NCT02741128

Safety and Immunogenicity of a Tetravalent Dengue Vaccine in HIV-Positive Adults Aged 18 to 50 Years in Brazil

A Phase II, randomized, observer-blind, placebo-controlled, multicenter study in 150 HIV-positive adults, treated with antiretrovirals, previously exposed to dengue, and aged 18 to 50 years in Brazil. Subjects will receive 3 injections of CYD dengue vaccine or placebo (0, 6, and 12 months) with a 6-month safety follow-up.

Clinical Study Protocol, Amendment 1

Health Authority File Number(s):	Not applicable
WHO Universal Trial Number (UTN):	U1111-1174-4398
Study Code:	CYD50
Development Phase:	Phase II
Sponsor:	Sanofi Pasteur SA 14 Espace Henry Vallée, 69007 Lyon, France Represented by: Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
Investigational Product(s):	CYD Dengue Vaccine
Form / Route:	Powder and solvent for suspension for injection / Subcutaneous
Indication For This Study:	Safety and immunogenicity evaluation of the CYD Dengue Vaccine in HIV-positive adults
Manufacturer:	Same as Sponsor
Coordinating Investigator	[redacted]
Sponsor's Responsible Medical Officer:	[redacted]

Global Safety Officer: [redacted]

Regional Clinical Trial Manager: [redacted]

Version and Date of the Protocol: Version 3.0 dated 10 December 2019

This protocol version 3.0 is the third version of the initial trial protocol version 1.0 dated 08 March 2016.

History of Protocol Versions

Version*	Date	Comments
1.0	08 March 2016	IEC/IRB-approved version not used in the study
2.0	06 December 2018	IEC/IRB-approved version used in the study
3.0	10 December 2019	Amendment 1

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

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Synopsis

Company:	Sanofi Pasteur
Investigational Product:	CYD Dengue Vaccine
Active Substance(s):	Live, attenuated, dengue serotype 1 virus Live, attenuated, dengue serotype 2 virus Live, attenuated, dengue serotype 3 virus Live, attenuated, dengue serotype 4 virus
Title of the Study:	Safety and Immunogenicity of a Tetravalent Dengue Vaccine in HIV-positive Adults Aged 18 to 50 Years in Brazil
Development Phase:	Phase II
Coordinating Investigator:	[redacted]
Study Centers:	This will be a multi-center study conducted in the Brazilian states of São Paulo and Rio de Janeiro. Investigators and sites are listed in the "List of Investigators, Study Centers, and Sponsor's Personnel Involved in the Study" document.
Planned Study Period:	November 2019 (First Visit of the First Subject) to March 2022 (Last Contact of the Last Subject)
Study Design and Methodology:	Randomized, observer-blind, placebo-controlled, multi-center, Phase II study in 150 HIV-positive adults, treated with antiretrovirals, and previously exposed to dengue Potential study participants will be screened for eligibility (see Screening Criteria below). Enrolled subjects will be randomized in a 2:1 ratio into 2 groups: • **CYD Dengue Vaccine Group** (N=100): CYD dengue vaccine • **Control Group** (N=50): placebo (NaCl 0.9%) Each subject will receive 3 injections of either CYD dengue vaccine or placebo at Day (D) 0, Month (M) 6, and M12. Subject's enrollment will occur in 2 steps: i) study recruitment will be paused after the first 20 randomized subjects (early safety data review); ii) vaccination will resume (first injection for the remaining 130 subjects; and second injection for the first 20 subjects, according to study timelines and study specifications) after the Independent Data Monitoring Committee (IDMC) has provided recommendation. <u>Randomization:</u> Interactive Response Technology (IRT) will be used to assign subject and dose number at each clinical site. <u>Blood sampling:</u> All subjects will provide several blood samples for screening tests, to determine dengue, yellow fever (YF), and Zika serostatus at baseline, for CYD dengue vaccine immunogenicity assessments, for dengue vaccinal viremia, and for Human Immunodeficiency Virus (HIV-1) status monitoring, depending on the time points. Additional biological samples may be collected in the event of serious adverse events

	(SAEs) (including serious adverse events of special interest [AESIs]), based upon the Investigator's judgment and as per local regulation. History of dengue infection/vaccination, YF infection/vaccination, and Zika infection will be collected. ***Collection of Safety data*** Immediate adverse events (AEs) observed to occur within 30 minutes post-vaccination will be collected. Solicited injection site reactions will be collected for Days 0–7 after each injection. Solicited systemic reactions will be collected for Days 0–14 after each injection. Unsolicited events will be collected for Days 0-28 after each injection. SAEs will be reported throughout the study (ie, from the Screening Visit to the 6 months safety follow-up phone call) and serious and non-serious AESIs will be collected in defined time windows according to the type of AESI. In addition, hospitalized suspected dengue cases occurring at any time in the study will be collected and documented. IDMC An IDMC will be involved in the regular review of hospitalized virologically-confirmed dengue cases (VCD), including assessment of severity. Related SAEs or deaths will be promptly reviewed by the IDMC. The IDMC will be involved in review of safety data of the first 20 subjects and will provide a recommendation on whether to continue enrollment and administration of the second dose of vaccine to the 20 already enrolled subjects, if no increased safety risk is concluded. Additionally, the IDMC will also be promptly notified of any significant confirmed modification of a subject's HIV infection status, as well as if any of the criteria for interruption of the study for safety reason are met (see next section for details).
Early Safety Data Review:	The safety of the CYD dengue vaccine will be continuously monitored by the Sponsor. In the context of this study (ie, HIV-positive adults), a blinded early safety data review (ESDR) will be performed, the goal of which is to allow for a cautious, stepwise approach to vaccine administration. An initial safety review for this study is planned when the first 20 subjects have received the first injection (Inj 1) and have provided safety data for Days 0-14 post-first injection. During the ESDR, study recruitment (and second injection for first 20 subjects, in case the safety review has not been completed at the time of second injection) will be paused. The safety data collected will be entered into the electronic case report book (CRB), and will be summarized and reviewed by the Sponsor. The safety evaluation team (SET) will conduct a blind review of the safety data. It is understood that this review will be based on preliminary data that have not been subject to validation and database lock. The SET will prepare a recommendation that will be reviewed by the IDMC. The IDMC will also review the safety data and then provide recommendation as to whether vaccination of the first enrollees and the enrollment of remaining subjects should resume or not. The ESDR will be assessing the following safety parameters: • Immediate (within 30 minutes) AEs • Solicited injection site and systemic reactions • Unsolicited AEs reported as related by the Investigator within 14 days after the first injection • SAEs and AESIs (including serious and non-serious AESIs) Case unblinding may be performed if required. Some unblinded analyses may be provided by an independent Sanofi Pasteur statistician (ie, not involved either in the study or in the Dengue project) upon IDMC request. The decision regarding the continuation of vaccination of the 20 first enrollees and of the remaining 130 subjects will be communicated to the Investigators and will be sent

	to the Independent Ethics Committees (IECs)/Institutional Review Boards (IRBs) and the IDMC for information.

Interruption of the Study for Safety Reason:	The detection/fulfillment of the following safety signals will put the study on a temporary hold:
	• With regard to investigational products:
	  • **15% of the total subjects included in the study** experience a Grade 3 solicited systemic reaction occurring within 14 days after any study injection and persisting for at least 48 hours and judged as related to the vaccination by the Investigator and not explained by any other possible etiology.
	  and/or
	  • **3 SAEs** (including serious AESI) considered as related to the vaccination by the Investigator or the Sponsor, except for the SAEs corresponding to the HIV condition included below.
	• With regard to HIV condition:
	  • **In 20% of the total subjects included in the study**: Confirmed plasma HIV-1 ribonucleic acid (RNA) > 1000 copies/mL 28 days post-injection after having been undetectable (< 50 copies/mL) pre-injection, not explained by non-adherence to ART and not explained by any other possible etiology.
	  or
	  • **In 10% of the total subjects included in the study**: Confirmed decrease in CD4+ T-cell (CD4) count greater than 30% assessed 28 days post-injection compared to the pre-injection value, not explained by non-adherence to ART and not explained by any other possible etiology.
	It is to be noted that any case of an increase in HIV-1 RNA >1000 copies/mL or decrease in CD4 count greater than 30% observed 28 days post-injection, not explained by non-adherence to ART and not explained by any other possible etiology, will be considered as an SAE. This increase in HIV viral load or decrease in CD4 count will be considered as "confirmed" if a second test taken 4 weeks after the first (ie, approximately 2 months post-injection) shows the same value/trend. The IDMC will be immediately notified if either or both criteria occur in any of the subjects enrolled in the study.
	The IDMC will also be immediately notified if the percentage of subjects defined in one of the 4 safety signals described above has been reached. The IDMC will then assess and recommend in an expedited way whether study enrollment and injections can continue, and/or whether other actions should be completed. In this scenario, additional biological tests may be required to assess the evolution of the subjects and to decide on the study continuation.
	Apart from the temporary hold following the enrollment of the first 20 subjects, and apart from a possible temporary hold following the detection of a safety signal, the study may be discontinued if new data about the investigational product resulting from this or any other studies become available; or for administrative reasons; or on advice of the Sponsor, the Investigators, the regulatory authorities in Brazil, and/or the IECs/IRBs.
	If the study is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators, the IECs/IRBs, and the regulatory authorities of the reason for termination or suspension. If the study is prematurely terminated for any reason, the Investigator will promptly inform the study subjects and assure appropriate therapy and follow-up.

Primary Objective:	Safety • To describe the safety of each injection of CYD dengue vaccine in HIV-positive adults previously exposed to dengue
Primary Endpoints:	Safety • Occurrence of any unsolicited systemic AE reported within 30 minutes after each injection • Occurrence of solicited (ie, pre-listed in the subject's diary card [DC] and in the CRB) injection site reactions (pain, erythema, and swelling) occurring up to 7 days after each injection • Occurrence of solicited systemic reactions (fever, headache, malaise, myalgia, and asthenia) occurring up to 14 days after each injection • Occurrence of unsolicited AEs occurring up to 28 days after each injection • Occurrence of serious and non-serious AESIs in defined time windows according to the type of AESI • Occurrence of SAEs throughout the study (ie, from D0 through the end of the study) • Occurrence of hospitalized VCD cases throughout the study (ie, from D0 through the end of the study) Other endpoints will be recorded or derived as described in the Statistical Analysis Plan. Depending on the item, these could include: nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), time of onset, duration, number of days of occurrence, intensity, relationship to vaccine, action taken, whether the event/reaction led to early termination from the study, seriousness, or outcome. Hospitalized suspected dengue disease is defined as an acute febrile illness with diagnosis of dengue requiring hospitalization (inpatient care). In such cases, 1 unplanned acute blood sample (within the first 5 days after fever onset) will be collected for virological confirmation of dengue disease. A suspected case will be considered VCD if there is a detection of wild-type (WT) dengue virus by dengue non-structural protein (NS) 1 antigen (Ag) enzyme-linked immunosorbent assay (ELISA) and/or dengue reverse transcriptase-polymerase chain reactions (RT-PCRs) (at the Global Clinical Immunology [GCI] or GCI designated laboratory). Note: Acute blood sample for all suspected hospitalized dengue cases should be collected within the pre-specified timeframe as described above. If this cannot be accomplished, this sample should still be obtained as soon as possible thereafter, for IDMC severity assessment
Secondary Objectives:	***Immunogenicity:*** • To describe the humoral immune response to each dengue serotype at baseline and after each injection of CYD dengue vaccine in HIV-positive adults previously exposed to dengue ***Safety:*** • To detect the CYD dengue vaccinal viremia post-injection 1 (post-Inj 1) in HIV-positive adults previously exposed to dengue • To describe changes in CD4 count and HIV RNA viral load after each injection of CYD dengue vaccine in HIV-positive adults previously exposed to dengue

Secondary Endpoints:	***Immunogenicity*** • Neutralizing antibody (Ab) levels (measured by dengue plaque reduction neutralization test [PRNT]) against each of the 4 parental dengue virus strains of CYD dengue vaccine construct at baseline and 28 days after each injection ***Safety*** • CYD dengue vaccinal viremia at 7 and 14 days post-Injection 1 • CD4 count and HIV RNA viral load 28 days after each injection of CYD dengue vaccine
Observational Objective:	***Immunogenicity*** • To describe the flavivirus (dengue, YF, and Zika) serological status in the study population at baseline
Observational Endpoints:	***Immunogenicity*** • Anti-NS1 IgG at baseline • Neutralizing Ab levels against YF at baseline • Neutralizing Ab levels against Zika at baseline
Planned Sample Size:	A total of 150 subjects are planned to be enrolled, in a 2:1 randomization ratio: • Group 1 – CYD Dengue Vaccine Group: 100 subjects • Group 2 – Placebo Group: 50 subjects
Schedule of Study Procedures:	<u>Visits/phone calls</u> During this study there will be 8 visits (V) and 5 phone contacts. <u>Vaccinations:</u> 3 injections at D0, M6, and M12 with either the CYD dengue vaccine or a Placebo <u>Blood sampling</u> All subjects will provide blood samples: • for screening tests (13 mL) (if results not already available from routine periodical tests; see Screening Criteria below) within 4 weeks before the first vaccination • for assessment of previous dengue exposure (1 mL) at screening • for dengue, YF, and Zika serostatus (5 mL) before the first injection at D0 • for vaccine immunogenicity assessments by PRNT (dengue neutralizing Abs) (4 mL) at baseline (prior to Inj 1) and 28 days after each injection at, respectively, Inj 1+28D, Inj 2+28D, and Inj 3+28D • for CD4 count (2x5mL) and HIV viral load (2x5 mL) prior to each injection in subjects for whom the tests have not been performed within 2 months before vaccination visit • for CD4 count (2x5 mL) and HIV viral load (2x5 mL) 28 days after each injection at, respectively, Inj 1+28D, Inj 2+28D, and Inj 3+28D • for dengue vaccinal viremia (4 mL) 7 and 14 days post-Inj 1 in all subjects • Unplanned blood samples (approximately 3 mL) will be collected in case of acute febrile illness with diagnosis of dengue requiring hospitalization (inpatient care) within the first 5 days after fever onset, for virological confirmation of dengue disease by NS1 antigen test (ELISA), and/or WT dengue RT-PCRs <u>Urine sampling</u> All female subjects will provide urine samples for urine pregnancy test before each injection

	Safety data: Staff will record information about immediate (within 30 minutes) AEs after each injection. Subjects will record in a DC information about solicited injection site reactions occurring up to 7 days after each injection, solicited systemic reactions occurring up to 14 days after each injection, and unsolicited AEs occurring up to 28 days after each injection. Information on 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. DCs will also be provided to the subjects to collect information on SAEs in-between injections (from 28 days post-injection until the next injection). Finally, a memory aid (MA) will be provided to collect information on SAEs during the safety follow-up (from 28 days after the last injection until 6 months after the last injection). Staff will review the safety data with the subjects during post-vaccination visits.
Duration of Participation in the Study:	The expected duration of each subject's participation in the study is approximately 18 months, including a safety follow-up period of 6 months post last vaccination. Subjects who are dengue-positive by rapid diagnostic test (RDT) or dengue IgG ELISA at Screening Visit and negative by PRNT (sample collected before the first injection [at Visit 1] and assayed before second injection) will be considered as not previously exposed. Therefore, they will not receive any further injections and will be followed for safety until 6 months after the last dose. In addition, if they received the vaccine, provisions for timely access to medical care will be offered for 10 years after the last dose, according to IDMC prior recommendations.
Investigational Products	
Study Product:	**CYD Dengue Vaccine**
Form:	Powder and solvent for suspension for injection
Composition:	Each individual 0.5 mL dose of reconstituted tetravalent vaccine contains: • 4.5-6.0 log10 cell-culture infectious dose 50% (CCID50) of each live, attenuated, dengue serotype 1, 2, 3, 4 virus • Excipients: essential amino acids, non-essential amino acids, L-arginine chlorhydrate, saccharose, D-trehalose dihydrate, D-sorbitol, Tris (hydroxymethyl) aminomethane, and urea • Solvent: NaCl 0.4%
Route:	Subcutaneous (SC)
Batch Number:	To be determined
Control Product:	**Placebo**
Form:	Solution
Composition:	NaCl 0.9%
Route:	SC
Batch Number:	To be determined
Screening Criteria:	The Screening Visit will take place within 4 weeks before the first injection. After a potential participant has signed an IEC/IRB-approved informed consent form (ICF), she/he will have a blood sample drawn in order to be screened for hepatitis B virus antigen (HBsAg)*, hepatitis C virus (HCV) antibodies*, assessment of previous dengue exposure, and for any hematological (hemoglobin, hematocrit, platelet count, white blood cells count) or biochemical (aspartate aminotransferase, alanine aminotransferase, urea, and creatinine) abnormalities. If serology, hematology, and

	biochemistry results are available and were obtained within 4 weeks before first injection, tests do not need to be performed again. Pre-injection HIV viral load and CD4 count will come from routine periodical tests of subjects as per local guidelines. For subjects for whom HIV viral load and CD4 count have not been assessed within 2 months before the Screening Visit, the Investigator will perform tests to ensure that results are available at the time of Visit 1. Subjects' previous exposure to dengue will be identified using a RDT (OnSite™ Dengue IgG/IgM3.0 Combo Rapid Test commercialized by CTK Biotech, Inc.) or an ELISA (Anti-Dengue IgG ELISA commercialized by Euroimmun) if the RDT result is negative. Only subjects identified as dengue seropositive will be eligible to participate in the study. Note: Subjects who previously tested negative by RDT can be re-screened using IgG ELISA. During the Screening Visit, applicable inclusion and exclusion criteria will be checked and a physical / clinical examination will be performed. * Unless documented information on previous infections is already available at the time of screening.
Inclusion Criteria:	Consent will be provided once at the Screening Visit. Inclusion criteria will be checked at Screening Visit and at the first vaccination Visit (V01 at D0). An individual must fulfill all of the following criteria in order to be eligible for study enrollment: 1) Aged 18 to 50 years on the day of first study vaccination (study product administration) ("18 to 50" means from the day of the 18th birthday to the day before the 51th birthday) 2) ICF has been signed and dated 3) Able to attend all scheduled visits and to comply with all study procedures 4) Documented seropositivity for HIV-1 infection based on the Brazilian HIV Guidelines' laboratory criteria (ie, 2 positive results obtained from different and independent determination methods) or detectable HIV-1 viral load results in the past 5) Stable HIV condition according to Brazilian HIV Guidelines (ie, with both CD4 count > 350 cells/mm^3 and sustainable and undetectable HIV viral load [< 50 copies/mL]) for at least 1 year before consent 6) Stable antiretroviral (ART) regimen based on local HIV protocol for at least 1 year before consent 7) Previous exposure to dengue confirmed by RDT or dengue IgG ELISA
Exclusion Criteria:	Exclusion criteria will be checked at Screening Visit and at the first vaccination Visit (V01 at D0). An individual fulfilling any of the following criteria is to be excluded from study enrollment: 1) Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be pre-menarche or post-menopausal for at least 1 year, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination until at least 4 weeks after the last vaccination) 2) Participation at the time of study enrollment (or in the 4 weeks preceding the first study vaccination) or planned participation during the present trial period in another clinical study investigating a vaccine, drug, medical device, or medical procedure 3) Receipt of any vaccine in the 4 weeks preceding the first study vaccination or planned receipt of any vaccine in the 4 weeks following any study vaccination

	4) Previous vaccination against dengue disease with either the study vaccine or another vaccine 5) Receipt of immune globulins, blood or blood-derived products in the past 3 months 6) Self-reported or suspected congenital or acquired immunodeficiency, except HIV; or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months) 7) Previous acquired immunodeficiency syndrome (AIDS), defined as the occurrence of opportunistic infection in the last 2 years before consent 8) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccines used in the study or to a vaccine containing any of the same substances 9) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily 10) Current alcohol abuse or drug addiction 11) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with study conduct or completion 12) Moderate or severe acute illness/infection (according to Investigator judgment) or febrile illness (temperature ≥ 38.0°C) on the day of vaccination. A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided 13) Identified as an Investigator or employee of the Investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (ie, parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study 14) Previous CD4 count < 200 cells/mm^3 (nadir) since diagnosis of HIV 15) History of chronic and active hepatitis B infection or HBsAg-positive 16) History of chronic and active hepatitis C infection or HCV Ab-positive 17) Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine > 3 times the upper limit of normal range (ULN) 18) Hemoglobin (Hb) < 10 g/dL 19) White blood cell count (WBC) < 1500 cells/mm^3 20) Platelets < 100 000 cells/mm^3

Statistical Methods:	All main analyses will be descriptive. For the main parameters, 95% confidence intervals (CIs) of point estimate will be calculated using normal approximation for quantitative data and exact binomial distribution (Clopper-Pearson method) for proportions. Two statistical analyses will be performed on unblinded data 28 days after the last injection (for safety and immunogenicity), and 6 months after the last injection of the last subject enrolled (for safety). Sample Size The objective of the study is to provide descriptive safety and immunogenicity results and therefore the sample size is arbitrarily set to 100 subjects for the CYD dengue vaccine Group and 50 subjects for the Placebo Group. There is a 95% probability of observing an event that has a true incidence of 3% for the CYD dengue vaccine Group.

