SANOFI PASTEUR

NCT Number: NCT02824198

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

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

Statistical Analysis Plan (SAP) - Core Body Part

Trial Code:	CYD63
Development Phase:	Phase II
Sponsor:	Sanofi Pasteur SA 2, avenue Pont Pasteur, F-69367 Lyon cedex 07, France
Investigational Product:	CYD Dengue Vaccine
Form / Route:	Powder and solvent for suspension for injection / Subcutaneous
Indication For This Study:	Prevention of dengue fever in adolescents and adults
Version and Date of the SAP core body part:	Version 2.0, 01 Mar 2017

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List of Abbreviations

Ab	antibody
AE	adverse event
AESI	adverse event of special interest
AIT	additional immunological test
AR	adverse reaction
ASC	Antibody secreting cells
BL	blood sample
CI	confidence interval
CMI	cell mediated immunity
CRF	ase report form
CSR	clinical study report
D	Day
DC	diary card
DF	dengue fever
DHF	dengue haemorrhagic fever
dil	dilution
ELISPOT	enzyme-linked immunospot
ENV	envelope
FAS	full analysis set
GCI	Global Clinical Immunology
GM	geometric mean
GMT	geometric mean of titer
GMTR	geometric mean of titer ratio
IVRS	interactive voice response system
IWRS	interactive web response system
LLOD	lower limit of detection
LLOQ	lower limit of quantification
LSMEAN	least squares means
MA	memory aids
MD	missing data
MED	medium
MedDRA	Medical Dictionary for Regulatory Activities
NM	non measurable
NR	not-reportable

PD3	post-dose 3
PRNT	plague reduction neutralization test
PPAS	per-protocol analysis set
PT	preferred term
RCDC	reverse cumulative distribution curve
RR	relative risk
SAE	serious adverse event
SafAS	safety analysis set
SAP	statistical analysis plan
SD	standard deviation
SOC	system organ class
ULOQ	upper limit of quantification
V	visit

1 Introduction

The present trial (CYD63) is to assess the immunogenicity and safety of sanofi pasteur's CYD dengue vaccine booster in healthy children, adolescents and adults who received 3 doses of the tetravalent dengue vaccine in the CYD28 trial conducted in Singapore from April 2009 to October 2010.

Dengue disease is caused by 4 closely related, but antigenically distinct, dengue virus serotypes (1, 2, 3, and 4) of the genus flavivirus. 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).

There is no licensed vaccine to prevent dengue infection or disease and no specific treatment exists. Sanofi Pasteur's tetravalent CYD dengue vaccine has been extensively evaluated in subjects from 9 to 60 years. Data regarding the levels of neutralizing Abs from long-term follow-up studies have shown a predictable decrease in the level of circulating Abs (geometric mean of titers [GMTs]) against all 4 serotypes 1 year after the third injection, regardless of age group, which was followed by a trend to stabilization during the subsequent years. However, long-term GMTs for each serotype remained overall higher than GMT values before vaccination.

Specifically in Singapore, a Phase II study (CYD28) was completed in 2014; 1198 subjects (2 to 45 years) received 3 injections of CYD dengue vaccine or a placebo/control vaccine at 0, 6, and 12 months with a 4 years Abs persistence and safety follow-up after the third injection (1). Study objectives were to describe safety and immunogenicity of CYD dengue vaccine. Overall, CYD dengue vaccine in Singapore showed satisfactory safety and immune responses against all 4 serotypes after 3 doses of CYD dengue vaccine. As the CYD dengue vaccine candidate claimed indication for the prevention of dengue disease is for individuals 9 years and above, the subjects enrolled in CYD63 will be a subset of CYD28 subjects that were aged ≥ 9 years on the day of the first injection of study vaccination. The general purpose of CYD63 is to assess and describe the booster effect of a CYD dengue vaccine dose administered approximately 5 years after the completion of a 3-dose vaccination schedule. To do so, this study will be looking at the non-inferiority of the neutralizing Ab responses 28 days after booster vaccination as compared to the Ab responses 28 days after the third dose of the primary vaccination, in a subset of subjects from CYD28. The assumption here is that booster vaccination should induce neutralizing Ab to at least the same levels as primary vaccination (i.e., post-Dose 3 [PD3] GMTs).

