Title: An open label, Phase 2 Study to Investigate Cell-mediated Immunity and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Healthy Children Aged 4 to 16 Years

NCT Number: NCT02948829

Protocol Approve Date: 01 July 2016

Certain information within this protocol has been redacted (ie, specific content is masked irreversibly from view with a black/blue bar) to protect either personally identifiable information (PPD) or company confidential information (CCI).

This may include, but is not limited to, redaction of the following:

- Named persons or organizations associated with the study.
- Proprietary information, such as scales or coding systems, which are considered confidential information under prior agreements with license holder.
- Other information as needed to protect confidentiality of Takeda or partners, personal information, or to otherwise protect the integrity of the clinical study.

PROTOCOL

An open label, Phase 2 Study to Investigate Cell-mediated Immunity and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Healthy Children Aged 4 to 16 Years

Safety and Immunogenicity of Takeda's TDV in Healthy Children

Sponsor:	Takeda Vaccines, Inc. One Takeda Parkway Deerfield, IL 60015 USA
Study Identifier:	DEN-313
IND Number:	Not applicable **EudraCT Number:** Not applicable
Vaccine Name:	Tetravalent Dengue Vaccine Candidate (TDV) (formerly DENVax) comprised of a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), a dengue serotypes 2/1 chimeric strain (TDV-1), a dengue serotypes 2/3 chimeric strain (TDV-3), and a dengue serotypes 2/4 chimeric strain (TDV-4).
Date:	01 July 2016
Version:	Version 1.0

1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

Table 1.a Contact Information

Issue	Contact
Serious adverse event and pregnancy reporting	PPD
Medical Monitor (medical advice on conduct of protocol or compound)	
Responsible Medical Officer (carries overall responsibility for the conduct of the study)	

1.2 Approval

REPRESENTATIVES OF TAKEDA

This study will be conducted with the highest respect for the individual participants in accordance with the requirements of this clinical study protocol and also in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonization E6 Good Clinical Practice: Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws, clinical study disclosure laws, and regulations.

SIGNATURES

PPD

INVESTIGATOR AGREEMENT

I confirm that I have read and that I understand this protocol, the Investigator's Brochure, and any other product information provided by the sponsor. I agree to conduct this study in accordance with the requirements of this protocol and also protect the rights, safety, privacy, and well-being of study subjects in accordance with the following:

- The ethical principles that have their origin in the Declaration of Helsinki.
- International Conference on Harmonization, E6 Good Clinical Practice: Consolidated Guideline.
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Regulatory requirements for reporting serious adverse events defined in Section 10.4 of this protocol.
- Terms outlined in the Clinical Study Site Agreement.
- Appendix A – Responsibilities of the investigator.

I further authorize that my personal information may be processed and transferred in accordance with the uses contemplated in Appendix B of this protocol.

Signature of Investigator Date

Investigator Name (print or type)

Investigator's Title

Location of Facility (City, State)

Location of Facility (Country)

1.3 Protocol Amendment Summary of Changes

Not applicable.

1.3.1 Summary of Changes

Not applicable.

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2.0 STUDY SUMMARY

Name of Sponsor: Takeda Vaccines, Inc. One Takeda Parkway Deerfield, IL 60015, USA		**Product Name:** Tetravalent dengue vaccine candidate (TDV)
Study Title: An open label, Phase 2 Study to Investigate Cell-mediated Immunity and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Healthy Children Aged 4 to 16 Years		
IND No.: Not applicable		**EudraCT No.:** Not applicable
Study Identifier: DEN-313	**Phase:** 2	**Study Blinding Schema:** Open Label

Background and Rationale:

Dengue fever is caused by infection with wild type dengue virus (DENV), a ribonucleic acid (RNA) virus that occurs as 4 recognized serotypes, DENV-1, DENV-2, DENV-3 or DENV-4. These dengue viruses are transmitted from human to human by mosquitoes (primarily *Aedes aegypti*). The 4 dengue viruses have spread worldwide and are endemic in Asia, Central and South America, the Caribbean, the Pacific Islands, and parts of Africa. There are an estimated 390 million dengue infections per year worldwide, which is more than 3 times the previous World Health Organization estimate of 50 to 100 million cases. Every year, around 500,000 cases of dengue hemorrhagic fever (DHF) require hospitalization with an estimated death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection.

Dengue fever is clinically defined as an acute febrile illness with 2 or more manifestations (headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leucopenia) and occurrence at the same location and time as other confirmed cases of dengue fever. The most severe forms of dengue infection (ie, DHF and dengue shock syndrome [DSS]) are life threatening. Primary infection with any 1 of the 4 dengue serotypes is thought to result in life-long protection from re-infection by the same serotype, but does not protect against a secondary infection by 1 of the other 3 dengue virus serotypes and may lead to an increased risk of severe disease (DHF/DSS).

Treatment of dengue fever is based solely on symptoms and signs, with fluid replacement required for hemorrhagic or shock cases. An antiviral therapy for dengue virus infection is not available. Preventive measures that rely on mosquito control and individual protection, are of limited efficacy, complex to implement and questionable in terms of cost-effectiveness. There is a great global public health need for a safe and effective vaccine that will protect against all serotypes of dengue infection, and thereby reduce the morbidity and mortality associated with dengue disease. A tetravalent dengue vaccine (CYD-TDV) has been recently approved in some countries in Asia and Latin America. However, the initial findings suggest an unfavorable risk-benefit profile for younger subjects with the approved vaccine. Vaccine efficacy was different between serotypes and depended on dengue Baseline seropositivity status. Hence, there is a continued unmet public health need for safer and more efficacious dengue vaccines. Additional vaccines are also important to ensure sufficient supply globally.

Tetravalent Dengue Vaccine Candidate (TDV) - Background:

Takeda's TDV consists of 1 molecularly characterized, attenuated dengue virus strain and 3 recombinant dengue virus strains expressing surface antigens corresponding to dengue serotypes 1-4. The dengue serotype 2 strain is based upon the attenuated laboratory-derived virus, DENV-2 Primary Dog Kidney (PDK)-53, originally isolated at Mahidol University, Bangkok, Thailand. The chimeric, attenuated vaccine strains for dengue serotypes 1, 3, and 4 were engineered by substituting the structural genes, pre-membrane (prM) and envelope (E), of TDV-2 with the prM and E genes of the DENV strains, DENV-1 16007, DENV-3 16562 or DENV-4 1036 virus, respectively. TDV is thus comprised of 4 dengue virus strains: a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), a dengue serotypes 2/1 chimeric strain (TDV-1), a dengue serotypes 2/3 chimeric strain (TDV-3), and a dengue serotypes 2/4 chimeric strain (TDV-4).

Nonclinical studies carried out in mice and nonhuman primates demonstrated an acceptable safety, immunogenicity, and efficacy profile of Takeda's TDV.

Data from 4 completed phase 1 studies, 1 completed phase 2 study, and 2 ongoing phase 2 studies have shown satisfactory reactogenicity, safety and immunogenicity of Takeda's TDV in adults in non-endemic areas as well as in

adults and children in endemic areas in Asia and Latin America. Currently ongoing and completed phase 2 studies have enabled the selection of a final TDV dose (lyophilized formulation), and a 2-dose vaccination regimen for subcutaneous (SC) administration 3 months apart for use in the pivotal program.

The current Investigator Brochure for Takeda's TDV contains additional product information and a more detailed review of pre-clinical and clinical studies.

Rationale for the Proposed Phase 2 Study:

Studies investigating the role of cell-mediated immunity (CMI) in natural infections have demonstrated roles in both virus clearance and potentiating disease. These investigations have been extended from the exploratory study of CMI in natural infection to the study of dengue vaccine recipients. Knowledge about specific CMI correlates of protection is incomplete. However, the exploration of CMI is encouraged by the WHO since specific CMI assays may be useful for the assessment of immunological memory and durability of protection. Assessments of cytokine responses may also assist in the evaluation of vaccine safety and may provide some indication of the potential risk that vaccination could predispose subjects to develop severe dengue febrile illness (DHF/DSS) during subsequent natural infection.

Studies in nonhuman primates have demonstrated that TDV elicits cluster of differentiation (CD)4+ and CD8+ T cell responses targeting the nonstructural (NS) proteins NS1, NS3, and NS5 of TDV-2. Both T cell subsets produced interleukin-2 (Il-2), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), and were multifunctional in nature. In addition, CD8+ T cells expressed the CD107α marker, and exhibited cross-reactivity with the NS proteins of the other 3TDV serotypes.

Subsequently, investigations of clinical samples from phase 1 studies have demonstrated generation of multi-lineage CMI after administration of TDV. Potent IFN-γ enzyme-linked immunospot (ELISPOT) responses were detected against serotype matched antigens which were confirmed by intracellular cytokine staining (ICS). The responses were predominantly mediated by CD8+ T cells, but a population of dengue-specific CD4+ T cells was also mobilized by the vaccine. Responses were directed against all regions of the TDV-2 proteome and also the TDV-1, -3, and -4 structural components of the vaccine. Cross-reactivity of responses to the TDV-2 proteome with DENV-1, -3 and -4 NS1, NS3, and NS5, which are not components of the vaccine, was also detected.

The DEN-313 study is planned to investigate cellular immune responses following TDV vaccination in dengue endemic regions and will contribute to immunogenicity data obtained from the ongoing phase 3 efficacy study (DEN-301) in a similar population comprised of subjects 4 to 16 years of age living in dengue endemic regions (Asia and Latin America).

This study will be conducted in compliance with the protocol, the International Conference on Harmonization and Good Clinical Practice (ICH-GCP) guidelines and applicable regulatory requirement(s).

Study Design:

This is an open-label phase 2 study with a single study group. The target sample size is 200 healthy subjects (100 subjects aged 4 to 16 years in Latin America; 100 subjects aged 4 to 8 years in Asia). Each subject will receive a 2-dose schedule of TDV by SC injection into the upper arm at Day 1 (Month 0) and at Day 90 (Month 3). Subjects will be screened prior to vaccination to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each study center. Any withdrawals or screen failures from enrolment until vaccination at Day 1 (Month 0) will be replaced.

Subjects will be followed up for immunogenicity and safety assessments for 3 years after the second vaccination at Day 90 (Month 3) including febrile illness surveillance to monitor any febrile illness with potential dengue etiology for long term safety.

Definition of febrile illness

The subjects and the subject's parent(s)/legally authorized representative (LAR), as applicable, will be asked to return to the study center for dengue fever evaluation of the subjects by the investigator in case of febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days).

Febrile illness surveillance

The subjects or the subject's parent(s)/LAR, as applicable, will be contacted at least weekly (eg, phone calls, home visits, or school-based surveillance) to ensure robust identification of febrile illness by reminding subjects or the

subject's parent(s)/LAR, as applicable, of their obligation to return to the site in case of febrile illness.

Handling of febrile illness cases (suspected dengue cases)

Subjects with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have 1 blood sample taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever) to confirm dengue infection. Testing will include dengue reverse transcriptase polymerase chain reaction (RT-PCR) and NS1 enzyme-linked immunosorbent assay (ELISA). Additional blood samples may be taken for diagnosis and clinical management of the subject as per standard medical practice. RT-PCR and NS1 ELISA results from the central laboratory will not be available for real time case management.

A new episode of febrile illness as described above will require an interval of at least 14 days from a previous febrile illness episode (ie, counting from the first day of febrile illness).

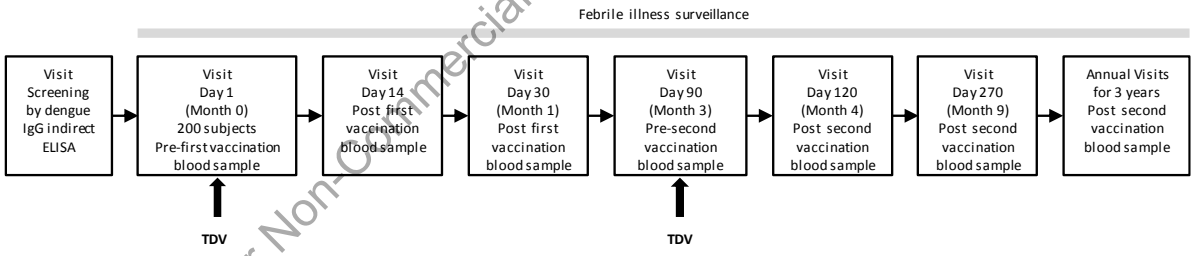
Duration of febrile illness surveillance

For each subject, surveillance for febrile illness will commence on the day of first vaccination (Day 1 [Month 0]) and will end 3 years after the second vaccination at Day 90 (Month 3).

Procedures

After informed consent/assent has been obtained, each subject will be assessed for eligibility to participate in the study. A dengue immunoglobulin G (IgG) indirect ELISA will be performed during the screening period to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each study center. Enrolment tracking of dengue naïve and dengue exposed subjects will be done using the interactive web response system. Subjects may proceed with vaccination in the study prior to availability of the screening dengue IgG indirect ELISA result until approximately 60% of subjects (ie, sample size at each study center) are of the same dengue Baseline seropositivity status. Once this limit is reached, the dengue Baseline seropositivity status (ie, screening dengue IgG ELISA result) will be reviewed before vaccination. At Day 1 (Month 0), a pre-vaccination blood sample will be taken and vaccination will occur. A second vaccination will be administered at Day 90 (Month 3). Subjects will be followed-up for 3 years post second vaccination. The study design is presented in Figure 1.

Figure 1: Schematic of Study DEN-313



ELISA=enzyme-linked immunosorbent assay, IgG=Immunoglobulin G, TDV=tetravalent dengue vaccine candidate
Notes:
(i) Approximately 40% or more subjects of either dengue Baseline seropositivity status at each study center.
(ii) Subjects presenting with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have 1 blood sample taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever).
(iii) Visits at Day 30 (Month 1) and Day 120 (Month 4) should occur at least 28 days after the first and second vaccination at Day 1 (Month 0) and Day 90 (Month 3), respectively. The window period (ie, -1/+7 days) for these visits will be calculated from the 30th day after each vaccination (ie, day of vaccination + 29 days).
(iv) Visit at Day 14 is for subjects >10 years of age.
(v) For each subject, additional blood samples will be taken annually for 3 years post-second vaccination at Day 90 (Month 3).
(vi) For each subject, surveillance for febrile illness and safety follow-up will end 3 years after the second vaccination at Day 90 (Month 3).

Assessment of dengue Baseline seropositivity status:

- A blood sample will be collected at Screening or pre-first vaccination at Day 1 (Month 0) if screening is performed the same day to determine dengue Baseline seropositivity status by dengue IgG indirect ELISA.

