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 dengue vaccine (CYD) 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).

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	CYD65
Development Phase:	Phase II
Sponsor:	Sanofi Pasteur SA 2, avenue Pont Pasteur, F-69367 Lyon cedex 07, France
Investigational Product(s):	CYD Dengue Vaccine
Form / Route:	Powder and solvent for suspension for injection / Subcutaneous
Indication For This Study:	Healthy subjects between 9 and 50 years of age
Version and Date of the SAP core body part:	Version 3.0 19 Mar 2019

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List of Abbreviations

Ab	antibody
AE	adverse event
AESI	adverse event of special interest
AF	assent form
Ag	antigen
AIT	Additional Immunological Test
AR	adverse reaction
BL	blood sample
CI	confidence interval
CMI	cell mediated immunity
CSR	clinical study report
D	day
DC	diary card
DF	dengue fever
DHF	dengue hemorrhagic fever
dil	dilution
Ecrf	electronic case report form
ELISPOT	Enzyme-linked immunospot
ELISA	enzyme-linked immunosorbent assay
FAS	full analysis set
GM	geometric mean
GMR	geometric mean ratio
GMT	geometric mean of titer
GMTR	geometric mean of titer ratio
ICF	informed consent form
LSMEANS	least squares means
IVRS	interactive voice response system
IWRS	interactive web response system
JE	Japanese encephalitis
LLOD	lower limit of detection
LLOQ	lower limit of quantification
MD	missing data
MedDRA	Medical Dictionary for Regulatory Activities
Nab	neutralizing antibodies

NS	non-structural protein
PC	phone call
PCR	polymerase chain reaction
PD3	post-dose 3
PPAS	per-protocol analysis set
PRNT	plaque reduction neutralizing test
PT	preferred term
RCDC	reverse cumulative distribution curve
SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SC	screening
SD	standard deviation
SOC	system organ class (primary)
ULOQ	upper limit of quantification
V	visit
VCD	virologically-confirmed dengue
WHO	World Health Organization
YF	Yellow fever

1 Introduction

This trial (CYD65) 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 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 is conducted in two endemic countries, one in Latin America (Colombia) and one in Asia (the Philippines) and includes 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.

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).

At present, no specific treatment exists for dengue disease. As of March 2019, the CYD dengue vaccine (commercial name Dengvaxia®) has been registered in 20 of : Argentina, Australia, Bangladesh, Bolivia, Brazil, Cambodia, Costa Rica, Dominican Republic, El Salvador, Europe, Guatemala, Honduras, Indonesia, Myanmar, Mexico, Paraguay, Peru, Singapore, Thailand, and Venezuela. Other than vaccination, preventive measures rely on mosquito control and personal protection. However, 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.

This statistical analysis plan (SAP) includes detailed procedures for executing statistical analysis of the primary and secondary variables and other data. Its purposes are the following:

- To state the objectives of this study
- To clearly define all variables (raw and derived), criteria and parameters that will be used for statistical analyses
- To describe statistical methods that will be used for analyses; descriptive statistics used are listed
- To define populations used for analyses

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 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.

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*

2.2 Secondary Objectives

Immunogenicity

STAGE I:

- To demonstrate the superiority of the immune response elicited by 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 GMR.

- To demonstrate the superiority of the immune response elicited by 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 seropositive subjects at baseline, 1 year after last 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 virologically-confirmed dengue (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 adverse events (AEs); serious adverse events (SAEs) throughout the trial and for 6-months following end of trial; and adverse events of special interest (AESIs) at defined time windows throughout the trial according to the type of AESI)

2.3 Additional Objectives

Immunogenicity

STAGE I:

- To describe the neutralizing Ab response of flavivirus (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 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)

STAGE I and STAGE II 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

Note: It is to be noted that the 1:1 ratio per subgroup and N=60 are unlikely to hold as only subjects dengue seropositive at baseline will be eligible to booster injection.

3 Description of the Overall Trial Design and Plan

3.1 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) stratified by site ([redacted], [redacted], [redacted], [redacted], [redacted] and [redacted]) and age group (Children 9-11 years, Adolescents 12-17 years, Adults 18-39 years and Adults 40-50 years) 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 (e.g., 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 Ab, and Ab specificity and affinity maturation assessments. More details are provided in Section 5.1.3 of the protocol.

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

3.2 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 (informed consent form [ICF] and assent form [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.

Vaccination

All subjects (N=1050) will receive 1 injection at D0, D180 and D365: a total of 350 subjects will receive 3 doses of CYD dengue vaccine (Group 1); 350 subjects will receive 1 PLA injection followed by 2 doses of CYD dengue vaccine (Group 2); and 350 subjects will receive 2 PLA injections followed by 1 dose of CYD dengue vaccine (Group 3).

Blood Sampling

All subjects will provide a blood sample (BL) 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

Safety Data Collection

Reactogenicity data will be collected in all subjects after the CYD or PLA injection: solicited injection site reactions will be collected for Days 0–7; immediate adverse events (AEs) observed to occur within 30 min post-injection will be collected; 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 adverse events (SAEs) will be reported throughout the study and serious and non-serious AESIs will be collected in defined time-windows according to the type of AESI.

STAGE II

Vaccination

Before the beginning of STAGE II, blood samples collected before the first injection of CYD dengue vaccine (at V01 for Group 1, V03 for Group 2, and V05 for Group 3) will be used for determining the baseline dengue serostatus for study participants in order to determine if booster injection can occur during STAGE II. 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 50 titer ≥ 1:10 to any dengue serotype at baseline, and a “seronegative” subject has been defined by a PRNT 50 titer <10 for all four dengue serotypes.

At 1 year following the last primary series vaccination, 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 in subgroup b (1b, 2b, and 3b) will receive a single booster injection of CYD dengue vaccine

Blood Sampling

Each STAGE II subject will provide blood samples for assessing neutralizing Ab titers against each of the 4 parental dengue virus strains immediately before and 28 days after receiving the booster injection

Safety Data Collection

For all STAGE II subjects, reactogenicity data will be collected in all subjects after the CYD 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 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 diagnosis of dengue requiring hospitalization (with bed attribution).

SAEs will be reported throughout STAGE II (and 6-month follow-up following booster injection) and serious and non-serious AESIs will be collected in defined time windows according to the type of AESI. Safety will also be followed up until 6 months after V07 for booster Y1 and for booster Y2

STAGE I and STAGE II

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 STAGE I subjects immediately before Injection 1 (specificity, affinity and CMI) and at 28 days following 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. 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.

See Table 3.1 for details of study procedures

Table 3.1: Study procedures

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 AIT only)**	**+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 AIT only)**	**+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‡‡	

	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 AIT only)**	**+20d**
CMI	BL1								BL6‡‡	BL10‡‡	BL7 or BL11‡‡	BL8 or BL12‡‡	BL9 or BL13‡‡	

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

* As describe 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 **Error! Reference source not found.** 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

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

4.1.1 Immunogenicity

4.1.1.1 Immunogenicity Endpoints

See Section 9.1.1.1 of the protocol.