Table of Study Procedures

Phase II Trial: a Screening Visit (Scr.), 8 Visits (V), and 5 Phone Calls (PC); 3 Injections; 7 to 9 Blood Samples; 18-month duration per subject

Visit (V) and Phone Call (PC) Number	Scr. *	V01*	V02	V03	V04	V05	PC1	PC2	V06	V07	PC3	PC4	V08	6-month Follow-up†
Trial Timelines Injection (Inj); Day (D); Month (M)	-D28 to –D0	Inj 1 (D0)	Inj 1 +7D	Inj 1 +14D	Inj 1 +28D	Inj 2 (M6)	Inj 2 +7D	Inj 2 +14D	Inj 2 +28D	Inj 3 (M12)	Inj 3 +7D	Inj 3 +14D	Inj 3 +28D	Last Inj +6 months
Time Windows (Days)			+3	+3	+14	±20	+3	+3	+14	±20	+3	+3	+14	+30
Phone calls							X†	X†			X†	X†		X†
Informed Consent	X													
Inclusion/Exclusion Criteria	X	X												
Demography/Body Stature		X												
Significant Medical History	X	X												
History of dengue infection/vaccination, YF infection/vaccination, or Zika infection	X	X												
Physical/Clinical Examinations ‡	X	X				X	†	†		X	†	†		†
Temperature §		X				X				X				
IRT Contact	X	X				X				X				
Randomization		X												
Concomitant Therapy **		X	X	X	X	X			X	X			X	
Urine Pregnancy Test ††		X				X				X				
Contraindications						X				X				
Blood Sampling														
Screening tests ‡‡	X													
Previous exposure to dengue using RDT or dengue IgG ELISA §§	X													
FV status (dengue, YF, and Zika) ***		BL1												
Dengue neutralizing Abs***		BL1			BL4				BL5				BL6	
Dengue vaccinal viremia			BL2	BL3										
CD4 count and HIV Viral Load †††		†††			BL4‡‡‡	†††			BL5‡‡‡	†††			BL6‡‡‡	
Virological confirmation of dengue by NS1 Ag ELISA and/or WT dengue RT-PCR		All acute febrile illness with diagnosis of dengue requiring hospitalization within the first 5 days after fever onset, occurring after the first visit (V01) and until the end of the 6-month safety follow-up												

Visit (V) and Phone Call (PC) Number	Scr. *	V01*	V02	V03	V04	V05	PC1	PC2	V06	V07	PC3	PC4	V08	6-month Follow-up†
Trial Timelines Injection (Inj); Day (D); Month (M)	-D28 to –D0	Inj 1 (D0)	Inj 1 +7D	Inj 1 +14D	Inj 1 +28D	Inj 2 (M6)	Inj 2 +7D	Inj 2 +14D	Inj 2 +28D	Inj 3 (M12)	Inj 3 +7D	Inj 3 +14D	Inj 3 +28D	Last Inj +6 months
Time Windows (Days)			+3	+3	+14	±20	+3	+3	+14	±20	+3	+3	+14	+30
Injection		Inj. 1				Inj 2				Inj 3				
30-Min. Observation Period – Collection of immediate events		X				X				X				
Injection Site Reactions & Systemic Events Assessment		Solicited injection site reactions will be collected for Days 0–7 after each injection. Solicited systemic reactions will be collected for Days 0–14 after each injection. Unsolicited events will be collected for Days 0-28 after each injection.												
Diary Card (DC) §§§														
Provided		DC1			DC2	DC3			DC4	DC5				
Checked			DC1	DC1	DC1	DC2			DC3	DC4			DC5	
Collected					DC1	DC2			DC3	DC4			DC5	
Memory Aid (MA)§§§														
Provided													MA	
Termination Record****													X	
SAEs and Serious AESIs ††††	‡‡‡‡	SAEs from the first visit (V01) and until the end of the 6-month safety follow-up; AESIs in defined time windows according to the type of AESI												

* Serology, hematology and biochemistry results, as well as all other information collected as part of the screening of potential subjects will be captured in the source document. Inclusion/exclusion criteria, significant medical history, history of dengue infection/vaccination, of YF infection/vaccination, and of Zika infection, physical/clinical examinations, CD4 count and HIV viral load results will be captured in the CRF at V01 for eligible subjects. The Screening Visit and Visit 1 can be simultaneous for subjects tested positive by RDT and whose tests have been performed in appropriate time windows (within 4 weeks before injection for hepatitis serology, hematology, and biochemistry; within 2 months for CD4 count and HIV viral load) as part of the subject's routine follow-up.

† A follow-up visit can be arranged depending on the information provided during the phone call, at the Investigator's discretion. In the case of the 6-month follow-up phone call, this can be anticipated to perform the 6 months follow up after the last vaccination in case of early termination.

‡ A full physical/clinical examination will be conducted on each vaccination visit (before vaccine injection), and at the Investigator's discretion at the time of other visits, based on the health status of the subject.

§ Subject's temperature is to be measured before each injection, and at the Investigator's discretion at the time of other visits, based on the health status of the subject. Temperatures will be recorded in source document only.

** Concomitant therapy will be collected for Days 7, 14 and 28 days post-Injection 1 as well as before and 28 days after Injection 2 and Injection 3.

†† In all female subjects, result of urine pregnancy test should be confirmed as negative before each vaccine injection.

‡‡ Screening tests will be performed at local laboratory within 4 weeks before the first vaccination. These tests will include: serology (HBsAg, HCV Ab), hematology (hemoglobin, hematocrit, platelet count, white blood cells count), and biochemistry (AST, ALT, urea and creatinine). HBsAg and HCV tests will not be performed in case the information is already available in subject's medical history. If serology, hematology, and biochemistry results are available and were obtained within 4 weeks before first injection, tests do not need to be performed again.

§§ Tests will be performed sequentially. RDT will be performed first, and IgG ELISA will only be performed on subjects who tested negative for RDT. Subjects who previously tested negative by RDT can be re-screened using dengue IgG ELISA.

*** Blood sample to be collected before vaccine injection.

††† Pre-injection CD4 count and HIV viral load will come from routine periodical tests of subjects as per local guidelines. For subjects for whom HIV viral load and CD4 count have not been assessed within 2 months before the Screening Visit, the Investigator will perform tests to ensure that results are available at the time of Visit 1. Likewise, if HIV viral load and CD4 count have not been tested within 2 months before Inj 2 or Inj 3 (for checking contraindication) the Investigator will perform tests to ensure that results are available before the corresponding vaccination visit.

‡‡‡ If an increase in HIV viral load (plasma HIV-1 RNA increase > 1000 copies/mL 28 days post-injection after having been undetectable [< 50 copies/mL] pre-injection) or a decrease in CD4 count (decrease in CD4 count greater than 30% assessed 28 days post-injection compared to pre-injection value), another blood sample is to be taken 4 weeks later for confirmation.

§§§ DCs are used for the collection of solicited and unsolicited AEs and concomitant medication. DC2 and DC4 are used for the collection of medical events and/or hospitalization and medications at the time of the event in the period between V04 and V05 and V06 and V07, respectively. Each DC is to be reviewed and collected by the site at the following visit. The MA is used for the collection of medical events and/or hospitalization and medications at the time of the event for 6 months after the third injection. The caller will ascertain whether any SAEs occurred since the last contact. During the follow-up period, subjects will be instructed to contact the clinical site if they are hospitalized or experience an AE that might be considered serious.

**** Termination record will be checked either during a planned study visit or a phone call.

†††† 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.

‡‡‡‡ SAEs related to study procedures (from Screening Visit to V01) will be reported on paper form; at V01, all SAE information for eligible subjects reported on paper SAE form should be transcribed into the EDC system. From V01 until the end of the safety follow-up, all SAEs and AESIs in defined time windows according to the type of AESI will be documented in the EDC.

List of Abbreviations

Ab	antibody
Ag	antigen
AE	adverse event
AESI	adverse event of special interest
AIDS	acquired immune deficiency syndrome
AR	adverse reaction
ART	antiretroviral therapy
$CCID_{50}$	cell-culture infectious dose 50%
CD4	CD4+ T-cell
CDM	Clinical Data Management
CI	confidence interval
C&MQO	Clinical and Medical Quality Operations
CRA	Clinical Research Associate
CRB	case report book
CRF	case report form
CTA	clinical trial agreement
CTL	Clinical Team Leader
DC	diary card
DENV	dengue virus
DF	dengue fever
DHF	dengue hemorrhagic fever
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ESDR	early safety data review
FAS	full analysis set
FDA	Food and Drug Administration
FV	flavivirus
FVFS	first visit, first subject
FVLS	first visit, last subject
GCI	Global Clinical Immunology
GCP	Good Clinical Practice
GMT	geometric mean titer
GPV	Global PharmacoVigilance
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus

HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
ICF	informed consent form
ICH	International Conference on Harmonisation
IDMC	independent data monitoring committee
IEC	Independent Ethics Committee
Ig	immune-globuline
IND	investigational new drug (application)
IRB	Institutional Review Board
IRT	interactive response technology
IVRS	interactive voice response system
IWRS	interactive web response system
JE	Japanese encephalitis
LCLS	last contact, last subject
LLT	lowest level term
LLOQ	lower limit of quantification
MA	memory aid
MedDRA	Medical Dictionary for Regulatory Activities
mL	milliliter
N/A	not applicable
NR	not reportable
NS	non-structural
post-Inj	post-Injection
PPAS	per-protocol analysis set
PRNT	plaque reduction neutralization test
PSO	Product Safety Officer
RTM	Regional Trial Manager
RDCS	Regional Director of Clinical Sciences
RDT	rapid diagnostic test
RMO	Responsible Medical Officer
RNA	ribonucleic acid
RT-PCR	reverse transcription-polymerase chain reaction
SAE	serious adverse event
SafAS	safety analysis set
SC	subcutaneous
SET	safety evaluation team
TMF	trial master file
UAR	unexpected adverse reaction

ULOQ	upper limit of quantification
VCD	virollogically-confirmed dengue case
WHO	World Health Organization
WT	wild-type
YF	yellow fever

1 Introduction

1.1 Background

This is a Phase II study assessing the safety and immunogenicity of the CYD dengue vaccine when administered in Human Immunodeficiency Virus (HIV)-positive adults, previously exposed to dengue, with clinically-stable condition under regular antiretroviral therapy (ART).

Dengue

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 (1-4). Dengue fever (DF) is characterized by biphasic fever, headache, and myalgia 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 platelets count are frequent. The incubation period of DF after the mosquito bite averages 4 days (range from 3 to 14 days) (2).

Dengue hemorrhagic fever (DHF) is characterized by abnormalities of homeostasis and increased vascular permeability that can lead to hypovolemia and hypotension (dengue shock syndrome), often complicated by severe internal bleeding. The case fatality rate of severe dengue can be as high as 20% without medical care, but is below 1% in most centers with modern intensive supportive therapy (2).

Human infection occurs by injection of the virus into the extravascular tissues during blood feeding by an infected *Aedes aegypti* mosquito or *Aedes albopictus* mosquito (1). Both these vectors are now present in all tropical and sub-tropical areas of the world and in some temperate areas of the USA, Europe, Africa, and the Middle East. Following its rapid spread in recent years, DF/DHF is now endemic/epidemic in Latin America, South East Asia, India, Africa, and the Caribbean, and Pacific regions.

According to the World Health Organization (WHO), over 2.5 billion people are now at risk from dengue in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South East Asia, and the Western Pacific. The Americas, South East Asia, and the Western Pacific regions are the most seriously affected. It is currently estimated that there may be 390 million dengue infections worldwide every year. An estimated 500 000 people with severe dengue require hospitalization, a large proportion of whom are children. About 2.5% of those affected die (2). Thus, according to WHO, there is an urgent need to develop a safe and effective method to prevent progression of this substantial public health issue (2) (3).

Routine laboratory diagnosis of dengue infection is based on the detection of dengue virus-specific antibodies (Abs), immunoglobulin (Ig) M and/or isolation of the virus or detection of viral ribonucleic acid (RNA) by reverse transcription-polymerase chain reaction (RT-PCR) or viral non-structural protein (NS) 1 antigen by enzyme-linked immunosorbent assay (ELISA) (4-6). The diagnosis of dengue falls into 2 stages: Stage I, the acute fever period lasting a few days when viremia may be detected; and Stage II, the early post-febrile period lasting a few weeks when IgM and IgG Abs are increased.

Until recently, vector control, and personal protection were the only measures to prevent dengue infection and disease. Although some prevention and vector control campaigns are well-documented successes, most of these successes have proved to be temporary as public health authorities were unable to sustain underlying control strategies in the face of social and/or environmental changes (7). It is now increasingly acknowledged that eliminating dengue as a public health burden will involve a safe and effective vaccine directed at the 4 serotypes of dengue virus responsible for the disease, in addition to preventive measures (7).

Human Immunodeficiency Virus

The HIV belongs to the family of retrovirus. It can be transmitted from human to human mostly through sexual intercourses, needle sharing among injecting drug users, and from mother to child during pregnancy (8).

During infection, virus particles bind to host cells expressing principally CD4 receptors but also a range of host proteins (9). The hallmark of HIV infection is the progressive depletion of CD4+ T cells (CD4) leading, after a highly variable period of time, to the acquired immune deficiency syndrome (AIDS). At this stage, the HIV infection becomes symptomatic and the infected person experiences opportunistic infections, eventually leading to death.

The development of ARTs regimen during the past 2 decades has transformed HIV infection from a progressive illness with a fatal outcome into a chronic manageable disease. In parallel, and following extensive national prevention campaigns, remarkable progresses have been made in terms of prevention-associated risk reduction. As for the development of an efficacious HIV vaccine, it is still facing important challenges, including the virus's genetic diversity, uncertainty about what constitutes protective immunity, and difficulty in the development of antigens that are highly immunogenic (8).

Despite advances on complementary fronts, HIV infection remains endemic worldwide, with higher incidence in low-income and middle-income countries, as a consequence of a limited access to health care (Asia, Sub-Saharan Africa, and Latin America). Globally, the number of new infections has decreased between 2000 and 2018. However, global prevalence of HIV has increased from 27.4 (23.1–32.6) million to 37.9 (32.7–44.0) million during the same period as ART has increased life expectancy of infected persons (10). In 2017, an estimated (10):

- 37.9 million (32.7 million–44.0 million) people globally were living with HIV
- 1.7 million (1.4 million–2.3 million) people became newly infected with HIV
- 770 000 (570 000–1.1 million) people died from AIDS-related illnesses

Dengue and HIV co-infection

Interactions between HIV and other infectious agents, particularly in tropical regions, has been associated with accelerated HIV/AIDS disease progression (11). However, the severity and characteristics of dengue and HIV co-infection and the reciprocal impact on disease progression remain elusive because of lack of systematic case–control analysis (12-14). An increased risk of severe dengue has not been described in HIV-positive subjects, but there are very limited data (11) (14-17). Thus far, case series have suggested that patients with dengue virus (DENV)-HIV co-infection have non-severe dengue outcome (18) and show no signs of accelerated progression of HIV disease (12-14). This may be due to the transient reduction of HIV viral load and no depletion of CD4+ T cells during acute dengue infection, plus the likely beneficial effect of the ART on the dengue infection course (13) (14) (17). Furthermore, it has also been observed that the DEN-2 NS5 protein inhibits HIV replication in CD4+ T cells in vitro (19). However, further research is still needed to understand DENV-HIV co-infection, especially since the number of cases is bound to increase in the coming years as life expectancy of HIV-positive persons increases.

1.2 Background of the Investigational Product

The CYD dengue vaccine

Sanofi Pasteur's tetravalent CYD dengue vaccine, using recombinant technology to obtain a live-attenuated vaccine, has been extensively evaluated in subjects from 9 months to 60 years.

