC9**	**PC10**	**PC11**	**PC12***	**PC13†**	**PC14†**	**PC15†**
Trial timelines (Days/Months)	**V01+ 2M**	**V01+ 4M**	**V01+ 8M**	**V01+ 10M**	**V01+ 14M**	**V01+ 16M**	**V01+ 18M**	**V01+ 20M**	**V01+ 22M**	**V05 + 14M**	**V05 + 16M**	**V05 + 18M**	**V05 + 20M**	**V05 + 22M**	**V05 + 30M**
Time Windows (Days)	**+8d**	**+8d**	**+8d**	**+8d**	**+8d**	**+8d**	**+20d**	**+8d**	**+8d**	**+8d**	**+8d**	**+8d**	**+8d**	**+8d**	**+20d**
Ask about SAEs not yet reported‡	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Remind subject / parent / legal acceptable representative to notify the site in case of an SAE	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
Review MA§															
subgroup a					MA1	MA1	MA1	MA1	MA1	DC4 or MA2	DC4	DC4			
subgroup b					MA1	MA1	MA1	MA1	MA1	DC4 Group b or MA2	DC4 Group b	DC4 Group b	DC4 Group b	DC4 Group b	MA2

* At PC7, all subjects will receive a post-Injection 3 safety follow-up phone call. At PC12, subgroup a subjects will receive the post-booster injection safety follow-up phone call, while subjects in subgroup b will receive a standard phone call from the study center.

† PC13 to P15 will be made only for subgroup b subjects. PC15 will be subgroup b post-booster injection safety follow-up phone call

‡ If an SAE occurred, instructions in Section 10 for reporting are to be followed

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

List of Abbreviations

Ab	antibody
ASC	antibody secreting cells
AE	adverse event
AESI	adverse event of special interest
AF	assent form
AIT	additional immunological tests
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AR	adverse reaction
AST	aspartate aminotransferase
BL	blood sample
BSA	bovine serum albumin
$CCID_{50}$	cell-culture infectious dose 50%
CDM	Clinical Data Management
CI	confidence interval
CMI	cell-mediated immunity
CQA	Clinical Quality Assessment
CRA	Clinical Research Associate
CRF; eCRF	case report form; electronic case report form
CRO	contract research organization
CTA	clinical trial agreement
CTL	Clinical Team Leader
CYD	chimeric yellow fever/dengue
D	day
DC	diary card
DF	dengue fever
DHF	dengue hemorrhagic fever
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
FAS	full analysis set
FDA	Food and Drug Administration
FUP	follow-up
FV	flavivirus
FVFS	first visit, first subject

FVLS	first visit, last subject
GCI	Global Clinical Immunology
GCP	Good Clinical Practice
GMR	geometric mean ratio
GMT	geometric mean titer
GMTR	geometric mean titer ratio
GPV	Global PharmacoVigilance
IATA	International Air Transport Association
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
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
JE	Japanese encephalitis
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	not-reportable
NS	non-structural
OD	optical density
PBMC	peripheral blood mononuclear cells
PC	phone call
PD	post-dose
PFU	plaque forming unit
PI	Principal Investigator
PLA	placebo
PPAS	Per-Protocol analysis set
PRNT	plaque reduction neutralization test

PSO	Product Safety Officer
RCTM	Regional Clinical Trial Manager
RDCD	Regional Director Clinical Development
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
SET	safety evaluation team
TMF	trial master file
ULOQ	upper limit of quantification
VCD	virologically-confirmed dengue
V	Visit
VE	vaccine efficacy
WHO	World Health Organization
WT	wild-type
YF	yellow fever

1 Introduction

1.1 Background

This study is a two-stage Phase II trial of Sanofi Pasteur's CYD dengue vaccine that will assess the immunogenicity and safety of CYD dengue vaccine in healthy adolescents and adults who will receive 3 primary doses of the tetravalent CYD dengue vaccine (each dose administered 6 months apart) (STAGE I) and a booster dose of the same vaccine either 1 or 2 years following the last dose in the primary series (STAGE II). The CYD65 study will be conducted in two endemic countries, one in Latin America (Colombia) and one in Asia (the Philippines) and will include subjects from 9 to 50 years of age. This age group was chosen based on the long-term follow-up from previous studies, where it was observed that not all subjects below 9 years of age benefitted from CYD dengue vaccine.

STAGE I will test the non-inferiority (NI) of neutralizing antibody (Ab) responses elicited by a 2-dose or 1-dose schedule when compared to a 3-dose schedule, 28 days after the last injection in the primary series. The comparisons that achieve NI will also be evaluated for superiority. STAGE II is designed to assess whether neutralizing Ab levels can be boosted through the stimulation of the immunological memory with a CYD dengue vaccine dose at 2 different time points, either 1 or 2 years after administration of the last dose in the primary series.

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), 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 (1). The primary cell subset infected after inoculation is composed of 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.

The mosquito vectors for dengue viruses, *Aedes aegypti* and *Aedes albopictus*, are present in all tropical and sub-tropical areas of the world and in some temperate areas of the 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 (2). 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 (3).

In endemic areas, DF is suspected in patients who develop sudden fever, headache, myalgia, 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, 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 (NS) protein 1 antigen by enzyme-linked immunosorbent assay (ELISA) (4) (5) (6). 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 (eg, dengue 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 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 FV-naïve and -immune populations were 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) was completed in 2012 (7). Subjects from CYD23 are being followed for safety in a long-term follow-up study (CYD57).

[a] As of October 2017, the CYD dengue vaccine (commercial name Dengvaxia®) has been licensed in 19 countries (in alphabetical order): Argentina, Australia, Bangladesh, Bolivia, Brazil, Cambodia, Costa Rica, El Salvador, Guatemala, Honduras, Indonesia, Malaysia, Mexico, Paraguay, Peru, the Philippines, Singapore, Thailand, and Venezuela for the prevention of dengue disease caused by all 4 virus serotypes (1, 2, 3, 4) in individuals 9 years and above living in endemic areas.

  - In Colombia, Honduras, Mexico and Puerto Rico, a Phase II study (CYD13) was completed in 2011; 600 subjects received 3 injections of CYD dengue vaccine or a placebo/control vaccine[a]. In Brazil, a Phase II study (CYD30) was completed in 2012; 150 subjects received 3 injections of CYD dengue vaccine or a control vaccine (Placebo). In both studies, subjects were aged between 9 and 16 years and were vaccinated at 0, 6, and 12 months with a 6 months safety follow-up after the third injection. Study objectives were to describe immunogenicity and safety of CYD dengue vaccine. Overall, CYD dengue vaccine in Latin America 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 (8) (9).

- In Phase III trials, approximately 694 adult subjects, 20,968 children aged between 2 and 17 years, and 1478 toddlers, or 23,140 subjects overall, received at least 1 dose of Phase III lots of CYD dengue vaccine (4.5 – 6.0 $\log_{10}$ $CCID_{50}$ per serotype). Two large efficacy studies (CYD14 (10) and CYD15 (11)), conducted in Asia Pacific (in children and adolescents from 2 to 14 years old) and Latin America (in children and adolescents from 9 to 16 years old) have completed the 2-year follow-up after the third injection (12). The 2 large efficacy studies were sufficiently powered to demonstrate significant efficacy of the CYD dengue vaccine in preventing the occurrence of virologically-confirmed dengue (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 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 (12).
  - In Colombia and Peru, a Phase III trial was completed in 2013; a total of 792 healthy toddlers received an injection of Yellow Fever (YF) vaccine concomitantly with the first dose of CYD dengue vaccine at 12-13 months of age. Study objectives were to describe immunogenicity and safety of YF vaccine administered concomitantly with the first dose of CYD dengue vaccine. This trial showed that the co-administration of YF with CYD dengue vaccine did not impact the immunogenicity and safety for CYD dengue vaccine in toddlers. This study also confirmed the good safety profile of CYD dengue vaccine in pediatric population as observed in previous Phase I and II trials in a 3-dose regimen (13)
  - In Mexico, a Phase III trial was completed in 2014; a total of 732 healthy toddlers received a booster injection of DTaP-IPV//Hib vaccine concomitantly with the second dose of CYD dengue vaccine at 15 to 18 months of age. Study objectives were to describe immunogenicity and safety of a booster injection of DTaP-IPV//Hib when administered concomitantly with the CYD dengue vaccine. This trial showed satisfactory safety and immune responses with the co-administration of DTaP-IPV//Hib and CYD dengue vaccines (14).

a Control Group received a placebo (NaCl) as first and second injections and ADACEL vaccine as third injection

Therefore, as of April 2015, around 28,500 subjects have received at least 1 dose of the final CYD dengue vaccine formulation, regardless of the administration schedule. Results demonstrates 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 milliliters (mL) to be administered at 6-month intervals.

As of December 2017, the claimed indication is in the process of being reassessed.

1.3 Potential Benefits and Risks

Detailed risk/benefit analysis is presented in the Investigator's Brochure (IB). As of December 2017, the IB is in the process of being amended with the most recent safety information.

1.3.1 Potential Benefits to Subjects

The subjects participating in the present clinical trial and receiving the CYD dengue vaccine may develop immunity and protection against dengue disease after vaccination with CYD dengue vaccine.

Moreover, being injected with a CYD dengue vaccine booster dose 1 or 2 years following last injection may bring back subjects' immune response against dengue to the level (or even above the level) following primary vaccination. This is certainly an important perspective in light of recently published data (15) (16).

Results from two large-scale Phase III efficacy trials showed the potential for CYD dengue vaccine to reduce the probability of a subject's having 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.

A number of supplemental analyses (see Section 1.4) have provided strong evidences that, for subjects that were dengue seropositive prior to CYD dengue vaccination, the vaccine protects against symptomatic dengue, hospitalized dengue, and severe dengue disease.

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 vaccine, a risk of allergic reaction (including anaphylactic reaction) cannot be excluded. Vasovagal malaise linked to the injection procedure may be observed in susceptible individuals. The full list of expected AEs can be found in the Investigator's Brochure.

As CYD dengue vaccine has a 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 (especially in children from 2 to 5 years of age), the combined analysis of the efficacy trials during the same year 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 because of dengue infection fully recovered after receiving appropriate supportive treatment (12).

Following a number of supplemental analyses (see Section 1.4), it was shown that subjects that were dengue seronegative prior to CYD dengue vaccination have an increased risk of hospitalized or severe disease, as compared to subjects who received the placebo.

Placebo

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

All Subjects

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

1.4 Rationale for the Trial

Dengue is endemic in most of Latin America, and in the Caribbean, Southeast Asian and Western Pacific countries. Despite dengue control programs, case management guidelines, and vector control efforts, dengue virus transmission remains high and prevention remains a public health priority.

Table 1.1 provides an overview of the number of suspected dengue cases and the incidence rate between 2014-2016 in Colombia and the Philippines.

Table 1.1: Number of suspected dengue cases in Colombia and the Philippines (2014-2016)

Country	Year	Number of suspected dengue cases*	Incidence rate (per 100,000 population)	Reference
Colombia	2014	105,356	215.32	(17)
	2015	96,444	194.72	(18)
	2016	103,822	209.62	(19)
Philippines	2014	113,485	122.90	(20)
	2015	200,415	196.81	(20)
	2016	220,518	219.0	(20)

* Suspected dengue case: person showing symptoms compatible with dengue with or without warning signs, or severe dengue, with no laboratory or epidemiological confirmation.

Trends in dengue infection change over time, and epidemiologic patterns observed vary not only across countries, but also within countries. As such, it should be noted that comparisons of dengue incidence rates between countries (and provinces) may be inappropriate because their reporting practices of dengue cases may vary.

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 VE was demonstrated in subjects from 2 to 16 years of age (15) (16). 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 the 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 (ie, higher Ab levels decrease the probability of dengue disease) (10) (11), and although 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.

Despite these favorable findings, there are still relevant and important issues to address: one is establishing efficacy persistence over time in vaccinated subjects; another is establishing the actual benefit of the third dose in the 3-dose vaccination schedule relative to the overall immunogenicity of the vaccine. While the CYD dengue vaccine demonstrated immune response to any dengue serotype of 64.7% (95% CI, 58.7-69.8) in the CYD15 trial (15) and 56.5% (95% CI 43.8-66.4) in the CYD14 trial (16), and in both trials the primary endpoint was achieved, data from follow-up trials studying levels of neutralizing Abs in a 3-dose primary vaccination series (with doses given at 6-month intervals) (15) (16) (21) (22) (8) (9) have shown a tendency for the GMTs to decrease over time. In CYD15, when measured at 28 days post-Dose 3, the level of GMTs had decreased by almost 20%, when compared to GMT levels measured 28 days post-Dose 2. The peak level of GMTs may have even been achieved 28 days post-Dose 2 or even

earlier, at 28 days post-Dose 1, but this is speculation, as there was no measurement made at this time point. Another factor to be considered for the continued development of the CYD dengue vaccine, due to its possible influence on GMT levels, is dengue baseline serological status. Subjects with baseline seropositivity have shown higher GMT levels than baseline seronegative subjects, when GMT levels were assessed 28 days after the last primary series dose.