4.1.1.2 Immunogenicity Assessment Methods

See Section 9.1.1.2 of the protocol.

4.1.2 Safety

There are no primary objectives for safety.

4.1.3 Efficacy

No clinical efficacy data will be obtained in the trial.

4.2 Secondary Endpoints and Assessment Methods

4.2.1 Immunogenicity

4.2.1.1 Immunogenicity Endpoints

See Section 9.2.1.1 of the protocol.

4.2.1.2 Immunogenicity Assessment Methods

See Section 9.2.1.2 of the protocol.

4.2.2 Safety

4.2.2.1 Safety Endpoints

See Section 9.2.2.2 of the protocol.

4.2.2.2 Safety Assessment Methods

See Section 9.2.2.3 of the protocol.

4.2.3 Efficacy

No clinical efficacy data will be obtained in the trial.

4.3 Additional Endpoints and Assessment Methods

4.3.1 Immunogenicity

4.3.1.1 Immunogenicity Endpoints

See Section 9.3.1.1 of the protocol.

4.3.1.2 Immunogenicity Assessment Methods

See Section 9.3.1.2 of the protocol.

The lower limit of detection (LLOD) for the 50% of neutralization titer by flow cytometry for Ab specificity is a titer of 20. To be able to calculate affinity, the LLOD for measuring the kinetic rates is an Ab concentration of 1 ng/mL and the lowest dissociation constant K_D that can reliably be measured on the instrument is 10 pM.

4.3.2 Safety

There are no additional objectives for safety.

4.3.3 Efficacy

No clinical efficacy data will be obtained in the trial.

4.4 Derived Endpoints: Calculation Methods

4.4.1 Immunogenicity

4.4.1.1 Computed Values for Analysis

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

For calculating geometric mean 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.

4.4.1.2 Seroconversion

If a pre-booster titer < 10 (1/dil) and a post-booster dose titer is ≥ 40 (1/dil) then the derived seroconversion indicator will be “Yes” for that test. Or, if a subject has a pre-booster titer ≥ 10 (1/dil) and achieves ≥ 4-fold increase from pre to post-booster dose titer then the seroconversion indicator will be “Yes” for the test. Otherwise, seroconversion will be “No”.

4.4.1.3 Calculation rules for the "at least X serotype(s)" tables

The criteria below will be computed for each subject and visit as soon as at least one of the four dengue serotype result is different from missing or not-reportable (NR) (i.e. coded no result in the serology database):

- Number and percentage of subjects with antibody titer ≥ 10 (1/dil) against at least 1, 2, 3, or 4 serotypes with the parental dengue virus strains.
- Number and percentages of subjects with antibody titer ≥ various titer thresholds (1/dil) against at least 1, 2, 3, or 4 serotypes with parental the dengue virus strains.

Titer(s) ≥ to a threshold for at least X serotype(s) with parental dengue virus strains is computed as a Yes/No/Missing variable (note: in the case no titer is available the variable will be missing). If at least X among the 4 serotype titers meet the threshold considered then the variable is derived to "Yes", otherwise if at least one titer is available and does not meet the threshold the variable is derived to "No". For the percentage calculation, all the subjects with at least one titer available regardless of the serotype will be considered in the denominator.

4.4.1.4 Flavivirus, Dengue and Yellow Fever/Japanese Encephalitis status

4.4.1.4.1 Baseline flavivirus (FV) status

Flavivirus (FV) status at baseline is defined as

- **Immune** for subjects with quantified (≥ LLOQ) neutralizing Abs against Janpanese Encephalitis (JE), Yellow Fever (YF), and/or against at least one dengue serotype in the baseline sample.
- **Non-immune** for subjects without quantified (< LLOQ) neutralizing Abs against JE, YF and with no Ab quantified against any of the four dengue serotypes in the baseline sample. To be classified in this group, *all* of the titers planned to be measured at baseline must be *available*, and *valid* i.e., not coded 'NR' in the serology database, otherwise the FV status will be classified as "*undetermined*."

The FV status will be **undetermined** for subjects that have a value < LLOQ and/or 'missing' and/or 'NR' results for all of the FVs planned to be measured. This category will not be presented separately but will be included in the 'All subjects' category.

The LLOQ for dengue, JE and YF neutralizing Abs is 10 (1/dilution [dil]).

4.4.1.4.2 Baseline Dengue Status

The dengue status at baseline is defined as the presence of Abs against at least one dengue serotype in the baseline blood sample. The baseline dengue blood sample closest to the first injection of CYD dengue vaccine will be assayed in priority as follows:

- Group 1 baseline dengue sample is the blood sample collected at V01 (BL1)
- Group 2 baseline dengue sample is the blood sample collected at V03 (BL2, or BL1 if BL2 is unavailable)

- Group 3 baseline dengue sample is the blood sample collected at V05 (BL4, or BL2 if BL4 is unavailable, or even BL1 if BL2 is unavailable)

The baseline dengue status variable will be calculated as follows:

- Immune for subjects with quantified (≥ LLOQ) neutralizing Abs against at least one dengue serotype in the baseline sample.
- Non-immune for subjects with no quantified (< LLOQ) neutralizing Abs against all of the four dengue serotypes in the baseline sample. In this group, all of the titers planned to be measured at baseline must be available and, valid i.e., different from no result ('NR') in the serology database, otherwise the baseline dengue status will be classified as "undetermined".

The baseline dengue status will be undetermined for subjects that have 'missing' and/or 'NR' results for all of the four dengue serotypes measured at baseline and for subjects who have values < LLOQ for some serotypes and a combination of 'missing' and 'NR' results for all others of the four dengue serotypes measured at baseline. Baseline dengue status will be used to identify the status of subjects at baseline.

4.4.1.4.3 Baseline Yellow Fever and Japanese Encephalitis Status

The following baseline statuses with identical categories as baseline dengue status (i.e., immune, non-immune and undetermined) are defined as follows:

- Baseline JE status for subjects in the Philippines: considering neutralizing Abs against JE
- Baseline YF status for subjects in Colombia: considering neutralizing Abs against YF

The baseline blood sample used for YF and JE status is at V01 (BL1) for all subjects.

4.4.2 Safety

Terms used in the clinical safety tables to describe the safety events are specified below:

- AE: Adverse event; includes immediate, solicited, and unsolicited non-serious or serious adverse events.
- AR: Adverse reaction; Adverse reaction corresponds to related AE
- Immediate AE/AR: Unsolicited non-serious AE ticked "immediate (within 30 minutes from the vaccination)" by the investigator in the eCRF or SAE with time of onset within 30 minutes
- Solicited reaction: Event pre-listed in the eCRF, and which occurred during the solicited period (period is 0 to 7 days for injection site reactions and 0 to 14 days for systemic reactions post-vaccination)
- Unsolicited AE: AE recorded in the eCRF unsolicited form and SAE form, excluding solicited reactions. Therefore, this term includes immediate AEs/ARs.
- Unsolicited non-serious injection site events are always recorded without relationship and analyzed as ARs.
- SAE: Unsolicited AE considered serious by the investigator.