As of October 2018, the CYD dengue vaccine clinical development plan includes 31 clinical studies, completed (25) or ongoing (6): 5 Phase I, 17 Phase II, and 9 Phase III. As of today, more than 30 000 subjects from 9 months through 60 years of age have received at least one injection of the final tetravalent CYD dengue vaccine formulation, regardless of the administration schedule.

Two large-scale Phase III efficacy studies were initiated during the clinical development of the CYD dengue vaccine: CYD14 and CYD15 (Phase III studies in multiple countries in Asia and Latin America, respectively). These efficacy studies were randomized, placebo-controlled, observer-blinded, and stratified by age. Each individual study was sufficiently powered to demonstrate significant efficacy of the CYD dengue vaccine in preventing the occurrence of virologically-confirmed dengue case (VCD) due to any serotype after 3 injections, given 6-month apart. Indeed, results from Phase III efficacy studies showed CYD dengue vaccine's potential to reduce probability for subjects to have symptomatic VCD and severe and/or hospitalized VCD due to any of the 4 serotypes.

Following per-protocol analysis, vaccine efficacy (VE) estimates against symptomatic VCD during the whole Active Phase[a] due to any of the 4 serotypes were 56.5% (95% confidence intervals (CI): 43.8; 66.4) for CYD14 and 60.8% (95% CI: 52.0; 68.0) for CYD15. In addition, efficacy against severe VCD cases and hospitalized VCD cases during the Active Phase was observed to be more than 70% (20) (21). VEs against dengue in participants who were 9 years of age or older were similar in the individual studies, with a pooled estimate of 65.6% (95% CI, 60.7

[a] From the first injection until 13 months after the third injection.

to 69.9), as compared with 44.6% (95% CI, 31.6 to 55.0) among participants under the age of 9 years (22). The reactivation of the active surveillance during the long-term follow-up in CYD14 and CYD15 allowed to estimate a pooled VE against symptomatic VCD of 38.7% (95% CI: 11.1; 57.8) 5 years after the first injection.

Overall, the CYD dengue vaccine was demonstrated to be immunogenic. Also, vaccinal viremia was observed in less than 6% of subjects (across all age groups) after the first dose, and in less than 1% of subjects after the subsequent doses, in the studies in which it was assessed (pooled data from CYD04, CYD05, CYD06, CYD10, CYD11, CYD12, CYD17, CYD23, and CYD24). Specifically in adults, the viremia was observed in 4.5% of subjects (pooled data from CYD04, CYD06, CYD10, CYD11, CYD12, and CYD17). No clinical relevance of the vaccinal viremia has been reported.

The first marketing authorization for the CYD Dengue vaccine (under the commercial name Dengvaxia®) was obtained in Mexico on 08 December 2015. As of November 2019 the CYD dengue vaccine is currently licensed in 21 countries in addition to the European Economic Area.

Dengue serostatus before injection with the CYD dengue vaccine

In July 2016, the WHO issued a position paper on Sanofi Pasteur's CYD dengue vaccine based on the Strategic Advisory Group of Experts' (SAGE) assessment that recognized its potential public health value when introduced in highly endemic countries. In addition, the SAGE also underlined the importance of addressing the question of the potential risk, over time, of hospitalized/severe dengue in individuals with no prior exposure to dengue before vaccination (23).

Analyzing long-term safety according to dengue serostatus at baseline presented an important challenge as serostatus had only been assessed in a subset of subjects (the so-called immunogenicity subset) in each of the 3 efficacy studies (CYD14, CYD15 and CYD23/57). In order to overcome this challenge Sanofi Pasteur leveraged a recently developed dengue anti-NS1 IgG ELISA assay to be able to infer the baseline dengue serostatus using specimens obtained approximately 1 month after the third vaccination collected from all study participants. In addition an efficient case-cohort design (including all subjects with outcomes of interest and a randomly selected "subcohort"), was used to obtain estimates of risk and efficacy with essentially the same power as the one it would have been obtained by including the entire study cohorts. This supplemental exploratory analysis was conducted on clinical data collected during the Phase IIb proof of concept efficacy study and the two Phase III efficacy studies.

The results of thus supplemental analysis confirmed the clear and sustained benefit of the CYD dengue vaccine in individuals 9-16 years of age living in endemic areas and previously infected by dengue prior to dengue vaccination. The results also showed an increased risk of hospitalization for dengue and clinically severe dengue (predominantly DHF Grade I or II [WHO 1997 (24)]) in seronegative individuals. Estimates from the long-term analysis suggest the onset of increased risk was mainly during the 3rd year following the first injection.

Sanofi Pasteur presented the full data of this supplemental analysis to the Independent Data Monitoring Committee (IDMC) in an *ad hoc* meeting held on 3-4 November 2017. During this meeting, the IDMC reviewed the data from these extended safety and efficacy analyses. It concluded that, in the case of subjects exposed to dengue prior to vaccination (henceforth, 'exposed subjects' or seropositive subjects), there is strong evidence that the vaccine protects

them from symptomatic dengue, hospitalized dengue and severe dengue. On the other hand, in the case of subjects not exposed to dengue before vaccination (henceforth, 'unexposed' subjects or seronegative subjects), the conclusion was that although vaccination may confer limited short-term benefit against symptomatic dengue, it also induces an increased risk of severe disease in the longer term.

Given these conclusions, the IDMC recommended that no further vaccination occur in unexposed subjects in ongoing or future trials, and on precautionary basis, including partially vaccinated subjects in ongoing trials.

In its most recent position paper, the WHO acknowledged that the CYD dengue vaccine had been shown to be efficacious and safe in persons who have had a dengue virus infection in the past (seropositive individuals), but carries an increased risk of severe dengue in those who experience their first natural dengue infection after vaccination (seronegative individuals). The WHO underlined that countries should consider introducing the CYD dengue vaccine only if risk for seronegative individuals can be minimized (2).

The WHO recommended strategy to minimize the risk of vaccinating seronegative persons is to perform a screening test on candidate vaccinees. It is acknowledged that laboratory-based serological assays testing for dengue virus IgG (eg, dengue IgG ELISA) would be impractical as results would not be available at the point of care in a timely manner. The WHO position paper underlines that rapid diagnostic tests (RDTs) would facilitate the implementation of the pre-vaccination screening strategy, and that, despite the fact that no such test has yet been validated or licensed specifically for the detection of past dengue infection, they could be used in high transmission settings until better RDTs become available (2) (25). Detection of anti-dengue IgG using enzyme-linked immunosorbent assay (ELISA) is generally used to determine previous dengue infection status and is routinely used in serosurveys to characterize dengue seroprevalence (26). In general, dengue ELISAs were found to be more sensitive than IgG RDTs for identifying prior dengue infection cases (27-30).

In this study, one RDT (*OnSite*™ Dengue IgG/IgM$^{3.0}$ Combo Rapid Test commercialized by CTK Biotech, Inc.) or one ELISA (Anti-Dengue IgG ELISA commercialized by Euroimmun) will be used to screen potential study participants (25) (31). These tests were selected because of their high specificity, following an internal evaluation of different options.

1.3 Potential Benefits and Risks

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

1.3.1 Potential Benefits to Subjects

HIV-positive subjects are typically excluded from clinical studies involving live-attenuated viral vaccines. However, vaccination against a number of pathogens is still recommended as HIV-positive individuals have an increased risk of getting some infections. For instance, the WHO recommends that HIV-positive subjects be vaccinated against diphtheria, pertussis, tetanus, measles, hepatitis B, *Haemophilus influenzae* type b, and *Streptococcus pneumoniae* (32). Thus, there could be a benefit for HIV-positive subjects living in areas endemic for dengue disease as subjects participating in the present clinical study and being injected with CYD dengue vaccine may acquire immunity and protection against dengue disease after vaccination.

Although efficacy has not been evaluated in subjects over 17 years, data from studies involving adults (18 to 45 years of age) (CYD22, and CYD47) suggest that adults respond well to the vaccine schedule used in efficacy studies as an increase of geometric mean of titers (GMTs) compared to baseline was observed. Indeed, post-injection (post-Inj) 3 Ab levels are generally higher than those seen in CYD14 and CYD15 where efficacy was demonstrated.

The supplemental analyses (see Section 1.2) have provided strong evidences that, for subjects that were dengue seropositive prior to CYD dengue vaccination, the vaccine protects against symptomatic dengue, hospitalized dengue, and severe dengue disease. In this study, only persons that have previously been exposed to dengue will be enrolled.

HIV infection has been associated with a reduced immunologic response to a number of inactivated and live-attenuated vaccines. For instance, the immune response following YF vaccination was found to be slightly inferior in HIV-positive individuals in comparison to that in uninfected persons. Nevertheless, higher CD4 counts as well as lower HIV viral load at the time of vaccination were predictors of higher neutralizing antibodies titers (33). Thus, HIV-positive persons with a clinically-stable condition under regular ART treatment that receive the CYD Dengue vaccination might develop an immunological response against the dengue virus.

1.3.2 Potential Risks to Subjects

CYD dengue vaccine

The data demonstrated that the reactogenicity profile after any injection of the CYD dengue vaccine is slightly increased compared to the placebo but appeared similar to licensed vaccines used in the age groups that have been studied.

As of June 2018 a total of 24 886 subjects aged ≥ 9 years have received at least 1 injection of the final formulation of the CYD dengue vaccine according to the final schedule. Among these subjects, a total of 2606 adults (aged 18 years and over) received at least 1 dose of the CYD dengue vaccine (see the Investigator Brochure) (34).

In the adult population, the most frequent solicited injection site reaction within 7 days after any CYD dengue vaccine injection was injection site pain (45.2%); erythema (7.9%) and swelling (2.4%) were less frequently reported. Injection site reactions tended to be reported with similar frequencies after each injection.

The most frequent solicited systemic reactions within 14 days after any CYD dengue vaccine injection were headache (51.4%), malaise (44.3%), and myalgia (42.2%) followed by asthenia (28.3%). Fever (4.9%) was less frequently reported. The frequency of all solicited systemic reactions tended to decrease with subsequent injections, except fever, which was reported with low incidence at each injection. The adverse reactions (ARs) were usually mild to moderate in severity and of short duration (0 to 3 days).

There were no anaphylactic reactions in adults. Allergic reactions based on the targeted list of preferred terms were reported in adults after any vaccine dose with frequency of 1.2%, versus 0.3%, any placebo injection. However, as with any vaccine, risk of allergic (including anaphylactic) reaction cannot be excluded.

Approximately three-quarter of adult subjects experienced at least one solicited reaction after any vaccination (73.2%), mainly solicited systemic reactions. Grade 3 solicited reactions were

reported by approximately 11% of adults and were mostly of the solicited systemic reactions category.

As CYD dengue vaccine has an YF vaccine backbone, and YF vaccination has been rarely associated with viscerotropic and neurotropic AEs, this risk has to be considered (35). However, it is important to underline that this risk has been associated with the YF virus 17D envelope protein (36), a protein that is absent from CYD dengue vaccine constructs. This theoretical risk linked to viscerotropism and neurotropism is further addressed in the "Guidelines for assessing viscerotropic and neurotropic AE" document. In the previous studies conducted with the CYD dengue vaccine, no confirmed viscerotropic, or neurotropic AEs were reported after any dose in any age groups.

It is to be noted that subjects not previously exposed to dengue are at an increased risk of hospitalization for dengue and clinically severe dengue (predominantly DHF Grade I or II [WHO 1997 (24)]). For this reason, individuals assessed as dengue seronegative are not to be enrolled in this study.

HIV-associated additional potential risks

In its *Guideline for the clinical evaluation of dengue vaccines in endemic areas* (37) the WHO advises to include HIV-positive subjects at the post-licensure stage of vaccine development.

As CYD dengue vaccine has YF vaccinal virus as backbone, HIV-associated potential risks can be expected to be similar to those observed following vaccination of HIV-positive subjects against other FV (ie, YF and Japanese encephalitis [JE]). The safety data from HIV-positive subjects vaccinated against YF is reportedly limited and insufficient to conclude on the vaccination's safety (33). However, no SAEs were reported in these studies (32); and as of today, no evidence points to an increase in the number of confirmed viscerotropic and neurotropic AEs in HIV patients following YF vaccination (33). Also, outside the clinical context, only a few SAEs were reported in HIV-positive individuals following the 2007-2010 YF vaccination campaign conducted in West and Central Africa (38). Overall, and although few data are available on the safety and efficacy of YF vaccination in HIV-positive persons, YF 17D vaccine is considered sufficiently safe in HIV-positive subjects who are not symptomatic or demonstrably immunosuppressed, and vaccination is recommended for persons with CD4 counts > 200/mm^3 who are deemed to be at risk of exposure to YF virus (39).

Likewise, the safety of vaccines against JE has been assessed in a very limited number of HIV-positive subjects, all of whom were children. In 2 independent studies involving HIV-positive Thai children, no severe AEs were reported (40) (41).

It was observed that the stimulation of the immune system with vaccination may transiently increase HIV viremia, through heterologous transactivation. This usually resolves within 4 weeks (42) (43). However, this increase does not have clinical implications, as it does not correspond to virologic failure and it is not related to ART resistance. Hence, there is a possibility that vaccination against dengue is associated with this phenomenon in some subjects (42). However, in patients who received a live-attenuated oral cholera vaccine, no such activation was observed and there were no significant changes in CD4 count and HIV-1 viral load between vaccine and placebo groups (44). As per Brazil National Recommendations on vaccination of HIV-positive population, CD4 cells count will be checked before each injection (43).

Thus, and based on the scarce data on the vaccination of HIV-positive subjects against other FV (YF and JE), no safety signals are expected to occur in this descriptive study.

Placebo

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

All subjects

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

1.4 Rationale for the Study

Dengue is endemic in most of Latin America and Caribbean. Despite the dengue control programs, case management guidelines, and vector control efforts, dengue virus transmission remains high and prevention remains a public health priority. The number of probable/suspected dengue cases and the incidence of dengue disease in Brazil have been among the highest of all Latin American countries. Table 1.1 offers an overview of the importance of dengue in Brazil in the last 6 years (45).

Table 1.1: Number of Dengue cases (total and confirmed), incidence rate, and number of deaths reported in Brazil during the 2014-2019 period

	Number of total dengue cases*	Number of laboratory-confirmed dengue cases	Incidence rate of all dengue cases (per 100 000 population)	Number of deaths	Severe dengue†
2019‡	2 070 170	1 131 455	992.91	702	1 321
2018	265 934	174 724	126.11	155	321
2017	252 054	42 101	120.43	133	378
2016	1 500 535	316 687	716.01	642	861
2015	1 649 008	500 972	809.70	863	1 569
2014	590 852	220 537	292.45	409	687

* Report of all dengue cases: suspected, probable, confirmed, non-severe and severe cases, and deaths. Probable dengue case: Person who has a fever or history of fever for 2-7 days duration, 2 or more symptoms of dengue and one serological test positive or epidemiological nexus with confirmed dengue case 14 days before onset of symptoms..

† Includes severe dengue and haemorrhagic dengue fever

‡ Data up to Week 42 for 2019.

Brazil is also plagued with a high burden of HIV/AIDS. In 2014, there were about 734 000 people living with HIV/AIDS with a prevalence of 0.4% in people above 15 years old. As regards HIV infection only, in Brazil, in 2017, 42 420 cases of HIV infection were reported to the Information System of Reportable Diseases (SINAN): 4306 of them from North region (10.2%), 9706 from

Northeast (22.9%), 16 859 from Southeast (39.7%), 8064 from South (19.0%) and 3485 from Midwest region (8.2%). From the beginning of the AIDS epidemic in 1980 through December 2017, 327 655 deaths related to AIDS have been reported in Brazil (46).

A recent publication has demonstrated that in a HIV-1 infected ART-naïve population in Brazil the mean reported value of CD4 count has slightly increased from 348 in the year 2003 to 389 in 2009. Nevertheless, the values have increased as the ART was initiated and maintained (means of 368 in the year 2001 to 414 in 2009 in HIV patients under ART). In terms of HIV viral load, the percentage of treated patients with undetectable viremia (< 50 copies/mL) has also increased from 48% in the year 2001 to 77% in 2007. Furthermore, the resistance to antiretrovirals in the general HIV population of the country has remained stable over the last decade (around 25%) (47).

In recent years, Brazil's National Ministry of Health has published guidelines for the management, diagnosis, treatment and follow-up of HIV-positive persons (48). Adherence to these guidelines has been made possible through the universal access (ie, government-guaranteed access at no cost) to treatment and medical assistance (including control laboratories, ie, CD4 count in some instances, HIV viral load, diagnosis exam) that is provided throughout the country, with laboratories available to follow patients in all regions of the country (48) (49). There are no major issues with shortages of antiviral drugs, and laboratories are available for the CD4 and HIV viral load assessments that are required for an appropriate follow-up of participants (49).

The tetravalent CYD dengue vaccine is a recombinant, live-attenuated vaccine cultured on serum free Vero cells. The purpose of the CYD dengue vaccine is to provide coverage against serotypes 1, 2, 3 and 4 and it has shown a positive benefits/risk balance in subjects aged 9 to 45 years living in endemic areas and previously exposed to dengue (20-22). So far, the CYD dengue vaccine has been tested on healthy subjects, and "at risk" populations have been excluded. As the product has been licensed in a number of countries, and as recommended by the WHO, it is now justified to widen clinical studies to all populations that may be exposed to dengue vaccination, and to evaluate whether the vaccine is safe and immunogenic in a population of special interest, such as HIV-positive adults.

It is thus of prime importance to assess whether the CYD dengue vaccine is safe in HIV subjects as dengue and HIV are both endemic in many areas of the world (15) (50-52). In countries where dengue is endemic and where mass vaccination campaigns may be implemented, there is a need to document the safety in this population as HIV-positive persons could be inadvertently vaccinated with the CYD dengue vaccine. This study will provide data on the use of the vaccine in a HIV-positive population and will be part of the Risk Management Plan.

It is also important to assess whether an immunological response mediated by the development of neutralizing Ab levels of HIV-positive subjects injected with the CYD dengue vaccine can be expected. Likely, and as observed with YF and JE vaccines, the CYD dengue vaccine may induce a lower immune response than that observed in healthy subjects (33) (40) (41). Indeed, 2 published studies providing immunogenicity data for YF vaccines in HIV-positive persons found lower rates of YF virus-specific neutralizing antibodies among HIV-positive persons, compared with uninfected controls at 10 to 12 months post-vaccination in both adults (53) and children (54). Although the mechanisms for the diminished immune response in HIV-positive persons are uncertain, an inverse correlation exists between immune response and HIV-1 RNA levels and a positive correlation with CD4 cell counts (55). It has also been suggested that the reduced and/or

less durable responses to YF vaccine in HIV-positive persons under ART are associated with lower CD4/CD8 cells ratio (56). More generally, the correlation between low CD4 count and low immunological response has also been observed following vaccination against other pathogens (57) (58).