Immunogenicity evaluation:

- Blood samples for assessment of cellular immune responses will be collected from all subjects at pre-first vaccination (Day 1 [Month 0]), 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and annually for 3 years post second vaccination. One additional blood sample will be collected at Day 14 from subjects >10 years of age.
- Blood samples for assessment of dengue neutralizing antibodies (microneutralization test [MNT]) will be collected from all subjects at pre-first vaccination (Day 1 [Month 0]), 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and annually for 3 years post second vaccination.

Safety evaluation:

- Unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be collected by interview and will be recorded for all subjects (ie, at Day 30 [Month 1] and Day 120 [Month 4]).
- Medically attended AEs* (MAAE) will be collected from first vaccination at Day 1 (Month 0) up to 6 months post second vaccination at Day 90 (Month 3) by interview and will be recorded (ie, at Day 30 [Month 1], Day 90 [Month 3], Day 120 [Month 4], and Day 270 [Month 9]).

  *AEs leading to a medical visit to or by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria.
- Collection of serious adverse events (SAE) and AEs leading to subject discontinuation or withdrawal for the entire study duration.
- Identification of febrile episodes with potential dengue etiology for the entire study duration.

Primary Objective:

- To assess the cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years at 1 month post second vaccination.

Secondary Objectives:

- To assess cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years up to 3 years post second vaccination.
- To assess cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years by region and dengue Baseline seropositivity status.
- To characterize phenotype of cellular immune responses to TDV by ICS in a subset of study subjects.
- To assess the post-vaccination neutralizing antibody response against each dengue serotype.
- To assess the post-vaccination neutralizing antibody response against multiple dengue serotypes.
- To describe the safety of 2 doses of TDV in healthy subjects aged 4 to 16 years.

Exploratory Objectives:

CCI [redacted]

Subject Population:

Healthy subjects: yes
Planned minimum age: 4 years
Planned number of subjects: 200
Planned number of Arms: 1; 2-dose regimen (1 dose at Day 1 [Month 0] and 1 dose at Day 90 [Month 3]), SC route.

Criteria for Inclusion:

1. The subject is aged 4 to 16 years, inclusive (Latin America) or 4 to 8 years, inclusive (Asia).
2. Individuals who are in good health at the time of entry into the study as determined by medical history, physical examination (including vital signs), and clinical judgment of the investigator.
3. The subject or the subject's parent(s)/LAR signs and dates a written informed consent form and any required privacy authorization prior to the initiation of any study procedures, after the nature of the study has been explained according to local regulatory requirements. Assent is obtained from the subject where required.
4. Individuals who can comply with study procedures and are available for the duration of follow-up.

Criteria for Exclusion:

1. Febrile illness (body temperature ≥38°C) or moderate or severe acute illness or infection at the time of enrolment.
2. History or any illness that, in the opinion of the investigator, might interfere with the results of the study or pose an additional risk to the subject due to participation in the study, including but not limited to:
    a. Known hypersensitivity or allergy to any of the vaccine components.
    b. Female subjects (post-menarche) who are pregnant or breastfeeding.
    c. Individuals with any serious chronic or progressive disease (eg, neoplasm, insulin-dependent diabetes, cardiac, renal or hepatic disease, neurologic or seizure disorder or Guillain-Barré syndrome).
    d. Known or suspected impairment/alteration of immune function, including:
        i. Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/ ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0) (use of inhaled, intranasal, or topical corticosteroids is allowed).
        ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks / ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0).
        iii. Administration of immunoglobulins and/or any blood products within the 3 months prior to Day 1 (Month 0) or planned administration during the study.
        iv. Receipt of immunostimulants within 60 days prior to Day 1 (Month 0).
        v. Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (Month 0).
        vi. Human immunodeficiency virus (HIV) infection or HIV-related disease.
        vii. Genetic immunodeficiency.
3. Receipt of any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 (Month 0) or planning to receive any vaccines within 28 days after Day 1 (Month 0).
4. Participation in any clinical study with another investigational product 30 days prior to Day 1 (Month 0) or intent to participate in another clinical study at any time during the conduct of this study.
5. Previous participation in any clinical study of a dengue candidate vaccine, or previous receipt of any dengue vaccines (investigational or licensed).
6. First degree relatives of individuals involved in the conduct of the study.
7. Females of childbearing potential[1] who are sexually active, and who have not used any acceptable contraceptive methods[2] for at least 2 months prior to Day 1 (Month 0).

[1] Defined as status post onset of menarche and not meeting any of the following conditions: bilateral tubal ligation (at least 1 year previously), bilateral oophorectomy (at least 1 year previously) or hysterectomy.

[2] Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring); barrier (condom with spermicide or diaphragm with spermicide) each and every time during intercourse; intrauterine device (IUD); monogamous relationship with vasectomized partner (partner must have been vasectomized for at least 6 months prior to Day 1 [Month 0]). Other contraceptive methods may be considered in agreement with the sponsor and will be approved by the appropriate ethics committee.

8. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method or avoid donation of ova up to 6 weeks post second vaccination.
9. Deprived of freedom by administrative or court order, or in an emergency setting, or hospitalized involuntarily.
10. Current alcohol abuse or drug addiction that may interfere with the subject's ability to comply with study procedures.
11. Identified as an employee of the investigator or study center, with direct involvement in the proposed study or other studies under the direction of that investigator or study center.

There may be instances when individuals meet all entry criteria except one that relates to transient clinical circumstances (eg, body temperature elevation or recent use of excluded medication or vaccine). Under these circumstances, eligibility for first vaccination may be considered if the appropriate window for delay has passed, inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

Subjects who are otherwise eligible but cannot receive the study vaccination because the limit of approximately 60% of subjects (ie, sample size at each study center) with the same dengue Baseline seropositivity status is reached will not continue in the study and will be deemed screen failures.

Criteria for delay of second vaccination at Day 90 (Month 3):

Subjects may encounter clinical circumstances that warrant a delay in the administration of second study vaccination. These situations are listed below. In the event that a subject meets a criterion for delay of vaccination, the subject may receive study vaccination once the window for delay has passed as long as the subject is otherwise eligible for study participation. The decision to vaccinate in those situations will be taken by the investigator.

1. Body temperature ≥38.0°C within 3 days of intended study vaccination.
2. Receipt of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation within 2 weeks of intended study vaccination.
3. Receipt of any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) of intended study vaccination.
4. Receipt of oral or parenteral steroids or immunosuppressive therapy within 1 month of intended study vaccination; dosage and duration of treatments for steroids are specified under the exclusion criteria.

Criteria for contraindication to vaccination at Day 90 (Month 3):

There are also circumstances under which receipt of second vaccination is a contraindication in this study. These circumstances include but are not limited to anaphylaxis or severe hypersensitivity reactions following the first vaccination. If these reactions occur, the subject must not receive second vaccination but is encouraged to continue study participation to enable continued surveillance for dengue and safety follow-up.

Study Vaccine:

The investigational vaccine is TDV, a tetravalent dengue vaccine comprised of 1 molecularly characterized, attenuated dengue virus strain, and 3 chimeric dengue virus strains: ~2 x 10^4, 5 x 10^3, 1 x 10^5, and 3 x 10^5 plaque forming units (PFU) of TDV-1, TDV-2, TDV-3, and TDV-4, respectively.

Duration of the Study:	**Period of Evaluation:**
The study duration for each subject will be approximately 3 years and 4 months including screening (up to 28 days prior to Day 1 [Month 0]), vaccination (Day 1 [Month 0] and Day 90 [Month 3]), and 3 years follow-up post second vaccination).	For the duration of a subject's participation.

Main Criteria for Evaluation and Analyses:

Primary Endpoint:

- Frequency of cellular immune responses to 2 doses of TDV at 1 month post second vaccination (Day 120 [Month 4]).
  Cellular immune response is defined as an IFN-γ ELISPOT response that is >3 times higher compared to Baseline (Day 1 [Month 0]) and ≥5 spots per well.

Secondary Endpoints:

- Magnitude of IFN-γ ELISPOT responses to 2 doses of TDV at 1 month post second vaccination (Day 120 [Month 4]).
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 6 months post second vaccination (Day 270 [Month 9]), and then annually for 3 years post second vaccination.
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV by region and dengue Baseline seropositivity status at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and then annually for 3 years post second vaccination.
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV at Day 14 in subjects >10 years of age.
- Phenotype characterization of cellular immune responses to TDV in a subset of study subjects by ICS at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and then annually for 3 years post second vaccination. Markers will include CD4, CD8, IFN-γ, TNF-α and IL-2. This subset of subjects will be selected from samples with IFN-γ ELISPOT responses >500 spot forming cells/10^6 cells and availability of sufficient cells.
- Phenotype characterization of cellular immune responses to TDV by region and dengue Baseline seropositivity status in a subset of study subjects by ICS at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and then annually for 3 years post second vaccination. Markers will include CD4, CD8, IFN-γ, TNF-α and IL-2. This subset of subjects will be selected from samples with IFN-γ ELISPOT responses >500 spot forming cells/10^6 cells and availability of sufficient cells.
- Geometric mean titer (GMT) of neutralizing antibodies for each of the 4 dengue serotypes.
- Seropositivity rates* (% of subjects) for each of the 4 dengue serotypes.
- Seropositivity rates* (% of subjects) for multiple (2, 3 or 4) dengue serotypes.

*Seropositivity is defined as a reciprocal neutralizing titer ≥10.

- Percentage of subjects experiencing non-serious unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose.
- Percentage of subjects with MAAEs from first vaccination up to 6 months post second vaccination.
- Percentage of subjects experiencing SAEs throughout the study.
- Percentage of subjects with virologically confirmed febrile illness with potential dengue etiology throughout the study.

Exploratory Endpoints:

CCI

Statistical Considerations:

All analyses will be descriptive; no statistical hypotheses will be tested in this study. Further details will be provided in the statistical analysis plan (SAP).

Analysis sets

Safety set: The safety set will consist of all subjects who received at least 1 dose of the study vaccine.

Full analysis set (FAS): The FAS will include all subjects who received at least 1 dose of the study vaccine and for whom a valid pre-dosing and at least 1 post-dosing blood sample have been received for immunogenicity.

Per-protocol set (PPS): The PPS will include all subjects in the FAS who have no major protocol violations. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving prohibited therapies, (3) not receiving 2 doses of study vaccine or receiving the second vaccination inadmissibly outside of the visit window, and (4) other major protocol violations that may be identified during data reviews.

Analysis of demographics and other Baseline characteristics

Age, gender, race, and other Baseline characteristics will be summarized descriptively for all enrolled subjects.

Immunogenicity analysis

For the primary immunogenicity endpoint (ie, frequency of cellular immune response), descriptive statistics will be provided by visit. Cellular immune response is defined as an IFN-γ ELISPOT response that is >3 times higher compared to Baseline (Day 1 [Month 0]) and ≥5 spots per well. Summaries will be provided based on all subjects, by region as well as dengue Baseline seropositivity status. The primary analyses will be based on the PPS; supportive analyses may be provided based on the FAS.

Similar analysis as for the primary immunogenicity endpoint will be provided for the secondary immunogenicity endpoints related to cellular immune response. For the secondary immunogenicity endpoints including seropositivity rates and GMTs for dengue neutralizing antibodies, descriptive statistics and 95% CIs will be provided by visit.

Seropositivity is defined as a reciprocal neutralizing titer ≥10. Dengue seropositivity at Baseline is defined as a reciprocal neutralizing titer ≥10 for one or more dengue serotypes.

CCI

Safety analysis

Safety data will be summarized descriptively based on the safety set. AEs will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA).

Unsolicited AEs, MAAEs, AEs leading to withdrawal, and SAEs will be summarized by system organ class (SOC) and preferred term (PT).

The percentage of subjects with virologically confirmed dengue will also be summarized.

Sample Size Justification:

This study is designed to be primarily descriptive and is not based on testing formal null hypotheses. Therefore, the sample size was not determined based on formal statistical power calculations. The number of subjects will provide a reasonable sample size for the evaluation of the objectives of the study.

Interim Analysis:

An interim analysis on safety and immunogenicity data is planned when all subjects have completed the Day 270 (Month 9) visit.

Data Monitoring Committee:

A data monitoring committee (DMC) will have oversight of this study. The DMC functions at a program level and further information is available in the DMC Charter.

2.1 Schedule of Study Procedures

Table 2.a presents the schedule of study procedures.

Table 2.a Schedule of Study procedures

	Screening (a)	Day 1 (Month 0)	Day 14 (b)	Day 30 (Month 1) (c)	Day 90 (Month 3)	Day 120 (Month 4) (c)	Day 270 (Month 9)	Annually for 3 years post second vaccination	Follow-up Visit (d)
Visit window	Up to 28 days prior to Day 1 (Month 0)		± 2 days	- 1/+ 7 days	± 15 days	- 1/+ 7 days	± 21 days	365 days from last vaccination ± 45 days	
Visits	X	X	X	X	X	X	X	X	
Signed informed consent/assent	X								
Assessment of eligibility criteria (e)	X	X							
Check contraindications to vaccination					X				
Check criteria for delay of vaccination					X				
Demographics	X								
Medical history	X	X			X				X
Concomitant medications/ vaccinations history (f)	X	X		X	X	X	X	X	X
Complete physical examination (g)	X	X			X				
Targeted physical examination (h)			X	X		X	X	X	X
Pregnancy test (i)	X	X			X				
Vaccine administration (j)		X			X				
Blood sample for screening by dengue IgG indirect ELISA (3 mL) (k)	X								
Blood sample (l)		X	X	X	X	X	X	X	
Surveillance for dengue fever (m)		X							
Febrile illness blood sample (n)		X							
Documentation of unsolicited adverse events (AEs) and medically attended AEs (o)		X	X	X	X	X	X		
Serious AEs and AEs leading to subject discontinuation or withdrawal (p)		X							