- Hospitalized suspected dengue case: Hospitalized suspected dengue disease is defined as an acute febrile illness with diagnosis of dengue requiring hospitalization (with bed attribution).
- VCD case: A VCD infection is defined as a suspected dengue which has positive sample for the dengue screen RT-PCR (i.e., ≥LLOQ) and/or the non-structural protein 1 (NS1) antigen (Ag) enzyme-linked immunosorbent assay (ELISA) is positive and/or the Simplexa™ dengue reverse transcription-polymerase chain reaction (RT-PCR) is positive. Dengue NS1 Ag ELISA positive is defined as sample ratio >1 by comparing the optical density reading of the sample to the optical density of the cutoff control serum. The Simplexa™ Dengue RT-PCR positive is defined as detected results for at least one dengue serotype.

4.4.2.1 Solicited Reactions

4.4.2.1.1 Daily Intensity

All daily records for solicited reactions will be derived into daily intensity according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

For the derivation of daily intensities the following sequential steps will be applied:

1) Solicited reactions (except Fever/Pyrexia) with an investigator presence recorded as "No" and with all daily records missing then all daily intensities will be derived as None.
2) For non-measurable solicited reactions, daily intensities will correspond to daily records reported in the clinical database. For measurable solicited reactions the daily measurements reported in the clinical database will be converted based upon the intensity scales defined in Section 9.2.2.3.2 of the protocol; this assumes a reaction that is too large to measure (non measurable, [NM]) is Grade 3. Note the intensity could be considered as "None" (i.e., not a reaction) in the analysis despite being considered a reaction by the Investigator (e.g., Injection site swelling measurement > 0 mm and < 25 mm in adolescents or adults).

Note: The maximum intensity on the ongoing period is derived from the record of the maximum intensity/measurement after the end of the solicited period following the rule described above.

4.4.2.1.2 Maximum Intensity

Maximum intensity is derived from the daily intensities computed as described in Section 4.4.2.1.1 and is calculated as the maximum of the daily intensities over the period considered.

4.4.2.1.3 Presence

Presence is derived from the maximum intensity on the period considered:

- None: No presence
- Grade 1, Grade 2, or Grade 3: Presence
- Missing: Missing presence

Subjects with at least one non-missing presence for a specific endpoint will be included in the analysis. Conversely, those without a non-missing presence will not be included in the analysis of the endpoint.

4.4.2.1.4 Time of Onset

Time of onset is derived from the daily intensities computed as described in Section 4.4.2.1.1. It corresponds to the first day with intensity of Grade 1, Grade 2, or Grade 3.

Note: If a reaction is not continuous (i.e., reaction occurs over two separate periods of time intervened by at least one daily intensity Missing or None) then the time of onset is the first day of the first occurrence.

Time of onset will be displayed by period as follows:

- Injection site reactions (D0-D7): D0-D3, D4-D7
- Systemic reactions (D0-D14): D0-D3, D4-D7, D8-D14

4.4.2.1.5 Number of Days of Occurrence

Number of days of occurrence over the period considered is derived from the daily intensities computed as described in Section 4.4.2.1.1. It corresponds to the number of days with daily intensities of Grade 1, Grade 2, or Grade 3. Number of days of occurrence on the solicited period with a specified intensity may also be derived.

Number of days of occurrence during the solicited period will be displayed by category (range) as follows:

- Injection site reactions (D0-D7): 1-3 days, 4-7 days, 8 days
- Systemic reactions (D0-D14): 1-3 days, 4-7 days, 8-14 days, 15 days

4.4.2.1.6 Overall Number of Days of Occurrence

If a reaction is ongoing at the end of the solicited period, then the overall number of days of occurrence is derived from the daily intensities and the stop date of the reaction after the end of the solicited period. The overall number of days of occurrence is:

- (stop date – last vaccination date) + (number of days of occurrence within the solicited period) – length of the solicited period + 1

If the stop date is missing or incomplete (contains missing data [MD]), the overall number of days of occurrence will be considered as Missing.

Overall number of days of occurrence will be displayed by category (range) as follows:

- Injection site reactions (D0-D7): 1-3 days, 4-7 days, ≥8 days, missing
- Systemic reactions (D0-D14): 1-3 days, 4-7 days, 8-14 days, ≥15 days, missing

4.4.2.1.7 Ongoing

Ongoing is derived from the last daily intensity of the solicited period computed as described in Section 4.4.2.1.1 and the maximum intensity on the ongoing period. The investigator's ongoing flag is not used because the measurement would determine the ongoing status of the reaction. Note the intensity could be considered as "None" (i.e., not a reaction) in the analysis despite being considered a reaction by the Investigator (e.g., Injection site swelling measurement > 0 mm and < 25 mm in adolescents or adults).

If the last daily intensity of the solicited period is at least Grade 1 and maximum intensity on the ongoing period is also at least Grade 1, then the reaction is considered ongoing. In any other cases the reaction will not be considered as ongoing.

4.4.2.2 Unsolicited Non-serious Adverse Events

4.4.2.2.1 Presence

An observation will be considered as an event if it has at least a verbatim term and is not a Grade 0 intensity event. Grade 0 events should be included in the listing "Unsolicited non-serious adverse events not included in the safety analysis".

4.4.2.2.2 Intensity

Intensity for unsolicited non-serious AE will be derived according to the following classification: None, Grade 1, Grade 2, Grade 3, or Missing.

If the unsolicited non-serious AE is measurable and its preferred term is part of the list of solicited reactions, then the measurement is derived based upon and following the same rule than the intensity scales defined in the protocol for that measurable injection site or systemic reaction. Note the intensity could be considered as "None" (i.e., not a reaction) in the analysis despite being considered a reaction by the Investigator (e.g., Injection site swelling measurement > 0 mm and < 25 mm in adolescents or adults).

Intensity for the other unsolicited non-serious AEs will correspond to the value reported in the eCRF.

The maximum intensity corresponds to the highest intensity for a unique term.

4.4.2.2.3 Last Vaccination

Last vaccination before an unsolicited non-serious AE is derived from the visit numbers provided in the clinical database and is calculated as follows:

- If an unsolicited non-serious AE has a non-missing visit number, the visit number should be used to determine the last vaccination before the unsolicited non-serious AE
- If the visit number is missing, then the start date should be used to determine the last vaccination before the unsolicited non-serious AE

4.4.2.2.4 Time of Onset

Time of onset is derived from the start date of the unsolicited non-serious AE provided in the clinical database and the date of last vaccination:

- Start date of the unsolicited non-serious AE - date of previous vaccination

The time of onset should be considered as missing only if one or both of the dates are missing or partially missing.

The unsolicited non-serious AEs will be analyzed "Within 28 days", which corresponds to AEs with a time to onset between 0 and 28 days after vaccination or missing. An AE with missing time of onset will be considered to have occurred just after the vaccination indicated by the visit number, so will be included in these tables.