This statistical analysis plan (SAP) includes detailed procedures for executing the 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 Objective

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

2.2 Secondary Objectives

Immunogenicity

1) If the primary objective of non-inferiority is achieved: To demonstrate the superiority, in terms of GMTRs, of a CYD dengue vaccine booster compared to the third CYD dengue vaccine injection in subjects from CYD28 trial (subjects from Group 1 only).
2) To describe the immune responses elicited by the CYD dengue vaccine booster or placebo injection in subjects who received three doses of the CYD dengue vaccine in the CYD28 trial in all subjects.
3) To describe the neutralizing antibody (Ab) levels of each dengue serotype PD3 (CYD28 subjects) and immediately prior to booster or placebo injection for all study subjects.
4) To describe the neutralizing Ab persistence 6 months, 1 year and 2 years post booster or placebo injection for all study subjects.

Safety

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

2.3 Additional Objectives

Only for the additional immunological tests (AIT) subset

1) To describe dengue neutralizing Ab levels (exploration of the Ab response's kinetics), Ab specificity and affinity maturation post-booster or placebo injection
2) To describe cell mediated immunity (CMI) responses post-booster or placebo injection.

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

In all subjects

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

3 Description of the Overall Trial Design and Plan

3.1 Trial Design

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

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

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

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

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

3.2 Trial Plan

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

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

Vaccination

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

Blood sampling

- For all subjects

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

- For the AIT subset

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

Collection of safety data

Clinical site personnel will record immediate adverse events (AEs) that occur within the 30 minutes after injection. Subjects or subjects' parent(s) / legally acceptable representative(s) will record in the diary card (DC) information about solicited injection site reactions from D0 to D7 post-injection, about solicited systemic reactions from D0 to D14 post-injection and unsolicited AEs from D0 to D28 post-injection. Information on non-serious adverse events of special interests (AESIs) will be collected within 7 days post-injection. Information on serious adverse events (SAEs) (including serious AESIs) will be collected throughout the trial. Subjects or subjects' parent(s) / legally acceptable representative(s) are to contact the Investigator in case of hospitalization for suspected dengue disease.

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

See Table 3.1 for details of study procedures.

Table 3.1: Study procedures

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

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

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

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

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

‡ In female subjects of childbearing potential (i.e. women and girls who have reached menarche and who had not surgical sterilization, or hysterectomy).

§ Blood samples planned during vaccination visit will be taken before vaccination

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

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

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

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

4 Endpoints and Assessment Methods

4.1 Primary Endpoints and Assessment Methods

4.1.1 Immunogenicity

4.1.1.1 Immunogenicity Endpoints

The primary endpoint for the evaluation of immunogenicity is:

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

4.1.1.2 Immunogenicity Assessment Methods

See Section 9.1.1.2 of the protocol for assessment methods.

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

The secondary endpoints for the evaluation of immunogenicity are:

1) Neutralizing Ab levels against each of the four parental dengue virus strains of CYD dengue vaccine as determined by PRNT measured 28 days after the third CYD dengue vaccine injection received in CYD28 trial and 28 days post-booster injection (subjects from Group 1 only).
2) Neutralizing Ab levels against each of the four parental dengue virus strains of the CYD dengue vaccine as determined by PRNT immediately prior and 28 days post-booster or placebo injection.

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

4.2.1.2 Immunogenicity Assessment Methods

See Section 9.2.1.2 of the protocol for assessment methods.

4.2.2 Safety

4.2.2.1 Safety Endpoints

The endpoints for the evaluation of safety are:

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

7) Occurrence of hospitalized virologically-confirmed dengue cases throughout the trial (i.e., from D0 through end of the study).

4.2.2.2 Safety Assessment Methods

See Section 9.2.2.3 of the protocol for assessment methods.

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

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

1) Dengue PRNT

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

2) Ab specificity and affinity maturation
   a) The neutralizing Abs in the wide-type dengue parental strains (DENV) depleted, BSA-control depleted sera and whether the Ab response is homotypic for each of the four parental dengue virus strains will be assessed qualitatively immediately prior to and at 7, 14 and 28 days post-injection. For the descriptive summary of Ab response, BSA-control depleted sera are considered as undepleted and DENV depleted as depleted. Homotypic Abs for individual serotypes will be defined as a neutralizing antibody titer above 20 (1/dil) following DENV depletion.
   b) Serotype-specific affinity (KA, nM^{-1}) and Ab concentration (µg/mL) will be measured against the parental wild-type strains in the sera immediately prior to and at 28 days post-injection.

3) CMI response

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

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

iii) Cytotoxic T-cell effector markers.

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

For all subjects

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

The assays, along with the corresponding samples, will be managed by the specific sanofi pasteur organization (e.g., Global Clinical Immunology [GCI], Research), or with an external laboratory, as it applies.