(a) Screening and the Day 1 (Month 0) visit may be performed on the same day depending on the availability and requirement of the dengue immunoglobulin G indirect enzyme-linked immunosorbent assay (dengue IgG indirect ELISA) result before first vaccination. All marked under either Screening or Day 1 (Month 0) is to be performed only once if screening is performed at Day 1 (Month 0).
(b) Visit only for subjects >10 years of age. See also footnote (l).
(c) Visits at Day 30 (Month 1) and Day 120 (Month 4) should occur at least 28 days after the day of the first and second vaccination at Day 1 (Month 0) and Day 90 (Month 3), respectively. The window period (ie, -1/+ 7 days) for these visits will be calculated from the 30th day after each vaccination (ie, day of vaccination + 29 days).
(d) Follow-up visit is only applicable if the subject terminates early. The follow-up visit should be performed as soon as possible and preferably at least 28 days after the last study vaccination.
(e) Eligibility by review of inclusion/exclusion criteria will be documented before entry into the study.
(f) History of vaccination against Japanese Encephalitis or Yellow Fever irrespective of time of administration and including the

vaccine type as well as any supportive documentation for these vaccinations, all concomitant medications and vaccine history from 1 month (minimum 28 days) prior to administration of each dose of TDV up to 1 month (minimum 28 days) thereafter, steroids and immunostimulants within 60 days prior to Day 1 (Month 0), immunoglobulins and blood products within 3 months prior to Day 1 (Month 0), and immunosuppressive therapy within 6 months prior to Day 1 (Month 0).
(g) Physical examination including measurement of weight and height; body mass index will be calculated automatically. Measurement of height is only required at Screening and at Day 1 (Month 0).
(h) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, body temperature, height and weight. Measurement of height is only required at Day 270 (Month 9), and annually for 3 years post second vaccination at Day 90 (Month 3).
(i) Pregnancy testing (serum or urine) for females of childbearing potential. Results must be confirmed and documented as negative prior to each study vaccine administration. Additional pregnancy tests may be performed during the study if deemed necessary by the investigator. Female of childbearing potential who are sexually active will be reminded during study visits to adhere to acceptable contraceptive methods up to 6 weeks post second vaccination.
(j) Subjects will be observed for at least 30 minutes after administration of each vaccine dose.
(k) A blood sample (approximately 3 mL) will be collected from all subjects to perform a dengue IgG indirect ELISA during the screening period to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each study center.
(l) Blood samples for assessment of dengue neutralizing antibodies and cellular immune responses will be collected from all subjects at pre-first vaccination (Day 1 [Month 0]), 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and annually for 3 years post second vaccination. One additional blood sample will be collected at Day 14 from subjects >10 years of age. Blood volumes for assessment of dengue neutralizing antibodies will be 2.5 mL at Day 30 (Month 1) and 5 mL at each of the other specified visits. The blood volume for assessment of cellular immune responses will be 20 mL at each specified visit. In subjects ≤10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 3 mL/kg or 50 mL in total (whichever is lower) within 8 weeks. In subjects >10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 5 mL/kg within 8 weeks.
(m) The subjects or the subject's parent(s)/legally authorized representative (LAR), as applicable, will be contacted at least weekly (eg, phone calls, home visits, or school-based surveillance) to ensure robust identification of febrile illness by reminding subjects or the subject's parent(s)/LAR, as applicable, of their obligation to return to the site in case of febrile illness.
(n) Subjects with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have a blood sample (4 mL) taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever). Additional blood samples may be taken for diagnosis and clinical management of the subject as per standard medical practice.
(o) Unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be collected by interview and will be recorded for all subjects at Day 30 (Month 1) and Day 120 (Month 4). Medically attended AEs occurring from first vaccination at Day 1 (Month 0) up to 6 months post second vaccination at Day 90 (Month 3) will be collected by interview and will be recorded at Day 30 (Month 1), Day 90 (Month 3), Day 120 (Month 4]) and Day 270 (Month 9).
(p) All serious AEs and AEs leading to subject discontinuation or withdrawal from first vaccination until the end of the study.

3.0 STUDY REFERENCE INFORMATION

3.1 Study-Related Responsibilities

The sponsor will perform all study-related activities with the exception of those identified in the Study-Related Responsibilities template. The identified vendors in the template for specific study-related activities will perform these activities in full or in partnership with the sponsor.

3.2 Principal Investigator/Coordinating Investigator

The sponsor will select a Signatory Principal Investigator / Coordinating Investigator from the investigators who participate in the study. Selection criteria for this investigator will include significant knowledge of the study protocol, the investigational vaccine, their expertise in the therapeutic area and the conduct of clinical research as well as study participation. The Signatory Principal Investigator / Coordinating Investigator will be required to review and sign the clinical study report and by doing so agrees that it accurately describes the results of the study.

3.3 List of Abbreviations

AE	Adverse event
CD	Cluster of differentiation
CMI	Cell-mediated immunity
Dengue IgG indirect ELISA	Dengue immunoglobulin G indirect enzyme-linked immunosorbent assay
DENV	Wild type dengue virus
DHF	Dengue hemorrhagic fever
DMC	Data monitoring committee
DSS	Dengue shock syndrome
E	Envelope
eCRF	electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMT	Geometric mean titer
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonization
ICS	Intracellular cytokine staining
IEC	Independent ethics committee
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IL-2	Interleukin-2
Inc	Incorporated
IRB	Institutional review board
IWRS	Interactive web response system
LAR	Legally authorized representative
MAAE	Medically attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
MNT	Microneutralization test
NS	Nonstructural
PBMC	Peripheral blood mononuclear cells
PPS	Per-protocol set

prM	pre-membrane
PT	Preferred term
RT-PCR	Reverse transcriptase polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SOC	System organ class
SUSAR	Suspected unexpected serious adverse reactions
TDV	Tetravalent dengue vaccine candidate
TDV-4	Dengue serotypes 2/4 chimeric strain
TDV-1	Dengue serotypes 2/1 chimeric strain
TDV-3	Dengue serotypes 2/3 chimeric strain
TDV-2	Molecularly characterized, attenuated dengue serotype 2 strain
TNF-α	Tumor necrosis factor-alpha
WHO	World Health Organization

3.4 Corporate Identification

TV Takeda Vaccines, Inc.

4.0 INTRODUCTION

4.1 Background

Dengue fever is caused by infection with wild type dengue virus (DENV), a ribonucleic acid (RNA) virus that occurs as 4 recognized serotypes, DENV-1, DENV-2, DENV-3 or DENV-4. These dengue viruses are transmitted from human to human by mosquitoes (primarily *Aedes aegypti*). The 4 dengue viruses have spread worldwide and are endemic in Asia, Central and South America, the Caribbean, the Pacific Islands, and parts of Africa. There are an estimated 390 million dengue infections per year worldwide, which is more than 3 times the previous World Health Organization estimate of 50 to 100 million cases. Every year, around 500,000 cases of dengue hemorrhagic fever (DHF) require hospitalization with an estimated death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection [1-4].

Dengue fever is clinically defined as an acute febrile illness with 2 or more manifestations (headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leucopenia) and occurrence at the same location and time as other confirmed cases of dengue fever. The most severe forms of dengue infection (ie, DHF and dengue shock syndrome [DSS]) are life threatening. Primary infection with any 1 of the 4 dengue serotypes is thought to result in life-long protection from re-infection by the same serotype, but does not protect against a secondary infection by 1 of the other 3 dengue virus serotypes and may lead to an increased risk of severe disease (DHF/DSS) [3-6].

Treatment of dengue fever is based solely on symptoms and signs, with fluid replacement required for hemorrhagic or shock cases. An antiviral therapy for dengue virus infection is not available. Preventive measures that rely on mosquito control and individual protection, are of limited efficacy, complex to implement and questionable in terms of cost-effectiveness. There is a great global public health need for a safe and effective vaccine that will protect against all serotypes of dengue infection, and thereby reduce the morbidity and mortality associated with dengue disease [1-7]. A tetravalent dengue vaccine (CYD-TDV) has been recently approved in some countries in Asia and Latin America [8]. However, the initial findings suggest an unfavorable risk-benefit profile for younger subjects with the approved vaccine. Vaccine efficacy was different between serotypes and depended on dengue Baseline seropositivity status. Hence, there is a continued unmet public health need for safer and more efficacious dengue vaccines. Additional vaccines are also important to ensure sufficient supply globally [9,10].

Tetravalent Dengue Vaccine Candidate (TDV) - Background:

Takeda's TDV consists of 1 molecularly characterized, attenuated dengue virus strain and 3 recombinant dengue virus strains expressing surface antigens corresponding to dengue serotypes 1-4. The dengue serotype 2 strain is based upon the attenuated laboratory-derived virus, DENV-2 Primary Dog Kidney (PDK)-53, originally isolated at Mahidol University, Bangkok, Thailand [11]. The chimeric, attenuated vaccine strains for dengue serotypes 1, 3, and 4 were engineered by substituting the structural genes, pre-membrane (prM) and envelope (E), of TDV-2 with the prM and E genes of the DENV strains, DENV-1 16007, DENV-3 16562 or DENV-4 1036 virus,

respectively [12]. TDV is thus comprised of 4 dengue virus strains: a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), a dengue serotypes 2/1 chimeric strain (TDV-1), a dengue serotypes 2/3 chimeric strain (TDV-3), and a dengue serotypes 2/4 chimeric strain (TDV-4).

Nonclinical studies carried out in mice and nonhuman primates demonstrated an acceptable safety, immunogenicity, and efficacy profile of Takeda's TDV.

Data from 4 completed phase 1 studies, 1 completed phase 2 study, and 2 ongoing phase 2 studies have shown satisfactory reactogenicity, safety and immunogenicity of Takeda's TDV in adults in non-endemic areas as well as in adults and children in endemic areas in Asia and Latin America. Currently ongoing and completed phase 2 studies have enabled the selection of a final TDV dose (lyophilized formulation), and a 2-dose vaccination regimen for subcutaneous (SC) administration 3 months apart for use in the pivotal program.

The current Investigator Brochure for Takeda's TDV contains additional product information and a more detailed review of pre-clinical and clinical studies [13].

4.2 Rationale for the Proposed Study

Studies investigating the role of cell-mediated immunity (CMI) in natural infections have demonstrated roles in both virus clearance and potentiating disease [14]. These investigations have been extended from the exploratory study of CMI in natural infection to the study of dengue vaccine recipients. Knowledge about specific CMI correlates of protection is incomplete. However, the exploration of CMI is encouraged by the World Health Organization (WHO) since specific CMI assays may be useful for the assessment of immunological memory and durability of protection. Assessments of cytokine responses may also assist in the evaluation of vaccine safety and may provide some indication of the potential risk that vaccination could predispose subjects to develop severe dengue febrile illness (DHF/DSS) during subsequent natural infection [15].

Studies in nonhuman primates have demonstrated that TDV elicits cluster of differentiation (CD)4+ and CD8+ T cell responses targeting the nonstructural (NS) proteins NS1, NS3, and NS5 of TDV-2. Both T cell subsets produced interleukin-2 (Il-2), interferon-gamma (IFN-γ), and tumor necrosis factor-alpha (TNF-α), and were multifunctional in nature. In addition, CD8+ T cells expressed the CD107α marker, and exhibited cross-reactivity with the NS proteins of the other 3 TDV serotypes.

Subsequently, investigations of clinical samples from phase 1 studies have demonstrated generation of multi-lineage CMI after administration of TDV. Potent IFN-γ enzyme-linked immunospot (ELISPOT) responses were detected against serotype matched antigens which were confirmed by intracellular cytokine staining (ICS). The responses were predominantly mediated by CD8+ T cells, but a population of dengue-specific CD4+ T cells was also mobilized by the vaccine. Responses were directed against all regions of the TDV-2 proteome and also the TDV-1, -3, and -4 structural components of the vaccine. Cross-reactivity of responses to the TDV-2 proteome with DENV-1, -3 and -4 NS1, NS3, and NS5, which are not components of the vaccine, was also detected.

The DEN-313 study is planned to investigate cellular immune responses following TDV vaccination in dengue endemic regions and will contribute to immunogenicity data obtained from the ongoing phase 3 efficacy study (DEN-301) in a similar population comprised of subjects 4 to 16 years of age living in dengue endemic regions (Asia and Latin America).

This study will be conducted in compliance with the protocol, the International Conference on Harmonization and Good Clinical Practice (ICH-GCP) guidelines and applicable regulatory requirement(s).

5.0 STUDY OBJECTIVES AND ENDPOINTS

5.1 Objectives

5.1.1 Primary Objective

- To assess the cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years at 1 month post second vaccination.

5.1.2 Secondary Objectives

- To assess cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years up to 3 years post second vaccination.
- To assess cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years by region and dengue Baseline seropositivity status.
- To characterize phenotype of cellular immune responses to TDV by ICS in a subset of study subjects.
- To assess the post-vaccination neutralizing antibody response against each dengue serotype.
- To assess the post-vaccination neutralizing antibody response against multiple dengue serotypes.
- To describe the safety of 2 doses of TDV in healthy subjects aged 4 to 16 years.

5.1.3 Exploratory Objectives

CCI [redacted]

5.2 Endpoints

5.2.1 Primary Endpoint

- Frequency of cellular immune responses to 2 doses of TDV at 1 month post second vaccination (Day 120 [Month 4]).

  Cellular immune response is defined as an IFN-γ ELISPOT response that is >3 times higher compared to Baseline (Day 1 [Month 0]) and ≥5 spots per well.

5.2.2 Secondary Endpoints

- Magnitude of IFN-γ ELISPOT responses to 2 doses of TDV at 1 month post second vaccination (Day 120 [Month 4]).
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 6 months post

second vaccination (Day 270 [Month 9]), and then annually for 3 years post second vaccination.

- Frequency and magnitude of IFN-γ ELISPOT responses to TDV by region and dengue Baseline seropositivity status at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and then annually for 3 years post second vaccination.
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV at Day 14 in subjects >10 years of age.
- Phenotype characterization of cellular immune responses to TDV in a subset of study subjects by ICS at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and then annually for 3 years post second vaccination. Markers will include CD4, CD8, IFN-γ, TNF-α and IL-2. This subset of subjects will be selected from samples with IFN-γ ELISPOT responses >500 spot forming cells/10^6 cells and availability of sufficient cells.
- Phenotype characterization of cellular immune responses to TDV by region and dengue Baseline seropositivity status in a subset of study subjects by ICS at 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and then annually for 3 years post second vaccination. Markers will include CD4, CD8, IFN- γ, TNF-α and IL-2. This subset of subjects will be selected from samples with IFN- γ ELISPOT responses >500 spot forming cells/10^6 cells and availability of sufficient cells.
- Geometric mean titer (GMT) of neutralizing antibodies for each of the 4 dengue serotypes.
- Seropositivity rates* (% of subjects) for each of the 4 dengue serotypes.
- Seropositivity rates* (% of subjects) for multiple (2, 3 or 4) dengue serotypes.
- *Seropositivity is defined as a reciprocal neutralizing titer ≥10.
- Percentage of subjects experiencing non-serious unsolicited adverse events (AE) during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose.
- Percentage of subjects with medically attended AEs (MAAEs) from first vaccination up to 6 months post second vaccination.
- Percentage of subjects experiencing serious adverse events (SAE) throughout the study.
- Percentage of subjects with virologically confirmed febrile illness with potential dengue etiology throughout the study.

5.2.3 Exploratory Endpoints

CCI

[redacted]

6.0 STUDY DESIGN AND DESCRIPTION

6.1 Study Design

This is an open-label phase 2 study with a single study group. The target sample size is 200 healthy subjects (100 subjects aged 4 to 16 years in Latin America; 100 subjects aged 4 to 8 years in Asia). Each subject will receive a 2-dose schedule of TDV by SC injection into the upper arm at Day 1 (Month 0) and at Day 90 (Month 3). Subjects will be screened prior to vaccination to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each study center. Any withdrawals or screen failures from enrolment until vaccination at Day 1 (Month 0) will be replaced.