Note: Unsolicited non-serious AE that occurred before vaccination (negative time of onset) or with a time of onset higher than defined above will not be included in analysis, but will be listed separately.

Time of onset will be displayed by period as follows: D0-D3, D4-D7, D8-D14, ≥ D15 and Missing.

4.4.2.2.5 Duration

Duration is derived from the start and stop dates of the unsolicited non-serious AE provided in the clinical database:

- Stop date of unsolicited non-serious AE - start date of unsolicited non-serious AE + 1.

The duration should be considered as missing only if one or both of the start and stop dates of the unsolicited non-serious AE is missing or partially missing.

Duration will be displayed by period as follows: 1-3 days, 4-7 days, 8-14 days, 15 days or more, Missing.

4.4.2.3 SAEs

4.4.2.3.1 Last Vaccination

Last vaccination before an SAE is derived from the last visit numbers provided in the clinical database and is calculated as follows:

- If an SAE has a non-missing visit number, the visit number should be used to determine the last vaccination before the SAE
- If the visit number is missing, then the start date should be used to determine the last vaccination before the SAE

4.4.2.3.2 Time of Onset

Time of onset will be computed using the same methodology than for unsolicited non-serious AEs described in Section 4.4.2.2.4.

SAEs will be analyzed throughout the study using the following periods:

- Within 28 days after injection
- During the study (i.e., all SAEs occurred during the study)

An SAE with missing time of onset will be considered to have occurred after the vaccination indicated by the visit number, so will be included in these tables. SAEs collected after Visits 1, 3, 5, 7, 8, or 9 with missing time of onset will be included in the table of SAEs analyzed "within 28 days" after the respective injection.

Note: SAEs that occurred before vaccination (negative time to onset) will not be included in analysis, but will be listed separately.

4.4.2.3.3 Duration

Duration will be computed using the same methodology than for unsolicited non-serious AEs described in Section 4.4.2.2.5.

4.4.2.4 Other Safety Endpoints

4.4.2.4.1 Pregnancy

This information will not be included in the analysis, but will be listed separately as collected.

4.4.2.4.2 Action taken

The information will be summarized as collected, including missing observations. No derivation or imputation will be done.

4.4.2.4.3 Seriousness

The information will be summarized as collected. No derivation or imputation will be done.

4.4.2.4.4 Outcome

The information will be summarized as collected. No derivation or imputation will be done.

4.4.2.4.5 Causality

The information will be summarized as collected. Missing causality (relationship) will be handled as described in Section 5.3.2.2.

4.4.2.4.6 AEs Leading to Study Discontinuation

A flag is available in the clinical database for all AEs in order to identify AEs leading to discontinuation.

The items that are counted are:

- Disposition table: A subject who has, on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" is checked
- Safety overview table: A subject who has either on the termination form, the reason for early termination "Serious Adverse Event" or "Other adverse event" is checked or lists an AE on an AE page (unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated
- System organ class (SOC)/PT frequency table: An event (unsolicited, or SAE) that has "Reaction Leading to Termination" or "Event Leading to Termination" or "Serious Adverse Event Leading to Termination" checked that is at least Grade 1 and is within the time period indicated

4.4.2.4.7 AESIs

The following serious AESIs (reported as SAEs) 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

The following non-serious AESI will be considered:

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

4.4.2.4.8 WHO Criteria

The WHO criteria will be derived for virologically-confirmed dengue cases.

A dengue case will be considered as meeting the WHO criteria if it is at least Grade I.

The definition of dengue hemorrhagic fever (DHF) Grade I, II, III, and IV will be consistent with the 1997 WHO definition:

- Clinical manifestations

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

a) Fever: acute onset, high (≥38°C) and continuous, lasting 2 to 7 days

b) Any of the following hemorrhagic manifestations: a positive tourniquet test, petechiae, purpura, ecchymosis, epistaxis, gum bleeding, and hematemesis and/or melena

- Laboratory findings:

a) Thrombocytopenia (platelet count ≤ 100×10^9 /L)

b) Plasma leakage as shown by hemoconcentration (hematocrit increased by 20% or more) or pleural effusion (seen on chest X-ray) and/or ascites and/or hypoalbuminemia

The first two clinical criteria, plus thrombocytopenia and signs of plasma leakage are sufficient to establish a clinical diagnosis of DHF. Pleural effusion (seen on chest X-ray) and/or hypoalbuminemia provide supporting evidence of plasma leakage.

DHF will be graded as follows:

- Grade I: Fever accompanied by non-specific constitutional symptoms; the only hemorrhagic manifestation is a positive tourniquet test.
- Grade II: Spontaneous bleeding in addition to the manifestations of Grade I patients, usually in the form of skin and/or other hemorrhages.
- Grade III: Circulatory failure manifested by rapid and weak pulse, narrowing of pulse pressure (20 mmHg or less) or hypotension, with the presence of cold clammy skin and restlessness
- Grade IV: Profound shock with undetectable blood pressure and pulse

Dengue cases may also be classified, as post-hoc analysis, by alternative definitions if and when they are available.

4.4.3 Efficacy

Not applicable.

4.4.4 Derived Viremia Variable

The following viremia endpoints will be calculated for each subject:

Presence of detectable (≥ LLOD) or quantified (≥ LLOQ) viremia (Yes, No) for the non-serotype specific dengue (by dengue screen RT-PCR) in hospitalized suspected dengue cases during the study.

4.4.5 Derived Other Variables

4.4.5.1 Age for Demographics

The age of a subject on the day of first injection is computed as follows:

Age in years: (Date of V01–Date of birth +1) / 365.25

4.4.5.2 Age Group

Age will be classified into the following groups:

- Children 9-11 years of age
- Adolescents 12-17 years of age
- Adults 18-39 years of age
- Adults 40-50 years of age
- 9-16 years of age
- 17-45 years of age
- 46-50 years of age

4.4.5.3 Duration of the Study

The duration of the study is computed in days as follows: Latest date of all subjects (termination date, last visit date, date of last contact) – earliest date of all subjects (date of visit V01) +1.

4.4.5.4 Subject Duration

The duration of a subject participation in the study is computed as follows:

Maximum (Visit dates, Termination date, Follow-up date, Last contact date) – V01 date + 1.

5 Statistical Methods and Determination of Sample Size

The statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics platform using SAS® Version 9.4 software or later.

The results of the statistical analysis will be available in the final clinical study report (CSR).

For descriptive purposes, the following statistics will be presented:

Table 5.1: Descriptive statistics produced

Baseline characteristics and follow-up description	**Categorical data**	Number of subjects. Percentage of subjects.
	Continuous data	Mean, standard deviation (SD), quartiles, minimum, and maximum.
Clinical safety results	**Categorical data**	Solicited: Number and percentage (95% CIs) of subjects. Unsolicited: Number and percentage (95% CIs) of subjects, and number of events.
Immunogenicity results	**Categorical data (seroprotection, seroconversion, cutoff)**	Number and percentage (95% CIs) of subjects.
	Continuous data (titer / titer ratio)	Log10: Mean and standard deviation. Anti-Log10 (work on Log10 distribution, and anti-Log10 applied): Geometric mean, 95% CI of the geometric mean, quartiles, minimum, and maximum. Graphical representation by Reverse Cumulative Distribution Curve (RCDC).
CMI, Ab specificity and affinity maturation	**Categorical data**	Number and percentage of subjects above or equal to the LLOD, between some ranges of values and number and percentage (95% CIs) of responders subjects.
	Continuous data	Log10: Mean and SD. Distribution: Geometric mean, 95% CI of the geometric mean, quartiles, minimum, and maximum of the corrected values

The CI for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (1), i.e., using the inverse of the beta integral with SAS.