4.3.1.2 Immunogenicity Assessment Methods

See Section 9.3.1.2 of the protocol for assessment methods.

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 the lowest dissociation constant K_D that can be 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 fold-rise and titer ratio (GMTR), < LLOQ will be converted to 0.5 LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator

There is no upper limit of quantification (ULOQ) with the PRNT method planned.

4.4.1.2 Fold-rise

The derived endpoint fold-rise is driven by computed titer values (as described in Section 4.4.1.1) and is computed as follows:

- the ratio of post-booster computed value divided by pre-booster computed

Note: If the titer value used for the above calculation is missing, then fold-rise is missing.

4.4.1.3 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".

4.4.1.4 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 4 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.

4.4.1.5 Pre-booster dengue status

The pre-booster dengue status is defined as the presence of Abs against at least one dengue serotype in the baseline sample in the blood sample collected at V01 in the present study from all subjects.

The pre-booster dengue status is computed based on computed values of serotypes with the parental dengue virus strain at V01. If the computed value is positive (≥ 10 1/ dil) for at least one serotype then the Dengue immune indicator will be "Immune". Else if the computed value is not positive (< 10 1/dil) and non-missing for all serotypes (i.e. all of the titers planned to be measured at baseline must be available, and valid [not coded "NR" in the serology database] then the Dengue immune indicator will be "Non-immune". Otherwise the baseline dengue status will be classified as "Undetermined".

In any other cases Dengue immune indicator will be missing.

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 an unsolicited related AE
- Immediate AE/AR: Unsolicited non-serious AE ticked "immediate (within 30 minutes from the vaccination)" by the investigator in the CRF or SAE with time of onset within 30 minutes
- Solicited reaction: Event pre-listed in the CRF, and which occurred during the solicited period (period is usually 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).
- Hospitalized virologically-confirmed dengue case: A virologically-confirmed dengue infection is defined as a suspected dengue which has positive sample for the dengue screen RT-PCR (i.e., ≥LLOQ) and/or the non-sturctural 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 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 Overall 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.

Note: The maximum 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 erythema measurement > 0 mm and < 25 mm, in adolescents).

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): 2-3 days, 4-7 days, ≥8 days, missing
- Systemic reactions (D0-D14): 2-3 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. 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.

Note: a reaction could be derived as not ongoing for the analysis despite being considered as ongoing by the Investigator (e.g. when the maximum Injection site erythema measurement after D7 for adolescents is > 0 mm but < 25 mm).

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.

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

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 2-year follow-up period (i.e., from 28 days after injection until the last subject contact)
- 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, 2 or 3 with missing time of onset will be included the table of SAEs analyzed "within 28 days", and SAEs collected after Visits 4, 5 or 6 with missing time of onset will be included in the table of SAEs analyzed "during the 2-year follow-up period".

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 haemorrhagic fever (DHF) grade I, II, III, and IV will be consistent with the 1997 WHO definition:

- Clinical manifestations
  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 X 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.

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

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 of CYD dengue vaccine in CYD28 is computed as follows:

Age in years: (Date of V01 in CYD28 –Date of birth +1) / 365.25

4.4.5.2 Age group

The age group code ("9 to 17 years of age" or "18 to 45 years of age" on the day of first injection in CYD28) is a part of the subject number. Nevertheless subjects with a wrong identifier due to misclassification at the time of randomization will be considered in there real strata of age at the time of analysis, based on the age definition in Section 4.4.5.1.

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

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, quartiles, minimum, and maximum.
Clinical safety results	**Categorical data**	Solicited reactions: Number and percentage (95% confidence interval [CI]) of subjects. Unsolicited AEs and SAEs: Number and percentage (95% CIs) of subjects, and number of events. Hospitalized virologically-confirmed dengue case: Number and percentage (95% CIs) of subjects
Immunogenicity results	**Categorical data (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 standard deviation. 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 (2), i.e., using the inverse of the beta integral with SAS®).

For immunogenicity, 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:

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

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").

5.1 Statistical Methods

5.1.1 Hypotheses and Statistical Methods for Primary Objective

If the number of evaluable subjects in Group 1 achieves 176 (i.e. per-protocol analysis set [PPAS] with valid immunogenicity titers) which would give 80.2% power for non-inferiority testing, the following statistical comparison will be performed for the primary objective. The primary hypothesis of non-inferiority will be tested on the PPAS and is to be confirmed using the full analysis set (FAS). If the number of evaluable subjects in Group 1 does not reach 176 for any serotype, hypotheses testing may not be performed for primary objective and the planned analyses may be descriptive for primary endpoints.