Subjects will be followed up for immunogenicity and safety assessments for 3 years after the second vaccination at Day 90 (Month 3) including febrile illness surveillance to monitor any febrile illness with potential dengue etiology for long term safety.

Definition of febrile illness:

The subjects and the subject's parent(s)/legally authorized representative (LAR), as applicable, will be asked to return to the study center for dengue fever evaluation of the subjects by the investigator in case of febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days).

Febrile illness surveillance:

The subjects or the subject's parent(s)/LAR, as applicable, will be contacted at least weekly (eg, phone calls, home visits, or school-based surveillance) to ensure robust identification of febrile illness by reminding subjects or the subject's parent(s)/LAR, as applicable, of their obligation to return to the site in case of febrile illness.

Handling of febrile illness cases (suspected dengue cases):

Subjects with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have 1 blood sample taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever) to confirm dengue infection. Testing will include dengue reverse transcriptase polymerase chain reaction (RT-PCR) and NS1 enzyme-linked immunosorbent assay (ELISA). Additional blood samples may be taken for diagnosis and clinical management of the subject as per standard medical practice. RT-PCR and NS1 ELISA results from the central laboratory will not be available for real time case management.

A new episode of febrile illness as described above will require an interval of at least 14 days from a previous febrile illness episode (ie, counting from the first day of febrile illness).

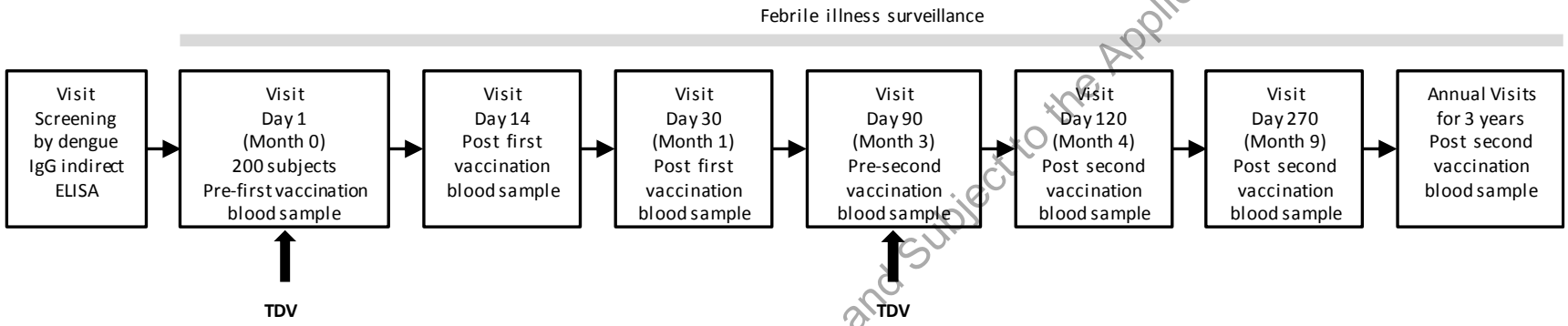
Duration of febrile illness surveillance:

For each subject, surveillance for febrile illness will commence on the day of first vaccination (Day 1 [Month 0]) and will end 3 years after the second vaccination at Day 90 (Month 3).

Procedures

After informed consent/assent has been obtained, each subject will be assessed for eligibility to participate in the study. A dengue immunoglobulin G (IgG) indirect ELISA will be performed during the screening period to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each study center. Enrolment tracking of dengue naïve and dengue exposed subjects will be done using the interactive web response system (IWRS). Subjects may proceed with vaccination in the study prior to availability of the screening IgG indirect ELISA result until approximately 60% of subjects (ie, sample size at each study center) are of the same dengue Baseline seropositivity status. Once this limit is reached, the dengue Baseline seropositivity status (ie, screening dengue IgG ELISA result) will be reviewed before vaccination. At Day 1 (Month 0), a pre-vaccination blood sample will be taken and vaccination will occur. A second vaccination will be administered at Day 90 (Month 3). Subjects will be followed-up for 3 years post second vaccination. The schematic of the study design is included as Figure 6.a. A schedule of study procedures is provided in Section 2.1.

Figure 6.a Schematic of Study Design



ELISA=enzyme-linked immunosorbent assay, IgG=Immunoglobulin G, TDV=tetravalent dengue vaccine candidate

Notes:

(i) Approximately 40% or more subjects of either dengue Baseline seropositivity status at each study center.

(ii) Subjects presenting with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have 1 blood sample taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever).

(iii) Visits at Day 30 (Month 1) and Day 120 (Month 4) should occur at least 28 days after the first and second vaccination at Day 1 (Month 0) and Day 90 (Month 3), respectively. The window period (ie, -1/+7 days) for these visits will be calculated from the 30th day after each vaccination (ie, day of vaccination + 29 days).

(iv) Visit at Day 14 is for subjects >10 years of age.

(v) For each subject, additional blood samples will be taken annually for 3 years post-second vaccination at Day 90 (Month 3).

(vi) For each subject, surveillance for febrile illness and safety follow-up will end 3 years after the second vaccination at Day 90 (Month 3).

Assessment of dengue Baseline seropositivity status:

- A blood sample will be collected at Screening or pre-first vaccination at Day 1 (Month 0) if screening is performed the same day to determine dengue Baseline seropositivity status by dengue IgG indirect ELISA.

Immunogenicity evaluation:

- Blood samples for assessment of cellular immune responses will be collected from all subjects at pre-first vaccination (Day 1 [Month 0]), 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and annually for 3 years post second vaccination. One additional blood sample will be collected at Day14 from subjects >10 years of age. Refer also to Appendix D.
- Blood samples for assessment of dengue neutralizing antibodies (microneutralization test [MNT]) will be collected from all subjects at pre-first vaccination (Day 1 [Month 0]), 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and annually for 3 years post second vaccination. Refer also to Appendix D.

Safety evaluation:

- Unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be collected by interview and will be recorded for all subjects (ie, at Day 30 [Month 1] and Day 120 [Month 4]).
- MAAEs* will be collected from first vaccination at Day 1 (Month 0) up to 6 months post second vaccination at Day 90 (Month 3) by interview and will be recorded (ie, at Day 30 [Month 1], Day 90 [Month 3], Day 120 [Month 4], and Day 270 [Month 9]).

  *AEs leading to a medical visit to or by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria.
- Collection of SAEs and AEs leading to subject discontinuation or withdrawal for the entire study duration.
- Identification of febrile episodes with potential dengue etiology for the entire study duration.

6.2 Justification for Study Design, Dose, and Endpoints

The present study (DEN-313) will contribute to immunogenicity data obtained from the ongoing phase 3 efficacy study (DEN-301); hence, the age range of study subjects (ie, 4 to 16 years of age), the study setting (ie, dengue endemic regions in Asia and Latin America countries), and the TDV dosing regimen (ie, 2-dose regimen 3 months apart) are similar for both studies.

The present study aims to have approximately 40% or more subjects of either dengue Baseline seropositivity status at each study center to ensure representative participation; hence, a dengue immunoglobulin G indirect enzyme-linked immunosorbent assay (dengue IgG indirect ELISA)

will be performed during the screening period. The upper age range in the study is 16 years; however, it is limited to 8 years in Asia to maximize chances of finding dengue naïve subjects.

In consideration of minimal variation over time and significant inter-individual variability of the Baseline cellular immune responses, a placebo group for comparison has not been included in this study. Baseline cellular immune responses will serve as the reference to evaluate vaccine effect. CMI will be evaluated before and 30 days after administration of each vaccine dose. In addition, CMI will be evaluated at Day 14 after first vaccination to capture early onset responses, but will be limited to subjects >10 years of age due to constraints of blood volume collection in younger subjects [16]. Blood samples will be collected at 6 months post second vaccination and thereafter annually for 3 years to evaluate the persistence of cellular immune responses induced by the vaccine. Phenotype characterization of cellular immune responses by ICS will be performed in a subset of subjects. This subset of subjects will be selected from samples with IFN-γ ELISPOT responses >500 spot forming cells/10^6 cells and availability of sufficient cells. In addition to CMI, blood samples will be collected to assess dengue neutralizing antibodies to explore correlation between neutralizing antibody response and cellular immune response.

Reactogenicity of TDV (ie, solicited local [injection site] and solicited systemic AEs) will be assessed in a sample of 4,000 subjects in the ongoing phase 3 efficacy study (DEN-301); hence, it is not planned in this study. Unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be recorded for all subjects, and MAAEs will be collected from the first vaccination at Day 1 (Month 0) up to 6 months post second vaccination at Day 90 (Month 3). SAEs will be collected for the entire study duration. This is consistent with general practice to collect AEs in vaccine studies.

Additionally, subjects will be actively followed up for 3 years post second vaccination to monitor any febrile illness with potential dengue etiology for long term safety. Surveillance for 3 years fulfils the WHO recommendation of long term safety follow-up in studies evaluating dengue vaccines in dengue endemic regions [6].

6.3 Duration of Subject's Expected Participation in the Entire Study

The study duration for each subject will be approximately 3 years and 4 months including screening (up to 28 days prior to Day 1 [Month 0]), vaccination (Day 1 [Month 0] and Day 90 [Month 3]), and 3 years follow-up post second vaccination).

6.4 Premature Termination or Suspension of Study or Investigational Site

6.4.1 Criteria for Premature Termination or Suspension of the Study

The study will be completed as planned unless one or more of the following criteria are satisfied that require temporary suspension or early termination of the study.

- New information or other evaluation regarding the safety or efficacy of the investigational vaccine that indicates a change in the known risk/benefit profile, such that the risk/benefit is no longer acceptable for subjects participating in the study.

- The data monitoring committee (DMC) recommends that the study should be suspended or terminated.
- Significant deviation from GCP that compromises the ability to achieve the primary study objectives or compromises subject safety.

6.4.2 Criteria for Premature Termination or Suspension of Investigational Sites

A study site may be terminated prematurely or suspended if the site (including the investigator) is found in significant deviation from GCP, protocol, or contractual agreement, is unable to ensure adequate performance of the study, or as otherwise permitted by the contractual agreement.

6.4.3 Procedures for Premature Termination or Suspension of the Study or the Participation of Investigational Site(s)

In the event that the sponsor, an institutional review board (IRB)/independent ethics committee (IEC) or regulatory authority elects to terminate or suspend the study or the participation of an investigational site, a study-specific procedure for early termination or suspension will be provided by the sponsor; the procedure will be followed by applicable investigational sites during the course of termination or study suspension.

7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS

All entry criteria, including test results, need to be confirmed prior to first vaccination.

7.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria:

1. The subject is aged 4 to 16 years, inclusive (Latin America) or 4 to 8 years, inclusive (Asia).
2. Individuals who are in good health at the time of entry into the study as determined by medical history, physical examination (including vital signs), and clinical judgment of the investigator.
3. The subject or the subject's parent(s)/LAR signs and dates a written informed consent form and any required privacy authorization prior to the initiation of any study procedures, after the nature of the study has been explained according to local regulatory requirements. Assent is obtained from the subject where required (Appendix C).
4. Individuals who can comply with study procedures and are available for the duration of follow-up.

7.2 Exclusion Criteria

Any subject who meets any of the following criteria will not qualify for entry into the study:

1. Febrile illness (body temperature ≥38°C) or moderate or severe acute illness or infection at the time of enrolment.
2. History or any illness that, in the opinion of the investigator, might interfere with the results of the study or pose an additional risk to the subject due to participation in the study, including but not limited to:
    a. Known hypersensitivity or allergy to any of the vaccine components.
    b. Female subjects (post-menarche) who are pregnant or breastfeeding.
    c. Individuals with any serious chronic or progressive disease (eg, neoplasm, insulin-dependent diabetes, cardiac, renal or hepatic disease, neurologic or seizure disorder or Guillain-Barré syndrome).
    d. Known or suspected impairment/alteration of immune function, including:
        i. Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/ ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0) (use of inhaled, intranasal, or topical corticosteroids is allowed).
        ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks / ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0).
        iii. Administration of immunoglobulins and/or any blood products within the 3 months prior to Day 1 (Month 0) or planned administration during the study.
        iv. Receipt of immunostimulants within 60 days prior to Day 1 (Month 0).

v. Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (Month 0).
vi. Human immunodeficiency virus (HIV) infection or HIV-related disease.
vii. Genetic immunodeficiency.

3. Receipt of any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 (Month 0) or planning to receive any vaccines within 28 days after Day 1 (Month 0).
4. Participation in any clinical study with another investigational product 30 days prior to Day 1 (Month 0) or intent to participate in another clinical study at any time during the conduct of this study.
5. Previous participation in any clinical study of a dengue candidate vaccine, or previous receipt of any dengue vaccines (investigational or licensed).
6. First degree relatives of individuals involved in the conduct of the study.
7. Females of childbearing potential who are sexually active, and who have not used any of the acceptable contraceptive methods for at least 2 months prior to Day 1 (Month 0).
    a. Of childbearing potential is defined as status post onset of menarche and not meeting any of the following conditions: bilateral tubal ligation (at least 1 year previously), bilateral oophorectomy (at least 1 year previously) or hysterectomy.
    b. Acceptable birth control methods are defined as 1 or more of the following:
        i. Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring).
        ii. Barrier (condom with spermicide or diaphragm with spermicide) each and every time during intercourse.
        iii. Intrauterine device (IUD).
        iv. Monogamous relationship with vasectomized partner (partner must have been vasectomized for at least 6 months prior to Day 1 [Month 0]).

    Other contraceptive methods may be considered in agreement with the sponsor and will be approved by the appropriate ethics committee.
8. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method or avoid donation of ova up to 6 weeks post second vaccination.
9. Deprived of freedom by administrative or court order, or in an emergency setting, or hospitalized involuntarily.
10. Current alcohol abuse or drug addiction that may interfere with the subject's ability to comply with study procedures.

11. Identified as an employee of the investigator or study center, with direct involvement in the proposed study or other studies under the direction of that investigator or study center.

There may be instances when individuals meet all entry criteria except one that relates to transient clinical circumstances (eg, body temperature elevation or recent use of excluded medication or vaccine). Under these circumstances, eligibility for first vaccination may be considered if the appropriate window for delay has passed, inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

Subjects who are otherwise eligible but cannot receive the study vaccination because the limit of approximately 60% of subjects (ie, sample size at each study center) with the same dengue Baseline seropositivity status is reached will not continue in the study and will be deemed screen failures.