In order to pursue the development of the CYD dengue vaccine, the present study will descriptively assess the safety and the immunogenicity of the CYD dengue vaccine in a HIV-positive population. It will involve 150 HIV-positive adults treated with ART (aged 18 to 50 years) who will receive either 3 injections of CYD dengue vaccine or placebo at 0, 6, and 12 months.

2 Study Objectives

2.1 Primary Objective

Safety

To describe the safety of each injection of CYD dengue vaccine in HIV-positive adults previously exposed to dengue.

The endpoints for the primary objectives are presented in Section 9.1.1.2.

2.2 Secondary Objectives

Immunogenicity:

- To describe the humoral immune response to each dengue serotype at baseline and after each injection of CYD dengue vaccine in HIV-positive adults previously exposed to dengue

Safety

- To detect the CYD dengue vaccinal viremia post-Inj 1 in HIV-positive adults previously exposed to dengue
- To describe changes in CD4 count and HIV RNA viral load after each injection of CYD dengue vaccine in HIV-positive adults previously exposed to dengue

The endpoints for the secondary objectives are presented in Section 9.2.2.1 and Section 9.2.1.2 respectively.

2.3 Observational Objective(s)

Immunogenicity

- To describe the FV (dengue, YF, and Zika) serological status in the study population at baseline.

The endpoints for the observational objective are presented in Section 9.3.1.1.

3 Investigators and Study Organization

This study will be conducted in Brazil. The Principal Investigators and any sub-investigators at the individual sites will be coordinated by 1 Coordinating Investigator. Details of the study centers, the Investigators at each center, and the Coordinating Investigator are provided in the "List of Investigators and Centers Involved in the Trial" document.

Safety evaluation team

An internal safety evaluation team (SET) will perform a blinded early safety analysis on safety data collected from Days 0-14 post-first injection for the first 20 subjects having received the first injection.

Independent Data Monitoring Committee

Following the SET assessment, an IDMC will review the early safety data of the first 20 subjects and will provide a recommendation on whether to continue enrollment and administration of the second dose of vaccine to the 20 already enrolled subjects (Section 5.1.6).

In addition, the IDMC will be involved in the regular review of hospitalized VCD cases, including assessment of severity. Related SAEs or deaths will be promptly reviewed by the IDMC.

Lastly, the IDMC will also be promptly notified of any significant confirmed modification of a subject's HIV infection status, as well as if any of the criteria for interruption of the study for safety reason are met (see Section 5.5).

Others

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

The laboratories involved in this study will be:

- Sanofi Pasteur Global Clinical Immunology (GCI), Swiftwater, Pennsylvania, USA or outsourced laboratory under the management of GCI for: dengue, YF, and Zika serological status, dengue neutralizing Ab titration, vaccine viremia testing, and virological confirmation of dengue
- Local laboratories for: screening tests (dengue IgG ELISA, hepatitis B surface antigen [HBsAg], hepatitis C virus [HCV] antibodies, hematology, and biochemistry), CD4 count, and HIV viral load assessments

The Sponsor's Responsible Medical Officer (the Responsible Medical Officer (RMO), the person authorized to sign this protocol and any amendments on behalf of the Sponsor) is Ana Paula de Almeida Salles Perroud, MD, MSc, and Clinical Team Leader (CTL) of this study.

4 Independent Ethics Committee / Institutional Review Board

Before the investigational product can be shipped to the investigational site and before the inclusion of the first subject, this protocol, the informed consent form (ICF), subject recruitment procedures, and any other written information to be provided to subjects must be approved by, and

/ or receive favorable opinion from, the appropriate Independent Ethics Committee (IEC) or Institutional Review Board (IRB).

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

The Investigator or the Sponsor will submit written summaries of the status of the study to the IEC / IRB annually, or more frequently if requested. All serious adverse events (SAEs) occurring during the study will be reported by the Investigator to the IEC / IRB, according to the IEC / IRB policy.

5 Investigational Plan

5.1 Description of the Overall Study Design and Plan

5.1.1 Study Design

This is a multi-center, observer-blind, randomized, placebo-controlled, Phase II study of the CYD dengue vaccine in 150 HIV-positive adults (18 to 50 years) in Brazil. Potential participants fulfilling all inclusion criteria, including a previous exposure to dengue according to the RDT or ELISA, will be enrolled. Subjects will receive 3 injections of either CYD dengue vaccine or placebo at 0, 6, and 12 months. The enrollment of subjects will be carried out in 2 steps, including an early safety data review (ESDR) before the second step.

Subjects HIV-positive and previously exposed to dengue according to RDT or ELISA will be randomized in a 2:1 ratio into 1 of 2 groups:

- Group 1 (N=100): subjects will receive 3 doses of CYD dengue vaccine (live, attenuated, dengue serotype 1, 2, 3, 4 virus)
- Group 2 (N=50): subjects will receive 3 doses of placebo (NaCl 0.9%)

Each subject will have her / his dengue serostatus confirmed using dengue PRNT and anti-NS1 IgG assay on the pre-injection blood sample. Subjects who are dengue-positive by RDT or ELISA at Screening Visit and dengue-negative by PRNT (sample collected before the first injection [at Visit 1] and assayed before second injection) will be considered as not previously exposed. Therefore, they will not receive any further injections and will be followed for safety until 6 months after the last dose. In addition, if they received the vaccine, provisions for timely access to medical care will be offered for 10 years after the last dose, according to IDMC prior recommendations.

All subjects are planned to be included in safety and immunogenicity analysis set. Blood samples will be taken at the Screening Visit (serology, hematology, biochemistry), and at several other time points throughout the study for baseline dengue, YF, and Zika serological status, CYD

dengue vaccine immunogenicity, dengue vaccinal viremia post-Inj 1, and HIV status monitoring assessments, depending on the time points. More details are provided in Section 5.1.3.

The duration of each subject's participation in the study will be approximately 18 months.

At the end of the trial, subjects will be informed on whether they received the CYD dengue vaccine or placebo. For those who received placebo, and who have a prescription, the CYD dengue vaccine will be offered free of charge by Sanofi Pasteur through the study doctor, in accordance with the Brazilian laws and with vaccine indication in Brazil. This will be a decision between the subject and the study doctor since the vaccine offered will not be part of the trial.

5.1.2 Justification of the Study Design

CYD50 study is designed to assess the CYD dengue vaccine safety and immunogenicity in a specific population: HIV-positive persons. The fact that the study will be randomized, observer-blind and placebo-controlled will help reducing bias in the selection of subjects within this particular population and in the safety assessments. It will also help insulating the study vaccine's safety and immunogenicity assessments from some confounding factors (eg, other underlying health issues, endemicity of other flavivirus in Brazil). It is noteworthy that this study will only be enrolling HIV-positive subjects with clinically-stable condition under regular ART regimen. Also, and in agreement with the WHO position paper (WHO 2018), the study will only be enrolling subjects with a previous exposure to dengue according to RDT or dengue IgG ELISA. These criteria will both help minimize potential risks to subjects' safety and allow for a more homogeneous evaluation of the vaccine's safety and immunogenicity.

Specific inclusion and exclusion criteria have been drawn in order to evaluate the CYD dengue vaccine in this special population. Only subjects who will have had stable CD4 count (> 350 cells/mm^3) and undetectable viral load (< 50 copies/mL) for at least 1 year before consent, and who will have been on a stable ART regimen based on local HIV guidelines for at least 1 year before consent, will be included in this study (48). The rationale is to be able to follow stable subjects who are less likely to have complications during the study period.

CD4 count and viral load will be monitored before the first injection to ensure that participants meet the requirements set by the Brazilian Guidelines for Vaccination in HIV population (48). Subjects who will have had previous CD4 count < 200 cells/mm^3 (nadir) since HIV-1 diagnosis will be excluded from this study.

All subjects will be screened according to a number of parameters (see Section 5.2.3) to ensure that *i)* they are dengue seropositive and *ii)* they do not have significantly altered hematological and biochemical parameters before the first injection in case of adverse drug reaction (from either ART or CYD dengue vaccine) and/or of dengue disease. Also, due to the risk of heterologous transactivation (ie, transitory increase of viral load after vaccination), subjects that are positive for HBsAg and/or HCV antibodies will be excluded from the study. Besides, subjects' HIV infection will continue to be monitored throughout the trial according to local HIV guidelines (48).

More specifically, this study will allow assessing 3 distinctive features of the vaccination of HIV-positive subjects under a stable ART regimen (henceforth, the ART-population) with the CYD dengue vaccine:

How well is the CYD dengue vaccine tolerated in the ART-population. The safety of the CYD dengue vaccine in an ART-population needs to be assessed in light of the vaccine's overall safety profile. Gathering data on the same safety endpoints assessed throughout dengue studies conducted by Sanofi Pasteur will be one of the key elements in weighing the potential risks of vaccination against the potential benefits in HIV-positive individuals.

How immunogenic is the CYD dengue vaccine in the ART-population. As HIV-positive subjects have an altered immune function, the CYD dengue vaccine may not be as immunogenic as in immunocompetent persons (see Section 1.4). In order to find out whether this is also the case for the CYD dengue vaccine, this study will assess the immunogenicity of the vaccine in the ART-population 28 days after each injection.

How is the CYD dengue vaccine impacting some features of the HIV-positive subjects' immune system. Whether the CYD dengue vaccine might raise safety concerns that are specific to the ART-population has to be assessed. In this study, 2 potential safety issues will be monitored. Firstly, and as the CYD dengue vaccine is a live-attenuated virus vaccine, there is the possibility that HIV-positive subjects develop a vaccinal viremia (viremia will be assessed at D7 and D14 post-Inj 1 in all subjects). Viremia previously observed across all age groups in healthy subjects (less than 6% after the first injection, and less than 1% after subsequent injections) has not had clinical significance in the safety of subjects enrolled in earlier trials. Secondly, there is also the possibility that vaccination against dengue exerts influence in the HIV infection of some subjects (Section 1.3.2). In order to evaluate this potential risk, CYD50 will be assessing CD4 count and HIV-1 viral load 28 days after each vaccination.

It is noteworthy that only HIV-1 viral load will be assessed throughout the trial. This decision is aligned with the current local practice due to the low prevalence of the HIV-2 in Brazil, and according to the procedures included in the local guidelines for the management of HIV subjects (48).

Since cross-reactivity has been observed between FV Abs after vaccination or natural infection, previous exposure to dengue, yellow fever (YF), and Zika will be assessed at baseline using the appropriate neutralizing assays (59-61). This will allow evaluating the possible impact of recent outbreaks of Zika and YF on study participants safety and immunogenicity results (62) (63). This is especially important as the majority of Zika and YF cases are asymptomatic (64-66).

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

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

5.1.3 Study Plan

An overview of assessments and study vaccinations is provided in the Table of Study Procedures.

Each potential subject will sign and date the ICF. In addition to the Screening Visit, included subjects will attend 8 study visits, will receive 4 follow-up phone calls, and will be contacted 6 months after the last Visit for a safety follow-up.

Vaccination

All subjects will receive 3 injections of either CYD dengue vaccine or placebo at Day (D) 0, Month (M) 6, and M12.

Blood sampling

A number of immunological, hematological and biochemical parameters (see Section 5.2.3) will be assessed at screening (unless the tests have already been performed as part of routine follow-up within the 4-week time window). The subject's dengue serostatus will be determined by a RDT or an ELISA during the Screening Visit.

Dengue, YF, and Zika baseline status will be assessed at D0.

Dengue vaccinal viremia will be assessed at D7 and D14 post-Inj 1.

Dengue neutralizing antibody (Ab) levels will be assessed at baseline (V01; prior to Inj 1) and 28 days after each injection.

If no HIV viral load and CD4 count results are available prior to each injection (ie, tests have not been performed within 2 months before injection visit), tests will be performed to ensure that results are available at the time of injection visit.

HIV viral load and CD4 count will be assessed 28 days after each injection. In case an increase in HIV viral load (plasma HIV-1 RNA > 1000 copies/mL 28 days post-injection after having been undetectable [< 50 copies/mL] pre-injection) or a decrease in CD4 count (decrease greater than 30% 28 days post-injection compared to the pre-injection value) is observed, a second blood sample is to be taken 4 weeks later for confirmation, as it is current local practice to confirm abnormal and significant deterioration of HIV condition related results (48) (see Section 5.5).

A summary of blood samplings is provided in Table 5.1.

Table 5.1: Blood Sampling Volume (mL) per Visit for all Assessments

Visit (V) Number	Scr.	V01	V02	V03	V04	V05	V06	V07	V08
Trial timelines (Days/Months)	-D28 to -D0	Inj 1 (D0)	Inj 1 +7D	Inj 1 +14D	Inj 1 +28D	Inj 2 (M6)	Inj 2 +28D	Inj 3 (M12)	Inj 3 +28D
Time Windows (Days)			**+3**	**+3**	**+14**	**±20**	**+14**	**±20**	**+14**
Approximate volume for each assessment (mL)									
Serology (HBsAg, HCV Ab)*	5								
Hematology (hemoglobin, hematocrit, platelet count, white blood cells count)*	3								
Biochemistry (AST, ALT, urea, creatinine)*	5								
Previous exposure to dengue using RDT or dengue ELISA	1†								
Dengue, YF, and Zika serostatus		5							
Dengue neutralizing Abs		2			2		2		2
Serum sample for retesting		2			2		2		2
Dengue vaccinal viremia			4	4					
CD4 count	‡				10	‡	10	‡	10
HIV-1 viral load	‡				10	‡	10	‡	10
Total volume for each subject	14§	9	4	4	24		24		24

* HBsAg and HCV tests will not be performed in case information is already available in subject's medical history. If serology, hematology, and biochemistry results are available and were obtain within 4 weeks before first injection, tests do not need to be performed again.

† Tests will be performed sequentially. RDT will be performed first, and IgG ELISA will only be performed on subjects who tested negative for RDT. Tests will be done by the Investigator, following the RDT or ELISA Manufacturer's instructions. Subjects who previously tested negative by RDT can be re-screened using dengue IgG ELISA.

‡ Pre-injection CD4 count and HIV viral load will come from routine periodical tests of subjects as per local guidelines. For subjects for whom CD4 count and HIV viral load have not been assessed within 2 months before the Screening Visit, the Investigator will perform tests to ensure that results are available at the time of Visit 1. Likewise, if CD4 count and HIV viral load have not been tested within 2 months before Inj 2 or Inj 3 (for checking contraindication), the Investigator will perform tests to ensure that results are available before the corresponding vaccination visit.

§ This volume is indicative only as it may differ between local laboratories. It does not include the volume that might possibly be required for the assessment of baseline CD4 count (10 mL) or HIV viral count (10 mL) in some subject (ie, subjects for whom CD4 count and HIV viral count have not been assessed within 2 months before the Screening Visit).

Collection of safety data

Safety data will be collected following each injection. Clinical site personnel will record immediate AEs that occur within the 30 minutes after injection. Subjects 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 occurring up to 28 days post-injection. Information on SAEs will be reported throughout the study (See Section 10.1 and Operating Guidelines for more information). Serious and non-serious AEs of special interest (AESIs) will be collected in defined time windows according to the type of AESI.

DCs will be provided to subjects to collect information on SAEs in-between injections (ie, from 28 days to 6 months post-Inj 1; and from 28 days to 6 months post-Inj 2) A memory aid (MA) will be provided to subjects to help them record SAEs during the 6 months safety follow-up (from 28 days post-Inj 3 to 6 months after last injection). Subjects are to contact the Investigator (within the first 5 days after fever onset) in case of hospitalization for suspected dengue disease at any time during the study for virological confirmation of the disease.

Clinical site personnel will review the safety data with the subjects during post-injection visits.

5.1.4 Visit Procedures

All information collected during the study visits must be reported into the source documents. For eligible subjects, the information collected will also be recorded in the electronic case report form (CRF) at V01.

Screening Visit (Scr.; D-28 to D0): Inclusion, Physical/Clinical Examination and Blood Sample of Potential Subjects

For all subjects (see Section 5.2.3)

1) Present the study to the subject in more details, answer any of her/his questions, and ensure that she/he has been informed of all aspects of the study that are relevant to their decision to participate.
2) Obtain the ICF signed and dated by the subject, and date and sign the ICF (only the Investigator or sub-Investigator). The Investigator will keep the original document and give the other one to the subject.
3) Review the inclusion and exclusion criteria for eligibility.
4) Check and collect the subject's significant medical history (including ART-related information).
5) Check and collect the subject's history of dengue infection/vaccination, YF vaccination/infection, and Zika infection.
6) Perform a physical examination.
7) Obtain the blood sample for screening tests (including assessment of previous exposure to dengue using the RDT or dengue IgG ELISA; see Section 7.1 for detailed instructions regarding the handling of blood samples), record the date of collection.

It is important to note that, if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt, even on another day within the Visit window. If ultimately a blood sample cannot be obtained, the reason will be recorded in the source document. In that case, the subject will not participate in the study.

8) Call the Interactive Response Technology (IRT) in order to get the subject's inclusion number
9) Remind the subject to call the study center if a serious medical event occurs
10) Arrange an appointment for Visit 1 (V01)

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

Notes:

- Subjects who previously tested negative by RDT for previous exposure to dengue can be re-screened using IgG ELISA. Subjects who are re-screened will be allocated a new subject number.
- If a subject is eligible but not randomized during the ESDR hold, the subject will be asked to come back to the site after the hold. The subject will then be re-screened and randomized. However, the subject will not be re-tested for previous exposure to dengue by RDT or IgG ELISA. It is to be noted that subjects that are re-screened will be allocated a new subject number.

Visit 1 (Inj 1 [D0]): Randomization, Blood Sample, and Vaccination of Eligible Subjects

Once dengue previous exposure has been identified using RDT or dengue IgG ELISA, the Visit 1 procedures may be undertaken.

1) Review the inclusion and exclusion criteria and confirm the eligibility
2) Collect demographic (year of birth and gender) and body stature (height and weight) data
3) Check and collect the subject's significant medical history (including ART-related information)
4) Check and collect the subject's history of dengue vaccination/infection, of YF vaccination/infection, and of Zika infection
5) Perform a physical examination and record the subject's axillary temperature before injection
6) Check concomitant medications and record every reportable medication ongoing at the time of vaccination
7) Perform a urine pregnancy test for all women enrolled in the study
8) Obtain a blood sample (see Section 7.1 for detailed instructions regarding the handling of blood samples), record the date of collection

It is important to note that, if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.