For immunogenicity results, assuming that Log10 transformation of the titers / data follows a normal distribution, at first, the mean and the 95% CI will be calculated on Log10 (titers / data) using the usual calculation for normal distribution (using Student's t distribution with n-1 degree of freedom), then antilog transformations will be applied to the results of calculations, in order to provide geometric means (GMs) and their 95% CI.

GM is defined as follows:

$$GM = \left(\prod_{i=1}^{n} y_i \right)^{1/n} = 10\text{^}\left(\frac{1}{n} \sum_{i=1}^{n} \log_{10}(y_i) \right)$$

where $(y_1, y_2, \ldots, y_n)$ are the observed titers or other data where applicable for each subject. Rounding rules on descriptive statistics will follow the Sanofi Pasteur standard technical guideline ("Conventions for the Presentation of Descriptive Statistics").

AUC-MB is defined as area under the magnitude-breadth curve and calculated as the average of the four log10 serotype-specific titers.

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Primary Objectives

5.1.1.1 Hypotheses

STAGE I:

Individual Hypotheses for Each Serotype:

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 vaccination) compared to the 3 dose of CYD dengue vaccine, in subjects seropositive at baseline.

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.

Individual hypotheses for each serotype will be as follows:

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

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

Where:

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

j=2

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

The overall non-inferiority of Group 2 to Group 1 at 28 days after last injection will be demonstrated if all 4 individual serotype null hypotheses are rejected. The same, overall non-inferiority of Group 2 to Group 1 at 1 year after last injection will be demonstrated if all 4 individual serotype null hypotheses are rejected.

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 Group 1 and Group 2 that achieve non-inferiority at 28 days after last vaccination in STAGE I will continue to be tested in STAGE II for the between Group 1 comparisons.

Individual Hypotheses for Each Serotype:

Paired Test (Within Group 1)

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), i.e., within Group 1 comparisons, 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:

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

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

Where:

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

j=1a or 1b (subgroup)

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

The overall non-inferiority of Group j (j=1a or 1b) at 28 days after booster injection to Group j (j=1a or 1b) at 28 days after last 3rd-injection will be demonstrated if all 4 individual serotype null hypothesis are rejected.

Two Sample Test (Between Group 1)

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 vaccination), i.e., between Group 1 and Group 2 comparison, in seropositive subjects at baseline. A Bonferonni alpha adjustment will be used to control for multiplicity.

Individual hypotheses for each serotype will be as follows:

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

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

Where:

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

j=2a or 2b (sub-group)

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

The overall non-inferiority of Group j (j=2a or 2b) to Group 1 at 28 days after booster injection will be demonstrated if all 4 individual serotype null hypotheses are rejected.

5.1.1.2 Statistical Methods

STAGE I:

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-vaccination titer between Group 2 and Group 1 (α=2.5% one sided) at 28 days and 1 year after last injection. For each serotype, non-inferiority will be demonstrated if the lower limit of the two sided adjusted 95% CI is greater than 1/2 (i.e., the log 10 of the difference should be above -0.301).

STAGE II:

Paired Test (Within Group 1)

The non-inferiority tests will be performed using the paired t-test adjusted 95% 2 sided CI (100*[1- α/total number of overall hypothesis tests to perform in STAGE II] CI [Bonferonni adjustment to control for multiplicity]) of the difference of the means of the log10 transformed post-vaccination 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 adjusted 95% CI is greater than 1/2 (i.e., 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 (Between Group 1 and Group 2)

The non-inferiority tests will be performed using the two sample t-test adjusted 95% 2 sided CI (100*[1- α/total number of overall hypothesis tests to perform in STAGE II] CI [Bonferonni adjustment to control for multiplicity]) of the difference of the means of the log10 transformed post-vaccination 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 adjusted 95% CI is greater than 1/2 (i.e., the log 10 of the difference should be above -0.301).

5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

5.1.2.1 Hypotheses

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

STAGE I

Individual Hypotheses for Each Serotype:

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 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:

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

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

Where:

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

j=2

The overall superiority of Group 2 to Group 1 at 28 days after last injection will be demonstrated if all 4 individual serotype null hypotheses are rejected. The same, overall superiority of Group 2 to Group 1 at 1 year after last injection will be demonstrated if all 4 individual serotype null hypotheses are rejected.

STAGE II:

Individual Hypotheses for Each Serotype:

Paired Test (Within Group 1)

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 last injection compared to the third CYD dose (28 days after third vaccination), i.e., within Group 1 comparisons, 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:

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

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

Where:

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

j=1a or 1b (sub-group)

The overall superiority of Group j (j=1a or 1b) at 28 days after booster injection to Group j (j=1a or 1b) at 28 days after last 3rd-injection will be demonstrated if all 4 individual serotype null hypotheses are rejected.

Two Sample Test (Between Group 1 and Group 2)

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 (28 days after third vaccination), i.e., between Group 1 and Group 2 comparisons, 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:

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

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

Where:

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

j=2a or 2b

The overall superiority of Group j (j=2a or 2b) to Group 1 at 28 days after booster injection will be demonstrated if all 4 individual serotype null hypotheses are rejected.

5.1.2.2 Statistical Methods

5.1.2.2.1 Statistical Methods for Hypotheses Objectives

STAGE I:

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-vaccination titer between Group 2 and Group 1 (α=2.5% one sided) at 28 days and 1 year after last injection. For each serotype, superiority will be demonstrated if the lower limit of the two sided adjusted 95% CI is greater than 1 (i.e., the log 10 of the difference should be above 0).

STAGE II:

Paired Test (Within Group 1)

The superiority tests will be performed using the paired t-test adjusted 95% 2 sided CI (100*[1-α/total number of overall hypothesis tests for non-inferiority to perform in STAGE II] CI [Bonferonni adjustment to control for multiplicity]) of the difference of the means of the log10 transformed post-vaccination 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 adjusted 95% CI is greater than 1 (i.e., 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 (Between Group 1)

The superiority tests will be performed using the two sample t-test adjusted 95% 2 sided CI (100*[1- α/number of overall hypothesis tests for non-inferiority to perform in STAGE II] CI [Bonferonni adjustment to control for multiplicity]) of the difference of the means of the log10 transformed post-vaccination 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 adjusted 95% CI is greater than 1 (i.e., the log 10 of the difference should be above 0).