7.3 Criteria for Delay of Second Vaccination at Day 90 (Month 3)

Subjects may encounter clinical circumstances that warrant a delay in the administration of second study vaccination. These situations are listed below. In the event that a subject meets a criterion for delay of vaccination, the subject may receive study vaccination once the window for delay has passed as long as the subject is otherwise eligible for study participation. The decision to vaccinate in those situations will be taken by the investigator.

1. Body temperature ≥38.0°C within 3 days of intended study vaccination.
2. Receipt of blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation within 2 weeks of intended study vaccination.
3. Receipt of any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) of intended study vaccination.
4. Receipt of oral or parenteral steroids or immunosuppressive therapy within 1 month of intended study vaccination; dosage and duration of treatments for steroids are specified under the exclusion criteria.

<u>Criteria for contraindication to vaccination at Day 90 (Month 3):</u>

There are also circumstances under which receipt of second vaccination is a contraindication in this study. These circumstances include but are not limited to anaphylaxis or severe hypersensitivity reactions following the first vaccination. If these reactions occur, the subject must not receive second vaccination but is encouraged to continue study participation to enable continued surveillance for dengue and safety follow-up.

7.4 Criteria for Discontinuation or Withdrawal of a Subject

The primary reason for discontinuation or withdrawal of the subject from the study should be recorded in the electronic Case Report Form (eCRF) using the following categories. For screen failure subjects, refer to Section 9.1.13.

1. Protocol deviation: The subject may remain in the study unless continuation in the study jeopardizes the subject's health, safety or rights.
2. Adverse Event: The subject has experienced an AE that requires early termination because continued participation imposes an unacceptable risk to the subject's health or the subject is unwilling to continue because of the AE.
3. Lost to follow-up: The subject did not return to the clinic and attempts to contact the subject or the subject's parent(s)/LAR were unsuccessful. Attempts to contact the subject or the subject's parent(s)/LAR must be documented. Lost to follow-up status will only be confirmed at the time of last subject last visit at the particular study site.
4. Withdrawal by subject: The subject (or subject's parent[s]/LAR) wishes to withdraw from the study. The reason for withdrawal, if provided, should be recorded in the eCRF.

   Note: All attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be recorded (ie, withdrawal due to an AE should not be recorded in the "voluntary withdrawal" category).

   Note: subjects will be considered as participating in the study up to 3 years post second vaccination unless they explicitly withdraw their consent (febrile surveillance will continue and febrile episodes will be recorded in the eCRF per protocol definition).
5. Study terminated by sponsor.
6. Pregnancy: Any subject who, despite the requirement for adequate contraception, becomes pregnant during the study will not receive further study vaccines. The site should maintain contact with the pregnant subject and complete a "Clinical Study Pregnancy Form" as soon as possible. In addition, the subject should be followed-up until the birth of the child, or spontaneous or voluntary termination; when pregnancy outcome information becomes available, the information should be captured using the same form. The subject should be reported as a withdrawal from study and the reason for withdrawal (eg, pregnancy) recorded in detail on the Study Termination" eCRF and subject' medical records.
7. Receipt of any other dengue vaccines (investigational or licensed) during the study.
8. Other.

   Note: The specific reasons should be recorded in the "specify" field of the eCRF.

7.5 Procedures for Discontinuation or Withdrawal of a Subject

The investigator may terminate a subject's study participation at any time during the study when the subject meets the study termination criteria described in Section 7.4. In addition, a subject may

discontinue his or her participation without giving a reason at any time during the study. Should a subject's participation be discontinued, the primary criterion for termination must be recorded. In addition, efforts should be made to perform all procedures as scheduled for the follow-up visit which should be performed as soon as possible and preferably at least 28 days after the last study vaccination. Discontinued or withdrawn subjects will not be replaced.

All withdrawn and discontinued subjects after vaccination at Day 1 (Month 0) will be followed for safety monitoring until the end of the study unless subjects are lost to follow-up or specifically withdrawn from febrile surveillance and safety follow-up. Those withdrawn or discontinued prior to vaccination will not be followed up for safety.

8.0 CLINICAL STUDY MATERIAL MANAGEMENT

This section contains information regarding all vaccines and materials provided directly by the sponsor, and/or sourced by other means, that are required by the study protocol, including important sections describing the management of clinical study material.

8.1 Study Vaccine(s) and Materials

The investigational vaccine is TDV, a tetravalent dengue vaccine comprised of 1 molecularly characterized, attenuated dengue virus strain, and 3 chimeric dengue virus strains: ~2 x 10^4, 5 x 10^3, 1 x 10^5, and 3 x 10^5 plaque forming units (PFU) of TDV-1, TDV-2, TDV-3, and TDV-4, respectively.

8.1.1 Dosage Form, Manufacturing, Packaging, and Labeling

TDV diluent:

TDV diluent (37 mM sodium chloride solution) is manufactured by CCI [redacted]. The diluent CCI [redacted] is provided in a single-use CCI [redacted] The vials will be labeled with a single panel or booklet label that will contain pertinent study information in local languages.

TDV:

CCI [redacted]

Lyophilized TDV is presented in a single-use, CCI [redacted]. The vials will be labeled with a single panel or booklet label that will contain pertinent study information in local languages.

Lyophilized TDV will be reconstituted by adding the entire extractable content of the diluent vial to yield a dose of CCI [redacted] vaccine. CCI [redacted]. TDV will be administered by SC route.

The TDV and diluent vials are packaged together into single dose dispensing cartons. The cartons will be labeled with a single panel or booklet label that will contain pertinent study information in local languages.

8.1.2 Storage

TDV and diluent will be shipped in refrigerated containers CCI [redacted] From receipt and prior to use, lyophilized TDV kits must be protected from light and stored at CCI [redacted] in a refrigerator.

All clinical study material must be kept in an appropriate, limited-access, secure place until it is used or returned to the sponsor or its designee for destruction. All sponsor-supplied vaccine(s) must be stored under the conditions specified on the label, and remain in the original container until dispensed. A daily temperature log of the vaccine storage area must be maintained every

working day. Temperature excursions must be reported to the sponsor as soon as possible and use of these vaccines requires sponsor approval.

8.1.3 Dose and Regimen

Subjects will receive a 2-dose regimen (Day 1 [Month 0]) and Day 90 [Month 3]) with TDV.

8.2 Study Vaccine Assignment and Dispensing Procedures

The investigator or its designee will access the IWRS at subject enrolment to obtain the subject number. This number will be used throughout the study.

The investigator or its designee will utilize the IWRS on the day of first dosing (Day 1 [Month 0]) to provide the necessary subject identifying information.

The investigator or its designee will access the IWRS at each dispensing visit to obtain the Vaccination Identification number for the vaccine dose. The vaccines will be prepared and administered by the pharmacist or vaccine administrator according to the instructions in the Pharmacy Manual or per sponsor instructions.

The investigator or its designee will be responsible for overseeing the administration of vaccine to subjects enrolled in the study according to the procedures stipulated in this study protocol. All vaccines will be administered only by personnel who are qualified to perform that function under applicable laws and regulations for that specific study.

If sponsor-supplied vaccine is lost or damaged, the site can request a replacement. Expired vaccines must not be administered.

Eligibility for vaccination prior to first study vaccine administration is determined by evaluating the entry criteria outlined in this protocol (Sections 7.1 and 7.2).

Eligibility for subsequent study vaccination is determined by following the criteria outlined in Section 7.3.

The study vaccine should not be administered to individuals with known hypersensitivity to any component of the vaccine.

As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccination. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

8.3 Randomization Code Creation and Storage

Not applicable.

8.4 Study Vaccine Blind Maintenance

Not applicable.

8.5 Unblinding Procedure

Not applicable.

8.6 Accountability and Destruction of Sponsor-Supplied Vaccine(s)

Vaccine supplies will be counted and reconciled at the site before being destroyed at the study sites or returned to the sponsor or its designee as noted below. Sites will maintain source documents in addition to entering data in the IWRS.

The investigator or its designee must ensure that the sponsor-supplied vaccine(s) are used in accordance with the approved protocol and is administered only to subjects enrolled in the study. To document appropriate use of sponsor-supplied vaccine (TDV), the investigator must maintain records of all sponsor-supplied vaccine(s) delivery to the site, site inventory, administration and use by each subject, and return to the sponsor or its designee.

Upon receipt of sponsor-supplied vaccine(s), the investigator or its designee must verify the contents of the shipments against the packing list. The verifier should ensure that the quantity is correct, the study vaccine is received within the labeled storage conditions (ie, no cold chain break has occurred during transit), and is in good condition. If quantity and conditions are acceptable, investigator or its designee will acknowledge receipt of shipment by recording in IWRS.

If there are any discrepancies between the packing list versus the actual product received, the sponsor or its designee must be contacted to resolve the issue. The packing list should be filed in the investigator's essential document file.

The investigator must maintain 100% accountability for all sponsor-supplied vaccine(s) received and administered during his or her entire participation in the study. Proper vaccine accountability includes, but is not limited to:

- Verifying that actual inventory matches documented inventory.
- Verifying that the log is completed for the vaccine lot number used to prepare each dose.
- Verifying that all containers used are documented accurately on the log.
- Verifying that required fields are completed accurately and legibly.

If any dispensing errors or discrepancies are discovered, the sponsor must be notified immediately.

The investigator must record the current inventory of all sponsor-supplied vaccine(s) (TDV) on a sponsor-approved vaccine accountability log. The following information will be recorded at a minimum: protocol number and title, name of investigator, site identifier and number, description of sponsor-supplied vaccine(s), expiry date and/or retest date, and amount. The log (IWRS) should include all required information as a separate entry for each subject to whom sponsor-supplied vaccine is administered.

The investigator will be notified of any expiry date or retest date extension of clinical study material during the study conduct. On expiry date notification from the sponsor or its designee, the site must complete all instructions outlined in the notification, including segregation of expired

clinical study material for return to the sponsor or its designee for destruction. In the event of expiry date extension of supplies already at the study site, supplies may be relabeled with the new expiry date at that study site. In such cases, the sponsor or its designee will prepare additional labels and all necessary documentation for completion of the procedure at the study site.

Prior to site closure or at appropriate intervals throughout the study, before any clinical study materials are destroyed or returned to the sponsor or its designee for destruction, a representative from the sponsor or its designee will perform clinical study material accountability and reconciliation. The investigator will retain a copy of the documentation regarding clinical study material accountability, return, and/or destruction, and originals will be sent to the sponsor or its designee.

The investigator (or designated individual) at each site will be responsible for vaccine accountability and will document receipt, use, return, or destruction of TDV. Vaccine accountability documentation will be reviewed by the monitor during clinical monitoring visits.

9.0 STUDY PLAN

9.1 Study Procedures

The following sections describe the study procedures and data to be collected. For each procedure, subjects are to be assessed by the same investigator or site personnel whenever possible. Screening and the Day 1 (Month 0) visit can be performed the same day. The Schedule of Study Procedures is located in Section 2.1.

9.1.1 Informed Consent/Assent

The requirements of the informed consent/assent are described in Section 15.2.

Informed consent/assent must be obtained prior to the subject entering into the study, and before any protocol-directed procedures are performed. Subjects who attain legal age during their study participation will be asked to provide consent as per local regulations.

A unique subject number will be assigned to each subject after informed consent/assent is obtained from the IWRS. If all eligibility criteria are fulfilled, this subject number will be used throughout the study. Subject numbers assigned to subjects who fail screening should not be reused (Section 9.1.13).

9.1.2 Demographics, Medical History and Prior Medications

Demographic information to be obtained will include date of birth, gender, race, and ethnicity as described by the subject or subject's parent(s)/LAR.

Medical History will also be collected, including but not limited to any medical history that may be relevant to subject eligibility for study participation such as prior vaccinations, concomitant medications, and previous and ongoing illnesses or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an AE that occurs during study participation, if it represents an exacerbation of an underlying disease/preexisting problem.

History of vaccination against Japanese Encephalitis or Yellow Fever will be recorded in the eCRF irrespective of time of administration and including the vaccine type as well as any supportive documentation for these vaccinations.

All concomitant medications and vaccine history from 1 month (minimum 28 days) prior to administration of each dose of TDV up to 1 month (minimum 28 days) thereafter, steroids and immunostimulants within 60 days prior to Day 1 (Month 0), immunoglobulins and blood products within 3 months prior to Day 1 (Month 0), and immunosuppressive therapy within 6 months prior to Day 1 (Month 0) are to be recorded on the relevant sections of the eCRF (See also Section 7.2). The use of antipyretics and/or analgesic medications within 24 hours prior to vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be described in the source documents or the eCRF. Study vaccination should be delayed if subjects have used antipyretics and/or analgesic medication within 24 hours prior to vaccine administration.

Medications taken for prophylaxis are those intended to prevent the onset of AEs following vaccination. Medications taken for treatment are intended to reduce or eliminate the presence of symptoms that are present.

Prohibited therapies (see also Section 7.2 and Section 7.3):

- Parenteral immunoglobulin preparation, blood products, and/or blood-derived products within the 3 months prior to Day 1 (Month 0).
- Immunosuppressive therapy within 6 months or systemic (eg, oral or parenteral) corticosteroid treatment within 60 days prior to Day 1 (Month 0) or immunostimulants within 60 days prior to Day 1 (Month 0).
- Any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 (Month 0) and Day 90 (Month 3), and 28 days after each study vaccination.
- Any other dengue vaccines (investigational or licensed) for the entire study period.
- Receipt of any other clinical study product within 30 days prior to Day 1 (Month 0).

These data must be written in the source documents.

Medical history (including corresponding medication) to be obtained will include any significant conditions or diseases that have disappeared or resolved at or prior to signing of informed consent/assent. Additionally, reasons for delay of second study vaccination and contraindications to second vaccination must be recorded in the eCRF (see Section 7.3).

9.1.3 Documentation of Study Entrance/Randomization

Only subjects for whom a signed informed consent form/assent form is obtained, meet all of the inclusion criteria and none of the exclusion criteria are eligible for entrance into the vaccination phase.

If the subject is found to be not eligible for the vaccination phase, the investigator should record the primary reason for failure on the subject enrolment log.

Subjects who are otherwise eligible but cannot receive the study vaccination because the limit of approximately 60% of subjects (ie, sample size at each study center) with the same dengue Baseline seropositivity status is reached will not continue in the study and will be deemed screen failures. Enrolment tracking of dengue naïve and dengue exposed subjects will be done using IWRS.

Any withdrawals or screen failures from enrolment until vaccination at Day 1 (Month 0) will be replaced.

9.1.4 Physical Examination

Physical examinations must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log. Complete physical examination will be performed at Screening, prior to vaccination at Day 1

(Month 0), and prior to vaccination at Day 90 (Month 3). A complete physical examination includes but is not limited to: auscultation of heart and lungs, palpation of the abdomen, inspection of extremities (including skin over intended vaccination site[s]), and a check of general appearance. Additional physical examinations may be performed if indicated by review of the subject's medical history. The findings should be documented in the subject's source document.