On the basis of these data, the effect of the third dose of the CYD dengue vaccine on immunogenicity has to be challenged by the possibility of a primary series vaccine schedule consisting of 1 or 2 doses.

Data generated by previous studies have also demonstrated the need to develop a booster vaccination strategy that will help to maintain acceptable levels of dengue neutralizing Abs over time. Although the humoral neutralizing immune response tends to wane after the third dose in the 3-dose vaccination schedule, neutralizing Ab levels continue to remain generally high after at least 1 year, when compared to baseline for each age group. In the CYD14 trial, although there was a decrease in neutralizing Ab GMT levels for all serotypes at 2 years post-injection, when compared to the levels after the third injection, the decrease was minimal compared to the decrease observed 1 year post-Dose 3 in the primary series.

Finally, the trial will assess the safety of the primary series vaccine schedules and the booster vaccination and ensure that booster vaccination does not raise safety issues that were not observed during and following the primary vaccination schedule. Serious adverse events (SAEs) will be assessed throughout the trial, up to 6 months following the booster (1 or 2-year injection). Serious and non-serious adverse events of special interest (AESIs) specific for dengue trials will be collected in defined time windows according to the type of AESI.

Protocol Amendment 1

This amended protocol introduces 3 important changes to the original protocol of the ongoing CYD65 clinical study.

The first change is related to the safety of the subjects included in the trial. In July 2016, the WHO issued a position paper on Sanofi Pasteur's CYD dengue vaccine based on the Strategic Advisory Group of Experts' (SAGE) assessment that recognized its potential public health value when introduced in highly endemic countries. In addition, the SAGE also underlined the importance of addressing the question of the potential risk, over time, of hospitalized/severe dengue in individuals with no prior exposure to dengue before vaccination (23) (24). Other scientific, public health, and regulatory leaders have expressed similar interest in obtaining more information on the long-term safety and efficacy of the CYD vaccine in seronegative individuals.

Sanofi Pasteur recognized this knowledge gap and remained committed to further evaluate the performance of the CYD vaccine.

Analyzing long-term safety according to dengue serostatus at baseline presented an important challenge as serostatus had only been assessed in a subset of subjects (the so-called immunogenicity subset) in each of the 3 efficacy studies (CYD14, CYD15 and CYD23/57). As a consequence of this, and in order to address the question of vaccine performance in seronegative individuals, Sanofi Pasteur decided to conduct an Exploratory Case-Cohort analysis using a time point for which a blood sample was collected in all study participants: approximately 1 month after the third injection of CYD dengue vaccine or placebo (month [M] 13). The rationale behind

this approach was that the classification of study participants according to dengue serostatus at this time point (as a surrogate of prior natural dengue exposure) could be used as a baseline for the evaluation of outcomes that occur later. However, the PRNT assay routinely used to quantify neutralizing Ab titers cannot discriminate between neutralizing Abs against wild-type dengue virus and chimeric dengue virus. Said otherwise, a positive PRNT assay at M13 can be the result of either prior dengue exposure or CYD dengue vaccination.

To overcome this challenge, Sanofi Pasteur leveraged an assay originally developed at University of Pittsburg (Pittsburg, PA, USA) and optimized by Sanofi Pasteur's Global Clinical Immunology (GCI) Department. This assay measures total immunoglobulin G (IgG) antibodies against the non-structural protein 1 (NS1) of the dengue virus by ELISA. Because the NS1 protein is not conserved between the dengue virus and the yellow-fever virus, previous exposure to CYD dengue vaccine is not expected to induce meaningful levels of antibody against the dengue NS1 protein. The application of the Dengue anti-NS1 IgG ELISA assay to M13 samples was therefore considered useful for expanding the existing data on both VE and potential risk of dengue hospitalization and/or severe dengue according to baseline serostatus in the CYD dengue vaccine efficacy trials. Thus, dengue serostatus was used in a supplemental case-cohort study as a covariate to assess the effects of CYD dengue vaccine for outcomes that occur after M13. In addition, the Dengue anti-NS1 IgG ELISA values were used in conjunction with multiple additional variables in imputation models to predict the D0 PRNT50 serostatus, to evaluate outcomes occurring after M0 and M13 by measured (where available), or imputed PRNT50 serostatus.

Sanofi Pasteur presented the full data of this supplemental analysis to the IDMC in an *ad hoc* meeting held on 3-4 November 2017. During this meeting, the IDMC reviewed the data from these extended safety and efficacy analyses. It concluded that, in the case of subjects exposed to dengue prior to vaccination (henceforth, 'exposed subjects' or seropositive subjects), there is strong evidence that the vaccine protects them from symptomatic dengue, hospitalized dengue and severe dengue. In the case of subjects not exposed to dengue before vaccination (henceforth, 'unexposed' subjects or seronegative subjects), the conclusion was that although vaccination may confer limited short-term benefit against symptomatic dengue, it also induces an increased risk of severe disease in the longer term. The IDMC stated that these findings are based on follow-up of dengue unexposed subjects having received 3 CYD dengue vaccine doses and no data exist to conclude if the risk in partially vaccinated dengue unexposed subjects is different from that in fully vaccinated dengue unexposed subjects.

Given these conclusions, the IDMC recommended that no further vaccination occur in unexposed subjects in ongoing or future trials, and on precautionary basis, including partially vaccinated subjects in ongoing trials. In addition, they recommended making available information on baseline serostatus for all vaccinated subjects whenever possible. Finally, for unexposed subjects that were vaccinated during a study, the IDMC recommended instituting mechanisms to provide timely access to appropriate care in the event of suspected dengue, for 10 years from the date of last vaccination.

Given the IDMC recommendations, Sanofi Pasteur is amending this study protocol. As a general rule, only subjects assessed as dengue exposed at baseline (ie, before receiving the first CYD dengue vaccine injection) will be eligible to receive any further dose of CYD dengue vaccine in an ongoing study. In CYD65, all subjects will be informed about their serostatus at baseline and

what it means. Moreover, all subjects will be asked about their willingness to continue participating in this study by signing an updated ICF and/or AF, as applicable. Subjects assessed as dengue exposed will confirm their participation in the study and will continue to be eligible to receive the booster injection at Year 1 (Y1) or Y2. Subjects classified as unexposed (seronegatives) at baseline, will only be able to continue in the study for safety follow-up and for the evaluation of antibody persistence at Y1 (this procedure was planned for subgroup a; subjects from subgroup b will provide a blood sample after giving their consent).

To determine the basal serostatus of subjects already included in the study, the Dengue PRNT will be used.

The second change to the original protocol consists in a shift of the study purpose. It consists of the addition of a co-primary objective, to determine the non-inferiority of the immune response to the vaccine 1 year after the last dose (3 doses versus 2 doses schedule). Initially, CYD65 aimed to assess how the CYD dengue vaccine performs when administered according to a 1-, 2-, or 3-dose regimen. This was reflected in the Primary Objectives as both the 1-dose and the 2-dose regimen were to be compared to the standard 3-dose regimen in terms of safety and immunogenicity. Through Amendment 1 of the protocol, the Sponsor re-centered the CYD65 study around the 2-dose versus 3-dose comparison. As a consequence of this decision, Primary Objectives for both Stage I and Stage II have been modified. In summary, a second non-inferiority test between Group 2 and Group1 will be performed using Ab titers at 1 year after the last injection in the Stage I. Conversely, non-inferiority tests between Group 3and Group 1 will no longer be performed. Rather, the immunogenicity of the 1-dose regimen will be described as part of the Additional Objectives.

The third and final change is related to the above-mentioned shift in the study purpose. It consists in the addition of a visit to the study centers for subjects who did not have such a visit planned in the original protocol. This corresponds to subjects initially assigned to subgroup b (approximately 525subjects). It will thus require the subjects and / or their parents / legally accepted representative(s) to provide their written consent. Subjects originally assigned to subgroup b will be asked to provide a blood sample at 1 year post last primary series injection, regardless of whether or not they are eligible to receive a booster vaccination. This will allow assessing whether there are differences in the persistence of neutralizing Abs between the 3 primary doses regimens.

2 Trial Objectives

2.1 Primary Objectives

STAGE I:

- To demonstrate the non-inferiority (NI) of the immune response elicited against each dengue serotype by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 28 days post-final injection, in terms of Geometric Mean Ratio (GMR).
- To demonstrate the NI of the immune response elicited against each dengue serotype by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 1 year after last injection, in terms of GMR.

STAGE II:

- To demonstrate the NI of the immune response elicited against each dengue serotype, in subjects seropositive at baseline, 28 days after administration of a booster dose of CYD dengue vaccine, in terms of geometric mean of titer ratio (GMTR, within a group) or GMR (between groups):

  Booster Dose at One Year

  - Post-Year 1 booster Group 1/post-Dose-3 Group 1 (GMTR)
  - Post-Year 1 booster Group 2/post-Dose 3 Group 1 (GMR)*

  Booster Dose at Two Years

  - Post-Year 2 booster Group 1/post-Dose 3 Group 1 (GMTR)
  - Post-Year 2 booster Group 2/post-Dose 3 Group 1 (GMR)*

** Note: The Stage II Primary Objectives involving Group 2 will apply only if NI has been achieved in Stage I; within Group 1 testing in STAGE II will be performed regardless of the STAGE I test results*

If non-inferiority is not achieved, immunogenicity-related analyses will be descriptive. The endpoints for the primary objectives are presented in Section 9.1.1.1.

2.2 Secondary Objectives

Immunogenicity

STAGE I:

- To demonstrate the superiority of the immune response elicited by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 28 days post-final injection, in terms of GMR.

- To demonstrate the superiority of the immune response elicited by the CYD dengue vaccine given as a 2-dose schedule (Group 2) compared to the immune response elicited by CYD dengue vaccine given as a 3-dose schedule (Group 1), in subjects seropositive at baseline, 1 year post-final injection, in terms of GMR

Note: NI has to be achieved before assessing for superiority

- To describe the neutralizing Ab levels of each dengue serotype at 28 days post-Injection 3 to the Ab levels immediately before receiving booster injection, by baseline serostatus, in all 3 groups.
- To describe the neutralizing Ab levels of each dengue serotype at 28 days post-Injection 2 and 28 days post-Injection 3 from Group 1 in a primary series schedule, by baseline serostatus.

STAGE II:

- To demonstrate the superiority of the immune response elicited against each dengue serotype, in subjects seropositive at baseline, 28 days after administration of a booster dose of CYD dengue vaccine in terms of GMTR (within group) or GMR (between groups):

Booster Dose at One Year

- Post-Year1 booster Group 1 compared to post-Dose 3 Group 1 (GMTR)
- Post-Year 1 booster Group 2 compared to post-Dose 3 Group 1 (GMR)

Note: NI has to be achieved before assessing for superiority

Booster Dose at Two Years

- Post-Year 2 booster Group 1 compared to post-Dose 3 Group 1 (GMTR)
- Post-Year 2 booster Group 2 compared to post-Dose 3 Group 1 (GMR)

Note: NI has to be achieved before assessing for superiority

- To describe the seroconversion rate 28 days after booster injection in all 3 groups.

Safety

STAGE I and STAGE II

- To describe all hospitalized VCD cases that have occurred at any time during the trial
- To evaluate the safety profile of CYD dengue vaccine after each and any injection (reactogenicity; unsolicited AEs; SAEs throughout the trial and for 6-months following any injection; and AESIs at defined time windows throughout the trial according to the type of AESI)

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

2.3 Additional Objectives

Immunogenicity

STAGE I:

- To describe the PRNT Ab response of FVs (dengue/Japanese Encephalitis [JE] in the Philippines, and dengue/YF in Colombia) serological status by age group and country at baseline in all 3 groups
- To describe the neutralizing Ab response to each dengue serotype at each available time point, by baseline serostatus, in all 3 groups
- To compare the immune response elicited against each dengue serotype by the CYD dengue vaccine given as a 2-dose schedule (Group 2) or a 1-dose schedule (Group 3) and given as a 3-dose schedule (Group 1) at 28 days after last injection, in subjects seropositive at baseline, in terms of GMR.
- To compare the immune response elicited against each dengue serotype by the CYD dengue vaccine given as a 2-dose schedule (Group 2) or a 1-dose schedule (Group 3) and given as a 3-dose schedule (Group 1) at 1 year after last injection, in subjects seropositive at baseline, in terms of GMR.

STAGE II:

Booster Dose at One and Two Years

- To describe the neutralizing Ab response to each dengue serotype 28 days after a booster injection

One Year Booster

- To describe the immune response elicited against each serotype 28 days after administration of a booster dose of CYD dengue vaccine 1 year after the last primary series injection in a 2-dose schedule (Group 2) or 1-dose schedule (Group 3) when compared to a 3-dose schedule (Group 1)

Two Year Booster

- To describe the immune response elicited against each serotype 28 days after administration of a booster dose of CYD dengue vaccine 2 years after the last primary series injection in a 2-dose schedule (Group 2) or 1-dose schedule (Group 3) when compared to a 3-dose schedule (Group 1)

Additional Immunological Tests (AIT) Subset Only:

- To describe dengue neutralization Ab levels (exploration of Ab response kinetics), Ab specificity and affinity maturation in a subset of subjects (N=60) in all groups (Ratio between subgroups 1:1 = 10 subjects per subgroup).
- To describe cell-mediated immune (CMI) responses at Day 0 (STAGE I) and at 0, 7, 14, 28 days post booster dose (Y1 and Y2) in this subset of subjects participating in all groups.