9) The person in charge of injections ("vaccinator") or authorized designee contacts the IRT to obtain the assigned product to be administered
10) Inject the appropriate investigational product
11) Record the date of injection, the site, and side of injection and the route of administration, as well as the dose number of the vaccine
12) Affix the vaccine labels in the subject's source documents
13) Keep the subject under observation for 30 minutes, and record any immediate AE reaction in the source document
14) Give the subject the first DC (DC1) to record any injection site reactions and systemic AEs, together with instructions for its completion, including explanations on the definition and use of intensity scales for collection of AEs
15) Give the subject a ruler to measure the size of any injection site reaction and a thermometer for temperature measurement, and instructions on how to use them
16) Remind the subject to call the study center if a serious medical event occurs
17) Arrange an appointment for the next visit (V02; at V01 + 7D [+ 3 day]).
18) Complete the relevant CRF pages for this Visit.

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

Note: The Screening Visit and Visit 1 can be simultaneous for subjects tested positive by RDT and whose tests have been performed in appropriate time windows (within 4 weeks before injection for hepatitis serology, hematology, and biochemistry; within 2 months for CD4 count and HIV viral load) as part of the subject's routine follow-up.

Visit 2 (Inj 1+7D [+3 days]): Collection of Safety Information and Blood Sample

1) Check the information entered into DC1 by interviewing the subject and request information concerning any medical event, serious, or not, that may have occurred since Visit 1
2) Record any injection site reactions or systemic AEs and any reportable concomitant medications
3) Perform a physical examination and record the subject's axillary temperature (if necessary)
4) Obtain a blood sample (see Section 7.1 for detailed instructions regarding the handling of blood samples), record the date of collection

It is important to note that, if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt within the Visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.

5) Remind the subject to call the study center if a serious medical event occurs.
6) Arrange an appointment for the next visit (V03; at V01 + 14D [+ 3 days]).
7) Complete the relevant CRF pages for this visit.

Visit 3 (Inj 1+14D [+3 days]): Collection of Safety Information and Blood Sample

1) Check the information entered into DC1 by interviewing the subject and request information concerning any medical event, serious, or not, that may have occurred since Visit 2
2) Record any AEs and any reportable concomitant medications
3) Perform a physical examination and record the subject's axillary temperature (if necessary)
4) Obtain a blood sample (see Section 7.1 for detailed instructions regarding the handling of blood samples), record the date of collection

It is important to note that, if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt within the Visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.

5) Remind the subject to call the study center if a serious medical event occurs.
6) Arrange an appointment for the next visit (V04; at V01 + 28D [+14 days]).
7) Complete the relevant CRF pages for this visit.

Visit 4 (Inj 1+28D [+14 days]): Collection of Safety Information and Blood Sample

1) Collect DC1 and check the information entered into it by interviewing the subject and request information concerning any medical event, serious, or not, that may have occurred since Visit 3
2) Record any AEs and any reportable concomitant medications
3) Perform a physical examination and record the subject's axillary temperature (if necessary)
4) Obtain a blood sample (see Section 7.1 for detailed instructions regarding the handling of blood samples), record the date of collection

It is important to note that, if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt within the Visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.

5) Remind the subject to call the study center if a serious medical event occurs
6) Provide the subject with DC2 and remind her/him to bring it back when she/he returns for Visit 5
7) Arrange an appointment for the next visit (V05; at V01 + 6 months (M6) [±20 days])
8) Complete the relevant CRF pages for this visit

Visit 5 (Inj 2 [M6; ±20 days]): Collection of safety information and vaccination

1) Collect DC2 and check the information entered into it by interviewing the subject and request information concerning any medical event, serious, or not, that may have occurred since last Visit

2) Record any reportable concomitant medications
3) Perform a physical examination and record the subject's axillary temperature before injection
4) Check any contraindication to vaccination
5) Check for confirmation of previous exposure to dengue (from PRNT laboratory results)
6) Perform a urine pregnancy test for all women enrolled in the study
7) The person in charge of injections ("vaccinator") or authorized designee contacts the IRT to obtain the assigned product to be administered
8) Inject the appropriate investigational product
9) Record the date of injection, the site, and side of injection and the route of administration, as well as the dose number of the vaccine
10) Affix the vaccine labels in the subject's source documents
11) Keep the subject under observation for 30 minutes, and record any immediate AE in the source document
12) Give the subject the DC3 to record any injection site reactions and systemic AEs, together with instructions for its completion, including explanations on the definition and use of intensity scales for collection of AEs
13) Ensure subject still has the ruler to measure the size of any injection site reaction and the thermometer for temperature measurement
14) Remind the subject to call the study center if a serious medical event occurs
15) Arrange an appointment for the next visit (V06; at V05 + 28D [+ 14 days]). Remind the subject that she/he will be contacted twice by phone (PC1; at V05+ 7D [+3 days]; PC2; at V05 + 14D [+3 days]) by study staff.
16) Complete the relevant CRF pages for this visit.

Phone Call 1 (Inj 2+7D [+3 days]): Safety Follow-up Phone Call

Note: If PC1 falls on a weekend or a holiday, the telephone call may be made on the following business day.

1) Record relevant information concerning the subject's health status on the source document. If a SAE occurred follow the instructions in Section 10 for reporting it.
2) Remind the subject to call the study center if a serious medical event occurs.
3) Remind the subject to record any AEs in the DC3.
4) Remind the subject of the appointment for the next visit (V06; at V05 + 28D [+14 days]).

Note: A follow-up visit can be arranged depending on the information provided during the phone call, at the Investigator's discretion.

Phone Call 2 (Inj 2+14D [+3 days]): Safety Follow-up Phone Call

Note: If PC2 falls on a weekend or a holiday, the telephone call may be made on the following business day.

1) Record relevant information concerning the subject's health status on the source document. If a SAE occurred follow the instructions in Section 10 for reporting it.
2) Remind the subject to call the study center if a serious medical event occurs.
3) Remind the subject to record any AEs in the DC3, and to bring it back when she/he returns for Visit 6.
4) Remind the subject of the appointment for the next Visit (V06; at V05 + 28D [+14 days]).

Note: A follow-up visit can be arranged depending on the information provided during the phone call, at the Investigator's discretion.

Visit 6 (Inj 2+28D [+14 days]): Collection of Safety Information and Blood Sample

1) Collect DC3 and check the information entered into it by interviewing the subject and request information concerning any medical event, serious, or not, that may have occurred since Visit 5
2) Record any AEs and any reportable concomitant medications
3) Perform a physical examination and record the subject's axillary temperature (if necessary)
4) Remind the subject to call the study center if a serious medical event occurs
5) Obtain a blood sample (see Section 7.1 for detailed instructions regarding the handling of blood samples), record the date of collection

It is important to note that, if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt within the Visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.

6) Provide the subject with DC4 and remind her/him to bring it back when she/he returns for Visit 7
7) Arrange an appointment for the next visit (V07; at V01 + 12 months (M12) [±20 days])
8) Complete the relevant CRF pages for this visit

Visit 7 (Inj 3 [M12; ±20 days]): Collection of safety information and vaccination

Same procedure as for Visit 5. Subject is provided with DC5.

Phone Call 3 (Inj 3+7D [+3 days]): Safety Follow-up Phone Call

Same procedures as for Phone Call 1.

Phone Call 4 (Inj 3+14D [+3 days]): Safety Follow-up Phone Call

Same procedures as for Phone Call 2.

Visit 8 (Inj 3+28D [+14 days]): Collection of Safety Information and Blood Sample

1) Collect DC5 and check the information entered into it by interviewing the subject and request information concerning any medical event, serious, or not, that may have occurred since Visit 7
2) Record any AEs and any reportable concomitant medications
3) Perform a physical examination and record the subject's axillary temperature (if necessary)
4) Obtain a blood sample (see Section 7.1 for detailed instructions regarding the handling of blood samples), record the date of collection

It is important to note that, if the attempt(s) to collect blood is (are) unsuccessful, the subject should be given the opportunity for another attempt within the Visit window. If a blood sample cannot be obtained, the reason will be recorded in the blood sampling page of the CRF.

5) Remind the subject to call the study center if a serious medical event occurs
6) Provide the subject with a MA
7) Remind the subject about the 6 months safety follow-up phone call (at M12 + 6 months [+30 days]).
8) Complete the relevant CRF pages for this visit and the End of Study CRF.

Safety Follow-up Telephone Call (6 months after last injection [+30 days]): Collection of SAEs

1) Ask the subject if she/he has experienced any SAE since the last injection. If an SAE occurred, follow the instructions in Section 10 for reporting it.
2) Complete the relevant case report book (CRB) form.

Note: A follow-up visit can be arranged depending on the information provided during the phone call.

Follow-up of subjects with Related AEs or with AEs That Led to Study/Vaccination Discontinuation:

A subject who experiences an AE (whether serious or non-serious) during the study must be followed until the condition resolves, becomes stable, or becomes chronic (even after the end of the subject's participation in the study) if *either* of the following is true:

- The AE is considered by the Investigator to be related to the product administered
- The AE caused the discontinuation of the subject from the study or from vaccination

5.1.5 Planned Study Calendar

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

Planned study period – FVFS (first Visit, first subject) to LCLS (last contact, last subject): November 2019 to March 2022

Planned inclusion period – FVFS to FVLS (first visit, last subject): November 2019 to September 2020

Planned vaccination period: November 2019 to September 2021

Planned end of study: March 2022

Planned date of final clinical study report: Q4 2022

5.1.6 Early Safety Data Review

The safety of the CYD dengue vaccine will be continuously monitored by the Sponsor. In the context of this study (ie, HIV-positive adults), a blinded ESDR will be performed, the goal of which is to allow for a cautious, stepwise approach to vaccine administration. An initial safety review for this study is planned when the first 20 subjects have received the first injection and have provided safety data for Days 0-14 post-injection 1. During the ESDR, study recruitment (and second injection for first 20 subjects, in case the safety review has not been completed at the time of second injection) will be paused.

The safety data collected will be entered into the CRF, and will be summarized, and reviewed by the Sponsor. The SET will review the blinded and unclean data and prepare a recommendation that will be reviewed by the IDMC. The IDMC will also review the safety data and then provide recommendation as to whether vaccination of the first enrollees and the enrollment of remaining subjects should resume or not. The ESDR will be assessing the following safety parameters:

- Immediate (within 30 minutes) AEs
- Solicited injection site and systemic reactions
- Unsolicited AEs reported as related by the Investigator within 14 days after the first injection
- SAEs and AESIs (including serious and non-serious AESIs)

Case unblinding may be performed if required. Moreover, some unblinded analyses may be provided by an independent Sanofi Pasteur statistician (ie, not involved either in the study or in the Dengue project) upon IDMC request.

The decision regarding the continuation of vaccination of the 20 first enrollees and the enrollment of the remaining 130 subjects will be communicated to the Investigators and will be sent to the involved IECs/IRBs and to the IDMC for information.

5.2 Enrollment and Retention of Study Population

5.2.1 Recruitment Procedures

Before the start of the study, Investigators and/or study staff will prepare a pre-screening data log listing eligible participants identified at least 4 weeks before the site begins recruitment. Using the most appropriate methods they will contact a pool of potential subjects and invite them to

participate in the study. Each site will ensure that all advertisements or materials planned to be used to recruit subjects (eg, letters, pamphlets, posters) are submitted to Sanofi Pasteur for review prior to submission to the IEC/IRB for approval.

Enrollment will begin once the first site enrolls the first subject into CYD50. A review of the enrollment at each site will be made by the Sponsor on a monthly basis with the aim of identifying potential issues with the completion of enrollment within the planned timelines. Should the Sponsor consider that a site is at risk of not meeting its expected target enrollment (ie, 50 subjects), this will be discussed with the site. If, after this discussion, there remains a risk to completion, Sanofi Pasteur reserves the right to re-allocate subject recruitment target from this site to another site.

5.2.2 Informed Consent Procedures

Informed consent is the process by which a subject voluntarily confirms his or her willingness to participate in a particular study. Informed consent must be obtained before any study procedures are performed. The process is documented by means of a written, signed, and dated ICF.

In accordance with GCP, prior to signing, and dating the consent form, the subject must be informed by appropriate study personnel about all aspects of the study that are relevant to making the decision to participate, and must have sufficient time and opportunity to ask any questions.

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

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

If new information becomes available that may be relevant to the subject's willingness to continue participation in the study, this will be communicated to him / her in a timely manner. Such information will be provided via a revised ICF or an addendum to the original ICF.

ICFs will be provided in duplicate of the signed consent will be made. The original will be kept by the Investigator, and the copy will be kept by the subject.

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

5.2.3 Screening Criteria

The Screening Visit will take place within 4 weeks before the first injection. After a potential participant has signed an IEC/IRB-approved ICF, she/he will have a blood sample drawn in order to be screened for previous exposure to dengue, HBsAg, HCV antibodies[a], and for any hematological (hemoglobin, hematocrit, platelet count, white blood cells count) or biochemical (aspartate aminotransferase, alanine aminotransferase, urea, and creatinine) abnormalities. If

[a] Unless documented information on previous infections is already available at the time of screening.

serology, hematology, and biochemistry results are available and were obtained within 4 weeks before first injection, tests do not need to be performed again.

Pre-injection HIV viral load and CD4 count will come from routine periodical tests of subjects as per local guidelines. For subjects for whom HIV viral load and CD4 count have not been assessed within 2 months before the Screening Visit, the Investigator will perform tests to ensure that results are available at the time of Visit 1.

Subjects' previous exposure to dengue will be assessed using a RDT (*OnSite*™ Dengue IgG/IgM3.0 Combo Rapid Test commercialized by CTK Biotech, Inc.) or an ELISA (Anti-Dengue IgG ELISA commercialized by Euroimmun) if the RDT result is negative. Only subjects identified as dengue seropositive will be eligible to participate in the study.

It is to be noted that subjects who previously tested negative by RDT for previous exposure to dengue can be re-screened using IgG ELISA.

During the Screening Visit, applicable inclusion and exclusion criteria will be checked and a physical / clinical examination will be performed.

5.2.4 Inclusion Criteria

Consent will be provided once at the Screening Visit. Inclusion criteria will be checked at Screening Visit and again at the first vaccination visit (V01 at D0).

An individual must fulfill all of the following criteria to be eligible for study enrollment:

1) Aged 18 to 50 years on the day of first study vaccination (study product administration) ("18 to 50" means from the day of the 18th birthday to the day before the 51th birthday)
2) ICF has been signed and dated
3) Able to attend all scheduled visits and to comply with all trial procedures
4) Documented seropositivity for HIV-1 infection based on the Brazilian HIV Guidelines' laboratory criteria (ie, 2 positive results obtained from different and independent determination methods) or detectable HIV-1 viral load results in the past
5) Stable HIV condition according to Brazilian HIV Guidelines (ie, with both CD4 count > 350 cells/mm3 and sustainable and undetectable HIV viral load [< 50 copies/mL]) for at least 1 year before consent
6) Stable ART regimen based on local HIV protocol for at least 1 year before consent
7) Previous exposure to dengue confirmed by RDT or dengue IgG ELISA

5.2.5 Exclusion Criteria

Exclusion criteria will be checked at Screening Visit and again at the first vaccination visit (V01 at D0). An individual fulfilling *any* of the following criteria is to be excluded from study enrollment:

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

1) Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be pre-menarche or post-menopausal for at least 1 year,

surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination until at least 4 weeks after the last vaccination).

2) Participation at the time of study enrollment (or in the 4 weeks preceding the first trial vaccination) or planned participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure.
3) Receipt of any vaccine in the 4 weeks preceding the first trial vaccination or planned receipt of any vaccine in the 4 weeks following any trial vaccination.
4) Previous vaccination against dengue disease with either the trial vaccine or another vaccine.
5) Receipt of immune globulins, blood or blood-derived products in the past 3 months.
6) Self-reported or suspected congenital or acquired immunodeficiency, except HIV; or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months).
7) Previous AIDS, defined as the occurrence of opportunistic infection in the last 2 years before consent.
8) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccines used in the trial or to a vaccine containing any of the same substances.
9) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily.
10) Current alcohol abuse or drug addiction.
11) Chronic illness that, in the opinion of the Investigator, is at a stage where it might interfere with trial conduct or completion.
12) Moderate or severe acute illness/infection (according to Investigator judgment) or febrile illness (temperature ≥ 38.0°C) on the day of vaccination. A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided.
13) Identified as an Investigator or employee of the Investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (ie, parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study.
14) Previous CD4 count < 200 cells/mm^3 (nadir) since diagnosis of HIV
15) History of chronic and active hepatitis B infection or HBsAg-positive
16) History of chronic and active hepatitis C infection or HCV Ab-positive
17) Aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine > 3 times the upper limit of normal range (ULN)
18) Hemoglobin (Hb) < 10 g/dL
19) White blood cell count (WBC) < 1500 cells/mm^3

20) Platelets < 100 000 cells/mm^3

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

5.2.6 Medical History

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

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

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

The reporting of signs and symptoms in lieu of a diagnosis is strongly discouraged.

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

5.2.7 Contraindications for Subsequent Vaccinations

The CYD dengue vaccine should not be administered to seronegative persons.

5.2.7.1 Temporary Contraindications

Should a subject experience one of the conditions listed below, the Investigator will postpone further vaccination until the condition is resolved. Postponement must still be within the timeframe for vaccination indicated in the Table of Study Procedures.

- Moderate or severe acute illness/infection (according to Investigator judgment) or febrile illness (temperature ≥ 38.0°C) or any clinical condition on the day of vaccination, that according to the Investigator's judgment could increase the risk of the subject in case of a new injection (this includes abnormal biological parameters [ie, hematic biometry, biochemistry] performed as per routine management of HIV assessment prior to injection). A subject should not receive a further injection until the condition has resolved or the febrile event has subsided.
- Receipt of any vaccine within the 4 weeks preceding vaccination.
- CD4 count pre-injection ≤ 350 cells/mm^3 and/or detectable HIV viral load (≥50 copies/mL) based on a second confirmatory test 4 weeks later. A subject should not receive a further

vaccination until the CD4 count is > 350 cells/mm3 and the HIV viral load is undetectable (<50 copies/mL). Time windows for the next vaccination should be respected.