5.1.1.1 Hypotheses

<u>Individual Hypotheses for Each Serotype to Demonstrate Non-inferiority:</u>

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

Individual hypotheses for each serotype will be as follows:

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

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

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

<u>Overall Hypothesis to Demonstrate Non-inferiority:</u>

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

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

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

5.1.1.2 Statistical Methods

A non-inferiority test will be performed using the 95% two sided CI of $GM(V_{Booster}/V_{PD3})$ for each serotype and the 95% CI will be calculated using paired t-test (2). Subjects with non-missing PD3 and post-booster dose titers will be included in this analysis.

For each serotype, the non-inferiority will be demonstrated if the lower limit of the two-sided 95% CI is greater than 1/2. If the null hypothesis is rejected, then the alternative hypothesis of non-inferiority will be supported. The overall null hypothesis will be rejected if the 4 individual null hypotheses are rejected simultaneously.

5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

5.1.2.1 Hypotheses and Statistical Methods for the First Secondary Objective

5.1.2.1.1 Hypotheses

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

Individual Hypothesis for Each Serotype to Demonstrate Superiority:

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

Individual hypotheses for each serotype will be as follows:

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

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

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

Overall Hypothesis to Demonstrate Superiority:

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

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

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

5.1.2.1.2 Statistical Methods

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

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

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

5.1.2.2 Hypotheses and Statistical Methods for the Other Secondary Objectives

5.1.2.2.1 Hypotheses

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

5.1.2.2.2 Statistical Methods

5.1.2.2.2.1 Immunogenicity

Response elicited by booster dose

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

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

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

Ab persistence

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

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

5.1.2.2.2.2 Safety

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

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

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

Unsolicited immediate systemic events will be described according to nature (MedDRA SOC, and PT) and relationship to vaccination.

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

All AEs leading to study termination will be described according to nature (MedDRA SOC, and PT) and relationship to vaccination.

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

Detection of hospitalized virologically-confirmed dengue cases

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

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

5.1.3 Statistical Methods for Observational Objectives

5.1.3.1 Hypotheses

There are no hypotheses.

5.1.3.2 Statistical Methods

5.1.3.2.1 All of the analyses in the AIT subset

The analyses for AIT subset are based on the following:

Dengue PRNT (at V02 and V03): neutralizing Ab levels against each of the four parental dengue virus strains as determined by PRNT.

CMI responses (at V01, V02, V03, V04, and V06):

- Cytokine secreting CD4 and CD8 T cells count (at V01, V04, and V06)

  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:

  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, V04, and V06)

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 values 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, V04, and V06)

  Note: The functional markers are 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 IFNγ/TNFα/IL-2/MIP-1β/CD107a for CD3+ CD8+ cells and IFNγ/TNFα/IL-2/MIP-1β/CD107a/CD154 for CD3+ CD4+ cells
  - Simple positive cells: Activated cells which express 1 of the following markers IFNγ/TNFα/IL-2/MIP-1β/CD107a for CD3+ CD8+ cells and IFNγ/TNFα/IL-2/MIP-1β/CD107a/CD154 for CD3+ CD4+ cells
  - Double positive cells: Activated cells which express 2 of the following markers IFNγ/TNFα/IL-2/MIP-1β/CD107a for CD3+ CD8+ cells and IFNγ/TNFα/IL-2/MIP-1β/CD107a/CD154 for CD3+ CD4+ cells
  - Triple positive cells: Activated cells which express 3 of the following markers IFNγ/TNFα/IL-2/MIP-1β/CD107a for CD3+ CD8+ cells and IFNγ/TNFα/IL-2/MIP-1β/CD107a/CD154 for CD3+ CD4+ cells
  - More than triple positive cells: Activated cells which express 3 of the following markers IFNγ/TNFα/IL-2/MIP-1β/CD107a for CD3+ CD8+ cells and IFNγ/TNFα/IL-2/MIP-1β/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, V02, and V03)

  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, V04, and V06)

  Note: Number of 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 (at V01, V02, V03, and V04):

- The neutralizing Abs in the 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, V02, V03, and V04).

  Note: Undepleted is defined as BSA-control depleted and depleted is DENV depleted. 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 and V04).

  Note: K_D (nM) will be collected from the lab and summarized in the final report, where $KA=1/K_D$.