5.1.2.2.2 Statistical Methods for Other Objectives

5.1.2.2.2.1 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 /28 days post-Dose 3)
- Group 1 only, GMTR for each serotype (parental strains) by baseline serostatus (28 days post-Dose 3 /post-Dose 2)

STAGE II:

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

5.1.2.2.2.2 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.

5.1.3 Statistical Methods for Additional Objectives

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

5.1.3.1 Immunogenicity

STAGES I / II

The immune response to each dengue serotype at each time point overall, by baseline dengue 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
- GMTR for each serotype (parental strains) 28 days after the second (Group 1 and 2), and third injections (Group 1, 2 and 3), and 28 days after booster (Group 1, 2, and 3) based on the baseline neutralizing Ab titer (see Section 4.4.1.4 for baseline dengue assessment for each group):
- 28 days after second injection: V04/V01 (Group 1) & V04/V03 (Group 2)
- 28 days after third injection: V06/V01 (Group 1), V06/V03 (Group 2) & V06/V05 (Group 3)
- 28 days after booster injection: V10/V01 (Group 1), V10/V03 (Group 2) & V10/V05 (Group 3)
- GMTR for each serotype (parental strains) 28 days after booster (Group 1, 2, and 3) based on the 28 days post third and pre-booster injection neutralizing Ab titer:
- Based on 28 days post third injection: V10/V06 (for Groups 1, 2 and 3)
- Based on pre-booster: V10/V07 (for Groups 1, 2 and 3)
- 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 RCDC
- Number and percentage of subjects who converted (based on pre- and 28 days post-booster time points)
- GM at baseline and 28 days after injection 3 for each serotype in subjects belonging to Groups 1, 2 and 3 (aggregate) who are seropositive at baseline and AUC-MB at baseline and 28 days after injection 3 for the average of the four serotypes, by age strata (9-16 years of age, 17-45 years of age, and 45-50 years of age), overall and by country.
- GM at baseline and 28 days after injection 3 for each serotype in subjects belonging to Group 1 who are seropositive at baseline, and AUC-MB at baseline and 28 days after injection 3 for

the average of the four serotypes, by age strata (9-16 years of age, 17-45 years of age, and 46-50 years of age), overall and by country.

- GM at baseline and 28 days after injection 3 for each serotype in subjects belonging to Group 2 who are seropositive at baseline, and AUC-MB at baseline and 28 days after injection 3 for the average of the four serotypes, by age strata (9-16 years of age, 17-45 years of age, and 46-50 years of age), overall and by country.
- GM at baseline and 28 days after injection 3 for each serotype in subjects belonging to Group 3 who are seropositive at baseline, and AUC-MB at baseline and 28 days after injection 3 for the average of the four serotypes, by age strata (9-16 years of age, 17-45 years of age, and 46-50 years of age), overall and by country.
- GM at pre-booster and 28 days post-booster for each serotype in subjects belonging to Groups 1a, 2a and 3a (aggregate) /1b, 2b and 3b (aggregate) who are seropositive at baseline and AUC-MB at pre-booster and 28 days post-booster for the average of the four serotypes, by age strata (9-16 years of age, 17-45 years of age, and 46-50 years of age), in Colombia and by 1a/1b, 2a/2b, 3a/3b separately.

Additionally, the number and percentage of subjects immune or non- immune to 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 age strata comparisons at baseline and 28 days after injection 3 (9-16 years vs 17-45 years vs 46-50 years) in STAGE I will be described using GMR (9-16y/17-45y; 9-16y/46-50y; 17-45y/46-50y) for each serotype, in subjects seropositive at baseline. Between age strata comparisons at pre-booster and 28 days post-booster (9-16 years vs 17-45 years vs 46-50 years) in STAGE II will be described using GMR (9-16y/17-45y; 9-16y/46-50y; 17-45y/46-50y) for each serotype, in subjects seropositive at baseline. Exploratory assessment of non-inferiority between age strata will be based on upper bound of 95% CI of the GMR being <2.

Between groups comparisons within the year of the booster (i.e., 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.

5.1.3.1.1 Analysis adjusting for baseline or pre-booster neutralizing Ab levels

Analysis of covariance (ANCOVA) also will be used to compare the mean response of neutralizing Ab levels against each dengue virus serotype 28 days after injection 3 between age strata adjusting for baseline neutralizing Ab levels against each dengue virus serotype based on the least squares means, in subjects seropositive at baseline.

For each serotype, the following model will be considered:

$Log10\ titer_i = log10\ baseline_i + country + age\ strata_i$

Where:

- $titer_i$ is the 28 days after injection 3 titer value for each subject
- $baseline_i$ is baseline titer value for each subject. Sample collected at V01 (BL1), V03 (BL2, or BL1 if BL2 is unavailable) and V05 (BL4, or BL2 if BL4 is unavailable, or even BL1 if BL2 is unavailable) will be used as baseline for Group 1, Group 2 and Group 3 respectively.
- country is the country for each subject
- age $strata_i$ is the age strata for each subject

Before applying the above ANCOVA model, the interactions will be assessed using the following model:

$$Log10\ titer_i = log10\ baseline_i + age\ strata_i + country + log10\ baseline_i \times age\ strata_i + age\ strata_i \times country$$

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 (i.e., Group 2a vs Group 1a and Group 3a vs Group 1a in Year 1, Group 2b vs Group 1b and Group 3b vs. Group 1b in Year 2) adjusting for the pre-booster neutralizing Ab levels against each dengue virus serotype based on the least squares means (LSMEANS), in subjects seropositive at baseline in Colombia. The confidence intervals and p-values from Type III sums of squares may also be presented. A Bonferroni adjustment will be used to control for multiplicity.

For each serotype, the following model will be considered:

$$Log10\ titer_i = log10\ pre\text{-}booster_i + group_i + age\ group_i$$

Where:

- $titer_i$ is the post-booster titer value for each subject
- pre-$booster_i$ is pre-booster titer value for each subject
- $group_i$ is Group 1a, Group 1b, Group 2a, Group 2b, Group 3a, and Group 3b for each subject
- age $group_i$ is the age group for each subject

Before applying the above ANCOVA model, the interactions will be assessed using the following model:

$$Log10\ titer_i = log10\ pre\text{-}booster_i + group_i + age\ group_i + log10\ pre\text{-}booster_i \times group_i + group_i \times age\ group_i$$

Analysis of covariance (ANCOVA) also will be used to compare the mean response of neutralizing Ab levels against each dengue virus serotype 28 days post-booster between age strata adjusting for the pre-booster neutralizing Ab levels against each dengue virus serotype based on the least squares means, in subjects seropositive at baseline, in Colombia.

For each serotype, the following model will be considered:

$$Log10\ titer_i = log10\ pre\text{-}booster_i + age\ strata_i$$

Where:

- $titer_i$ is the 28 days post-booster titer value for each subject

- pre-booster$_i$ is pre-booster titer value for each subject.
- age strata$_i$ is the age strata for each subject

Before applying the above ANCOVA model, the interactions will be assessed using the following model:

Log10 titer$_i$=log10 pre-booster$_i$ + age strata$_i$ +log10 pre-booster$_i$ × age strata$_i$

5.1.3.2 AIT Subset

The analyses for AIT subset are based on the following:

Dengue PRNT (at V01, V06, V07, V08, V09 and V10): neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT.