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

3 Investigators and Trial Organization

This study will be conducted in approximately 6 centers in 2 countries: Colombia (3 sites) and the Philippines (3 sites). The Principal Investigators and any sub-investigators at the individual sites will be coordinated by one Coordinating Investigator. Details of the trial centers, the Investigators at each center, and the Coordinating Investigators 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 VCD cases[a], including assessment of severity. Additionally, any related SAE or death or serious AESIs will be promptly reviewed by the IDMC.

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

The laboratories involved in this study will be:

- 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 contract research organization (CRO) or performed in-house by the Sponsor.

The Sponsor's Responsible Medical Officer (RMO) (the person authorized to sign this version of the protocol and future amendments on behalf of the Sponsor) is [redacted].

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 (ICF), and assent form (AF), 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).

[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 serious VCD dengue case if there is a detection of wild type (WT) dengue virus by NS1 antigen ELISA and/or wild-type dengue RT-PCR.

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 / IRB annually, or more frequently if requested. According to the local IEC / IRB policy, either all SAEs occurring during the trial that are related to vaccination will be reported by the Investigator to the IEC / IRB.

5 Investigational Plan

5.1 Description of the Overall Trial Design and Plan

5.1.1 Trial Design

This observer-blind, randomized, Phase II non-inferiority trial will be conducted in 2 sequential stages.

At enrollment, for STAGE I, approximately 1050 healthy subjects between 9 and 50 years of age will be randomized (1:1:1) to 1 of 3 treatments arms (Groups 1, 2, or 3) to receive 3 injections (in various schedules of CYD dengue vaccine and placebo administration) over a 12-month period (administered 6 months apart: at D0; D0 + 6 months; and D0 + 12 months).

For STAGE II, subjects randomized to each of the 3 treatment arms in STAGE I will also be randomized (1:1) to 1 of 2 subgroups (eg, subgroup a or b). The subject's dengue serostatus at baseline, based on plaque reduction neutralization test (PRNT), will determine her/his eligibility to receive the booster injection. Only the subjects identified as dengue seropositive at baseline will receive a booster injection of CYD dengue vaccine at either 12 months (Subgroup a, Injection 3 + 12 months) or 24 months (Subgroup b, Injection 3 + 24 months) following the last primary series injection.

At each injection visit in STAGE I, subjects will receive a single dose of either CYD dengue vaccine (CYD) or placebo (PLA): Group 1 subjects (N = 350) will receive CYD dengue vaccine at all 3 injection visits; Group 2 subjects (N = 350) will receive PLA at the first injection visit and CYD dengue vaccine at the last 2 visits; and Group 3 subjects (N = 350) will receive PLA at the first 2 visits and CYD dengue vaccine at the last injection visit.

A total of 60 subjects (10 subjects in each of 6 subgroups) who consent to participate in additional immunological testing will also be included in a specific subset (AIT subset). It is to be noted that the 1:1 ratio per subgroup is unlikely to hold as only subjects dengue seropositive at baseline will be eligible to booster injection.

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

The duration of each subject's participation in the trial will be approximately 30 to 42 months.

5.1.2 Justification of the Trial Design

The trial design will enable a thorough evaluation of the administration of alternate dosing regimens (1- and 2-dose regimens) when compared to a 3-dose schedule which is considered the optimum prophylactic intervention against dengue infection in endemic countries. This design will also help evaluating the benefits provided to subjects after booster vaccination with the CYD dengue vaccine 1 or 2 years after receipt of the final dose in the primary series.

Sanofi Pasteur's Clinical Development Plan for dengue includes a High Priority Trials repertoire consisting of 3 trials – CYD63, CYD64 and CYD65. The aim of the first 2 trials, is to assess a booster dose of CYD dengue vaccine 4 to 5 years after administration of the last dose of a 3-dose schedule (21) (22) (9). The aim of the current two-stage CYD65 trial is to evaluate the possibility of achieving the same immune response achieved by the 3-dose vaccine schedule with 2 doses or even 1 dose; it is also to demonstrate the benefits of booster vaccination with CYD dengue vaccine 1 or 2 years after receipt of the final dose in the primary series. The selection of these specific time points for booster injection stemmed to a large extent from results seen in the CYD05 trial. When reductions in GMT levels were measured at 28 days post-Dose 3 and again at 1 year following this third dose, subjects who had lower GMTs at 28 days post-Dose 2 had the highest increases of GMTs 28 days after Dose 3 (6-month interval between doses). When assessed 1 and 2 years after the last primary injection, subjects in Group 2 (who had received 2 CYD doses with an interval of 8.5 months), had reductions in titers when compared to the levels 28 days after dose 2, but the GMT levels were still higher than GMT levels measured at pre-injection 1 for all serotypes. In CYD05, it was not until 3 years after the final primary injection, that GMT levels for serotypes 1 and 2, in particular, were lower than they had been at pre-injection 1.

Based on these data, administration of a booster injection 1 year or 2 years after the last primary dose might benefit populations in endemic regions in terms of maintaining neutralizing Ab levels.

Also, knowing that in endemic countries the possibility of having previous contact with FV is extremely high, and knowing also that baseline seropositivity is associated with higher Ab response to the vaccine, the CYD65 trial will evaluate the baseline serostatus for previous exposure to dengue/YF in Colombia, and for dengue/JE in the Philippines before the administration of all 3 dosing schedules in the primary vaccination series, as well as the effect of exposure to FVs might have on the immunogenicity of the vaccine.

5.1.3 Trial Plan

STAGE I:

Before inclusion, the Investigators will inform potentially eligible subjects and / or their parents / legally accepted representative(s) about the trial and will give them an oral description of the trial design, with the general risks and benefits that have been associated with the trial and the

injections. Then, each subject / subjects' parent(s) / legally acceptable representative(s) will sign and date consent forms (ICF and AF, as applicable). An interactive voice response system / interactive web response system (IVRS/IWRS) will be used to assign treatment groups and subject numbers at each clinic site. Table 5.1 presents the number of subjects that will be randomized to each treatment group and the schedule for the administration of CYD / PLA injections for each group.

Vaccination

All subjects (N=1050) will receive 1 injection at Day (D) 0, D180 and D365: a total of 350 subjects will receive 3 doses of CYD vaccine (Group 1); 350 subjects will receive 1 PLA injection followed by 2 doses of CYD vaccine (Group 2); and 350 subjects will receive 2 PLA injections followed by 1 dose of CYD vaccine (Group 3).

Table 5.1: STAGE I group assignments

Treatment Group	STAGE I		
	Schedule*	**N**	**Product Received**
Group 1	CYD1/CYD2/CYD3	350	CYD
Group 2	PLA1/CYD1/CYD2	350	PLA/CYD
Group 3	PLA1/PLA2/CYD1	350	PLA/CYD
Total		**1050**	

* Injections are given at D0, D0 + 6 months, and D0 + 12 months
CYD: CYD dengue vaccine; PLA: placebo.

Blood Sampling

All subjects will provide a blood sample at enrollment V01 (pre-Injection 1, BL1), at V03 (pre-Injection 2, BL2) at V04 (V03 + 28 days, BL3), at V05 (pre-Injection 3, BL4), at V06 (V05 + 28 days, BL5), and at Y1 V07 (V05 + 12M, BL6) for dengue immunogenicity (Table 5.2).

Table 5.2: STAGE I injection and blood sampling schedules

Procedure	**Product or Test**	**V01 (D0)**	**V02 (V01 + 28d)**	**V03 (V01 + 6M)**	**V04 (V03 + 28d)**	**V05 (V01 + 12M)**	**V06 (V05 + 28d)**	**Y1 V07 (V05+ 12M)**
Injection	**CYD or PLA**	Inj.1	Contact visit	Inj. 2		Inj. 3		
Collection of blood sample:	**Neutralizing Abs (all subjects)**	BL1* (pre-inj. 1.) 5 mL	N/A	BL2* (pre-inj. 2) 5 ml	BL3 5 mL	BL4* (pre-inj. 3) 5 mL	BL5 5 ml	BL6† 5 mL

* Dengue status at baseline will be assessed for each group prior to the first CYD injection (Group 1 at V01, Group 2 at V03, and Group 3 at V05)

† In the context of STAGE I, BL6 will be used to assess neutralizing Abs persistence at 1 year post last primary series injection. In STAGE II, BL6 will be used to assess neutralizing Ab levels at pre-booster injection for subjects from subgroup a that were seropositive at baseline

D or d=day; M=month; N/A=not applicable;

Blood samples will be taken from each subject for assessing neutralizing Ab titers against each of the 4 parental dengue virus strains at study baseline (immediately prior to first injection) and at pre-Injection 2, 28 days post-Injection 2, pre-Injection 3, 28 days post-Injection 3, and 1 year post-Injection 3. In addition to helping to maintain the blind, blood samples taken pre-Injection 2 and pre-Injection 3 will be used to assess the immunological response to the first dose in Group 1, and to identify possible natural infections in Groups 2 and 3 (2-dose and 1-dose regimen subjects, respectively). Blood samples taken at pre-Injection 2 will be used as baseline for Group 2 subjects receiving their first CYD dengue vaccine injection. Blood samples taken 28 days post-Injection 2 will provide information on post-Injection 2 GMTs in Group 2 (first CYD dengue vaccine administration); and blood samples taken at pre-Injection 3 will serve as baseline for Group 3 subjects receiving their first and only CYD dengue vaccine injection and to identify possible natural infections in Group 3. Finally, the blood sample taken 1 year post-injection 3 in all subjects will be used to assess Ab persistence.

To further understand the effects of exposure to wild-type dengue on vaccination against dengue in endemic areas, 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 will be collected for virological confirmation: an acute sample (within the first 5 days after fever onset[a]). A suspected hospitalized dengue case will be considered a VCD case if there is a detection of wild-type dengue virus by NS1 antigen ELISA and/or wild-type dengue RT-PCR.

STAGE II (Booster Year 1 or Year 2)

Before the beginning of STAGE II, blood samples collected before the first injection of CYD dengue vaccine will have been assayed with PRNT to determine each subject's dengue serostatus at baseline. The blood sample closest to the first injection of CYD dengue vaccine will be assayed in priority (ie, BL1 for Group 1; BL2 for Group 2 [or BL1 if BL2 in unavailable]; BL4 for Group 3 [or BL2 if BL4 is unavailable, or even BL1 if BL2 is unavailable]). Only subjects identified as dengue seropositive will be eligible to receive the booster injection. Dengue serostatus will be determined using the Dengue PRNT assay. A "seropositive" subject has been generally defined by a PRNT titer ≥ 1:10 to any dengue serotype at baseline, and a "seronegative" subject has been defined by a PRNT titer <10 for all four dengue serotypes. As the prevalence of

[a] Acute blood sample for all suspected hospitalized dengue cases should be collected within the pre-specified time frame described. If this cannot be accomplished, this sample should be obtained as soon as possible thereafter for IDMC severity assessment.

Zika was low in the regions where subjects were enrolled, at the time of the primary series, the potential cross-reactivity between Zika and Dengue virus in the Dengue PRNT assay is not expected to confound the determination of the participants' dengue serostatus at baseline.

At 1 year following the last primary series injection, subjects dengue seropositive at baseline from subgroup a (1a, 2a, and 3a) will receive a single booster dose of CYD dengue vaccine. At 2 years following their last primary series injection, dengue seropositive subjects from subgroup b (1b, 2b, and 3b) will receive a single booster injection of CYD dengue vaccine (Table 5.3).

Table 5.3: STAGE II subgroup assignments (only in subjects identified as seropositive at baseline)

Stage I Group	STAGE II		
	Subgroup	Post Inj 3 + 1 year	Post Inj 3 + 2 years
Group 1	1a	CYD booster	--
	1b	--	CYD booster
Group 2	2a	CYD booster	--
	2b	--	CYD booster
Group 3	3a	CYD booster	--
	3b	--	CYD booster

Each STAGE II subject will provide blood samples for assessing neutralizing Ab titers against each of the 4 parental dengue virus strains. The first blood sample will be collected in all subjects approximately 1 year post last primary series injection. This blood sample will be used to assess both the Ab persistence at 1 year in all subjects and the pre-booster Ab levels in subgroup a. Subjects receiving the booster injection will provide additional blood samples. Subjects from subgroup a will provide a second blood sample 28 days after receiving the booster injection. Subjects from subgroup b will provide blood samples immediately before and 28 days after receiving the booster injection.

After a dengue seronegative subject consented to continue in the study, the blood sample collected 1 year after the last primary series injection will be the last clinical procedure required from her/him. After this blood sample, a dengue seronegative subject will no longer be asked to come to the study site. However, the telephone contacts already planned in the study will be maintained as a safety follow-up.