5.2.7.2 Definitive Contraindications

Should a subject experience 1 of the conditions listed below, the Investigator will discontinue vaccination:

1) Pregnancy, as indicated by a positive urine test
2) Dengvaxia® administered inadvertently during the study (eg, as part of a vaccination campaign)
3) An anaphylactic or other significant allergic reaction to the previous dose of vaccine
4) Abnormal laboratory parameter of Grade 2 or 3 and assessed by the Investigator as related to the previous dose of vaccine
5) Subject deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized without her/his consent
6) Self-reported or suspected congenital or acquired immunodeficiency, except HIV, or receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months, or long-term systemic corticosteroids therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months)
7) Ongoing clinical AE related to the previous study vaccination, and in the Investigator's opinion, contraindicating further vaccination
8) SAE related to the study vaccine following the previous study vaccination
9) Confirmed CD4 count < 200 cells/mm^3

Subjects will not be withdrawn due to contraindication but will be followed-up for safety. Subjects will not be replaced.

If a female subject who has already received at least one injection becomes pregnant during the study, she will continue to be followed for safety assessments until delivery or until 6 months after the last injection, whichever is the latest. No additional injections will be administered.

Subjects with a definitive contraindication will continue to be followed-up for the study-defined safety and immunogenicity assessments, as applicable.

5.2.8 Conditions for Withdrawal

Subjects will be informed that they have the right to withdraw from the study at any time.

For example, a subject may be withdrawn from the study:

- At the discretion of the Investigator or Sponsor due to safety concerns (withdrawal) or to significant non-compliance with the protocol (based on the Investigator’s judgment), without the subject’s permission
- At the request of the subject (dropout)

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

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

Withdrawn subjects will not be replaced.

5.2.9 Lost to Follow-up Procedures

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

5.2.10 Classification of Subjects Who Discontinue the Study

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

Serious Adverse Event	To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an SAE, as defined in Section 9.1.1.1
Adverse Event	To be used when the subject is permanently terminated from the study because of an AE (including an SAE), as defined in Section 9.1.1.1. This category also applies if the subject experiences a definitive contraindication that is an SAE or AE.
Non Compliance with Protocol	To be used when the Investigator withdraws a subject from the study because of failure to follow the protocol, including when it is retrospectively discovered that a subject did not fulfill the eligibility criteria. The Investigator will provide a comment as to the specific cause of non-compliance.
Lost to Follow-up	To be used when the subject cannot be found or contacted in spite of efforts to locate him/her before the date of his/her planned last visit, as outlined in Section 5.2.9. The certified letter was sent by the Investigator and returned unsigned, and the subject or parent/guardian did not give any other news and did not come to any following visit.
Protocol Deviation	To be used: • In case of significant non-compliance with the protocol (eg, deviation of the Inclusion / Exclusion criteria, non-compliance with time windows, blood sampling, or vaccination refusal, missed injection/treatment, or error in the vaccine/treatment administration) • If the subject experiences a definitive contraindication that is not an SAE or AE • The subject or the parent/guardian signed the certified letter sent by the Investigator but did not give any other news and did not come to any following visit

Withdrawal by Subject	To be used: • When the subject or parent/guardian indicated unwillingness to continue in the study • When the subject or parent/guardian made the decision to discontinue participation in the study for any personal reason other than an SAE/AE (eg, subject is relocating, inform consent withdrawal, etc.)

5.2.11 Follow-up of Discontinuations

The site should complete all scheduled safety follow-ups and contact any subject who has prematurely terminated the study because of an AE or a protocol deviation.

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

If the subject's status at the end of the study is "Withdrawal by Subject", the site will attempt to contact them for the 6-month follow-up except if they specified that they do not want to be contacted again and it is documented in the source document.

5.2.12 Follow-up and Reporting of Pregnancies

Pregnancy is an exclusion criterion for enrollment in this study, but a subject could potentially become pregnant during her participation. In case of pregnancy during the study and if at least 1 dose of the study vaccine(s) has been administered, the subject will not be discontinued from the study, but no further vaccination will be administered until after delivery (if applicable and still within the study vaccination window). However, the subject will be followed for safety assessment (and may be followed for immunogenicity assessment, if applicable).

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

If the EDC system is not available, the Investigator must fill out a paper Pregnancy Reporting Form (provided by the Sponsor at the start of the study) and inform the Sponsor within 1 month of identifying a pregnancy case.

Study staff must then maintain contact with the subject to obtain information about the outcome (ie, details about the delivery and the newborn, or about pregnancy termination) and must update the Pregnancy Reporting forms even after the end of the study. This information should be provided to the Sponsor within 1 month of delivery.

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

5.3 Safety Emergency Call

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

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

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

5.4 Modification of the Study and Protocol

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

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

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

5.5 Interruption of the Study

The detection/fulfillment of the following safety signals will put the study on a temporary hold:

- With regard to investigational products:
    - **15% of the total subjects included in the study** experience a Grade 3 solicited systemic reaction occurring within 14 days after any study injection and persisting for at least 48 hours and judged as related to the vaccination by the Investigator and not explained by any other possible etiology.

    and/or

    - 3 SAE (including serious AESI) considered as related to the vaccination by the Investigator or the Sponsor, except for the SAEs corresponding to the HIV condition included below.
- With regard to HIV condition:

- **In 20% of the total subjects included in the study:** Confirmed plasma HIV-1 RNA > 1000 copies/mL 28 days post-injection after having been undetectable (< 50 copies/mL) pre-injection, not explained by non-adherence to ART and not explained by any other possible etiology.

or

- **In 10% of the total subjects included in the study:** Confirmed decrease in CD4 count greater than 30% assessed 28 days post-injection compared to the pre-injection value, not explained by non-adherence to ART and not explained by any other possible etiology.

It is to be noted that any case of an increase in HIV-1 RNA > 1000 copies/mL or decrease in CD4 count greater than 30% observed 28 days post-injection, not explained by non-adherence to ART and not explained by any other possible etiology, will be considered as an SAE. This increase in HIV viral load or decrease in CD4 count will be considered as "confirmed" if a second test taken 4 weeks after the first (ie, approximately 2 months post-injection) shows the same value/trend. The IDMC will be immediately notified if either or both criteria occur in any of the subjects enrolled in the study.

The IDMC will also be immediately notified when the percentage of subjects defined in one of the 4 safety signals described above has been reached. The IDMC will then assess and recommend in an expedited way whether study enrollment and injections can continue, and/or whether other actions should be completed. In this scenario, additional biological tests may be required to assess the evolution of the subjects and to decide on the study continuation.

Apart from the temporary hold following the enrollment of the first 20 subjects (see Section 5.1.6), and apart from a possible temporary hold following the detection of a safety signal, the study may be discontinued if new data about the investigational product resulting from this or any other studies become available; or for administrative reasons; or on advice of the Sponsor, the Investigators, the regulatory authorities in Brazil, and/or the IECs/IRBs.

If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the regulatory authorities, and the IECs/IRBs of the reason for termination or suspension, as specified by the applicable regulatory requirements.

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

6 Vaccines Administered

6.1 Identity of the Investigational Products

Subjects will receive either 3 doses of the trial product, CYD dengue vaccine, or 3 doses of placebo as control product.

These products will be administered on D0, D0 + 6 months, and D0 + 12 months. This study will be using the monodose presentation of the CYD dengue vaccine.

6.1.1 Identity of Study Products

Product:	CYD Dengue Vaccine
Vaccine:	Live, attenuated, tetravalent dengue virus vaccine
Presentation:	Monodose
Form:	powder and solvent for suspension for injection
Dose volume:	0.5 milliliters (mL) of the reconstituted vaccine
Route:	Subcutaneous (SC) injection
Batch number:	To be defined

6.1.1.1 Composition

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

- **Active Ingredients:** 4.5–6 $\log_{10}$ cell-culture infectious dose 50% ($CCID_{50}$) of each live, attenuated, recombinant, dengue virus serotype 1, 2, 3, 4
- **Excipients**: essential amino acids, non-essential amino acids, L-arginine chlorhydrate, saccharose, D-trehalose dihydrate, D-sorbitol, Tris (hydroxymethyl) aminomethane, and urea
- **Solvent:** NaCl 0.4%

6.1.1.2 Preparation and Administration

Sanofi Pasteur's CYD dengue vaccine consists of a powder and solvent for suspension for injection and must be stored between +2°C and +8°C (ie, in a refrigerator) and protected from light.

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

The vaccine is to be administered subcutaneously in the deltoid region of the upper arm in a volume of 0.5 mL.

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

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

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

6.1.1.3 Dose Selection and Timing

The CYD dengue vaccine will be administered in 3 doses with a 6-month interval between each dose as per the indication obtained for the vaccine licensed in Brazil.

6.1.2 Identity of Control Product

6.1.2.1 Composition

Product:	Placebo
Form:	Solution
Vaccine:	NaCl 0.9%
Route:	SC
Batch number:	To be determined

6.1.2.2 Preparation and Administration

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

The placebo must be removed from refrigerator. The placebo should not be used if particles are present in the solution. The placebo will be administered subcutaneously in the deltoid region of the upper arm in a volume of 0.5 mL.

6.1.2.3 Dose Selection and Timing

Not applicable.

6.2 Identity of Other Product

Not applicable.

6.3 Product Logistics

6.3.1 Labeling and Packaging

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

- Study code
- Name of product and Group assignment
- Route of injection
- Investigational use only statement
- Storage conditions
- Batch number
- Name of Sponsor

- Expiry date

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

6.3.2 Product Shipment, Storage, and Accountability

6.3.2.1 Product Shipment

The Clinical Logistics Coordinator or designee will contact the Investigator or a designee to determine the dates and times of delivery of products.

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

6.3.2.2 Product Storage

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

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

6.3.2.3 Product Accountability

The person in charge of product management at the site will maintain records of product delivery to the study site, product inventory at the site, the dose(s) given to each subject, and the disposal of, or return to the Sponsor of unused doses.

The necessary information on the product labels is to be entered into the source document and the CRB. If applicable, information may also be entered into the subject's vaccination card.

The Sponsor's monitoring staff will verify the study site's product accountability records against the record of administered doses in the CRBs and the communication from the IRT (if applicable).

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

6.3.3 Replacement Doses

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

6.3.4 Disposal of Unused Products

Unused or wasted products will be either disposed of or returned to the Sponsor in accordance with the instructions in the Operating Guidelines. Product accountability will be verified throughout the study period.

6.3.5 Recall of Products

If the Sponsor makes a decision to launch a retrieval procedure, the Investigators will be informed of what needs to be done.

6.4 Blinding and Code-breaking Procedures

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

The code may be broken in the event of an AE only when the identification of the vaccine received could influence the treatment of the subject. Code-breaking should be limited to the subject(s) experiencing the AE.

The blind can be broken by the Investigator or a delegate through the IRT system, as explained in the code-breaking procedures described in the Operating Guidelines. Once the emergency has been addressed by the site, the Investigator, or a delegate must notify the Sanofi Pasteur RMO if a subject's code was broken. All contact attempts with the Sponsor prior to unblinding are to be documented in the source documents, and the code-breaking CRF is to be completed.

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

- by the GPV Department through an internal system for reporting to health authorities in the case of an SAE as described in International Conference on Harmonisation (ICH) E2A. In this case, the code will be broken only for the subject(s) in question. The information resulting from code-breaking (ie, the subject's vaccine or Group assignment) will not be communicated to either the Investigator or the immediate team working on the study, except for the GPV representative.
- by the IDMC if needed to facilitate their assessment of safety.

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

Although the ESDR performed when the 20 first subjects have received the first injection does not require the unblinding of data, unblinding can be performed at this review, if required. Moreover, some unblinded analyses may be provided by an independent Sanofi Pasteur statistician (ie, not involved either in the study or in the Dengue project) upon IDMC request.

A planned analysis will be performed on data collected up to 28 days post-Inj 3 of the last subject enrolled. This analysis will require the unblinding of data.

A second and final analysis will be performed at the end of the study.

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

6.5 Randomization and Allocation Procedures

Each eligible subject (ie, meets inclusion criteria, and does not fulfill any exclusion criteria) will be randomly assigned to 1 of the 2 groups via an IRT, according to a 2:1 ratio (2 subjects included in the CYD Dengue Vaccine Group for 1 subject included in the control Group). Site staff will connect to the IRT, enter identification and security information, and confirm a minimal amount of data in response to IRT prompts. The IRT will then state the vaccine assignment (code number). Subject numbers will be recorded in the CRB and will not be reassigned for any reason. The full detailed procedures for randomization are described in the Operating Guidelines.

Subject numbers that are assigned by the IRT system will consist of a 12-digit string (a 3-digit country code, a 4 digit study center identifier, and a 5-digit subject identifier connected by "-"). For example, Subject 0760001-00001 is the first subject enrolled in center number 1 in Brazil (076 being Brazil country code). Subject numbers should not be reassigned for any reason. The randomization codes will be kept securely in the IRT.

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

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

The R&D Site Quality Operations Department at Sanofi Pasteur will hold the randomization codes in a secured location.

Notes:

- Subjects who previously tested negative by RDT for previous exposure to dengue can be re-screened using IgG ELISA. Subjects who are re-screened will be allocated a new subject number.
- If a subject is eligible but not randomized during the ESDR hold, the subject will be asked to come back to the site after the hold. The subject will then be re-screened and randomized.

However, the subject will not be re-tested for previous exposure to dengue by RDT or IgG ELISA. The subject will be allocated a new subject number.

6.6 Treatment Compliance

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

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

6.7 Concomitant Medications and Other Therapies

At the time of enrollment, ongoing medications and other therapies (eg, blood products) should be recorded in the source document as well as new medications prescribed for new medical conditions / AEs during study participation.

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

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

Reportable medications include medications that impact or may impact the consistency of the safety information collected after any vaccination and/or the immune response to vaccination. Three standard categories of reportable medications are defined:

- Category 1: medications impacting or that may have an impact on the evaluation of the safety (eg, antipyretics, analgesics, and non-steroidal anti-inflammatory drugs [NSAIDs], steroids/corticosteroids). *Note: inhaled and topical steroids should not be captured.*
- Category 2: medications impacting or that may have an impact on the immune response.
    - Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy or long-term systemic corticosteroids (for more than 2 consecutive weeks) in the 4 weeks after study injection
    - Immune globulins, blood or blood-derived products in the 3 months before the first study injection, in the 4 weeks before second and third injection, and in the 4 weeks after any injection
    - Any vaccine (other than the study vaccine) in the 4 weeks before second and third injection, and in the 4 weeks after any injection
- Category 3: medications impacting or that may have an impact on both the safety and the immune response (eg, steroids/corticosteroids)

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

- Trade name.
- Origin of prescription: prophylaxis Yes/No. Medication(s) prescribed for AE prophylaxis will be recorded in the Action Taken of the AE collection tables.
- Medication category (1, 2, or 3).
- Start and stop dates.

Dosage and administration route, homeopathic medication, topical, and inhaled steroids, as well as topical, ophthalmic, and ear treatments will not be recorded. Topical analgesics should not be applied at the site of vaccination; however, if they are applied inadvertently to the vaccination site, they should be recorded as a Category 1 medication in this specific instance.

Medications given in response to an AE will be captured in the "Action Taken" section of the AE CRF only. No details will be recorded in the concomitant medication CRF unless the medication(s) received belongs to one of the pre-listed categories. Medications will not be coded.

7 Management of Samples

Blood samples for the assessment of:

- Screening characteristics will be collected at Screening Visit (if results not already available from routine periodical tests)
- Dengue, YF, and Zika baseline status will be collected at Visit 1
- Dengue neutralizing Abs, CD4 count, and HIV viral load will be collected at Visits 4, 6, and 8
- Dengue vaccinal viremia will be collected at Visits 2 and 3

See the Table of Study Procedures and Section 5.1 for details of the sampling schedule.

7.1 Sample Collection

7.1.1 Blood Samples

All the following blood samples will be collected in tubes provided by or recommended by the Sponsor. Immediately prior to the blood draw, the staff member performing the procedure will verify the subject's identity; will write the assigned subject's number on the pre-printed label that contains that subject's number and the sampling stage; and will attach the label to the tube. When vaccination and blood sample collection occur at the same Visit and vaccine is given only in one of the arms, blood is to be taken from the limb opposite to the one that will be used for vaccination.

7.1.1.1 Blood Sample for Screening Characteristics Assessment

Approximately 14 mL (according to local laboratories) of blood will be collected from potential subjects in order to perform laboratory tests, including assessment of previous exposure to dengue, HBsAg, HCV Ab, hematology (hemoglobin, hematocrit, platelet count, white blood cells

count), biochemistry (AST, ALT, urea, and creatinine)[a]. For subjects for whom HIV viral load and CD4 count have not been assessed within 2 months before the Screening Visit, an additional volume of blood (10 mL per test) will be required before the Screening Visit to perform the tests. The screening analyses will be performed at local laboratories. For subjects for whom these tests have been performed in appropriate time windows (within 4 weeks before injection for hepatitis serology, hematology, and biochemistry; within 2 months for HIV viral load and CD4 count) as part of the subject's routine follow-up, no new tests will be required.

Also, subject' previous exposure to dengue will be assessed using a RDT (OnSite™ Dengue IgG/IgM3.0 Combo Rapid Test commercialized by CTK Biotech, Inc.) or ELISA (Anti-Dengue IgG ELISA commercialized by Euroimmun).

7.1.1.2 Blood Sample for Dengue, YF, and Zika Serostatus, Dengue Neutralizing Antibodies, CD4 Count, and HIV Viral Load Assessments

At V01 (before first vaccination injection) for all subjects, 9 mL of blood will be collected in order to assess dengue, YF, and Zika status, as well as baseline dengue neutralizing Ab levels (PRNT).

At V04, V06, and V08 for all subjects, 24 mL of blood will be collected in order to assess dengue neutralizing Ab levels, CD4 count, and HIV viral load, 28 days post-injection. See Table 5.1 for blood samples volumes for each laboratory test.

In addition, for subjects for whom HIV viral load and CD4 count have not been assessed within 2 months before Inj 2 (Visit 5) or Inj 3 (Visit 7), an additional volume of blood (10 mL per test) will be required before injection visits to perform the tests and check the contraindication to further vaccination. The tests will be performed at local laboratories.

7.1.1.3 Blood Sample for Dengue Vaccinal Viremia

At V02 and V03 for all subjects, 4 ml of blood will be collected in order to evaluate post-Inj 1 vaccinal viremia.