CMI responses (at V01, V07, V08, V09 and V10):

- Cytokine secreting CD4 and CD8 T cells count (at V01, V07, V08, V09 and V10)

  Note: The raw individual results will not be directly described in the analysis: the results of all stimulations will be corrected with the corresponding medium result. The detailed calculation is below:

  For envelope (ENV) CYD 1, ENV CYD 2, ENV CYD 3, ENV CYD 4, NS3 peptide pools from YF 17D and DEN, and positive control, the medium (MED) = value of the negative control medium.

  Therefore, the following corrected endpoints will be used:

  - Before vaccination: X_{D0} - MED_{D0} where *X* is the value of ENV CYD 1, ENV CYD 2, ENV CYD 3, ENV CYD 4, positive control or NS3 peptide pools from YF 17D and DEN, etc.
  - 28 days after vaccination: $X_{D28} – MED_{D28,}$
  - 12 months after vaccination: $X_{M12} – MED_{M12,}$
  - Change from baseline endpoints (ratio): $(X_{D28} – MED_{D28})/(X_{D0} - MED_{D0})$ and $(X_{M12} – MED_{M12})/(X_{D0} - MED_{D0})$.
  - Change from D28 endpoints (ratio): $(X_{M12} – MED_{M12})/(X_{D28} - MED_{D28})$.
- T cell subpopulation (naïve, effector, central and terminally differentiated memory T cells) count (at V01, V07, V08, V09 and V10)

  Note: Naïve T cells are defined as CD45RA+ and CCR7+, effector memory T cells defined as CD45RA- and CCR7-, central memory T cells defined as CD45RA- and CCR7+, and terminally differentiated memory T cells as CD45RA+ and CCR7-.
- No corrected value will be used for the subpopulation analyses. Only the raw value of the medium, ENV CYD1, ENV CYD 2, ENV CYD 3, ENV CYD 4, positive control or NS3 peptide pools from YF 17D and DEN will be summarized.
- Cytotoxic T cell effector markers (at V01, V07, V08, V09 and V10)

  Note: The functional markers are including IFNγ, TNFα, IL-2, MIP-1β and CD107a for CD3+ CD8+ T cells, and have one more marker CD154 for CD3+ CD4+ T cells. The

cytotoxic T cell will be also summarized for activated cells, simple positive cells, double positive cells, triple positive cells and more than triple positive cells. The definition for different cells are:

- Activated cells: Positive for at least one of the following markers IFN γ /TNF α /IL-2/MIP-1 β/CD107a for CD3+ CD8+ cells and IFN γ /TNF α /IL-2/MIP-1 β /CD107a/CD154 for CD3+ CD4+ cells
- Simple positive cells: Activated cells which express 1 of the following markers IFN γ /TNF α /IL-2/MIP-1 β /CD107a for CD3+ CD8+ cells and IFN γ /TNF α /IL-2/MIP- 1 β /CD107a/CD154 for CD3+ CD4+ cells
- Double positive cells: Activated cells which express 2 of the following markers IFN γ /TNF α /IL-2/MIP-1 β /CD107a for CD3+ CD8+ cells and IFN γ /TNF α /IL-2/MIP- 1 β /CD107a/CD154 for CD3+ CD4+ cells
- Triple positive cells: Activated cells which express 3 of the following markers IFN γ /TNF α /IL-2/MIP-1 β /CD107a for CD3+ CD8+ cells and IFN γ /TNF α /IL-2/MIP- 1 β /CD107a/CD154 for CD3+ CD4+ cells
- More than triple positive cells: Activated cells which express 3 of the following markers IFN γ /TNF α /IL-2/MIP-1 β /CD107a for CD3+ CD8+ cells and IFN γ /TNF α /IL- 2/MIP-1 β /CD107a/CD154 for CD3+ CD4+ cells

In addition, the number of responders to the ICS test by stimulation will be computed. To be considered as responders the two following conditions must be filled:

$$\frac{X_{post-booster} - MED_{post-booster}}{X_{D0} - MED_{D0}} \geq 2$$

$$[X_{post\text{-}booster}\text{-}MED_{post\text{-}booster}] - [X_{D0}\text{-}MED_{D0}] \geq 0.05\%$$

- The post-booster visits here are D28 and M12
- Ex vivo B cells (plasmablast) count (measured by ELISPOT) (at V01, V07, V08 and V09)
- Number of antibody secreting cells (ASC) for each of the four specific parental dengue virus strains IgG and the total IgG will be summarized.
- Memory B cells count (measured by ELISPOT) (at V01, V07, and V10)

  Note: Antibody secreting cells (ASC) for each of the four specific parental dengue virus strains IgG and the total IgG will be summarized. In addition, for each of the four specific parental dengue virus strains IgG, the ratio will be also calculated. The ratio (%) can be obtained by:

$$\text{Ratio} = \frac{\text{ASC for CYD1/2/3/4 IgG}}{\text{ASC for Total IgG}} \times 100\%$$

Ab specificity and affinity maturation:

- The neutralizing Abs in the wide-type dengue parental strains (DENV) depleted, BSA-control depleted sera and whether the response in homotypic for each of the four parental dengue virus strains will be assessed (at V01, V06, V07, V08, V09 and V10).

Note: Undepleted is defined as BSA-control depleted sera and depleted is DENV depleted samples. Homotypic response is defined as a neutralizing antibody titer above 20 (1/dil) following DENV depletion.

- Serotype-specific affinity (KA, nM-1) and Ab concentration (µg/mL) for each of the four parental dengue virus strains will be measured (at V01, V06, V07, and V10).

All the analyses will be descriptive at each available time point by treatment subgroups (Group 1a/1b/2a/2b/3a/3b), by subgroups in Y1 (Group 1a/2a/3a), by subgroups in Y2 (Group 1b/2b/3b) and by treatment groups (Group 1/2/3)..

- For categorical data, the number and percentage of subjects above or equal to the 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

As this objective is exploratory, other analyses might be performed.

Note: 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). The 1:1 ratio and 10 subjects per subgroup is unlikely to hold as only subjects dengue seropositive at baseline will be eligible to booster injection.

5.1.4 Complementary output

Additional analyses by baseline dengue and YF/JE statuses will be provided in Appendix 15 of the CSR.

Safety analyses:

- Overview and summary tables of AEs
- SAEs including serious AESIs by SOC and PT
- Non-serious AESIs by SOC and PT

5.2 Analysis Sets

Six analysis sets will be used: PPAS, FAS, SafAS, the enrolled subjects set, the randomized subjects set, and the AIT subset.