STAGE I and STAGE II

Safety Data Collection

During both STAGE I and STAGE II, reactogenicity data will be collected in all subjects after any injection: immediate adverse events (AEs) observed to occur within 30 min post-injection will be collected; solicited injection site reactions will be collected for Days 0–7; solicited systemic reactions will be collected for Days 0–14; and unsolicited non-serious events will be collected for Days 0-28.

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 a diagnosis of dengue requiring hospitalization (with bed attribution).

Serious and non-serious AESIs will be collected in defined time windows according to the type of AESI. SAEs will be reported throughout the study and until 6 months after booster injection in both subgroups.

Additional Immunological Tests (AIT) Subset

In addition to the blood samples described above, samples will also be drawn from 60 subjects from a specific site in Colombia who consent to participate in the AIT subset.

For evaluations of CMI, Ab specificity and affinity maturation, blood samples will be taken from subjects immediately before Injection 1 (specificity, affinity and CMI) and at 28 days following Injection 3 (specificity and affinity) during STAGE I.

During STAGE II, only subjects identified as seropositive at baseline and thus eligible to booster injection will continue to be part of the AIT subset. The sampling from these subjects will continue into STAGE II when samples will be drawn immediately before the booster injection, and at 7 days, 14 days, and 28 days post-booster injection for Ab specificity and CMI; Ab affinity maturation will be assessed only prior to and 28 days post-booster injection.

Table 5.4 presents the subset of AIT study subjects to be tested for Ab specificity, affinity and CMI, following the administration of Year 1 or Year 2 boosters.

Table 5.4: Subset of subjects by age group and volume of blood samples taken for AIT

		Children and Adolescents (9 to 17 Years of Age)			Adults (18 to 50 Years of Age)		
STAGE	**Timeline**	**Neutralizing Abs**	**CMI**	**Total Volume**	**Neutralizing Abs**	**CMI**	**Total Volume / Visit**
		Volume for each assessment (mL)					
STAGE I	**D0 (pre-Injection 1)**	5*	25	30	5*	35	40
	28 days post-Injection 3	5*	--	5	5*	--	5
STAGE II (For booster dose at Y1 or Y2, as applicable)†	**D0 (pre- Booster)**	5*	25	30	5*	35	40
	Booster + 7 days	5‡	15	20	5†	25	30
	Booster + 14 days	5†	15	20	5†	25	30
	Booster + 28 days	5*	15	20	5*	25	30

* These samples will also be assessed for Ab specificity and affinity maturation.

† Besides the pre-booster injection blood sample, STAGE II blood samples will be drawn from subjects dengue seropositive at baseline alone.

‡ These sample will also be assessment for Ab specificity if required

5.1.4 Visit Procedures

Note: SAEs and hospitalized suspected dengue cases are to be reported throughout the study period. AESIs will be collected in defined time windows according to the type of AESI.

STAGE I

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

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

The Investigator or designated study personnel will:

1) Present the trial to the subject / subject's parent (s) / legally acceptable representative(s) 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 (ICF and AF, as applicable) signed and dated by the subject and / or the subject's parent (s) / legally acceptable representative(s), date and sign the ICF (only the Investigator). The Investigator will provide a copy to the subject (or

parent[s] / legally acceptable representative[s]) and retain the original document according to local policies or regulations

3) Collect demographic data (date of birth and gender)
4) Review the contraindications
5) Check and collect the subject's significant medical history
6) Check and collect the subject's history of JE or YF vaccination / infection and / or dengue infection / vaccination
7) Check concomitant medications and record every reportable medication ongoing at the time of vaccination
8) Perform a physical examination and record the subject's axillary temperature
9) Perform a urine pregnancy test (pre-injection) (women of childbearing potential[a] only)
10) Review the inclusion / exclusion criteria
11) If the subject is eligible, contact the Interactive Voice / Web Response System (IVRS / IWRS) to obtain a subject number and vaccine dose to be administered at V01
12) **For all subjects:** obtain the first blood sample (BL1, 5mL) for neutralizing Abs, record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of Ab neutralizing blood samples); the volume used for specificity and affinity maturation testing in the AIT subset will use this aliquot as applicable
13) **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 CMI 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 eCRF. In that case, and if the subject wants to participate in the trial, he/she will be vaccinated.

14) Inject the appropriate study vaccine
15) Record the date of injection, the site and side of injection and the route of administration, as well as the dose number of the vaccine
16) Affix the vaccine labels in the subject's source documents
17) Keep the subject under observation for 30 minutes, and record any adverse reaction in the source documents
18) Give the subject / subject's parent (s) / legally acceptable representative(s) the diary card 1 (DC1) to record any injection site reactions and systemic AEs, together with

[a] To be considered of non-child-bearing potential, a female must be 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 and until at least 3 weeks after vaccination

instructions for its completion, including explanations on the definition and use of intensity scales for collection of AEs (Remind the parent / legally acceptable representative to bring back the DC when they return for Visit 02 at a specified date)

19) 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
20) Remind the subject / subject's parent (s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
21) Arrange an appointment for the second visit (28 days [+ 14 days])
22) Complete the source documents and relevant eCRF pages for this visit

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

Visit 2 (28 days [+14-day time window] after Visit 1): Collection of Safety Information

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature
2) Collect and check the information entered into the DC1 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) Check concomitant medications and record every reportable medication
4) Remind the subject / subject's parent(s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
5) Arrange an appointment for Visit 3 (365 days [+/- 20 days] after Visit 1)
6) Complete the source documents and relevant CRF pages for the visit

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

The Investigator or authorized designee will:

1) Ask if the subject has experienced any SAEs not yet reported. If an SAE occurred, follow the instructions in Section 10 for reporting it
2) Remind the subject / parent / legally acceptable representative to notify the site in case of an SAE

Visit 3 (180 Days [± 20-day Window] After Visit 1)

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature
2) Review for any contraindications
3) Perform a urine pregnancy test (pre-injection) (women of childbearing potential only)
4) Check concomitant medications and record every reportable medication ongoing at the time of vaccination

5) **For all subjects:** collect the blood sample (BL2, 5 mL) for neutralizing Abs and record the date of collection (see Section 7.1.1 for detailed instructions on 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 eCRF.*

6) Contact IVRS/IWRS to obtain the dose number to be administered
7) Inject the appropriate study vaccine into the opposite limb from the limb the vaccination was administered to in Visit 1
8) Record the date of injection, the site and side of injection and the route of administration, as well as the dose number of the vaccine
9) Affix the vaccine labels in the subject's source documents
10) Keep the subject under observation for 30 minutes, and record any injection site reactions or systemic events in the source documents
11) Give the subject / subject's parent (s) / legally acceptable representative(s) the DC2 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 and remind subject / subject's parent(s) / legally acceptable representative(s) to bring back the DC when they return for Visit 4 at a specified date
12) Remind the subject / subject's parent(s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
13) Arrange an appointment for Visit 4 (28 days [+14-day window] after Visit 3)
14) Complete the source documents and relevant eCRF pages for this visit

Visit 4 (28 days [+14-day window] after Visit 3): 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
2) Check and collect the information entered into DC2 by interviewing the subject and request information concerning any medical event, serious or not, that may have occurred since Visit 3
3) Check concomitant medications and record every reportable medication
4) **For all subjects:** collect the third blood sample (BL3, 5mL) 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 eCRF.*

5) Remind the subject / subject's parent(s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
6) Arrange an appointment for the Visit 5 (365 days [+/- 20 days] after Visit 1)
7) Complete the source documents and relevant eCRF pages for this visit

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

Same procedures as for PC1 and PC2

Visit 5 (365 Days [± 20-Day Window] After Visit 1): Vaccination, 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
2) Review for any contraindications
3) Urine pregnancy test (pre-injection) (women of childbearing potential only)
4) Check concomitant medications and record every reportable medication ongoing at the time of injection
5) **For All Subjects:** Collect the fourth blood sample (BL4 for neutralizing Abs; see Section 7.1.1 for detailed instructions regarding the handling of blood samples) and record the date of collection
6) Contact IVRS/IWRS to obtain the dose number to be administered
7) Inject the appropriate study vaccine into the opposite limb from the limb the injection was administered to in Visit 3
8) Record the date of injection, the site and side of injection and the route of administration, as well as the dose number of the vaccine
9) Affix the vaccine labels in the subject's source documents
10) Keep the subject under observation for 30 minutes, and record any injection site reactions or systemic events in the source documents and any reportable concomitant medications
11) Give the subject / subject's parent (s) / legally acceptable representative(s) the DC3 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
12) Remind the parent/legally acceptable representative to bring back the DC when they return for Visit 6 at a specified date
13) Remind the subject / subject's parent (s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
14) Arrange an appointment for Visit 6 (28 days [+ 14 days] after Visit 5)
15) Complete the source documents and relevant eCRF pages for this visit

Visit 6 (+ 28 days [+14-day window] after V05): 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
2) Check and collect the information entered into DC3 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) Check concomitant medications and record every reportable medication
4) **For all subjects:** Collect the fifth blood sample (BL5, 5mL) for neutralizing Abs and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples); the volume used for specificity and affinity maturation testing in the AIT subset will also be used from this aliquot as applicable.
5) Give the parent / legally acceptable representative Memory Aid 1 (MA1)
6) Remind the parent / legally acceptable representative to have the MA1 with them at the 6 month follow-up telephone contact
7) Remind the subject / subject's parent(s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
8) Arrange an appointment for Visit 7 (12 months r 24 months [± 20-day window] after Visit 5)
9) Complete the source documents and relevant eCRF pages for this visit

Telephone Calls: **PC5** (V01 + 14 months [+8-day window]); **PC6** (V01 + 16 months [+8-day window]; **PC7** (V01 + 18 months [+20-day window]); **PC8** (V01 + 20 months [+8-day window]); **PC9** (V01 + 22 months [+8-day window]); **PC10** (V05 + 14 months [+8-day window]), subjects from both subgroups; **PC11** (V05 + 16 months[+8-day window]), subjects from both subgroups; **PC12** (V05 + 18 months [+20-day window]), subgroup a subjects 6-month FUP contact, subgroup b subjects standard phone call contact; **PC13** (V05 + 20 months [+8-day window]), subgroup b subjects; **PC14** (V05 + 22 months [+8-day window] subgroup b subjects); and **PC15** (V05 + 30 months [+20-day window] subgroup b 6-month FUP contact)**.**

1) Same procedures as for PC1 and PC2
2) Review the information entered into 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 the previous visit or phone call

Visit 6-Inf (unscheduled visit, between PC7 [6 months after last injection in Stage I] and Visit 7–Year 1 [12 Months After Visit 5]): Information of subject on vaccine safety (subgroup a and b)

This unscheduled visit is to take place as soon as possible between V06 and V07. During this visit, subjects will be informed about the increased risk of hospitalized or severe dengue for subjects not exposed to dengue infection prior to the first injection with the CYD dengue vaccine.

At the latest, this visit will take place at the same time as Y1 V07. In the event the subjects' baseline dengue serostatus is available at the time of V06-Inf, and the Amendment 1 of the protocol is already approved by ECs/IRBs and Health Authorities, subjects could be asked to confirm their willingness to continue participating in the study during this unscheduled visit. If it is not possible to ask for subjects' consent at V06-Inf, subjects will be asked to confirm their participation in the study at Y1 V07.

STAGE II

For each subject, the blood sample collected before first CYD dengue vaccine injection (ie, at V01 for subjects in Group 1, V03 for subjects in Group 2, and V05 in subject in Group 3) will have been assayed with PRNT to determine dengue serostatus at baseline. Only subjects identified as dengue seropositive will be eligible to receive the booster injection.

The blood sample collected 1 year after the last primary series injection will be the last clinical procedure required from dengue seronegative subjects at baseline. Besides the BL6 at Y1 V07, the clinical procedures described hereafter (including the blood samples in the AIT subset) will be for dengue seropositive subjects alone.

One Year Booster (BL6-BL9) or Two Year Booster (BL6; BL10-BL13)

Visit 7–Year 1 (12 Months [+60-day Window] After Visit 5): Booster Vaccination (subgroup a), Collection of Safety Information (subgroup a and b), and Blood Sample (subgroup a and b)

The Investigator or designated study personnel will:

For all subjects:

1) Present the changes brought to the study design to the subject / subject's parent (s) / legally acceptable representative(s) in more detail, answer any of his/her/their questions, and ensure that he/she/they has/have been informed of all aspects that are relevant to their decision to continue participating in the study
2) Have the subject and / or the subject's parent (s) / legally acceptable representative(s), date and sign the new AF and / or ICF version, as applicable
3) Review the pages of the MA with the parent / legally acceptable representative
4) **For all subjects having provide their consent:** Collect 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); the volume used for specificity and affinity maturation testing in the AIT subset will also be used from this aliquot as applicable.
5) **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 CMI blood samples).