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

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

Additionally, and for all hospitalized suspected dengue cases, the Investigator must ensure that key biological parameters (hematocrit, platelet count, AST, and ALT) have been checked or are

[a] HBsAg and HCV tests will not be performed in case documented information is already available in subject's medical history

planned to be checked as part of local standard of care at the hospital (ideally within the 5 days after the fever onset). If these parameters have not been measured, additional blood specimens will be taken[a]. The aim of these tests is the assessment of severity according to the WHO/IDMC classification.

Table 7.1 presents the additional serum aliquots in the event of a suspected hospitalized dengue disease at any time during the study.

Table 7.1: Blood Sampling Volume (mL) for Suspected Hospitalized Dengue Case

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

*The volume of blood samples will depend on local laboratory needs.

More detailed instructions are provided in the Operating Guidelines.

7.1.3 Urine Samples for Pregnancy Test

A minimum of 6 mL of first-stream urine will be collected directly in 2 sterile tubes before any vaccination (ie, at V01, V05, and V07). Labeling procedures will be done the same way as for blood samples (see Section 7.1.1).

7.2 Sample Preparation

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

7.2.1 Blood Sample Preparation

7.2.1.1 Screening Characteristics Assessment

The preparation of the blood samples for the assessment of screening characteristics (ie, serology, hematology, biochemistry) will be done according to local laboratories. The determination of previous dengue exposure will be done according to the RDT and ELISA Manufacturer's instructions.

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

7.2.1.2 CD4 Count and and HIV-1 Viral Load

The preparation of the blood samples for the CD4 count and for the HIV viral load assessment will be done according to local laboratories.

7.2.1.3 Dengue, YF, and Zika Serostatus at Baseline; Dengue Neutralizing Abs, Vaccinal Viremia, and Wild-Type Dengue Viremia

Following the blood draw, the sampling tube should be stored at room temperature for a minimum of 60 minutes and a maximum of 2 hours to allow the blood to clot before centrifugation. The tube must be stored vertically and will not be shaken. Beyond 2 hours, the sampling tube must be refrigerated at a temperature of 2°C to 8°C and must be centrifuged within a maximum of 24 hours.

After being allowed to clot, blood samples for serum Ab response, vaccinal viremia, and WT dengue viremia assessments will be centrifuged before being divided into appropriate aliquots of serum. Samples will then be handled one subject at a time to avoid a mix-up of subjects' blood tubes. Serum will be transferred to the appropriate number of tubes, pre-labeled with adhesive labels that clearly identify the subject's number and sampling stage or Visit number.

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

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

7.2.2 Urine Samples

Urine samples for pregnancy tests at the vaccination visits will be taken and analyzed at the study center.

7.3 Sample Storage and Shipment

During storage, serum tubes are to be kept in a freezer whose temperature is set and maintained at -70°C or below. The temperature will be monitored and documented on the appropriate form during the entire study. If it rises above -10°C for any period of time, the Clinical Logistics Coordinator must be notified. See the Operating Guidelines for further details.

Shipments to the laboratories will be made only after appropriate monitoring, and following notification of the Clinical Logistics Coordinator. Sera will be shipped frozen, using dry ice to maintain them in a frozen state, in the packaging container provided by the carrier. Again, temperatures will be monitored. Shipments must be compliant with the United Nations Class 6.2 specifications and the International Air Transport Association (IATA) 602 packaging instructions.

Samples will be shipped to GCI at Sanofi Pasteur. The address is provided in the Operating Guidelines.

7.4 Future Use of Stored Biological Samples for Research

Any unused part of the serum samples may be used for additional tests related to dengue immune response that may not be available at the writing of this protocol. These serum samples will be

securely stored at the Sanofi Pasteur serology laboratory (GCI) for up to 10 years. Storage for a longer period is possible but will require approbation from Brazilian Authorities. In agreement with Brazilian local regulation, these samples will not be used for any testing other than that related to the present study (eg, retesting of dengue neutralizing Abs, assessment of FV status). These samples are being retained in long-term storage to support answers to regulatory questions related to the product's licensure and the potential revalidation of the study results.

The other biological samples collected to qualify the subject for inclusion in the study or to monitor the subject's health are dedicated for immediate use. In case they are not completely used up, they will be destroyed at the latest at the end of the study or after the time requested by local law.

Any new research to be done using the biological samples collected in the study will be submitted for IEC/IRB approval. The subject will have to provide her/his consent to every new research that might be performed with their stored samples. When it is not possible to obtain the subject's consent, Sanofi Pasteur will request the IEC/IRB's authorization to use biological samples stored at GCI.

8 Clinical Supplies

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

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

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

In the event that additional supplies are required, study staff must contact Sanofi Pasteur, indicating the quantity required. Contact information is provided in the Operating Guidelines.

9 Endpoints and Assessment Methods

9.1 Primary Endpoints and Assessment Methods

9.1.1 Safety

9.1.1.1 Safety Definitions

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

Adverse event (AE):

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

Therefore an AE may be:

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

All AEs include serious and non-serious AEs.

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

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

Serious adverse event (SAE):

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

An SAE is any untoward medical occurrence that at any dose

- Results in death

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

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

Adverse reaction:

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

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

The following additional definitions are used by Sanofi Pasteur:

Immediate event/reaction:

Immediate events are recorded to capture medically relevant unsolicited systemic AEs (including those related to the product administered) that occur within the first 30 minutes after vaccination.

Solicited reaction:

A solicited reaction is an "expected" adverse reaction (sign or symptom) observed and reported under the conditions (nature and onset) pre-listed in the protocol and CRB (eg, injection site pain between D0 and D7 post-vaccination, or headache between D0, and D7).

By definition, solicited reactions are to be considered as being related to the product administered.

For injectable vaccines, solicited reactions can either be solicited injection site reactions or solicited systemic reactions.

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

[b] All medical events leading to hospitalizations will be recorded and reported as SAEs, with the exception of: hospitalization planned before inclusion into the study or outpatient treatment with no hospitalization.

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

Unsolicited AE / AR:

An unsolicited AE is an observed AE that does not fulfill the conditions pre-listed in the CRB in terms of diagnosis and/or onset window post-vaccination. For example, if headache between D0 and D7 is a solicited reaction (ie, pre-listed in the protocol and CRB), then a headache starting on D7 is a solicited reaction, whereas headache starting on D8 post-vaccination is an unsolicited AE. Unsolicited AEs includes both serious (SAEs) and non-serious unsolicited AEs.

Injection site reaction:

An injection site reaction is an AR at and around the injection site. Injection site reactions are commonly inflammatory reactions. They are considered to be related to the product administered.

Systemic AE:

Systemic AEs are all AEs that are not injection or administration site reactions. They therefore include systemic manifestations such as headache, fever, as well as localized or topical manifestations that are not associated with the vaccination or administration site (eg, erythema that is localized but that is not occurring at the injection site).

Adverse event of special interest (AESI):

An adverse event of special interest is one of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring, and rapid communication by the Investigator to the Sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the study Sponsor to other parties (eg, regulators) might also be warranted.

9.1.1.2 Safety Endpoints

The primary endpoints for the evaluation of safety are:

- Occurrence of any unsolicited systemic AE reported within 30 minutes after each injection
- Occurrence of solicited (ie, pre-listed in the subject's DC and in the CRB) injection site reactions (pain, erythema, and swelling) occurring up to 7 days after each injection
- Occurrence of solicited systemic reactions (fever, headache, malaise, myalgia, and asthenia) occurring up to 14 days after each injection
- Occurrence of unsolicited AEs occurring up to 28 days after each injection
- Occurrence of serious and non-serious AESIs in defined time windows according to the type of AESI
- Occurrence of SAEs throughout the trial (ie, from D0 through the end of the study)
- Occurrence of hospitalized VCD cases throughout the study (ie, from D0 through the end of the study)

Other endpoints will be recorded or derived as described in the Statistical Analysis Plan. Depending on the item, these could include: nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), time of onset, duration, number of days of occurrence, Grade of severity, relationship to vaccine, action taken, whether the event/reaction led to early termination from the study, seriousness, or outcome.

9.1.1.3 Safety Assessment Methods

At each vaccination Visit, the Investigator or a delegate will perform a clinical or medically-driven physical examination, and will ask the subject about any solicited reactions and unsolicited AEs recorded in the diary card, as well as about any other AEs that may have occurred since the previous Visit. All relevant data will be transcribed into the CRB according to the instructions provided by the Sponsor.

9.1.1.3.1 Immediate Post-vaccination Observation Period

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

- Unsolicited systemic AEs will be recorded as immediate AEs in the CRB (presence marked as "yes" and details collected)
- Solicited and unsolicited injection site reactions and solicited systemic reactions will be recorded in the CRB in the same way as any reactions starting on the day of vaccination
- SAEs will be recorded in the CRB and reported to the Sponsor in the same way as any other SAEs, according to the procedures described in Section 10.

9.1.1.3.2 Reactogenicity (Solicited Reactions From Day 0 to Day 7/D14 After Each Vaccination)

After the first vaccination, subjects will be provided with a diary card, a digital thermometer, and a flexible ruler, and will be instructed how to use them. The following items will be recorded by the subjects in the diary card on the day of vaccination and for the next 7 days (ie, D0 to D7) until resolution:

- Daily temperature, with the route by which it was taken
- Daily measurement or intensity Grade of all other solicited injection site and systemic reactions
- Action taken for each event (eg, medication)

  The action(s) taken by the subject to treat and/or manage any **solicited reactions** will be classified in the CRB using the following list (all applicable items should be checked):

  - None
  - Medication
  - Health care provider contact
  - Hospitalized
  - Discontinuation of study vaccination

Table 9.1 and Table 9.2 present, respectively, the injection site reactions and systemic reactions that are pre-listed in the diary cards and CRB, together with the intensity scales.

Table 9.1: Solicited injection site reactions: terminology, definitions, and intensity scales

CRB term (MedDRA lowest level term [LLT])	Injection site pain	Injection site erythema	Injection site swelling
Diary card term	Pain	Redness	Swelling
Definition	Pain either present spontaneously or when the injection site is touched or injected limb is mobilized	Presence of a redness including the approximate point of needle entry	Swelling at or near the injection site Swelling or edema is caused by a fluid infiltration in tissue or cavity and, depending on the space available for the fluid to disperse, swelling may be either soft (typically) or firm (less typical) to touch and thus can be best described by looking at the size of the swelling
Intensity scale*	Grade 1: A type of AEs that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living. Grade 2: A type of AEs that is usually alleviated with additional therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant. Grade 3: A type of AEs that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm	Grade 1: ≥ 25 to ≤ 50 mm Grade 2: ≥ 51 to ≤ 100 mm Grade 3: > 100 mm

* For the subjective reaction of pain, subjects will record the intensity level (Grade 1, 2, or 3) in the diary card. For the measurable reactions of redness and swelling, they will record just the size of the reaction, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis

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

CRB term (MedDRA lowest level term [LLT])	Fever	Headache	Malaise	Myalgia	Asthenia
Diary card term	Temperature	Headache	Feeling unwell	Muscle aches and pains	Weakness
Definition	Elevation of temperature to ≥°38.0°C	Pain or discomfort in the head or scalp. Does not include migraine	General ill feeling. Malaise is a generalized feeling of discomfort, illness, or lack of well-being that can be associated with a disease state. It can be accompanied by a sensation of exhaustion or inadequate energy to accomplish usual activities.	Muscle aches and pains are common and can involve more than one muscle at the same time. Muscle pain can also involve the soft tissues that surround muscles. These structures, which are often referred to as connective tissues, include ligaments, tendons, and fascia (thick bands of tendons). Does not apply to muscle pain at the injection site which should be reported as injection site pain	Generalized weakness
Intensity scale*	Grade 1: ≥ 38.0°C to ≤ 38.4°C	Grade 1: A type of AEs that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living	Grade 1: A type of AEs that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.	Grade 1: A type of AEs that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living	Grade 1: No interference with activity.

CRB term (MedDRA lowest level term [LLT])	Fever	Headache	Malaise	Myalgia	Asthenia
	Grade 2: ≥ 38.5°C to ≤ 38.9°C	Grade 2: A type of AEs that is usually alleviated with additional therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant	Grade 2: A type of AEs that is usually alleviated with additional therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.	Grade 2: A type of AEs that is usually alleviated with additional therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant	Grade 2: Some interference with activity.
	Grade 3: ≥ 39.0°C	Grade 3: A type of AEs that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention	Grade 3: A type of AEs that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.	Grade 3: A type of AEs that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention	Grade 3: Significant; prevents daily activity

* For all reactions but fever, subjects will record the intensity level (Grade 1, 2, or 3) in the diary card. For fever, they will record the body temperature, and the classification as Grade 1, 2, or 3 will be assigned at the time of the statistical analysis based on the unit used to measure the temperature and the intensity scale.

Important notes for the accurate assessment of temperature:

Subjects are to measure body temperature once per day, preferably always at the same time. The optimal time for measurement is the evening, when body temperature is the highest. Temperature is also to be measured at the time of any apparent fever. The observed daily temperature and the route of measurement are to be recorded in the diary card, and the highest temperature will be recorded by the site in the CRB. The preferred route for this study is the axillary route. Pre-vaccination temperature is also systematically collected by the Investigator on the source document. Tympanic thermometers must not be used.

9.1.1.3.3 Unsolicited Adverse Events

In addition to recording solicited reactions, subjects will be instructed to record any other medical events that may occur during the 28-day period after each injection. Space will be provided in the diary card for this purpose.

Information on SAEs will be collected and assessed throughout the study. SAEs related to study procedures and occurring between the Screening Visit and the first injection visit (V01), if applicable, will be first reported using a paper SAE Reporting Form, and then reported in the CRB for enrolled subjects. SAEs occurring from V01 until 6 months after the last injection will be directly reported in the CRB of subjects. Any SAE occurring at any time during the study will be reported by the Investigator in the CRB according to the completion instructions provided by the Sponsor; this includes checking the "Serious" box on the AE CRF and completing the appropriate Safety Complementary Information CRFs. All information concerning the SAE is to be reported either as part of the initial reporting or during follow-up reporting if relevant information became available later (eg, outcome, medical history, results of investigations, copy of hospitalization reports, and verbal autopsy questionnaire if used.).See Section 10 for further details on SAE reporting.

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

- Start and stop dates[a]
- Intensity of the event:

  For measurable unsolicited AEs that are part of the list of solicited reactions, the size of the AE as well as the temperature for fever will be collected and analyzed based on the corresponding scale used for solicited reactions (see Table 9.1 and Table 9.2).

  All other unsolicited AEs will be classified according to the following intensity scale:

  - Grade 1: A type of AEs that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.

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

  - Grade 2: A type of AEs that is usually alleviated with additional therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research participant.
  - Grade 3: A type of AEs that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.
- Whether the AE was related to the investigational product (for unsolicited systemic AEs)

  The Investigator will assess the causal relationship between the AE and the investigational product as either "Not related" or "Related", as described in Section 9.1.1.3.5.
- Action taken for each AE (eg, medication)

  The action(s) taken by the subject to treat and/or manage any unsolicited AEs will be classified in the CRB using the following list (all applicable items should be checked):
  - None
  - Medication
  - Health care provider contact
  - Hospitalized
  - Discontinuation of study vaccination
- Whether the AE was serious

  For each SAE, the Investigator will complete all seriousness criteria that apply (outcome, elapsed time, and relationship to study procedures)
- Whether the AE caused study discontinuation

9.1.1.3.4 Adverse Events of Special Interest

Serious AESIs

The following serious AESIs will be considered:

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

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

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

Non-Serious AESIs

The following non-serious AESIs will be considered:

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

9.1.1.3.5 Assessment of Causality

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

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

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

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

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

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

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

Dengue Screen RT-PCR

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

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

Simplexa™ Dengue RT-PCR

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

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

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

Dengue NS1 Ag ELISA

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

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

Hematology – Biochemistry

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

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

Interpretation of results

If a sample is positive for the Dengue Screen RT-PCR (ie, ≥lower limit of quantification [LLOQ]) and/or the NS1 assay is positive and/or the Simplexa™ dengue RT-PCR is positive, this will be classified as a VCD infection.

9.1.2 Immunogenicity

There are no primary objectives for immunogenicity.

9.1.3 Efficacy

There are no primary objectives for efficacy.

9.2 Secondary Endpoints and Assessment Methods

9.2.1 Safety

9.2.1.1 Safety Definitions

The safety definitions are presented in Section 9.1.1.1.

9.2.1.2 Safety Endpoints

- CYD dengue vaccinal viremia at 7 and 14 days post-Inj 1
- CD4 count and HIV RNA viral load 28 days after each injection of CYD dengue vaccine

9.2.1.3 Safety Assessment Methods

Vaccinal viremia

YF RT-PCR (Non-Serotype-Specific Vaccine Viremia)

Quantitation of non-serotype-specific vaccine viremia is determined by testing serum samples with a nucleic-acid based assay. YF virus RNA is extracted from the serum to discard potential Taq polymerase inhibitors or interfering factors, using a commercial kit. Then, a RT-PCR is carried out with primers from the YF core gene sequence. The inclusion of a standard in each run allows results to be expressed as a concentration of log10 PFU equivalents/mL or log10 Genomic Equivalents (GEq)/mL.

This assay will be performed on blood samples taken at V02 and V03 on all subjects.

CYD RT-PCR (Serotype-Specific Vaccine Viremia)

Four RT-PCRs will be used to perform quantitation and serotype identification of post-vaccinal serotype-specific dengue vaccine viremia from serum samples displaying a positive YF RT-PCR result. Virus RNA is extracted from the serum to discard potential Taq polymerase inhibitors or interfering factors, using a commercial kit. Then a RT-PCR is carried out with serotype-specific primers from the envelope-NS1 junction gene sequence. Based on the dengue virus plasmidic standards included in each run, results can be expressed as a concentration of log10 GEq/mL.

This assay will be performed only for YF RT-PCR-positive samples.

CD4+ T-cell count

Numeration of CD4 is determined by flow cytometry on unlyzed whole blood. When whole blood is added to the reagent tube (commercial kit), fluorochrome-labeled antibodies in the reagents bind specifically to white blood cell surface CD4+ antigens, and a fluorescent nuclear dye binds to the nucleated blood cells. After a fixative solution is added, the sample is run on the flow cytometer. As reagent tubes contain a known number of fluorescent reference beads, it is possible

to calculate the CD4 counts (cells/mm^3) by comparing cellular events to bead events. This test will be performed in local laboratories.