Targeted physical examination including but not limited to measurement of vital signs (see Section 9.1.5) will be performed for all subjects at Day 30 (Month 1), Day 120 (Month 4), Day 270 (Month 9), and annually for 3 years post second vaccination or at the follow-up visit (ie, when the subject terminates early). Targeted physical examination will also be performed at Day 14 for subjects >10 years of age.

9.1.5 Vital Signs

These will include (but are not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, body temperature, height and weight. Measurement of height is required at Screening, Day 1 (Month 0), Day 270 (Month 9), and annually for 3 years post second vaccination.

Body temperature measurement will be described in the Procedures Manual.

9.1.6 Dengue Baseline Seropositivity Status

A blood sample for evaluation of the dengue Baseline seropositivity status by dengue IgG indirect ELISA to be performed locally will be taken at Screening or pre-first vaccination at Day 1 (Month 0) if screening is performed the same day. Refer also to Appendix D.

The analysis of study endpoints by dengue Baseline seropositivity status will however be based on the MNT result of the blood sample taken pre-first vaccination at Day 1 (Month 0) to ensure consistent assessments across the program.

9.1.7 Immunogenicity and CMI Assessments

All subjects will undergo blood sampling for assessment of cellular immune responses at pre-first vaccination (Day 1 [Month 0]), 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and annually for 3 years post second vaccination. One additional blood sample will be collected at Day 14 from subjects >10 years of age. Refer also to Appendix D.

All subjects will undergo blood sampling for assessment of neutralizing antibodies (MNT) at pre-first vaccination (Day 1 [Month 0]), 1 month post first vaccination (Day 30 [Month 1]), pre-second vaccination (Day 90 [Month 3]), 1 month and 6 months post second vaccination (Day 120 [Month 4] and Day 270 [Month 9], respectively), and annually for 3 years post second vaccination. Refer also to Appendix D.

In subjects ≤10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 3 mL/kg or 50 mL in total (whichever is lower) within 8 weeks. In subjects >10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 5 mL/kg within 8 weeks [16]. Refer also to Appendix D.

9.1.8 Processing, Labeling and Storage of Peripheral Blood Mononuclear Cell Samples

Peripheral blood mononuclear cells (PBMC) will be collected, processed, labeled and stored according to study site standard operating procedures (SOP). Refer to the SOP for detailed instructions.

All blood samples will be processed, labeled and stored according to the laboratory guideline or other appropriate guideline provided to the study site.

9.1.9 Handling of Febrile Illness Cases (Suspected Dengue Cases)

Subjects with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have 1 blood sample taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever) to confirm dengue infection. Testing will include dengue RT-PCR and NS1 ELISA. Additional blood samples may be taken for diagnosis and clinical management of the subject as per standard medical practice. RT-PCR and NS1 ELISA results from central the laboratory will not be available for real time case management. Refer also to Appendix D.

A new episode of febrile illness as described above will require an interval of at least 14 days from a previous febrile illness episode (ie, counting from the first day of febrile illness).

9.1.10 Safety Assessments

Safety assessments will include collection and recording of unsolicited AEs (serious and non-serious) and MAAEs. Refer to Section 10.1 for safety definitions. Details on collection and reporting of AEs are in Section 10.4.

9.1.11 Contraception and Pregnancy Avoidance Procedure

For female subjects of childbearing potential, pregnancy testing (serum or urine) will be performed at Screening, prior to vaccination at Day 1 (Month 0), and prior to vaccination at Day 90 (Month 3). Additional pregnancy tests may be performed during the study if deemed necessary by the investigator. Female of childbearing potential who are sexually active will be reminded during study visits to adhere to acceptable contraceptive methods (see Section 7.2) up to 6 weeks post second vaccination. Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and will be asked to sign a consent form/assent form stating that they understand the requirements for avoidance of pregnancy, donation of ova. Refer also to Section 7.2.

9.1.12 Pregnancy

To ensure subject safety and the safety of the unborn child, each pregnancy in a subject having received the investigational vaccine must be reported to the sponsor within 24 hours of the site learning of its occurrence. If the subject becomes pregnant during the study, she will not receive any further doses of any sponsor-supplied investigational vaccine. The pregnancy must be followed to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. This follow-up should occur even if the intended duration of safety follow-up for the study has ended.

Any pregnancy occurring following study vaccine administration should be reported immediately, using a pregnancy notification form, to the contact listed in the Investigator Site File.

9.1.13 Documentation of Enrolled Subjects who are not Vaccinated

Investigators must account for all subjects for whom a signed informed consent/assent form has been obtained. If a previously enrolled subject is found to be not eligible at Day 1 (Month 0), the investigator should complete the eCRF. The IWRS should be contacted as a notification for not receiving the study vaccine.

The primary reason for not receiving the study vaccine is to be recorded in the eCRF using the following categories:

- AEs prior to receipt of investigational vaccine,
- Screen failure (did not meet one or more inclusion criteria or did meet one or more exclusion criteria),
- Withdrawal by subject and/or subject's parent(s)/LAR,
- Site terminated by sponsor,
- Study terminated by sponsor,
- Other (specify reason).

Subject numbers assigned to subjects who fail screening including eligible subjects not vaccinated because the limit of approximately 60% of subjects (ie, sample size at each study center) with the same dengue Baseline seropositivity status is reached at the study center, or who are withdrawn or discontinued between enrolment and Day 1 (Month 0) should not be reused.

9.2 Monitoring Subject Vaccination Compliance

The investigator must record each administration of investigational vaccine (TDV) into the subject's source documents and eCRF.

9.3 Schedule of Observations and Procedures

The schedule for all study-related procedures for all evaluations is shown in Section 2.1. Assessments should be completed at the designated visit/time point(s).

9.3.1 Enrolment (Screening: up to 28 days prior to Day 1 [Month 0]) and Vaccination Procedures (Day 1 [Month 0] and Day 90 [Month 3])

Prior to vaccination:

Note: Procedures at either Screening or Day 1 (Month 0) are to be performed only once if Screening is performed at Day 1 (Month 0).

- Before performing any other study procedure, the signed informed consent/assent needs to be obtained (Screening). Refer to Section 9.1.1.
- Collect demographic data (Screening). Refer to Section 9.1.2.
- Collect medical history and concomitant medications/ vaccinations history (Screening, prior to vaccination at Day 1 [Month 0], and prior to vaccination at Day 90 [Month 3]). Refer to Section 9.1.2.
- Perform a complete physical examination (Screening, prior to vaccination at Day 1 [Month 0], and prior to vaccination at Day 90 [Month 3]). Refer to Section 9.1.4.
- Perform pregnancy testing (serum or urine) for female subjects of childbearing potential (Screening, prior to vaccination at Day 1 [Month 0], and prior to vaccination at Day 90 [Month 3]). Refer to Section 9.1.11.
- Remind female subjects of childbearing potential who are sexually active to adhere to acceptable contraceptive methods (see Section 7.2) up to 6 weeks post second vaccination (Screening, Day 1 [Month 0], and Day 90 [Month 3]). Refer to Section 9.1.11.
- Review of systems: Review of systems is a structured interview that queries the subject or the subject's parent(s)/LAR as to any complaints the subject has experienced across each organ system (Screening, prior to vaccination at Day 1 [Month 0]).
- Check inclusion and exclusion criteria (Screening, prior to vaccination at Day 1 [Month 0]). Refer to Sections 7.1 and 7.2.
- Check criteria for delay of study vaccination at Day 90 (Month 3). Refer to Section 7.3.
- Check contraindications to second study vaccination at Day 90 (Month 3). Refer to Section 7.3.
- Record MAAEs at Day 90 (Month 3). Refer to Section 10.4.3.
- Collect blood sample (Screening). Refer to Section 9.1.6.

  Blood should be taken from the subject using aseptic venipuncture technique. Refer to the detailed collection and handling procedures outlined in the Procedures Manuals.

If subject meets all eligibility criteria:

- Collect blood sample (prior to vaccination at Day 1 [Month 0] and prior to vaccination at Day 90 [Month 3]). Refer to Sections 9.1.6 through 9.1.8.

  Blood should be taken from the subject using aseptic venipuncture technique. Refer to the detailed collection and handling procedures outlined in the Procedures Manuals.

- Vaccinate subject (Day 1 [Month 0] and Day 90 [Month 3]). Refer to Section 8.1.3.
- Observe subject for at least 30 minutes following vaccination (Day 1 [Month 0] and Day 90 [Month 3]). All safety data will be collected in the subject's source documents.

The subjects or the subject's parent(s)/LAR, as applicable, will be instructed to come to the study site if the subject experiences febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) for dengue fever evaluation by the investigator. Refer to Section 9.1.9.

The subjects or the subject's parent(s)/LAR, as applicable, will receive a written reminder of the next planned study activity and will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious.

9.3.2 Clinic Visits after Vaccination (Day 1 [Month 0] and Day 90 [Month 3]) for all Subjects at Day 30 (Month 1), Day 120 (Month 4), Day 270 (Month 9), and Annually for 3 Years Post Second Vaccination.

- Collect concomitant medications/vaccinations history (Day 30 [Month 1], Day 120 [Month 4]), Day 270 [Month 9], and annually for 3 years post second vaccination). Refer to Section 9.1.2.
- Record unsolicited AEs (Day 30 [Month 1] and Day 120 [Month 4]). Refer to Section 10.4.2.
- Record MAAEs (Day 30 [Month 1], Day 120 [Month 4], and Day 270 [Month 9]). Refer to Section 10.4.3.
- Perform a targeted physical examination (Day 30 [Month 1], Day 120 [Month 4], Day 270 [Month 9], and annually for 3 years post second vaccination). Refer to Sections 9.1.4 and 9.1.5.
- Remind female subjects of childbearing potential who are sexually active to adhere to acceptable contraceptive methods (see Section 7.2) up to 6 weeks post second vaccination (Day 30 [Month 1] and Day 120 [Month 4]). Refer to Section 9.1.11.
- Collect blood sample (Day 30 [Month 1], Day 120 [Month 4], Day 270 [Month 9], and annually for 3 years post second vaccination). Refer to Sections 9.1.7 and 9.1.8.

  Blood should be taken from the subject using aseptic venipuncture technique. Refer to the detailed collection and handling procedures outlined in the Procedures Manuals.

The subjects or the subject's parent(s)/LAR, as applicable, will be instructed to come to the study site if the subject experiences febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) for dengue fever evaluation by the investigator. Refer to Section 9.1.9.

The subjects or the subject's parent(s)/LAR, as applicable, will receive a written reminder of the next planned study activity and will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious.

9.3.3 Additional Clinic Visit after Vaccination (Day 1 [Month 0]) for Subjects Aged Older than 10 Years (Day 14)

- Perform a targeted physical examination ((Day 14). Refer to Section 9.1.4.
- Collect blood sample (Day 14). Refer to Sections 9.1.7 and 9.1.8.

Blood should be taken from the subject using aseptic venipuncture technique. Refer to the detailed collection and handling procedures outlined in the Procedures Manuals.

The subjects or the subject's parent(s)/LAR, as applicable, will be instructed to come to the study site if the subject experiences febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) for dengue fever evaluation by the investigator. Refer to Section 9.1.9.

The subjects or the subject's parent(s)/LAR, as applicable, will receive a written reminder of the next planned study activity and will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious.

9.3.4 Contacts During Febrile Illness Surveillance

The subjects or the subject's parent(s)/LAR, as applicable, will be contacted at least weekly (eg, phone calls, home visits, or school-based surveillance) to ensure robust identification of febrile illness by reminding subjects or the subject's parent(s)/LAR, as applicable, of their obligation to return to the site in case of febrile illness.

The subjects or the subject's parent(s)/LAR, as applicable, will be instructed to come to the study site if the subject experiences febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) for dengue fever evaluation by the investigator. Refer to Section 9.1.9.

The subjects or the subject's parent(s)/LAR, as applicable, will receive a written reminder of the next planned study activity and will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or is otherwise perceived as serious.

9.3.5 Follow-Up Visit

If a subject is withdrawn or discontinues after vaccination, standard visit procedures should be performed if possible. The follow-up visit should be performed as soon as possible and preferably at least 28 days after the last study vaccination.

9.3.6 Post-Study Care

No post-study care will be provided.

9.4 Biological Sample Retention and Destruction

In this study, specimens for immune response testing will be collected as described in Section 9.1.6 through Section 9.1.8. Specimen to confirm dengue infection will be collected from subjects with febrile illness as described in Section 9.1.9. Biological samples will be processed and stored per the Laboratory Guidelines as provided in the Procedures Manual/Laboratory Manual. The samples will be preserved and retained at a central laboratory that was contracted by the sponsor for this purpose for up to but not longer than 20 years or as required by applicable law. The sponsor has put into place a system to protect the subjects' personal information to ensure optimal confidentiality and defined standard processes for sample and data collection, storage, analysis, and destruction.

Serum and PBMC samples will be used for the analyses defined in this protocol, but can also, with permission from the subject or subject's parent(s)/LAR, be used for research related or unrelated to the disease or the vaccine under study.

10.0 ADVERSE EVENTS

10.1 Definitions

10.1.1 AEs

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered a study vaccine; it does not necessarily have to have a causal relationship with study vaccine administration.

An AE can therefore be any unfavorable and unintended sign (eg, a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the administration of a study vaccine whether or not it is considered related to the study vaccine.

AEs will be graded by the investigator in the following manner:

Mild	Grade 1	Awareness of symptoms that are easily tolerated, causing minimal discomfort and not interfering with everyday activities. Relieved with or without symptomatic treatment.
Moderate	Grade 2	Sufficient discomfort is present to cause interference with normal activity. Only partially relieved with symptomatic treatment.
Severe	Grade 3	Extreme distress, causing significant impairment of functioning or incapacitation. Prevents normal everyday activities. Not relieved with symptomatic treatment

10.1.2 Unsolicited AEs

Unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be collected by interview (ie, at Day 30 [Month 1] and Day 120 [Month 4]) and will be recorded for all subjects onto the relevant sections of the eCRF. Subjects will be observed for at least 30 minutes after administration of each vaccine dose (Day 1 [Month 0] and Day 90 [Month 3]). All safety data will be collected in the subject's source documents.

10.1.3 MAAEs

MAAEs defined as AEs leading to a medical visit to or by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria occurring from first vaccination at Day 1 (Month 0) up to 6 months post second vaccination at Day 90 (Month 3) will be collected by interview (ie, at Day 30 [Month 1], Day 90 [Month 3], Day 120 [Month 4], and Day 270 [Month 9]) and will be recorded onto the relevant section of the eCRF.