For subjects from subgroup a:

a. Review for any contraindications, including dengue serostatus at baseline
b. Perform a physical examination and record the subject's axillary temperature
c. Urine pregnancy test (pre-injection) (women of childbearing potential only)

d. Check concomitant medications and record every reportable medication ongoing at the time of vaccination
e. Contact IVRS/IWRS to obtain the dose number to be administered
f. Inject the appropriate study vaccine into the opposite limb from the limb the vaccination was administered to in Visit 5
g. Record the date of injection, the site and side of injection and the route of administration, as well as the dose number of the vaccine
h. Affix the vaccine labels in the subject's source documents
i. Keep the subject under observation for 30 minutes, and record any injection site reactions or systemic events in the source documents and any reportable concomitant medications

6) For subgroup a: Give the subject / subject's parent (s) / legally acceptable representative(s) the DC4 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.

   For subgroup b: Give the subject / subject's parent (s) / legally acceptable representative (s) the DC4 Group b to collect SAEs until the V07 (Y2) visit

   Dengue seronegative subjects at baseline: Give the subject / subject's parent (s) / legally acceptable representative(s) the MA2

7) Remind the parent / legally acceptable representative to bring back the DC4 or the DC4 Group b when they return for the next visit at a specified date
8) Remind the subject /subject's parent(s) / legally acceptable representative(s) to call the study center a serious medical event occurs
9) Arrange an appointment for the next visit (V08 for subgroup a AIT subset; V10 for other subgroup a subjects; V07 Year 2 for subgroup b subjects)
10) Complete the source documents and relevant eCRF pages for this visit

Visit 7–Year 2 (24 Months [+60-day Window] After Visit 5): Booster Vaccination, Collection of Safety Information, and Blood Sample

The Investigator or designated study personnel will:

For subjects from subgroup b:

1) Check the information entered into DC4 Group b by interviewing the subject / subject's parent / legally acceptable representative
2) Perform a physical examination and record the subject's axillary temperature
3) Collect blood sample (BL10, 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); the volume used for specificity and affinity maturation testing in the AIT subset will also be used from this aliquot as applicable.

4) **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 CMI blood samples).
5) Review for any contraindications
6) Urine pregnancy test (pre-injection) (women of childbearing potential only)
7) Check concomitant medications and record every reportable medication ongoing at the time of vaccination
8) Contact IVRS/IWRS to obtain the dose number to be administered
9) Inject the appropriate study vaccine into the opposite limb from the limb the vaccination was administered to in Visit 5
10) Record the date of injection, the site and side of injection and the route of administration, as well as the dose number of the vaccine
11) Affix the vaccine labels in the subject's source documents
12) Keep the subject under observation for 30 minutes, and record any injection site reactions or systemic events in the source documents and any reportable concomitant medications
13) Give the subject / subject's parent (s) / legally acceptable representative(s) the DC5 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.
14) Remind the parent / legally acceptable representative to bring back the DC5 when they return for the next visit at a specified date
15) Remind the subject /subject's parent(s) / legally acceptable representative(s) to call the study center a serious medical event occurs
16) Arrange an appointment for the next visit (V08 for AIT subset; V10 for other subjects)
17) Complete the source documents and relevant eCRF pages for this visit

Visit 8 (Visit 7 +7 days [+ 3-day window]): Only for AIT Subset

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature
2) Check concomitant medications and record every reportable medication
3) At V08 (Y1) check the information entered into DC4 for subgroup a;

   At V08 (Y2) check the information entered into DC5 for subgroup b

   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 7

4) Obtain a blood sample (BL7 or BL11[a]) for neutralizing Abs, Ab specificity (if assessment required) and CMI, and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples)
5) Remind the parent / legally acceptable representative to bring back the DC4 (subgroup a) or DC5 (subgroup b) when they return for the next visit at a specified date
6) Remind the subject / subject's parent(s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
7) Arrange an appointment for Visit 9
8) Complete the source documents and relevant eCRF pages for this visit

Visit 9 (Visit 7 + 14 days [+7-day window]): Only for AIT Subset

The Investigator or designated study personnel will:

1) Perform a physical examination and record the subject's axillary temperature
2) Check concomitant medications and record every reportable medication
3) At V09 (Y1) check the information entered into DC4 for subgroup a;

   At V09 (Y2) check the information entered into DC5 for subgroup b

   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 8
4) Check the information entered into DC4 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 8
5) Obtain a blood sample (BL8 or BL12[b]) for neutralizing Abs, Ab specificity (if assessment required) and CMI, and record the date of collection (see Section 7.1.1 for detailed instructions regarding the handling of blood samples).
6) Remind the subject / subject's parent / legally acceptable representative to bring back the DC4 (subgroup a) or DC5 (subgroup b) when they return for the next visit at a specified date
6) Remind the subject / subject's parent(s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
7) Arrange an appointment for the next visit
8) Complete the source documents and relevant eCRF pages for this visit

[a] BL7 or BL11: 20 mL will be drawn in adolescent subjects and 30 mL in adult subjects.

[b] BL8 or BL12: 20 mL will be drawn in adolescent subjects and 30 mL in adult subjects.

Visit 10 (Visit 7 + 28 days [+ 7-day window for AIT subset; + 14-day window for all subjects]): 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
2) Check concomitant medications and record every reportable medication
3) At V10 (Y1) check the information entered into DC4 for subgroup a;

   At V10 (Y2) check the information entered into DC5 for subgroup b

   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 7 or Visit 9
4) Record any injection site reactions or systemic events and reportable concomitant medications
5) Collect blood sample (BL9 or BL13, 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). The volume used for specificity and affinity testing in the AIT subset will be used also from this aliquot as applicable.
6) **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 CMI blood samples).
7) Provide MA2 to record any medical events from the booster vaccination, together with instructions for its completion
8) Remind the subject / subject's parent(s) / legally acceptable representative(s) to call the study center if a serious medical event occurs
9) Complete the source documents, relevant eCRF pages and the termination record for this visit

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 until resolution of the event 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]: 26 April 2016 to 20 February 2020
- Planned inclusion period - FVFS to FVLS[b]: April 2016 to August 2016
- Planned vaccination period: April 2016 to August 2016
- Planned end of trial: February 2020
- Planned date of final clinical study report: July 2020

5.1.6 Early Safety Data Review

The 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 countries 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' parent(s) / legally acceptable representative(s) and should assure appropriate therapy and follow-up.

An internal SET 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 AE of interest will be promptly reviewed by the IDMC.

5.2 Enrollment and Retention of Trial Population

5.2.1 Recruitment Procedures

Recruitment procedures and materials will be submitted to the IECs/IRB for approval before implementation. Recruitment will be conducted in a competitive manner.

5.2.2 Informed Consent Procedures

Informed consent is the process by which a subject and / or an appropriate and legally acceptable representative voluntarily confirms his or her willingness to 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. Depending on age, the subject may be required to sign and date the AF, which varies according to local or regional regulations. The AF is in addition to, not in place of, an ICF that is signed by the parent / legally acceptable representative

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

[b] FVLS: first visit, last subject

One AF is to be signed by subjects < 18 years of age (or based on local regulations) and one ICF is to be signed by subjects ≥ 18 years of age (or based on local regulations) and by any parents / legally acceptable representatives of subjects (or based on local regulations).

An ICF template specific for each ethics committee will be used. In Colombia, the ICF for minors must be signed by both parents and a psychological evaluation of minors must be conducted to assess the subject's level of understanding. Any other local regulations applicable in the Philippines will also be taken into account.

Following the amendment of the original protocol, subject / subject's parent(s) / legally acceptable representative(s) is to sign a new version of the AF and / or the ICF, as per local regulations.

In accordance with GCP, prior to signing and dating the consent form, the subject or representative 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 or legal representative is 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 IECs / 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 IECs / IRB prior to the form being used.

If new information becomes available that may be relevant to the subject's or legally acceptable representative's 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.

To ensure compliance with local or regional regulatory and institutional authorities, ICFs and AFs will be provided according to local guidelines.

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) Aged 9 to 50 years on the day of enrollment[a]

[a] "9 to 50 years" means from the day of the 9th birthday to the day before the 51st birthday.

2) Subject in good health, based on medical history and physical examination
3) AF or ICF has been signed and dated by the subject (based on local regulations), and informed consent form 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 is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be pre-menarche[a], surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination until at least 4 weeks after the last vaccination)
2) Participation at the time of study enrollment (or in the 4 weeks preceding the first trial vaccination) or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device or medical procedure
3) Self-reported 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)
4) Self-reported systemic hypersensitivity to any of the vaccine components[b], or history of a life-threatening reaction to the vaccine used in the trial or to a vaccine containing any of the same substances
5) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion[c]
6) Receipt of blood or blood-derived products in the past 3 months, which might interfere with assessment of the immune response
7) Planned receipt of any vaccine in the 4 weeks following any trial vaccination
8) Previous vaccination against dengue disease with either the trial vaccine or another vaccine
9) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily

[a] For pre-menarche females, young female subjects will declare if they have not yet started menstruation; if a young female subject reaches menarche during the study, she is to be considered as a woman of childbearing potential from that time forward.

[b] The components of CYD dengue vaccine and placebo are listed in Section 6.1 and in the Investigator's Brochure.

[c] Chronic illness may include, but is not limited to, cardiac disorders, renal disorders, auto-immune disorders, diabetes, psychiatric disorders, or chronic infection.

10) Current alcohol abuse or drug addiction that, based on Investigator's judgment, may interfere with the subject´s ability to comply with trial procedures
11) Identified as a site employee of the Investigator, with direct involvement in the proposed study or other studies under the direction of that Investigator or study center, as well as family members (ie, immediate, husband, wife, and their children, adopted or natural) of the employees or the Investigator
12) A prospective subject must not be included in the study until the following conditions and/or symptoms are resolved:
    - Febrile illness (temperature ≥ 38.0°C) or moderate or severe acute illness/infection (according to Investigator's judgment) on the day of vaccination
    - Receipt of any vaccine in the 4 weeks preceding the first trial vaccination

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 eCRF. The significant medical history section of the eCRF 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 further vaccination until the condition is resolved. Postponement will continue to fall within the timeframe for vaccination specified in the Table of Study Procedures.

- 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

Should a subject experience one of the conditions listed below, the Investigator will discontinue vaccination:

- Pregnancy, as indicated by a positive urine test
- An anaphylactic or other significant allergic reaction to the previous dose of vaccine
- For STAGE II: classified as a dengue unexposed subject (seronegative)

If a subject has been classified as a dengue unexposed subject at the time of V07, she/he will not be withdrawn from the study, unless subject voluntarily withdraws. The subject will have the possibility to provide the Y1 blood sample (BL6) and to continue participating in the safety follow-up.

In the event of a local or national immunization program with for example, a pandemic influenza vaccine or any other vaccine as needed, subjects who receive this vaccine at any time during the trial will not be withdrawn from the trial.

5.2.8 Conditions for Withdrawal

Subjects / Subject's Parents / Legally acceptable representatives will be informed that they have the right 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 eCRF.

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 follow-up examination, documented reasonable effort (ie, 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 eCRF 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 eCRF. Reasons are listed below from the most significant to the least significant (refer to the eCRF 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 adverse event:** 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 (ie, 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 follow-ups 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 for the 6-month follow-up after vaccination, except if they specified that they do not want to be contacted again and it is documented in the source document.

State the follow-up duration in the event of discontinuation: eg, 6 months after the last injection.

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 vaccination or PLA injection, the subject will not be discontinued from the trial and will be followed for safety assessment (and may be followed for immunogenicity assessment). However, no additional vaccination will be administered.

All pregnancy cases should be reported if they occurred during the study and during the follow-up period. 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—ie, 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 (eg, 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 (eg, that affect the conduct of the trial or the safety of subjects), require IECs / 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 IECs / IRB approval has already been given, are not initiated without IECs / 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 / IRB. 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

Subjects included in the study will either receive 1, 2, or 3 injections of the CYD dengue vaccine in a primary series, each 6 months apart, followed after 1 or 2 years by a booster injection of the CYD dengue vaccine. Other subjects may receive 1 or 2 doses of PLA as part of the 3-dose regimen and will receive the remaining dose(s) of CYD dengue vaccine.

6.1 Identity of the Investigational Product

6.1.1 Identity of Trial Product

CYD dengue vaccine:	Live, attenuated, tetravalent dengue virus vaccine
Form:	Powder and solvent for suspension for injection
Dose:	0.5 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 to 6.0 log10 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 chlorhydrate, saccharose, D-trehalose dihydrate, D-sorbitol, tris (hydroxymethyl) aminomethane, and urea
- **Solvent:** NaCl 0.4%

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.

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 injection to ensure their safety, and any reactions during this period will be documented in the eCRF. 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 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 4.5 to 6.0 $\log_{10}$ $CCID_{50}$ per serotype (1,2,3,4) 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, 1 to 2 years 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

Vaccine:	NaCl 0.9% (Placebo)
Form:	Liquid
Dose:	0.5 mL
Route:	SC in the deltoid region of the upper arm
Batch number:	TBD

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.

6.2 Identity of Other Product(s)

Not applicable.