All the analyses will be descriptive.

- 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.

5.1.3.2.2 Analysis adjusting for baseline neutralizing Ab levels

Analysis of covariance (ANCOVA) will be used to compare the post-booster mean response of neutralizing Ab levels against each dengue virus serotype of Groups 1 and 2 adjusting for the baseline neutralizing Ab levels against each dengue virus serotype based on the least squares means (LSMEANS).

For each serotype, the following model will be considered:

$Log10\ titer_i = log10\ baseline_i + group_i$

Where:

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

$baseline_i$ is pre-booster titer value for each subject

$group_i$ is Group 1 or Group 2 for each subject

Before applying the above ANCOVA model, the interaction between the pre-booster titer value and the group will be assessed using the following model:

$Log10\ titer_i = log10\ baseline_i + group_i + log10\ baseline_i \times group_i$

5.1.4 Complementary output

Additional analyses of the immune responses elicited by CYD dengue vaccine booster by age group (9-17 years and 18-45 years) and by pre-booster dengue status will be provided in Appendix 15 of the CSR.

Immunogenicity analyses:

- GMTRs (post-booster / PD3) against each of the 4 parental dengue serotypes in Group 1 only
- GMTs against each of the 4 parental dengue serotypes
- GMTRs (post-booster / pre-booster) against each of the 4 parental dengue serotypes
- Number and percentages of subjects ≥ 10 (1/dil) against each dengue serotype with the parental dengue virus strains at each available time point
- Seroconversion rates based on pre- and post-booster titers

Safety analyses:

- Overview and summary tables of AEs after booster dose
- 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 set.

5.2.1 Per-Protocol Analysis Set

The PPAS is a subset of the FAS. It will include all subjects who had no protocol deviations from the present study. Subjects will be excluded from the PPAS for the following reasons:

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

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

5.2.2 Full Analysis Set

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

5.2.3 Safety Analysis Set

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

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

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 Group 1 or Group 2.

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 the first 60 randomized subjects from two specific sites (30 subjects per site): 45 subjects from Group 1 and 15 subjects in Group 2.

5.2.5 Populations Used in Analyses

The PPAS will be used for the analysis for the primary objective. The FAS will be used for the superiority hypotheses testing for first secondary objective. All the immunogenicity analyses for immune response elicited by booster dose will be performed on the PPAS, and will be confirmed on the FAS. The immunogenicity Ab persistence analysis will be performed on the FAS. Subjects will be analyzed according to the vaccine group they were randomized to.

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

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. Subjects will be analyzed by the vaccine group to which they were randomized.

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

5.3 Handling of Missing Data and Outliers

5.3.1 Immunogenicity

The LLOQ 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. The values assessed as aberrant by the Sanofi Pasteur Research Department in Marcy l'Etoile, France will be excluded from the statistical analysis.

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 three planned statistical analyses.

A first statistical analysis will be performed on all available data collected, cleaned and locked up to Day 28 post-booster injection (Visit 4).

A second statistical analysis of all the available data obtained up to 1 year post-booster injection will be conducted.

These two planned analyses 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 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.

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

5.5 Determination of Sample Size and Power Calculation

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

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

With 176 evaluable subjects in Group 1, for each serotype, there is 80.2% overall power (see Table 5.2) using paired t-test to reject the 4 individual null hypotheses simultaneously; calculation assumed a non-inferiority margin (delta) =2, one-sided type I error =0.025 and correlation between the responses PD3 and post-booster dose of the same serotype in the same subject = 0.6.

Table 5.2: Power/Sample size calculation summary table for primary endpoint (only for Group 1 subjects)

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

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

Since 4 individual null hypotheses should be rejected simultaneously to reject the overall null hypothesis, so no multiplicity adjustment for alpha is necessary.

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

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

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 includes a statistical review.

5.7 Changes in the Conduct of the Trial or Planned Analyses

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

The definition of depleted and undepleted 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. The new definition of the homotypic Ab response is a neutralizing antibody titer above 20 (1/dil) following DENV depletion. Heterotypic antibodies will not be analyzed.

6 References List

1 CYD28, Immunogenicity and Large-Scale Safety of Tetravalent Dengue Vaccine in Healthy Subjects Aged 2 to 45 Years in Singapore. Final Clinical Study Report - Year 4 Follow-Up, version 4.0, dated 17 June 2015

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

3 Breslow NE, Day NE. Statistical methods in cancer research. Volume II: The design and analysis of cohort studies. Oxford (UK): Oxford University Press; 1987