10.1.4 SAEs

An SAE is defined as any untoward medical occurrence that:

1. Results in DEATH.
2. Is LIFE THREATENING.
    - 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 that hypothetically might have caused death if it were more severe.
3. Requires inpatient HOSPITALIZATION or prolongation of existing hospitalization.
4. Results in persistent or significant DISABILITY/INCAPACITY.
5. Leads to a CONGENITAL ANOMALY/BIRTH DEFECT in the offspring of a subject.
6. Is an IMPORTANT MEDICAL EVENT that satisfies any of the following:
    - May require intervention to prevent items 1 through 5 above.
    - May expose the subject to danger, even though the event is not immediately life threatening or fatal or does not result in hospitalization.

10.2 Causality of AEs

Relatedness (causality) to vaccine will also be assessed by the investigator. The relationship of each AE to study vaccine(s) will be assessed using the following categories:

Related:	There is suspicion that there is a relationship between the study vaccine and the AE (without determining the extent of probability); there is a reasonable possibility that the study vaccine contributed to the AE.
Not Related:	There is no suspicion that there is a relationship between the study vaccine and the AE; there are other more likely causes and administration of the study vaccine is not suspected to have contributed to the AE.

10.2.1 Relationship to Study Procedures

Relationship (causality) to study procedures should be determined for all AEs.

The relationship (causality) should be assessed as "Yes" if the investigator considers that there is reasonable possibility that an event is due to a study procedure. Otherwise, the relationship should be assessed as "No".

10.2.2 Outcome of AEs

Resolved:	The subject has fully recovered from the event or the condition has returned to the level observed at Baseline.
Resolving:	The event is improving but the subject is still not fully recovered.
Not resolved:	The event is ongoing at the time of reporting and the subject has still not recovered.
Resolved with sequelae:	As a result of the AE, the subject suffered persistent and significant disability/incapacity (eg, became blind, deaf or paralysed).
Fatal:	The subject died due to the event. If the subject died due to other circumstances than the event, the outcome of the event per se should be stated otherwise (eg, not resolved or resolving).
Unknown:	If outcome is not known or not reported.

10.3 Additional Points to Consider for AEs

An untoward occurrence generally may:

- Indicate a new diagnosis or unexpected worsening of a pre-existing condition. Intermittent events for pre-existing conditions or underlying disease should not be considered as AEs.
- Necessitate therapeutic intervention.
- Require an invasive diagnostic procedure.
- Require vaccine discontinuation or a change in concomitant medication.
- Be considered unfavorable by the investigator for any reason.

Diagnoses vs signs and symptoms:

- Each event should be recorded to represent a single diagnosis. Accompanying signs (including abnormal laboratory values) or symptoms should NOT be recorded as additional AEs. If a diagnosis is unknown, signs or symptoms should be recorded appropriately as AEs.

Worsening of AEs:

- If the subject experiences a worsening or complication of an AE after starting administration of the study vaccine, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").
- If the subject experiences a worsening or complication of an AE after any change in study vaccine, the worsening or complication should be recorded as a new AE. Investigators should ensure that the AE term recorded captures the change in the condition (eg, "worsening of…").

Changes in severity of AEs:

- If the subject experiences changes in severity of an AE, the event should be captured once with the maximum severity recorded.

Preplanned surgeries or procedures:

- Preplanned procedures (surgeries or therapies) that were scheduled prior to signing of informed consent/assent are not considered AEs. Complications resulting from any planned surgery should be reported as AEs.

Elective surgeries or procedures:

- Elective procedures performed where there is no change in the subject's medical condition should not be recorded as AEs, but should be documented in the subject's source documents. Complications resulting from an elective surgery should be reported as AEs.

10.4 Procedures

10.4.1 Collection and Reporting of AEs

All AEs, whether considered related with the use of the study vaccine or not, must be monitored until symptoms subside and any abnormal laboratory values have returned to Baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. All findings must be reported on an Adverse Event eCRF and on the SAE form, if necessary (see Section 10.4.2). All findings in subjects experiencing AEs must also be recorded in the subject's source documents. Any unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be collected by interview and will be recorded for all subjects (ie, at Day 30 [Month 1] and Day 120 [Month 4]). MAAEs will be collected from first vaccination at Day 1 (Month 0) up to 6 months post second vaccination at Day 90 (Month 3) by interview and will be recorded (ie, at Day 30 [Month 1], Day 90 [Month 3], Day 120 [Month 4], and Day 270 [Month 9]). AEs leading to subject discontinuation or withdrawal will be collected from first vaccination until the end of the study.

The following information will be documented for each event:

- Reported term for the Adverse Event,
- Start and end date,
- Serious (Y/N),
- Severity,
- Investigator's opinion of the causality (relationship) between the event and administration of study vaccine(s) ("related" or "not related"),

- Investigator's opinion of the causality (relationship) to study procedure(s), including the details of the suspected procedure,
- Action taken with the study vaccine,
- Outcome of event.

10.4.2 Collection and Reporting of Unsolicited AEs

Subjects will be observed for at least 30 minutes after administration of each vaccine dose (Day 1 [Month 0] and Day 90 [Month 3]). Unsolicited AEs during the 28-day period (day of vaccination +27 subsequent days) after administration of each vaccine dose will be collected by interview for all subjects (ie, at Day 30 [Month 1] and Day 120 [Month 4]) and must be recorded onto the Adverse Event eCRF.

10.4.3 Collection and Reporting of MAAEs and AEs Leading to Subject Discontinuation or Withdrawal

MAAEs occurring from first vaccination at Day 1 (Month 0) up to 6 months post second vaccination at Day 90 (Month 3) will be collected by interview (ie, at Day 30 [Month 1], Day 90 [Month 3], Day 120 [Month 4], and Day 270 [Month 9]) and must be recorded as an AE onto the Adverse Event eCRF.

AEs leading to subject discontinuation or withdrawal will be collected from first vaccination until the end of the study and must be recorded as an AE onto the Adverse Event eCRF.

10.4.4 Collection and Reporting of SAEs

Collection of SAEs will commence from the time the subject is administered the first study vaccination (Day 1 [Month 0]) until the end of the study.

SAEs should be reported according to the following procedure:

A sponsor SAE form must be completed, in English, and signed by the investigator immediately or within 24 hours of first onset or notification of the event. The information should be completed as fully as possible but contain, at a minimum:

- A short description of the event and the reason why the event is categorized as serious.
- Subject identification number.
- Investigator's name.
- Name of the study vaccine(s).
- Causality assessment.

The SAE form should be transmitted within 24 hours to the attention of the contact(s) in the list provided to each site.

10.5 Follow-Up Procedures

10.5.1 AEs

All AEs will be monitored until resolution or a stable status is reached or until a formal diagnosis can be made or until the end of the study, whichever occurs first. This could potentially be followed outside of this study or in a planned extension study.

10.5.2 SAEs

If information not available at the time of the first report becomes available at a later date, the investigator should complete a follow-up SAE form or provide other written documentation and fax it immediately within 24 hours of receipt. Copies of any relevant data from the hospital notes (eg, laboratory tests, discharge summary, post-mortem results) should be sent to the sponsor.

All SAEs should be followed up until resolution or permanent outcome of the event or is otherwise explained. The timelines and procedure for follow-up reports are the same as those for the initial report.

10.5.3 Safety Reporting to Investigators, IRBs or IECs, and Regulatory Authorities

The sponsor or its designee will be responsible for the reporting of all suspected unexpected serious adverse reactions (SUSARs) and any other applicable SAEs to regulatory authorities, investigators and IRBs or IECs, as applicable in accordance with national regulations in the countries where the study is conducted. Relative to the first awareness of the event by/or further provision to the sponsor or sponsor's designee, SUSARs will be submitted within 7 days for fatal and life-threatening events and 15 days for other SUSARs, unless otherwise required by national regulations. The sponsor will also prepare an expedited report for other safety issues where these might materially alter the current benefit-risk assessment of an investigational medicinal product or that would be sufficient to consider changes in the study vaccine administration or in the overall conduct of the study. The investigational site also will forward a copy of all expedited reports to their IRB or IEC in accordance with national regulations.

10.5.4 Post-Study Events

Any AE that occurs outside of the protocol-specified observation period or after the end of the study but considered to be caused by the study vaccine(s) must be reported to the sponsor. These AEs will be processed by the sponsor's Pharmacovigilance Department. Instructions for how to submit these AEs will be provided in a handout in the Investigator Site File.

11.0 STUDY-SPECIFIC REQUIREMENT(S)

11.1 Study-Specific Committees

11.1.1 Data Monitoring Committee

A DMC will have oversight of this study. The DMC functions at a program level and further information is available in the DMC Charter.

12.0 DATA HANDLING AND RECORDKEEPING

The full details of procedures for data handling will be documented in the Data Management Plan. AEs, medical history, and concurrent conditions will be coded using the medical dictionary for regulatory activities (MedDRA), system organ class (SOC), high level group term (HLGT), high level term (HLT), low level term (LLT), preferred term (PT), and their corresponding descriptive terms. Drugs will be coded using the WHO Drug Dictionary.

12.1 Electronic CRFs (eCRF)

Completed eCRFs are required for each subject for whom a signed informed assent/consent has been obtained.

The sponsor or its designee will supply investigative sites with access to eCRFs. The sponsor will make arrangements to train appropriate site staff in the use of the eCRF. These eCRFs are used to transmit the information collected in the performance of this study to the sponsor and regulatory authorities. eCRFs must be completed in English. Data are entered directly onto eCRFs.

After completion of the entry process, computer logic checks will be run to identify items, such as inconsistent dates, missing data, and questionable values. Queries may be issued by sponsor personnel (or designees) and will be answered by the site.

Corrections to eCRFs are recorded in an audit trail that captures the old information, the new information, identification of the person making the correction, the date the correction was made, and the reason for change.

The principal investigator or its designee must review the eCRFs for completeness and accuracy and must sign and date the appropriate eCRFs as indicated. Furthermore, the investigator must retain full responsibility for the accuracy and authenticity of all data entered on the eCRFs.

Electronic CRFs will be reviewed for completeness and acceptability at the study site during periodic visits by study monitors. The sponsor or its designee will be permitted to review the subject's medical and hospital records pertinent to the study to ensure accuracy of the eCRFs. The completed eCRFs are the sole property of the sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate governmental health or regulatory authorities, without written permission of the sponsor.

12.2 Record Retention

The investigator agrees to keep the records stipulated in Section 12.0 and those documents that include (but are not limited to) the study-specific documents, the identification log of all participating subjects, medical records. Temporary media such as thermal sensitive paper should be copied and certified, source worksheets, all original signed and dated informed consent forms and assent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent forms), electronic copy of eCRFs, including the audit trail, and detailed records of vaccine disposition to enable evaluations or audits from regulatory authorities, the sponsor or its designees. Furthermore, ICH E6 Section 4.9.5 requires the investigator to retain

essential documents specified in ICH E6 (Section 8) until at least 2 years after the last approval of a marketing application for a specified vaccine indication being investigated or, if an application is not approved, until at least 2 years after the investigation is discontinued and regulatory authorities are notified. In addition, ICH E6 Section 4.9.5 states that the study records should be retained until an amount of time specified by applicable regulatory requirements or for a time specified in the Clinical Study Site Agreement between the investigator and sponsor.

Refer to the Clinical Study Site Agreement for the sponsor's requirements on record retention. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.

13.0 STATISTICAL METHODS

13.1 Statistical and Analytical Plans

A statistical analysis plan (SAP) will be prepared and finalized prior to the planned interim analysis. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all study objectives.

Data reviews will be conducted prior to the planned interim analysis and final analysis. This review will assess the accuracy and completeness of the study database, subject evaluability, and appropriateness of the planned statistical methods.

All analyses will be descriptive; no statistical hypotheses will be tested in this study. Further details will be provided in the SAP.

13.1.1 Analysis Sets

Safety set: The safety set will consist of all subjects who received at least 1 dose of the study vaccine.

Full analysis set (FAS): The FAS will include all subjects who received at least 1 dose of the study vaccine and for whom a valid pre-dosing and at least 1 post-dosing blood sample have been received for immunogenicity.

Per-protocol set (PPS): The PPS will include all subjects in the FAS who have no major protocol violations. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving prohibited therapies, (3) not receiving 2 doses of study vaccine or receiving the second vaccination inadmissibly outside of the visit window, and (4) other major protocol violations that may be identified during data reviews.

13.1.2 Analysis of Demographics and Other Baseline Characteristics

Age, gender, race, and other Baseline characteristics will be summarized descriptively for all enrolled subjects.

13.1.3 Immunogenicity Analysis

For the primary immunogenicity endpoint (ie, frequency of cellular immune response), descriptive statistics will be provided by visit. Cellular immune response is defined as an IFN-γ ELISPOT response that is >3 times higher compared to Baseline (Day 1 [Month 0]) and ≥5 spots per well. Summaries will be provided based on all subjects, by region as well as dengue Baseline seropositivity status. The primary analyses will be based on the PPS; supportive analyses may be provided based on the FAS.

Similar analysis as for the primary immunogenicity endpoint will be provided for the secondary immunogenicity endpoints related to cellular immune response. For the secondary immunogenicity endpoints including seropositivity rates and GMTs for dengue neutralizing antibodies, descriptive statistics and 95% CIs will be provided by visit.

Seropositivity is defined as a reciprocal neutralizing titer ≥10. Dengue seropositivity at Baseline is defined as a reciprocal neutralizing titer ≥10 for one or more dengue serotypes.

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13.1.4 Safety Analysis

Safety data will be summarized descriptively based on the safety set. AEs will be coded according to MedDRA.

Unsolicited AEs, MAAEs, AEs leading to withdrawal, and SAEs will be summarized by SOC and PT.

The percentage of subjects with virologically confirmed dengue will also be summarized.

13.2 Interim Analysis and Criteria for Early Termination

An interim analysis on safety and immunogenicity data is planned when all subjects have completed the Day 270 (Month 9) visit.

13.3 Determination of Sample Size

This study is designed to be primarily descriptive and is not based on testing formal null hypotheses. Therefore, the sample size was not determined based on formal statistical power calculations. The number of subjects will provide a reasonable sample size for the evaluation of the objectives of the study.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Study-Site Monitoring Visits

Monitoring visits to the study site will be made periodically during the study to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data recorded on the eCRFs. Source documents are defined as original documents, data, and records. The investigator and institution guarantee access to source documents by the sponsor or its designee (Clinical Research Organization [CRO]) and by the IRB or IEC.

All aspects of the study and its documentation will be subject to review by the sponsor or its designee, including but not limited to the Investigator's Binder, study vaccine, subject medical records, informed consent documentation, documentation of subject authorization to use personal health information (if separate from the informed consent forms), and review of eCRFs and associated source documents. It is important that the investigator and other study personnel are available during the monitoring visits and that sufficient time is devoted to the process.