6.3 Product Logistics

6.3.1 Labeling and Packaging

CYD dengue vaccine and placebo will be supplied in single-dose vials 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 Clinical Logistics Coordinator or designee 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 (ie, 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 vaccination study staff at the site will maintain records of product delivery to the trial site, product inventory at the site, the doses given to each subject, and the disposal of or return to the Sponsor of unused doses.

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

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 (eg, 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 either disposed of or returned to the Sponsor 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 booster or placebo. Neither the observer Investigator, nor the Sponsor study staff interacting with the Investigator, nor the subjects / subjects' parent(s) / legally acceptable representative(s) 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 RMO 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 information resulting from code-breaking (ie, 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.
- IDMC, if needed, to facilitate the assessment of safety of VCD.

The IECs / 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.

Three planned analyses will be performed on data collected up to 1 year post-injection 3, up to 28 days post Y1 booster injection, and up to 28 days post Y2 booster injection. 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 fourth 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.

In order to determine the baseline serostatus of subjects assigned to each treatment group, a specific process will be implemented to maintain the blind. This process will involve an unblinded statistician, independent from the study statistician, and of an unblinded GCI representative.

6.5 Randomization and Allocation Procedures

Each subject who meets the inclusion/exclusion criteria and signs an ICF/AF will be randomly assigned to one of the 3 groups (6 subgroups) via an IVRS / IWRS, according to a 1:1:1:1:1:1 enrollment ratio (350 subjects per group [175 subjects per subgroup]).

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 subject number and the vaccine assignment (code number). Subject numbers will be

[a] according to local regulations

recorded on the eCRFs and will not be reassigned for any reason. The full detailed procedures for randomization are described in the Operating Guidelines.

Sixty subjects from a specific site in Colombia who consent to participation in additional immune response testing 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 the AIT subset and "0" for all other subjects. The second digit of the subject identifier will be a pre-defined figure (1 or 2); "1" will be used for subjects randomized to subgroup "a" (receive booster at 1 Year) and "2" for subjects randomized to subgroup "b" (receive booster at 2 Years). For example, Subject 001-12001 will be the first subject randomized to the AIT subset enrolled in center number 1 and will receive the booster at 24 months (Y2) following administration of the third dose in the primary series.

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

- Children 9 through 11 years of age
- Adolescents 12 through 17 years of age
- Adults 18 through 39 years of age
- Adults 40 through 50 years of age

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 eCRF. 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 eg, 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 eCRF 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 eCRF from the day of vaccination to the end of the solicited and unsolicited follow-up period (eg, 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, 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 eCRF 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 eCRF 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 samples and subjects included in the AIT subset will have 9 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 against the laboratory request form. 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, Antibodies Specificity and Affinity Maturation Assessment

At V01, V03, V04, V05, V06, and V07 (STAGE I) and at V07[a] and V10 (STAGE II) for all subjects. At V08 and V09, subjects included in the AIT subset will provide 5 mL of blood 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 and will attach the pre-printed 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 to the AIT subset, these blood samples will also be used to assess Ab specificity and affinity maturation.

7.1.2 Blood Sample for Cellular Immunity Assessment

In addition to the above, blood samples for CMI assessment will be collected at V01, V07, V08, V09, and V10. Between 15 and 35 mL (depending on both the study visit and the subject's age; see Table 5.4) of blood will be collected in sodium 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 Sample for Virological Confirmation of Suspected Hospitalized Dengue Disease and Assessment of Disease Severity

In case a subject is 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.

For all hospitalized suspected dengue cases, the Investigator must ensure that key biological parameters (aspartate aminotransferase [AST], alanine aminotransferase [ALT], hematocrit and platelet count) 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. The aim of these tests is the assessment of severity according to the WHO/IDMC classification.

[a] Subjects from subgroup a will provide a blood sample at Y1 V07 while subjects from subgroup b will provide a blood sample at both Y1 V07 and Y2 V07. Thus, the blood sample at V07 will be used both to assess Ab persistence (subgroup a and subgroup b) and Ab titers pre-booster injection (subgroup a).

Table 7.1 presents the additional serum aliquots to be taken if dengue disease is suspected in a hospitalized subject at any time during the trial. Additional details are found in the Operating Guidelines.

Table 7.1: Blood sampling volume 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*

Ag: antigen.
Note: Further details are provided in the Operating Guidelines

7.2 Sample Preparation

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

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.

The subject's identification number, the date of sampling, and the number of aliquots obtained 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 centrifuged 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 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, Antibodies 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, Ab 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 neutralizing Abs assessment 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 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 Mediated 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 / subjects' parents / legally acceptable representatives 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, or Sponsor representative, will supply the trial sites with protocols, ICFs, AFs, CRFs, SAE reporting forms 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 2 weeks 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(s) for the evaluation of immunogenicity are:

STAGE I:

- Neutralizing Ab titers against each dengue virus serotype 28 days after the last CYD dengue vaccine injection in the Group 1 and Group 2 primary series schedules
- Neutralizing Ab titers against each dengue virus serotype 1 year after the last CYD dengue vaccine injection in the Group 1 and Group 2primary series schedules

STAGE II:

- Neutralizing Ab titers against each dengue virus serotype 28 days post-booster dose (Year 1 and Year 2, respectively, for each group that will be tested for NI in STAGE II)

9.1.1.2 Immunogenicity Assessment Methods

Dengue Neutralizing Abs

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

Dengue PRNT

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).

For STAGE I, all subjects will provide blood samples for assessing neutralizing Ab titers against each of the 4 parental dengue virus strains at baseline V01 (pre-Injection 1, BL1), at V03 (pre-Injection 2, BL2) at V04 (V03 + 28 days, BL3), at V05 (pre-Injection 3, BL4), at V06 (V05 + 28 days, BL5), and at Y1 V07 (1 year post-Injection 3, BL6) for dengue immunogenicity.

For STAGE II, subjects will provide blood samples for assessing neutralizing Ab titers against each of the 4 parental dengue virus strains. The first blood sample will be collected in all subjects approximately 1 year post last primary series injection (BL6; to assess Ab persistence but also pre-booster Ab levels in subgroup a). Subjects receiving the booster injection will provide additional blood samples. Subjects from subgroup a will provide a blood sample 28 days after receiving the booster injection (BL9). Subjects from subgroup b will provide blood samples immediately before booster injection (BL10) and 28 days after booster injection (BL13).

Additional assays and other analyses might be used to assess and characterize dengue immune response if required.

9.1.2 Safety

There are no primary objectives for safety.

9.1.3 Efficacy

No clinical efficacy data will be collected in this 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:

STAGE I:

- Neutralizing Ab titers against each dengue virus serotype 28 days post-Injection 2 in Group 1, 28 days post-Injection 3, and 1 year post-injection 3 in all 3 groups

STAGE II:

- Neutralizing Ab titers against each dengue virus serotype 28 days post-Injection 3 in Group 1, immediately prior to booster injection and 28 days post-booster injection in all 3 groups
- Seroconversion rates 28 days after injection for each of the 4 parental dengue virus strains of CYD dengue vaccine: percentages of subjects with either a pre-booster titer < 10 (1/dil) and a post-booster titer ≥ 40 (1/dil), or a pre-booster titer ≥ 10 (1/dil) and a ≥ 4-fold increase in post-booster titer as determined immediately prior to and 28 days post-booster in all 3 groups.

9.2.1.2 Immunogenicity Assessment Methods

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

For each particular immunogenicity endpoint, the assay will be performed on the blood samples taken at:

STAGE I Endpoints:

- 28 days post-Injection 2 (Group 1), and 28 days post-Injection 3 (all groups)

STAGE II Endpoints:

- Immediately prior to and 28 days post-booster injection in all 3 groups

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 (eg, asthma) if the frequency or intensity increases post-injection.

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:

- Serious 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 SAEs, 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:

An unexpected adverse reaction is an AR, the nature or severity of which is not consistent with the applicable product information (eg, 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 eCRF**.** The assessment of these AEs post-vaccination is mandatory. A solicited reaction is defined by a combination of:

- Symptom and
- Onset post-vaccination

eg, injection site pain between D0 and D7 post-vaccination, or headache between D0 and D14.

A solicited reaction is therefore an AR observed and reported under the conditions (symptom and onset) prelisted (ie, solicited) in the eCRF 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 eCRF in terms of diagnosis and / or onset post-vaccination, ie, excluding solicited reactions, eg, if headache between D0 and D14 is a solicited reaction (ie, prelisted in the eCRF), 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.

[a] All injection site AEs are considered to be related to vaccination and are therefore all *injection site 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 associated with the vaccination site, eg, 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 primary endpoints for the evaluation of safety are:

STAGE I and STAGE II

- 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 injection.
- Occurrence, time to onset, number of days of occurrence, intensity, whether it leads to discontinuation or not, and action taken of solicited (prelisted in the subject's diary card and electronic case report form [eCRF]) injection site reactions (pain, erythema, and swelling) occurring up to 7 days after injection.
- 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.
- 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 up to 28 days after injection.
- 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 injection.
- Occurrence of SAEs, including serious AESIs (with specific time windows according to the nature of the event) throughout the trial).
- Occurrence of hospitalized VCD cases throughout the trial (ie, from D0 through end of study).

9.2.2.3 Safety Assessment Methods

At V01, V03, V04, V05, V06, V07 and V10 (and also V08 and V09 for subjects in the AIT subset), the Investigator or an observer-blind delegate will perform a clinical or medically-driven physical examination, and will ask the subject / or parent or / legally acceptable representative 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 eCRF 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 injection to ensure their safety. The post-injection 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 eCRF, as follows:

- Any unsolicited systemic AE occurring during the first 30 minutes post-injection will be recorded on the eCRF 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 occurring during the first 30 minutes post-injection will be reported in the same way as any other SAE and to the Sponsor, according to the procedures described in Section 10.

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

After injection, subjects / subjects' parents / legally acceptable representatives 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 / subjects' parents / legally acceptable representatives in the DC on the day of vaccination and for the next 7 days (ie, D0 to D7) for the solicited injection site reactions and for the next 14 days (ie, D0 to D14) for the solicited systemic reactions, 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 (eg, medication)

The action taken by the subjects / subjects' parents / legally acceptable representatives to treat any **solicited reactions** will be classified in the eCRF 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 / subjects' parents / legally acceptable representatives 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 DCs and eCRF, together with the intensity scales.

Table 9.1: Solicited injection site reactions: terminology, definitions, and intensity scales for trials involving children, adolescents and adults

eCRF 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: Easily tolerated Grade 2: Sufficiently discomforting to interfere with normal behavior or activities Grade 3: Incapacitating, unable to perform usual activities	Grade 1: > 0 to < 25 mm Grade 2: ≥ 25 to < 50 mm Grade 3: ≥ 50 mm	Grade 1: > 0 to < 25 mm Grade 2: ≥ 25 to < 50 mm Grade 3: ≥ 50 mm
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 / parents / legally acceptable representatives will record the intensity level (Grade 1, 2, or 3) in the diary card. 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

† Intensity scale for children aged 9 to 11 years

‡ Intensity scale for adolescents and adults ≥ 12 years of age

Table 9.2: Solicited systemic reactions: terminology, definitions, and intensity scales

eCRF 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	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 parents / legally acceptable representatives will record the intensity level (Grade 1, 2, or 3) in the diary card. 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 / Parents / Legally acceptable representatives 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 eCRF. The preferred route for this trial is axillary. Pre-vaccination temperature is also systematically collected by the Investigator on the source document for all subjects. Tympanic thermometers must not be used.

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

In addition to recording solicited reactions, subjects / subjects' parents / legally acceptable representatives will be instructed to record any other medical events that may occur during the 28-day period after injection. 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 (eg, medication)

The action taken by the subject / subjects' parent / legally acceptable representative to treat any **unsolicited AEs** will be classified in the eCRF 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 6 months after booster injection.

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 (eg, 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 Section 10 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 Method for Assessing 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 each country. 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 eCRF.

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 eCRF.

Interpretation of Results

If a sample is positive for the dengue screen RT-PCR (ie, ≥LLOQ) and/or the NS1 assay is positive and/or the Simplexa dengue RT-PCR is positive, this will be classified as a VCD 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

STAGE I:

- Neutralizing Ab titers against each dengue virus serotype at all STAGE I time points
- Neutralizing Ab titers against FVs at baseline prior to first injection of CYD dengue vaccine (JE in the Philippines, YF in Colombia)

STAGE II:

- Neutralizing Ab titers against each dengue virus 28 days post booster injection (Y1 for subgroup a and Y2 for subgroup b)

AIT Subset Only:

The immune response against the 4 dengue serotypes elicited by the boosting dose of dengue vaccine will be assessed by the measurement of neutralizing antibodies, to include:

1) Neutralizing Ab responsse
    a. Neutralizing Ab levels against each of the 4 parental dengue virus strains immediately prior to and at 7, 14, and 28 days post-booster 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 3 (STAGE I); and immediately prior to and at 28 days post-booster injection as a priority, and at 7 and 14 days post-injection if necessary (STAGE II). 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 (STAGE I) and immediately prior to and at 28 days post-booster injection (STAGE II).
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:

    - Cytokine secreting CD4 and CD8 T cells count immediately prior to the primary dose schedules (STAGE I) and immediately prior to and 7, 14, and 28 days after the Year 1 and Year 2 booster dose for a subset of subjects in Groups 1, 2, and 3.
    - T-cell subclasses (naïve, effector, central and terminally differentiated memory T cells) count immediately prior to the primary dose schedules (STAGE I), and

immediately prior to and 7, 14, 28 days after the Year 1 or year 2 booster dose for a subset of subjects in Groups 1, 2, and 3.