5.2.1 Per-Protocol Analysis Set

The per-protocol analysis set (PPAS) will include all subjects who had no protocol violations as defined below 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 PP set 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 (V01 +365 days ± 20 days) defined in the tables of the study procedures
- Subject did not provide a V06 post-dose serology sample in the proper time window (V05 +28 days ± 14 days)
- Subject received a protocol-restricted medication, therapy, or vaccine in STAGE I as defined in Section 6.7 of the protocol.
- Subject's post-injection serology samples at V06 did not produce a valid test result (i.e. 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 dengue serostatus at baseline.
- 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 (i.e. a result different from "not-reportable" ['NR'] or missing, for at least one dengue serotype).
- Subjects randomized to Group 1 not included in the PP set in STAGE I

5.2.2 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 (i.e. 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.

5.2.3 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 safety analysis set 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).

5.2.4 Other Analysis Sets

Enrolled subjects set

Enrolled subjects are subjects for whom a CRF has been created.

Randomized subjects set

Randomized subjects are subjects who were randomly assigned to Group1a, Group1b, Group2a, Group2b, Group3a and Group3b.

AIT subset

The AIT subset is defined as those subjects who belong to the Randomized subjects set but had additional blood samples drawn for the assessment of CMI, additional neutralizing Ab titers (for exploration of the Ab response's kinetics), and Ab specificity and affinity maturation in the present trial. The AIT subset will include 60 randomized subjects from a specific site. Subjects will be analyzed by the vaccine group to which they were randomized.

5.2.5 Populations Used in Analyses

The PPAS will be used for the analysis for the primary objectives. 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.

Enrolled subjects and randomized subjects will be used for various standard population tables including duration of the study, disposition of participants and deviations. Demographic and baseline characteristics will be presented on the PPAS and FAS.

The AIT subset will be used to describe additional neutralizing Ab levels in this subset, Ab specificity and affinity maturation, and CMI responses.

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

5.3 Handling of Missing Data and Outliers

5.3.1 Immunogenicity

The LLOQ and ULOQ management will be performed as described in Section 4.4.1.1. Missing data will not be imputed. No test or search for outliers will be performed.

5.3.2 Safety

No replacement will be done unless state otherwise. No search for outliers will be performed.

In all subject listings, partial and missing data will be clearly indicated as missing.

5.3.2.1 Immediate

For unsolicited non-serious systemic AEs, a missing response to the "Immediate" field is assumed to have occurred after the 30-minute surveillance period and will not be imputed.

For SAEs, missing or partially missing elapsed time from last vaccination recorded if within 24 hours will remain missing and not be imputed. Such SAEs will not be considered as immediate.

5.3.2.2 Causality

Missing causality (relationship) for unsolicited non-serious AEs and SAEs will be considered at the time of analysis as related to vaccination.

5.3.2.3 Measurements

Missing measurement (for temperature or length) will not be replaced.

5.3.2.4 Intensity

For solicited reactions, missing intensities will be handled as described in Section 4.4.2.1.1. For unsolicited non-serious AEs, missing intensities will remain missing and will not be imputed.

5.3.2.5 Start Date and Stop Date

Missing or partially missing start dates for unsolicited AEs will remain missing and not be imputed. If either the start or stop date is missing or partially missing, the time to onset will be considered to be missing. Nevertheless unsolicited AEs with missing time to onset will be included in analyses according to the visit collected.

Missing or partially missing stop dates for AEs (solicited reactions and unsolicited AEs) will remain missing and not be imputed.

5.3.2.6 Action taken

Missing actions taken will remain missing and not be imputed.

5.3.3 Efficacy

Not applicable.

5.4 Interim / Preliminary Analysis

No interim analyses are planned. However, there will be four planned statistical analyses.

The first statistical analysis will be performed on all available data collected, cleaned and locked up to 1 year post-Injection 3.

This planned analysis will require the unblinding of data. Once the interim database lock has been conducted, the trial statistician will break the blind and will conduct the statistical analysis. A specific process will be implemented to maintain the blind at both the subject and Investigator levels. Thus, the data and results generated will not be communicated either to the subjects or to the Investigators and study centers.

The second statistical analysis will be performed on all available data collected, cleaned and locked up to Day 28 post-first year booster.

The third statistical analysis will be performed on all available data collected, cleaned and locked up to Day 28 post-second year booster.

The fourth and final statistical analysis will be conducted on all data once the study has ended (6 months after the second year booster) and the final database lock has occurred.

5.5 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]). [redacted]

Table 5.2: 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	99.52
	296	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj 3	2	95.20
90%	266	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	98.94
	266	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj 3	2	93.26
80%	235	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	97.76
	235	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj 3	2	89.22
70%	207	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	95.78
	207	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj 3	2	83.54
60%	178	Group 2 vs Group 1	28 days post-Inj 3 vs 28 days post-Inj 3	2	92.92
	178	Group 2 vs Group 1	1year post-Inj 3 vs 1 year post-Inj 3	2	75.64

Table 5.3: [redacted]

[redacted]

The following assumptions were considered:

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

5.6 Data Review for Statistical Purposes

A treatment blind review of the data has been anticipated through the data review process led by data management before database lock. This review of the data included a statistical review. An unblinding statistician is to support the project before first DBL, and all other team members are keeping blinding until first DBL.

5.7 Changes in the Conduct of the Trial or Planned Analyses

The following parts written in SAP are the changes different from Protocol Amendment 1:

The definition of j had been clarified for the Overall hypothesis.

The definition of WHO criteria and the corresponding derivation method had been added for virologically-confirmed dengue cases.

Instead of presenting the number and percentage of subjects with hospitalized suspected dengue case, only the hospitalized virologically-confirmed dengue cases will be summarized.

The ANCOVA model will be tested in Year 1 (Group 2a and Group 3a vs. Group 1a) and Year 2 (Group 2b and Group 3b vs. Group 1b).

The definition of depleted and undepleted for Ab specificity had been added: undepleted is defined as BSA-control depleted sera and depleted is DENV depleted samples.

The definition of homotypic Abs had been changed based on recent research findings to a neutralizing antibody titer above 20 (1/dil) following DENV depletion. Heterotypic antibodies will not be analyzed.

Delete "If non-inferiority is not achieved in Primary Objective, assessments of immunogenicity will be descriptive." The descriptive analyses will conduct anyway regardless of the non-inferiority is achieved or not.

The definition of successful conclusion at Stage I and Stage II separately was added for clarity. Bonferroni adjustment for multiple testing at 28 days and 1 year post-Inj3 between Group 2 and Group 1 was removed and the adjustment of power was added as illustrated in Section 5.5.

The age stratus (9-16, 17-45 and 46-50 years of age) were added in definition of age group.

The definition of AUC-MB was added.

Description and comparison of GM in seropositive subjects between age strata were added, both overall and within each Group. These will allow the assessment of immunogenicity profile by age strata.

The ANCOVA model was added for 28 days after injection 3 between age strata adjusting for baseline neutralizing Ab levels.

The ANCOVA model was added for 28 days post-booster between age strata adjusting for the pre-booster neutralizing Ab levels.

6 References List

1 Newcombe R.G., Two-sided confidence intervals for the single proportion: comparison of seven methods, Statistics in Medicine, (1998) 17, 857-872