HIV-1 RNA viral load

RT-PCR will be used to perform quantitation of HIV-1 viral load from serum samples using a commercial kit. After the amplification of a HIV-1 conserved target region, a fluorescent probe hybridizing with the target region allows quantifying the number of amplified HIV-1 RNA copies. Based on standards included in each run, results can be expressed as a number of copies/mL. This test will be performed in local laboratories following the local regulations on routine management of HIV-positive patients (48).

9.2.2 Immunogenicity

9.2.2.1 Immunogenicity Endpoints

- Neutralizing Ab levels (measured by plaque reduction neutralization test [PRNT]) against each of the 4 parental dengue virus strains of CYD dengue vaccine construct at baseline and 28 days after each injection

9.2.2.2 Immunogenicity Assessment Methods

Dengue Neutralizing Abs

Dengue neutralizing Ab levels will be measured by $PRNT_{50}$ (using parental dengue virus strains of CYD dengue vaccine constructs) by Sanofi Pasteur GCI, Swiftwater, USA (or outsourced with a GCI selected external laboratory).

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

This assay will be performed on blood samples taken at baseline and 1 month after each vaccine injection (V01, V04, V06, and V08).

9.2.3 Efficacy

There are no secondary objectives for efficacy.

9.3 Observational Endpoints and Assessment Methods

9.3.1 Immunogenicity

9.3.1.1 Immunogenicity Endpoints

- Anti-NS1 IgG at baseline
- Neutralizing Ab levels against YF at baseline
- Neutralizing Ab levels against Zika at baseline

9.3.1.2 Immunogenicity Assessment Methods

Anti-NS1 IgG

Antibodies to dengue NS1 antigen will be measured using the Dengue anti-NS1 IgG ELISA performed at the Sponsor's laboratory in Swiftwater, PA, USA.

Briefly, equimolar concentrations of all 4 serotypes of Dengue NS1 antigen is coated onto a microtiter plate and serial 2-fold dilutions of human serum samples are added and incubated to allow binding to NS1. Then an HRP-conjugated anti-human IgG detection antibody is added followed by colorimetric substrate. The concentration of IgG antibodies to Dengue NS1 are calculated over 8-serial fold dilutions relative to assay reference with an assigned value (ELISA Units /mL).

YF Neutralizing Abs

YF neutralizing Ab levels will be measured by a neutralization test by Sanofi Pasteur GCI, Swiftwater, USA, or GCI outsourced laboratory.

Briefly, serial twofold dilutions of serum to be tested (previously heat-inactivated) are mixed with a constant concentration of the YF vaccinal strain 17D. The mixtures are inoculated in duplicate into wells of a plate of confluent Vero cells. After adsorption, cell monolayers are overlaid, and incubated for a few days. The reported value (end point neutralization titer) represents the highest dilution of serum at which ≥ 50% of YF challenge virus is neutralized when compared to the negative control wells, which represents the 100% virus load. The LLOQ of this assay is 10 (1/dilution). This method will be performed on blood sample taken at baseline (V01) to determine FV status.

Zika Neutralizing abs (Microneutralization assay)

Zika neutralizing Ab levels will be measured by Sanofi Pasteur GCI, Swiftwater, USA or GCI outsourced laboratory.

Serial, two-fold dilutions of serum to be tested (previously heat-inactivated) are mixed with a constant concentration of Zika virus. The mixtures are inoculated in duplicate into wells of a 96-well microplate with permissive cells. After adsorption, cell monolayers are incubated for a few days. A reduction in virus infectivity (viral antigen production) due to neutralization by antibody present in serum samples is detected by ELISA. After washing and fixation, Zika viral antigen production in cells is detected by successive incubations with Zika-specific MAbs, anti-mouse Ig conjugate, and a chromogenic substrate. The resulting OD is measured using a microplate reader.

The reduction in Zika virus infectivity as compared to that in the virus control wells constitutes a positive neutralization reaction indicating the presence of neutralizing antibodies in the serum sample. On the other hand, the infection of cells indicates the absence of neutralizing antibodies in the serum sample.

10 Reporting of Serious Adverse Events

To comply with current regulations on SAE reporting to health authorities, the Investigator must document all SAEs regardless of causal relationship, and notify the Sponsor and the Clinical Research Associate (CRA) within the notification timelines stated in the following sections. The Investigator will give access and provide the Sponsor and the CRA with all necessary information to allow the Sponsor to conduct a detailed analysis of the safety of the investigational product(s). It is the responsibility of the Investigator to request all necessary documentation (eg, medical records, discharge summary, physical autopsy report if performed and verbal autopsy questionnaire if used) in order to provide comprehensive safety information. All relevant information must then be transcribed onto the AE CRF and the appropriate Safety Complementary Information CRFs.

10.1 Initial Reporting by the Investigator

SAEs occurring during a subject's participation in the study or experiment must be reported within 24 hours to the Sponsor's GPV Department and to the CRA. Every SAE must be reported, even if the Investigator considers that it is not related to the vaccine. The Investigator (licensed physician [MD or D.O.]) must validate the information entered on the AE CRF by completing the Investigator validation form.

The Investigator must indicate on the AE CRF that the event was serious and must complete the relevant SAE section of this form as well as the appropriate Safety Complementary Information CRFs. An e-mail alert will automatically be sent by the EDC system to the GPV mailbox, the CRA and the CTL with relevant SAE information details.

If the EDC system is unavailable, the site must notify the Sponsor, using the paper version of the CRB, as described in the Operating Guidelines.

The Investigator must complete the paper copies of the AE CRF and of the appropriate Safety Complementary Information CRFs and send them to the Sponsor by one of the following means:

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

  Sanofi Pasteur
  Global PharmacoVigilance Department
  14, Espace Henry Vallée
  69007 Lyon
  France

When the EDC system becomes available, the Investigator must transcribe the information from the paper forms into the EDC system.

If there is need for urgent consultation, the Investigator is to contact the RMO. If the RMO cannot be reached, the Investigator may contact the Call Center as described in Section 5.3.

10.2 Follow-up Reporting by the Investigator

The AE CRF completed initially must be updated within 24 hours after the Investigator has become aware of any new relevant information concerning the SAE (eg, outcome, precise description of medical history, results of the investigation). All relevant information must be included directly in the AE CRF and the appropriate Safety Complementary Information CRFs. An e-mail alert will be sent automatically to the GPV Department and to the CRA. Copies of documents (eg, medical records, discharge summary, autopsy) may be requested by the GPV Department.

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

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

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

10.4 Assessment of Causality

The causal relationship between the SAE and the product administered will be evaluated by the Investigator as described in Section 9.1.1.3.5.

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

The causal relationship to study procedures will be also assessed in the CRB.

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

10.5 Reporting SAEs to Health Authorities and IECs / IRBs

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

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

10.6 Using a Verbal Autopsy Questionnaire to Aid in Determining the Cause of Death

In case of the absence or inadequacy of health information that would allow a thorough evaluation of the causes of the death of a subject participating in the study, the verbal autopsy procedure may be triggered by either the Investigator or the Sponsor. Detailed instructions on the use of the verbal autopsy questionnaire, as well as the questionnaire itself, are provided in the Operating Guidelines.

10.7 Special Considerations on CD4 Count- and HIV-1 Viral Load Safety Signals

Reporting

In this study, an increase in HIV viral load (plasma HIV-1 RNA > 1000 copies/mL 28 days post-injection after having been undetectable [< 50 copies/mL] pre-injection) or a decrease in CD4 count (decrease in CD4 greater than 30% assessed 28 days post-injection compared to the pre-injection value), not explained by non-adherence to ART and not explained by any other possible etiology, is to be reported as an SAE according to the procedure described above (Section 10.1).

Follow-up reporting

The eSAE Form completed initially will be updated within 24 hours after the Investigator has received the results from the second blood sample. After validation, an e-mail alert will be sent automatically to the GPV Department and to the CRA. All relevant information must be included directly in the eSAE form. Copies of documents (eg, medical records, discharge summary, autopsy) may be requested by the GPV Department. Upon reception of second blood sample confirmatory results, criteria for holding the study will be checked (see Section 5.5).

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

Assessment of causality

The causal relationship between the SAE and the product will be assessed as described above (Section 10.4). Upon meeting a safety criterion for holding the study (see Section 5.5), the Sponsor alongside the IDMC and the Investigator(s) will decide whether to modify or discontinue the study.

Reporting to health authorities and IECs/IRBs

See Section 10.5.

11 Data Collection and Management

11.1 Data Collection and CRB Completion

Individual diary cards, specifically designed for this study by the Sponsor and provided to the study sites, will be given to study participants for the recording of daily safety information as described in Section 9.1.1.3. These diary cards will include pre-listed terms and intensity scales (see Table 9.1 and Table 9.2) as well as areas for free text to capture additional safety information or other relevant details. Subjects will also be provided with rulers for measuring the size of

injection site reactions, and with standard digital thermometers for measuring daily temperatures. To ensure consistency of reporting, the study sites will instruct subjects on how to correctly use these tools.

The 6-month follow-up will be done by interviewing subjects either during a Visit or over the telephone using a questionnaire to capture SAEs and AESIs, if applicable. A MA will be provided to the subjects at the preceding study Visit to help them record information on events occurring between this Visit and the 6-month follow-up. In case a follow-up Visit is planned, a diary card may be provided to the subject.

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

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

To ensure the correct and consistent completion of the CRBs, the Sponsor or authorized representative will provide all necessary tools, instructions, and training to all site staff involved in data entry prior to study start. Additional instructional documents such as training manuals and completion instructions will be provided to assist with data entry during the course of the study.

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

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

11.2 Data Management

Management of SAE and pregnancy data

During the study, SAE data (reported on the AE, Death, and Safety Complementary Information CRFs) and pregnancy data (reported by the Investigator on ePregnancy Forms) will be integrated into the Sponsor's centralized GPV database upon receipt of these forms and after a duplicate check. Each case will be assigned a case identification number. Each case will be assessed by the case management platform or its delegate before being reported to the relevant authorities as

necessary. The assessment of related cases will be done in collaboration with the Global Safety Officer and the RMO. Follow-up information concerning a completed case will be entered into the GPV database, and a new version of the case will be created.

The information from the GPV database cases will be reconciled with that in the clinical database.

Management of clinical and laboratory data

Clinical data, defined as all data reported in the CRB, and laboratory data will be handled by the Sponsor's Clinical Data Management (CDM) platform or authorized representative.

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

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

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

11.3 Data Review

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

12 Statistical Methods and Determination of Sample Size

12.1 Statistical Methods

All statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics Platform using the SAS® software, at least version 9.3 (SAS Institute, Cary, NC, USA).

A statistical analysis plan (SAP) will be written and peer reviewed before any analyses. In accordance with the protocol, the SAP will describe all analyses to be performed under the responsibility of the Sponsor and all the conventions to be taken.

A statistical analysis of the safety and immunogenicity data obtained up to Day 28 after the last injection of the last subject enrolled will be conducted once data are available and an interim database lock has been conducted. A final analysis will be conducted once the 6-month safety data have been collected and the final database lock has occurred. No multiplicity adjustment is necessary because no hypotheses will be tested.

12.1.1 Hypotheses and Statistical Methods for Primary Objective

12.1.1.1 Hypotheses

No hypotheses will be tested.

12.1.1.2 Statistical Methods

All analyses will be descriptive. Safety will be assessed after each and any dose of CYD dengue vaccine.

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

The analysis of safety will address the number and percentage of subjects with injection site or systemic AEs until 28 days following each injection (solicited systemic reactions from D0 to D14, solicited injection site reactions from D0 and D7 and unsolicited AEs until D28, including AESIs, and immediate reactions, ie, occurring within 30 minutes after each vaccination).

Solicited reactions will be described according to their intensity and according to time to onset, number of days of occurrence, action taken, and according to whether they lead to trial discontinuation.

Unsolicited AEs will be described by MedDRA System-Organ Class and preferred term definition according to their relationship, severity, time to onset, and duration.

Serious AESIs (in defined time windows according to the type of AESI) and SAEs (throughout the trial; including the 6-month follow-up) will be described by MedDRA preferred term, outcome, and relationship to vaccination.

12.1.2 Hypotheses and Statistical Methods for Secondary Objective(s)

12.1.2.1 Hypotheses

No hypotheses will be tested.

12.1.2.2 Statistical Methods

The proportion of subjects with a detected and quantified CYD dengue vaccinal viremia (ie, above the detection level) whatever the serotype (as assessed by YF RT-PCR) and for each of the 4 dengue serotypes (by serotype-specific RT-PCR method) after the first CYD dengue vaccine injection will be presented.

A summary of CD4 count and HIV-1 RNA viral load over time will be presented.

Analysis of dengue neutralizing antibody levels will be performed in each vaccine Group before first injection (at baseline) and 28 days after each injection using:

- GMTs against each serotype with the parental dengue virus strains
- GM of individual titer ratio (GMTR) against each serotype with the parental dengue virus strains (post-Injection 2/pre-Injection 1, and post-Injection 3/pre-Injection 1)

12.2 Analysis Sets

12.2.1 Full Analysis Set

The full analysis set (FAS) is defined as the subset of randomized subjects who received either CYD dengue vaccine or placebo and had blood sample drawn and valid post-injection serology results (ie, a result different from "not reportable" ["NR"] or missing, for at least one dengue serotype).

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

12.2.2 Safety Analysis Set

The safety analysis set (SafAS) is defined as those subjects who have received at least one dose of the study vaccine[a]. All subjects will have their safety analyzed after each dose according to the vaccine they actually received.

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

12.2.3 Per-Protocol Analysis Set

The per-protocol analysis set (PPAS) is a subset of the FAS. The subjects presenting with at least one of the following relevant protocol deviations will be excluded from the PPAS:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not complete the vaccination schedule
- 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 receive vaccine in the proper time window
- Subject did not provide post-dose serology sample in the proper time window or a post-dose serology sample was not drawn
- Subject received a protocol-prohibited medications (see Section 6.7)
- Subject's post-injection serology sample did not produce a valid test result (ie, a result different from "NR" or missing, for at least one dengue serotype)

A PP set will be defined for each vaccination. Subjects will remain in the corresponding population as long as they do not meet one of the above criteria.

12.2.4 Other Analysis Set

Not applicable

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

12.2.5 Populations Used in Analyses

12.2.6 Populations Used in Analyses

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

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

12.3 Handling of Missing Data and Outliers

12.3.1 Safety

No replacement will be done.

12.3.2 Immunogenicity

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

For calculating GMTR, < LLOQ will be converted to 0.5 LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator. Any titer reported as > upper limit of quantitation (ULOQ) will be converted to ULOQ.

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

12.3.3 Efficacy

No clinical efficacy data will be obtained in the trial.

12.4 Interim / Preliminary Analysis

No analyses are planned.

12.5 Determination of Sample Size and Power Calculation

The objective of the trial is to provide descriptive safety and immunogenicity results and therefore the sample size is arbitrarily set to 100 subjects for the CYD dengue vaccine Group and 50 subjects for the Placebo Group. There is a 95% probability of observing an event that has a true incidence of 3% for the CYD dengue vaccine Group.

13 Ethical and Legal Issues and Investigator / Sponsor Responsibilities

13.1 Ethical Conduct of the Study / Good Clinical Practice

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

13.2 Source Data and Source Documents

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

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

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

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

Good Documentation Practice should be followed by the Investigator and the site staff managing source documents.

13.3 Confidentiality of Data, Data Protection, and Access to Subject Records

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

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

All personal data collected related to subjects, Investigators, or any person involved in the study, which may be included in the Sponsor's databases, shall be treated in compliance with all applicable laws and regulations, including the GDPR (Global Data Protection Regulation). Data collected must be adequate, relevant and not excessive, in relation to the purposes for which they are collected. Each category of data must be properly justified and in line with the study objective.

Subjects will be assigned a unique identifier by the Sponsor. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information that would make the subject identifiable will not be transferred.

The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject.

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

The subject must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

When archiving or processing personal data pertaining to the Investigator and/or to the subjects, the Sponsor shall take all appropriate measures to safeguard and prevent access to this data by any unauthorized third party.

13.4 Monitoring, Auditing, and Archiving

13.4.1 Monitoring

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

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

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

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

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

- The center has all the documents necessary for archiving
- All samples have been shipped to the appropriate laboratories

- All unused materials and products have been either destroyed or returned to the Sponsor

13.4.2 Audits and Inspections

A quality assurance audit may be performed at any time by the Sponsor's Clinical Quality Assessment department (CQA) or by independent auditors to verify that the study has been conducted according to the protocol, GCP and ICH requirements, and other applicable regulations. An inspection may be conducted by regulatory authorities. The Investigator must allow direct access to study documents during these inspections and audits.

13.4.3 Archiving

The Investigator and the study site shall retain and preserve 1 copy of the study file containing the essential documents related to the study and records generated during the study ("Study File") for the longer of the 2 following periods ("Retention Period"):

- 25 years after the signature of the final study report or
- such longer period as required by applicable regulatory requirements

If during the Retention Period, the study site is no longer able to retain the Study File due to exceptional circumstances (such as bankruptcy), the study site shall contact the Sponsor to organize the transfer of the Study File to the Sponsor's designee at the Sponsor's expense. Following the Retention Period, the Investigator and/or the study site are responsible to dispose of the Study File according to the applicable regulations. Patient medical records shall be retained in compliance with local regulations.

Archived data may be held on Electronic Records, provided that a back-up exists and that a hard copy can be obtained if required. The protocol, documentation, approvals, and all other documents related to the study will be kept by the Sponsor in the Trial Master File (TMF). Data on AEs are included in the TMF. All data and documents will be made available if requested by relevant authorities.

13.5 Financial Contract and Insurance Coverage

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

13.6 Stipends for Participation

Expenses that are directly related to the subject's participation in the study (for example cost of transportation for attending visits) will be compensated. Subjects will not receive any remuneration for participation in the study.

13.7 Publication Policy

Data derived from this study are the exclusive property of Sanofi Pasteur. Any publication or presentation related to the study must be submitted to Sanofi Pasteur for review before submission

of the manuscript. After publication of the results of the study, any participating center may publish or otherwise use its own data provided that any publication of data from the study gives recognition to the study Group. In addition, Sanofi Pasteur shall be offered an association with all such publications, it being understood that Sanofi Pasteur is entitled to refuse the association.

Sanofi Pasteur must have the opportunity to review all proposed abstracts, manuscripts, or presentations regarding this study at least 90 days prior to submission for publication / presentation. Any information identified by Sanofi Pasteur as confidential must be deleted prior to submission, it being understood that the results of this study are not to be considered confidential.

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

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15 Signature Page

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