- Cytotoxic T-cell effector markers immediately prior to the primary dose schedules (STAGE I), and immediately prior to and 7, 14, and 28 days after the Year 1 or Year 2 booster dose for a subset of subjects in Groups 1, 2, and 3.

b. B-cell response:

- Ex vivo B-cells (plasmablast) count (measured by ELISPOT) immediately prior to the primary dose schedules and immediately prior to and 7 and 14 days after the Year 1 or Year 2 booster dose for a subset of subjects in Groups 1, 2, and 3.
- Memory B-cells count (measured by ELISPOT) immediately prior to the primary dose schedules and immediately prior to and 28 days after the year 1 or year 2 booster dose for a subset of subjects in Groups 1, 2, and 3.

The assays, along with the corresponding samples, will be managed by the specific Sanofi Pasteur organization (eg, Research), or with an external laboratory, whichever applies.

9.3.1.2 Immunogenicity Assessment Methods

9.3.1.2.1 Flavivirus Neutralizing Abs

Japanese Encephalitis (JE) Neutralizing Abs

JE virus neutralizing Ab measurement will be assessed by PRNT by Sanofi Pasteur GCI, Swiftwater, USA, or a GCI outsourced laboratory. Serial 10-fold dilutions of serum to be tested (previously heat-inactivated) are mixed with a constant challenge dose (expressed as PFU/mL) of the JE Beijing strain (genotype III). The mixtures are inoculated into wells of a plate of confluent LLC-MK2 cells. After adsorption, cell monolayers are overlaid and incubated for several days. The reported value (end point neutralization titer) represents the highest dilution of serum at which ≥ 50% of JE challenge virus is neutralized when compared to the negative control wells, which represents the 100% virus load. The LLOQ for this study will be 10 (1/dil).

JE Neutralizing Ab measurement will be assessed at V01 (BL1) only in subjects from the Philippines.

Yellow Fever (YF) Neutralizing Abs

YF neutralizing Ab levels will be measured using PRNT by Sanofi Pasteur GCI, Swiftwater, USA, or GCI outsourced laboratory. Briefly, serial two-fold dilutions of serum to be tested (previously heat-inactivated) are mixed with a constant concentration of the YF vaccinal strain 17D (expressed as PFU/mL). The mixtures are inoculated in duplicate into wells of a plate of confluent Vero cells. After adsorption, cell monolayers are overlaid and incubated for a few days. The reported value (end point neutralization titer) represents the highest dilution of serum at which ≥ 50% of YF challenge virus (in plaque counts) is neutralized when compared to the negative control wells, which represents the 100% virus load. The LLOQ for this study will be 10 (1/dil).

YF Neutralizing Ab measurement will be assessed at V01 (BL1) only in subjects from Colombia.

9.3.1.2.2 Additional Immunology Testing

Blood samples will also be drawn from subjects randomly selected in the AIT subset. For evaluation of CMI, Ab specificity and affinity maturation, samples will be drawn from STAGE I subjects immediately before Injection 1 (specificity, affinity and CMI) and at 28 days after Injection 3 (specificity and affinity). During STAGE II, only subjects identified as seropositive at baseline and thus eligible to booster injection will continue to be part of the AIT subset. Sampling from these subjects will continue into STAGE II, when samples will be drawn immediately before the booster injection and at 7 days, 14 days, and 28 days post-booster injection (specificity and CMI at all time points and affinity at D0 and D28 post-booster).

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 (DENV01/3/4 and DENV02) and control bovine serum albumin (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. Ab 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).

Dengue Neutralizing Abs

The immunogenicity assessment methods of dengue neutralizing Abs are assays, along with the same as those presented in Section 9.1.1.2.

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 peripheral blood mononuclear cells (PBMCs). Cells are incubated with antigens (pools of peptides from YF 17D or dengue viruses), an Ab 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 hours at 37°C. Plates will be washed and incubated with biotinylated mouse anti-human pan-IgG Fc Ab 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(s). It is the responsibility of the Investigator to request all necessary documentation (eg, 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 (MD 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 local 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:

The Investigator must complete the SAE Reporting Form, check off the "Initial Reporting Form" box, and send it to the Sponsor by one of the following means:

- By fax, to the following number: + 33 4 37 37 71 32
- In PDF format to the following e-mail address, using a method of transmission that includes password protection: PV.outsourcing@sanofipasteur.com
- By express mail, to the following address:

  Sanofi Pasteur
  Global PharmacoVigilance Department
  14, Espace Henry Vallée
  69007 Lyon – 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 the RMO or other designated Sponsor representative. The contact information is provided in the "List of Investigators and Centers Involved in the Trial". If a representative cannot be reached, the Investigator may contact the Call Center as described in Section 5.3.

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 (eg, 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 of the RCTM. All relevant information must be included directly in the eSAE form. Copies of documents (eg, 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 to be adapted according to local regulations will be responsible for informing the IECs or IRBs that reviewed the trial protocol.

11 Data Collection and Management

11.1 Data Collection and eCRF Completion

Individual safety diary cards, 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 / Parents or legally acceptable representatives 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 / parents / legally acceptable representatives on how to correctly use these tools.

The 6-month follow-up will be done by interviewing subjects either during a visit or over the telephone using a questionnaire to capture SAEs and AEs of particular interest, if applicable. A MAs will be provided to the subjects at the preceding trial visit to help them record information on events occurring between this visit and the 6-month follow-up.

Relevant information will be transcribed into the eCRF. Any SAEs captured during this 6-month follow-up period will be reported and followed up as per the normal process for reporting SAEs.

The clinical team may decide to replace the MAs by a diary card if a follow-up visit is planned for the subjects.

At specified intervals, the Investigator or an authorized designee will interview the subjects / parents / legally acceptable representatives to collect the information recorded in the diary card, 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 eCRF. (Any information that was not documented in the diary card will first be captured in the source document and then reported electronically.) The eCRF 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 eCRF 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 eCRF, 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 9.3 or higher (SAS Institute, Cary, North Carolina, USA).

Assuming that log10 transformation of the titers/ratios follows a normal distribution, first, the mean and 95% CI will be calculated on log10 (titers/ratios) 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.

12.1.1 Hypotheses and Statistical Methods for Primary Objective(s)

STAGE I:

A non-inferiority testing approach will be performed for each serotype to show the non-inferiority of 2 doses of CYD dengue vaccine (28 days after last injection) compared to 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

The non-inferiority testing approach will also be performed for each serotype to show the non-inferiority of 2 doses of CYD dengue vaccine (1 year after last injection) compared to the 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

If non-inferiority is not achieved, assessments of immunogenicity will be descriptive.

Individual hypotheses for each serotype will be as follows:

$$H_0^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} \leq \frac{1}{\delta}$$

$$H_1^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} > \frac{1}{\delta}$$

Where:

i=1, 2, 3, and 4 (each serotype),

j=2

δ the non-inferiority margin, is set to 2, ie 0.301 (=log10 [2]).

The non-inferiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, non-inferiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1/2 (ie, the log 10 of the difference should be above -0.301).

STAGE II:

The non-inferiority testing for within Group 1 comparisons will be performed in Stage II regardless of whether non-inferiority is achieved or not for either test in STAGE I. However, only those Group(s) that achieve non-inferiority in STAGE I will continue to be tested in STAGE II for the between Group 1 comparisons.

Paired Test

$$H_0^i: GM\left(V^i_{Booster\,j}/V^i_{PD3j}\right) \leq \frac{1}{\delta}$$

$$H_0^i: GM\left(V^i_{Booster\,j}/V^i_{PD3j}\right) > \frac{1}{\delta}$$

Where:

i=1, 2, 3, and 4 (each serotype),

j=1a, and 1b

δ the non-inferiority margin, is set to 2, ie, 0.301 (=log10 [2]).

The non-inferiority tests will be performed using the paired t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Booster j and Post Dose 3 j (α=2.5% one sided). For each serotype, non-inferiority will be demonstrated if the lower limit of the 2-sided 95% CI is greater than 1/2 (ie, the log 10 of the difference should be above -0.301). Subjects with non-missing post dose 3 and post booster titer will be included in this analysis.

Two Sample Test

A non-inferiority testing approach will be performed for each serotype to show the non-inferiority of a CYD booster dose (28 days after booster) either 1 year and/or 2 years after a 2-dose schedule compared to the third CYD dose (28 days after third injection), ie, between Group 1 and Group 2 comparison, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

Individual hypotheses for each serotype will be as follows:

$$H_0^i: GMT^i_{Group\,j}/GMT^i_{Group\,1} \leq \frac{1}{\delta}$$

$$H_1^i: GMT^i_{Group\,j}/GMT^i_{Group\,1} > \frac{1}{\delta}$$

Where:

i=1, 2, 3, and 4 (each serotype),

j=2a and 2b

δ the non-inferiority margin, is set to 2, ie, 0.301 (=log10 [2]).

The non-inferiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, non-inferiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1/2 (ie, the log 10 of the difference should be above -0.301).

12.1.2 Hypotheses and Statistical Methods for Secondary Objective(s)

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

STAGE I

A superiority testing approach will be performed for each serotype to show the superiority of 2 doses of CYD dengue vaccine (28 days after last injection) compared to 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

A superiority testing approach will also be performed for each serotype to show the superiority of 2 doses of CYD dengue vaccine (1 year after last injection) compared to the 3 doses of CYD dengue vaccine, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

Individual hypotheses for each serotype will be as follows:

$$H_0^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} \leq 1$$

$$H_1^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} > 1$$

Where:

i=1, 2, 3, and 4 (each serotype),

j=2

The superiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, superiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1 (ie, the log 10 of the difference should be above 0).

STAGE II:

Paired Test

A superiority testing approach will be performed for each serotype to show the superiority of a CYD booster dose (28 days after booster) either 1 year and / or 2 years after last injection compared to the third CYD dose (28 days after third injection), ie, within Group 1 comparisons, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

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

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

Where:

i=1, 2, 3, and 4 (each serotype),

j=1a, and 1b

The superiority tests will be performed using the paired t-test 95% 2 sided CI of the difference of the means of the log10 transformed post-injection titer between Booster j and post Dose 3 j (α=2.5% one sided). For each serotype, superiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1 (ie, the log 10 of the difference should be above 0). Subjects with non-missing post Dose 3 and post Booster titer will be included in this analysis.

Two Sample Test

A superiority testing approach will be performed for each serotype to show the superiority of a CYD dengue vaccine booster dose (28 days after booster) either 1 year and/or 2 years after a 2-dose schedule compared to the third CYD dengue vaccine dose (28 days after third injection), ie, between Group 1 and Group 2 comparison, in subjects seropositive at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

Individual hypotheses for each serotype will be as follows:

$$H_0^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} \leq 1$$

$$H_1^i: GMT^i_{Group\ j} / GMT^i_{Group\ 1} > 1$$

Where:

i=1, 2, 3, and 4 (each serotype),

j=2a, 2b, 3a and 3b

The superiority tests will be performed using the two sample t-test 95% 2 sided CI of the difference

of the means of the log10 transformed post-injection titer between Group j and Group 1 (α=2.5% one sided). For each serotype, superiority will be demonstrated if the lower limit of the two sided 95% CI is greater than 1 (ie, the log 10 of the difference should be above 0).

B.1 Immunogenicity

STAGE I:

In addition, immunogenicity will be assessed using the following parameters:

- Geometric means of the individual titer ratios (GMTR) for each serotype (parental strains) by baseline serostatus (1 year post-Dose 3 /post-Dose 3)
- Group 1 only, geometric mean of the individual titer ratios (GMTR) for each serotype (parental strains) by baseline serostatus (post-Dose 3/post-Dose 2)

STAGE II:

- Seroconversion rates 28 days after the booster for each serotype (parental strains) of CYD dengue vaccine

B.2 Safety

STAGES I AND II:

Safety profile will be described after each injection for primary schedules and for booster dose.

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 from D0 to D07, and unsolicited immediate systemic event occurring within 30 minutes after each injection. 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.

Detection of Symptomatic Dengue Cases

The number and percentage of subjects with a hospitalized, VCD cases occurring during the trial after the injection will be described using safety analysis set.

12.1.3 Statistical Methods for Additional Objectives

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

***Immunogenicity*:**

STAGES I and II

The immune response to each dengue serotype at each time point overall, by baseline serostatus (only in Stage I), and by age group and country will be assessed descriptively using the following parameters:

- GMT for each serotype (parental strains) at each time point
- Geometric mean of the individual titer ratios (GMTR) for each serotype (parental strains)
- Number and percentage of subjects with dengue neutralizing Ab titer ≥ 10 (1/dil) (parental strains) at each time point against each serotype
- Number and percentage of subjects with dengue neutralizing Ab titer ≥ 10 (1/dil) (parental strains) at each time point against at least one, two, three, or the four dengue serotypes
- 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 time point

- Distribution of titers against each of the 4 serotypes at each time point and corresponding reverse cumulative distribution curve
- Number and percentage of subjects who converted

Additionally, the number and percentage of subjects immune or non- immune to FV (dengue, and JE or YF) status at baseline overall and by age group and country.

Between groups comparisons at 28 days and 1 year after injection 3 (ie, Group 2 vs Group 1, Group 3 vs Group 1) in Stage I will be described using GMR at each time point for each serotype, in subjects seropositive at baseline.

Between groups comparisons within the year of the booster (ie, Group 2a vs Group 1a, Group 3a vs Group 1a, Group 2b vs Group 1b and Group 3b vs. Group 1b) will be described using GMR for each serotype 28 days after booster based on subjects seropositive at baseline.

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 (eg, Group 2 versus Group 1 or Group 3 versus Group 1) controlling for the pre-booster neutralizing Ab levels against each dengue virus serotype, in subjects seropositive at baseline. Further details regarding the ANCOVA model will be outlined in the SAP.

AIT Subset:

- 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.

12.2 Analysis Sets

12.2.1 Per-Protocol Analysis Set

The Per-Protocol analysis set (PPAS) will include all subjects who had no protocol deviations and will be defined for both stages as follows:

STAGE I:

The subjects who will be vaccinated but who meet any of the following study violations will be excluded from the PPAS in STAGE I:

- Subject did not meet all protocol-specified inclusion/exclusion criteria or definitive contraindications
- Subject did not receive the correct number of doses or injections
- Subject received a vaccine other than the one that he/she was randomized to receive
- Administration of vaccine at V05 was not done as per protocol (site and route of administration)

- Subject did not receive vaccine at V05 in the proper time window defined in the tables of the study procedures
- Subject did not provide a V06 post-dose serology sample in the proper time window
- Subject received a protocol-restricted medication, therapy, or vaccine in STAGE I as defined in Section 6.7.
- Subject's post-injection serology samples at V06 did not produce a valid test result (ie, a result different from "not-reportable" ['NR'] or missing, for at least one dengue serotype)

STAGE II:

The subjects who will be vaccinated but who meet any of the following study violations will be excluded from the PPAS in STAGE II:

- Subject did not meet all protocol-specified inclusion/exclusion criteria or definitive contraindications
- Subject did not receive the correct number of doses or injections
- Subject had negative serostatus at baseline dengue status.
- Subject received a vaccine other than the one that he/she was randomized to receive
- Administration of vaccine at V07 was not done as per protocol (site and route of administration)
- Subject did not receive vaccine at V07 in the proper time window defined in the tables of the study procedures
- Subject did not provide a V10 post-dose serology sample in the proper time window
- Subject received a protocol-restricted medication, therapy, or vaccine in STAGE II as defined in Section 6.7 of the protocol
- Subject's post-injection serology samples at V10 did not produce a valid test result (ie, a result different from "not-reportable" ['NR'] or missing, for at least one dengue serotype)
- Subjects randomized to Group 1 not included in the PPAS in STAGE I

12.2.2 Full Analysis Set

The FAS is defined as the subjects who received either at least one injection of CYD dengue vaccine or placebo and had at least one blood sample drawn and valid post-injection serology results (ie, a result different from "not-reportable" ['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 at least one injection of either CYD dengue vaccine or placebo[a]. A SafAS is defined for each dose as the subset of subjects having received this dose; subjects will be analyzed according to the treatment received at this dose. For the analysis at any dose, subjects will be analyzed according to the treatment received at the first dose.

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 PPAS will be used for the analysis for the primary objective. The FAS will be used for the superiority hypotheses testing for secondary objectives. All other immunogenicity analyses will be performed on the FAS.

The SafAS will be used for the description of clinical safety.

12.3 Handling of Missing Data and Outliers

12.3.1 Immunogenicity

For the computation of GMTs, a titer reported as < LLOQ will be converted to a value of 0.5 LLOQ.

For calculating titer ratio (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. Details will be described in the SAP.

12.4 Interim / Preliminary Analysis

No interim analyses are planned.

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

12.5 Determination of Sample Size and Power Calculation

A total of 1050 subjects are planned to be enrolled with a 1:1:1 enrollment ratio (350 per group [or 1:1:1:1:1:1 randomization ratio, 175 per subgroup]) (Table S4). Considering a potential dropout rate of 15%, this sample size would provide 888 evaluable subjects.

Table 12.1: Power of the study based on the Primary Objectives - Stage I

Baseline Seropositive (%)	Population per group	Objectives	Endpoints	NI Margin	Power (%)
100%	296	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	98.9
	296	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	90.64
		Overall			88.6
90%	266	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	97.86
	266	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	88.52
		Overall			86.6
80%	235	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	95.56
	235	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	82.3
		Overall			78.64
70%	207	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	92.06
	207	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	74.52
		Overall			68.60
60%	178	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	86.00
	178	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj	2	63.12
		Overall			54.28

Table 12.2: [redacted]

[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

The following assumptions were considered:

- Alpha level of 2.5% (one sided) with a Bonferonni adjustment for multiplicity
- Non-inferiority margin of 2 (log10 = 0.301)
- [redacted]
- Dropout rate of 15%

The sample size will also provide a 95% probability of observing an AE that has a true incidence of >0.85% in each group (N=350).

For the measurement of the Cellular Immune Response, a convenience number of 60 subjects from a specific site in Colombia who consent to participate in additional immune response testing, the AIT subset, was selected, 10 subjects from each subgroup.

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 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, ICFs / AFs, 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 DC, the study coordinator or designee will obtain verbal clarification from the subject, enter the response into the "Investigator’s comment" page of the DC, and transfer the information to the eCRF.

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 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.

13.4 Monitoring, Auditing, and Archiving

13.4.1 Monitoring

Before the start of the trial (ie, 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, eCRF 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 eCRF Completion Guidelines for entering data into the eCRF, 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:

[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.

- 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 (eg, 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 eCRF, 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 Quality Assessment (CQA) department 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 CTA 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.

Specifically for the subjects identified as seronegative at baseline, Sanofi Pasteur will also cover reasonable expenses related to healthcare for dengue illness for 10 years after the last dose received. Details will be communicated to the Ethics Committees/Institutional Review boards and to study participants.

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) will be reimbursed. 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

1 Gubler DJ. Dengue. In: Epidemiology of arthropod-borne viral disease. Monath TPM, editor, Boca Raton (FL): CRC Press, 1988:223-60.

2 World Health Organization. Dengue and dengue haemorrhagic fever, Fact sheet N°117, Updated May 2015. [Accessed on 08 July 2015. Available from URL: http://www.who.int/mediacentre/factsheets/fs117/en/.

3 World Health Organization. Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated). WHO Expert Committee on Biological Standardization. WHO Technical Report Series No. 979, 2013. Annex 2.

4 Dengue Guidelines For Diagnosis,Treatment, Prevention and Control, 2009, WHO. WHO/HTM/NTD/DEN/2009.1.

5 Kumaria R, Chakravarti A. Molecular detection and serotypic characterization of dengue viruses by single-tube multiplex reverse transcriptase-polymerase chain reaction. Diagn Microbiol Infect Dis. 2005 Aug;52(4):311-6.

6 Dussart P, Petit L, Labeau B, Bremand L, Leduc A, Moua D et al. Evaluation of Two New Commercial Tests for the Diagnosis of Acute Dengue Virus Infection Using NS1 Antigen Detection in Human Serum. PLoS Negl Trop Dis. August 2008. Volume 2/Issue 8/e280.

7 Sabchareon A, Wallace D, Sitivichayakul C, et al. Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. Lancet. 2012;380(9853):1559-67.

8 Villar LÁ, Rivera-Medina DM, Arredondo-García JL, Boaz M, Starr-Spires L, Thakur M, and al. Safety and immunogenicity of a recombinant tetravalent dengue vaccine in 9-16 year olds: a randomized, controlled, phase II trial in Latin America. Pediatr Infect Dis J. 2013;32(10):1102-9.

9 Dayan GH, Garbes P, Noriega F, Izoton de Sadovsky AD, Rodrigues PM, Giuberti C, and al. Immunogenicity and safety of a recombinant tetravalent dengue vaccine in children and adolescents ages 9-16 years in Brazil. Am J Trop Med Hyg. 2013;89(6):1058-65.

10 CYD14, Efficacy and safety of a novel tetravalent dengue vaccine in healthy children aged 2 to 14 years in Asia. Interim Clinical Study Report - Up to 24 months after the third vaccination (Year 1 Hospital Phase), Version 2.0, dated 17 June 2015.

11 CYD15, Efficacy and safety of a novel tetravalent dengue vaccine in healthy children and adolescents aged 9 to 16 years in Latin America. Interim Clinical Study Report - Up to 13 months post-injection 3, Version 1.0, dated 16 January 2015.

12 Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and Long-Term Safety of a Dengue Vaccine in Regions of Endemic Disease. N Engl J Med. 2015;373(3):1195-1206.

13 CYD29, Immunogenicity and Safety of Yellow Fever Vaccine (Stamaril®) Administered Concomitantly with Tetravalent Dengue Vaccine in Healthy Toddlers at 12-13 Months of Age in Colombia and Peru. Final Clinical Study report, version 1.0, dated 27 March 2013.

14 CYD33, Immunogenicity and Safety of a Booster Injection of DTaP-IPV//Hib (PentaximTM) Administered Concomitantly with tetravalent Dengue Vaccine in Healthy Toddlers Aged 15 to 18 Months in Mexico. Final Clinical Study Report, version 1.0, dated 29 July 2014.

15 Capeding MR, Tran NH, Hadinegoro SR, Ismail HI, Chotpitayasunondh T, Chua MN, et al. Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. Lancet 2014;384(9951):1358-65.

16 Villar L, Dayan, GH, Arredondo-García, JL, Rivera DM, Cunha R, Deseda C, et al. Efficacy of a tetravalent dengue vaccine in Children in Latin America. N Engl J Med. 2015;372(2):113-23.

17 Pan American Health Organization (PAHO). Number of Reported Cases of Dengue and Severe Dengue (SD) in the Americas, by Country; Figures for 2014. 05 May 2015 [cited 2015 July 14]. Available from: http://bit.ly/1J8zn1v

18 Pan American Health Organization (PAHO). Number of Reported Cases of Dengue and Severe Dengue (SD) in the Americas, by Country; Figures for 2015. 26 August 2017 [cited 2017 November 30]. Available from: file:///C:/Users/i0243906/Downloads/2016-cha-dengue-cases-jan-15-ew-52.pdf

19 Pan American Health Organization (PAHO). Number of Reported Cases of Dengue and Severe Dengue (SD) in the Americas, by Country; Figures for 2016. 06 February 2017 [cited 2017 November 30]. Available from: file:///C:/Users/i0243906/Downloads/2016-cha-dengue-cases-jan-26-ew-52.pdf

20 National Epidemiology Center, Republic of the Philippines. Morbidity Week, 2014-2016. [Accessed on 01 December 2017. Available from URL: http://www.doh.gov.ph/taxonomy/term/119?page=1.

21 Capeding RZ, Luna IA, Bomasang E, Lupisan S, Lang J, Forrat R, Wartel A, Crevat D. Live-attenuated, tetravalent dengue vaccine in children, adolescents and adults in a dengue endemic country: randomized controlled phase I trial in the Philippines.Vaccine 2011;29(22):3863-72. doi: 10.1016/j.vaccine.2011.03.057. Epub 2011 Apr 6.

22 Leo YS, Wilder-Smith A. Archuleta S, Shek LP, Chong CY, Leong HN, et al. Immunogenicity and safety of recombinant tetravalent dengue vaccine (CYD-TDV) in individuals aged 2-45 y: Phase II randomized controlled trial in Singapore. Human Vaccin Immunother. 2012;8(9):1259-71.

23 World Health Organization. Dengue vaccine: WHO Position paper - July 2016. Weekly epidemiological record. 2016;91(30):349-64. Available at URL: http://www.who.int/wer/2016/wer9130.pdf?ua=1

24 The SAGE Working Group on Dengue Vaccines and WHO Secretariat. Background paper on dengue vaccines. World Health Organization. 17 March 2016. Available at URL: http://www.who.int/immunization/sage/meetings/2016/april/1_Background_Paper_Dengue_Vaccines_2016_03_17.pdf?ua=1

15 Signature Pages

Sponsor Signature

I confirm that this protocol (version 4.0 dated 12 December 2017) is in accordance with applicable regulations and Good Clinical Practice.

Function	Name	Date	Signature
Sponsor's Responsible Medical Officer Clinical Team Leader Clinical Department Sanofi Pasteur SA	[redacted]	19 December 2017	[redacted]