14.2 Protocol Deviations

The investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to study subjects. Should other unexpected circumstances arise that will require deviation from protocol-specified procedures, the investigator should consult with the medical monitor (and IRB or IEC, as required) to determine the appropriate course of action. There will be no exemptions (a prospective approved deviation) from the inclusion or exclusion criteria.

14.3 Quality Assurance Audits and Regulatory Agency Inspections

The study site also may be subject to quality assurance audits by the sponsor or its designees. In this circumstance, the sponsor-designated auditor will contact the site in advance to arrange an auditing visit. The auditor may ask to visit the facilities where laboratory samples are collected, where the vaccine is stored and prepared, and any other facility used during the study. In addition, there is the possibility that this study may be inspected by regulatory agencies, including those of foreign governments (eg, the Food and Drug Administration [FDA], the United Kingdom Medicines and Healthcare Products Regulatory Agency, the Pharmaceuticals and Medical Devices Agency of Japan). If the study site is contacted for an inspection by a regulatory body, the sponsor should be notified immediately. The investigator and institution guarantee access for quality assurance auditors to all study documents as described in Section 14.1.

15.0 ETHICAL ASPECTS OF THE STUDY

This study will be conducted with the highest respect for the individual participants (ie, subjects) according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki, and the ICH Harmonized Tripartite Guideline for GCP. Each investigator will conduct the study according to applicable local or regional regulatory requirements and align his or her conduct in accordance with the "Responsibilities of the Investigator" that are listed in Appendix A. The principles of Helsinki are addressed through the protocol and through appendices containing requirements for informed consent and investigator responsibilities.

15.1 IRB and/or IEC Approval

IRBs and IECs must be constituted according to the applicable federal/local requirements of each participating region. The sponsor or its designee will require documentation noting all names and titles of members who make up the respective IRB or IEC. If any member of the IRB or IEC has direct participation in this study, written notification regarding his or her abstinence from voting must also be obtained.

The sponsor or its designee will supply relevant documents for submission to the respective IRB or IEC for the protocol's review and approval. This protocol, the Investigator's Brochure, a copy of the informed consent form/assent form, and, if applicable, subject recruitment materials and/or advertisements and other documents required by all applicable laws and regulations, must be submitted to a central or local IRB or IEC for approval. The IRB's or IEC's written approval of the protocol and subject informed consent/assent form must be obtained and submitted to the sponsor or its designee before commencement of the study (ie, before shipment of the sponsor-supplied vaccine(s) or study specific screening activity). The IRB or IEC approval must refer to the study by exact protocol title, number, and version date; identify versions of other documents (eg, informed consent form/assent form) reviewed; and state the approval date. The sponsor will notify the site once the sponsor has confirmed the adequacy of site regulatory documentation and, when applicable, the sponsor has received permission from the competent authority to begin the study. Until the site receives [notification] no protocol activities, including screening may occur.

Sites must adhere to all requirements stipulated by their respective IRB or IEC. This may include notification to the IRB or IEC regarding protocol amendments, updates to the informed consent form/assent form, recruitment materials intended for viewing by subjects, local safety reporting requirements, reports and updates regarding the ongoing review of the study at intervals specified by the respective IRB or IEC, and submission of the investigator's final status report to IRB or IEC. All IRB and IEC approvals and relevant documentation for these items must be provided to the sponsor or its designee.

Subject incentives should not exert undue influence for participation. Payments to subjects must be approved by the IRB or IEC and sponsor.

15.2 Subject Information, Informed Consent/Assent, and Subject Authorization

Written consent documents will embody the elements of informed consent as described in the Declaration of Helsinki and the ICH guidelines for GCP and will be in accordance with all applicable laws and regulations. The informed consent form/assent form, subject authorization form (if applicable), and subject information sheet (if applicable) describe the planned and permitted uses, transfers, and disclosures of the subject's personal and personal health information for purposes of conducting the study. The informed consent form/assent form and the subject information sheet (if applicable) further explain the nature of the study, its objectives, and potential risks and benefits, as well as the date informed consent is given. The informed consent form will detail the requirements of the participant and the fact that he or she is free to withdraw/ the subject's parent(s)/LAR is free to withdraw their child/ward at any time without giving a reason and without prejudice to his or her further medical care.

The investigator is responsible for the preparation, content, and IRB or IEC approval of the informed consent form/assent form and if applicable, the subject authorization form. The informed consent form/assent form, subject authorization form (if applicable), and subject information sheet (if applicable) must be approved by both the IRB or IEC and the sponsor prior to use.

The informed consent form/assent form, subject authorization form (if applicable), and subject information sheet (if applicable) must be written in a language fully comprehensible to the prospective subject/subject's parent(s)/LAR. It is the responsibility of the investigator to explain the detailed elements of the informed consent form/assent form, subject authorization form (if applicable), and subject information sheet (if applicable) to the subject/subject's parent(s)/LAR. Information should be given in both oral and written form whenever possible and in the manner deemed appropriate by the IRB or IEC. In the event the subject is not capable of rendering adequate written informed consent, then the subject's parent(s)/LAR may provide such consent for the subject in accordance with applicable laws and regulations.

The subject, or the subject's parent(s)/LAR, must be given ample opportunity to: (1) inquire about details of the study and (2) decide whether or not to allow their child/ward to participate in the study. If the subject, or the subject's parent(s)/LAR, determines he or she/their child/their ward will participate in the study, then the informed consent form/assent form and subject authorization form (if applicable) must be signed and dated by the subject, or the subject's parent(s)/LAR, at the time of consent and prior to the subject entering into the study. The subject or the subject's parent(s)/LAR should be instructed to sign using their legal names, not nicknames, using blue or black ballpoint ink. The investigator must also sign and date the informed consent form and subject authorization (if applicable) at the time of consent and prior to subject entering into the study; however, the sponsor may allow a designee of the investigator to sign to the extent permitted by applicable law.

Once signed, the original informed consent form/assent form, subject authorization form (if applicable), and subject information sheet (if applicable) will be stored in the investigator's site file. The investigator must document the date the subject or subject's parent(s)/LAR signs the informed consent in the subject's medical record and eCRF. Copies of the signed informed consent

form/assent form, the signed subject authorization form (if applicable), and subject information sheet (if applicable) shall be given to the subject.

All revised informed consent forms/assent forms must be reviewed and signed by the relevant subject/the relevant subject's parent(s)/LAR in the same manner as the original informed consent/assent form. The date the revised consent/assent was obtained should be recorded in the subject's medical record and eCRF, and the subject should receive a copy of the revised informed consent form/assent form.

15.3 Subject Confidentiality

The sponsor and its designees affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this study, a subject's source data will only be linked to the sponsor's clinical study database or documentation via a unique identification number. As permitted by all applicable laws and regulations, limited subject attributes, such as sex, age, or date of birth, and subject initials may be used to verify the subject and accuracy of the subject's unique identification number.

To comply with ICH guidelines for GCP and to verify compliance with this protocol, the sponsor requires the investigator to permit its monitor or its designee's monitor, representatives from any regulatory authority (eg, FDA, Medicines and Healthcare products Regulatory Agency, Pharmaceuticals and Medical Devices Agency), the sponsor's designated auditors, and the appropriate IRBs and IECs to review the subject's original medical records (source data or documents), including, but not limited to, laboratory test result reports, ECG reports, admission and discharge summaries for hospital admissions occurring during a subject's study participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject as part of the informed consent process (see Section 15.2).

Copies of any subject source documents that are provided to the sponsor must have certain personally identifiable information removed (ie, subject name, address, and other identifier fields not collected on the subject's eCRF).

15.4 Publication, Disclosure, and Clinical Study Registration Policy

15.4.1 Publication and Disclosure

The results of this study are expected to be published in a scientific journal. It is anticipated that clinical and laboratory co-investigators will participate in authorship. The order of authorship and choice of journal will be determined by the Principal Investigators and the sponsor. The data analysis center for this study will provide the analyses needed for publication. Information regarding this study will be posted on ClinicalTrials.gov.

15.4.2 Clinical Study Registration

In order to ensure that information on clinical studies reaches the public in a timely manner and to comply with applicable law, regulation and guidance, the sponsor will, at a minimum register all

clinical studies conducted in subjects that it sponsors anywhere in the world on ClinicalTrials.gov or other publicly accessible websites before study initiation. The sponsor contact information, along with investigator's city, country, and recruiting status will be registered and available for public viewing.

15.4.3 Clinical Study Results Disclosure

The sponsor will post the results of this clinical study, regardless of outcome, on ClinicalTrials.gov or other publicly accessible websites, as required by applicable laws and/or regulations.

Study completion corresponds to the date on which the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical study concluded according to the pre-specified protocol or was terminated.

In line with EC Regulation N° 1901/2006, the sponsor will submit a pediatric study within 6 months of their completion and irrespective of whether it is part of a pediatric investigational plan (completed or not yet completed) or not, or whether it is intended for submission later on as part of a variation, extension or new stand-alone marketing-authorization application or not.

15.5 Insurance and Compensation for Injury

Each subject in the study must be insured in accordance with the regulations applicable to the site where the subject is participating. If a local underwriter is required, then the sponsor or sponsor's designee will obtain clinical study insurance against the risk of injury to clinical study subjects. Refer to the Clinical Study Site Agreement regarding the sponsor's policy on subject compensation and treatment for injury. If the investigator has questions regarding this policy, he or she should contact the sponsor or sponsor's designee.

16.0 REFERENCES

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Appendix A Responsibilities of the Investigator

Clinical research studies sponsored by the sponsor are subject to ICH GCP and all the applicable local laws and regulations.

The investigator agrees to assume the following responsibilities:

1. Conduct the study in accordance with the protocol.
2. Personally conduct or supervise the staff who will assist in the protocol.
3. Ensure that study related procedures, including study specific (non-routine/non-standard panel) screening assessments are NOT performed on potential subjects, prior to the receipt of written approval from relevant governing bodies/authorities.
4. Ensure that all colleagues and employees assisting in the conduct of the study are informed of these obligations.
5. Secure prior approval of the study and any changes by an appropriate IRB/IEC that conform to 21 CFR Part 56, ICH, and local regulatory requirements.
6. Ensure that the IRB/IEC will be responsible for initial review, continuing review, and approval of the protocol. Promptly report to the IRB/IEC all changes in research activity and all anticipated risks to subjects. Make at least yearly reports on the progress of the study to the IRB/IEC, and issue a final report within 3 months of study completion.
7. Ensure that requirements for informed consent, as outlined in 21 CFR Part 56, ICH and local regulations, are met.
8. Obtain valid informed consent from the parent(s)/LAR of each subject/each subject who participates in the study, and document the date of consent in the subject's medical chart. Valid informed consent is the most current version approved by the IRB/IEC. Each informed consent form should contain a subject authorization section that describes the uses and disclosures of a subject's personal information (including personal health information) that will take place in connection with the study. If an informed consent form does not include such a subject authorization, then the investigator must obtain a separate subject authorization form from each subject or the subject's parent(s)/LAR.
9. Prepare and maintain adequate case histories of all persons entered into the study, including eCRFs, hospital records, laboratory results, etc., and maintain these data for a minimum of 2 years following notification by the sponsor that all investigations have been discontinued or that the regulatory authority has approved the marketing application. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.
10. Allow possible inspection and copying by the regulatory authority of GCP-specified essential documents.
11. Maintain current records of the receipt, administration, and disposition of sponsor-supplied vaccines, and return all unused sponsor-supplied vaccines to the sponsor.

12. Report AEs to the sponsor promptly. In the event of an SAE, notify the sponsor within 24 hours.
13. Review and provide a signature as approval of the content of the clinical study report.

Appendix B Investigator Consent to Use of Personal Information

Takeda will collect and retain personal information of investigator, including his or her name, address, and other personally identifiable information. In addition, investigator's personal information may be transferred to other parties located in countries throughout the world (eg, the United Kingdom, United States, and Japan), including the following:

- Takeda, its affiliates, and licensing partners.
- Business partners assisting Takeda, its affiliates, and licensing partners.
- Regulatory agencies and other health authorities.
- IRBs and IECs.

Investigator's personal information may be retained, processed, and transferred by Takeda and these other parties for research purposes including the following:

- Assessment of the suitability of investigator for the study and/or other clinical studies.
- Management, monitoring, inspection, and audit of the study.
- Analysis, review, and verification of the study results.
- Safety reporting and pharmacovigilance relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to the study.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to other vaccines used in other clinical studies that may contain the same chemical compound present in the investigational vaccine.
- Inspections and investigations by regulatory authorities relating to the study.
- Self-inspection and internal audit within Takeda, its affiliates, and licensing partners.
- Archiving and audit of study records.
- Posting investigator site contact information, study details and results on publicly accessible clinical study registries, databases, and websites.

Investigator's personal information may be transferred to other countries that do not have data protection laws that offer the same level of protection as data protection laws in investigator's own country. Investigator acknowledges and consents to the use of his or her personal information by Takeda and other parties for the purposes described above.

Appendix C Elements of the Subject Informed Consent

In seeking informed consent, the following information shall be provided to each subject/subject's parent(s)/LAR:

1. A statement that the study involves research.
2. An explanation of the purposes of the research.
3. The expected duration of the subject's participation.
4. A description of the procedures to be followed, including invasive procedures.
5. The identification of any procedures that are experimental.
6. The estimated number of subjects involved in the study.
7. A description of the subject's/subject's parent(s)/LAR responsibilities.
8. A description of the conduct of the study.
9. A statement describing the vaccination(s) and the probability for random assignment to each vaccination.
10. A description of the possible side effects following vaccine administration that the subject may receive.
11. A description of any reasonably foreseeable risks or discomforts to the subject and, when applicable, to an embryo, fetus, or nursing infant.
12. A description of any benefits to the subject or to others that reasonably may be expected from the research. When there is no intended clinical benefit to the subject, the subject/subject's parent(s)/LAR should be made aware of this.
13. Disclosures of appropriate alternative procedures or courses of treatment, if any, that might be advantageous to the subject and their important potential risks and benefits.
14. A statement describing the extent to which confidentiality of records identifying the subject will be maintained, and a note of the possibility that regulatory agencies, auditor(s), IRB/IEC, and the monitor may inspect the records. By signing a written informed consent form, the subject/the subject's parent(s)/LAR is authorizing such access.
15. For research involving more than minimal risk, an explanation as to whether any compensation and an explanation as to whether any medical treatments are available if injury occurs and, if so, what they consist of or where further information may be obtained.
16. The anticipated prorated payment(s), if any, to the subject for participating in the study.
17. The anticipated expenses, if any, to the subject for participating in the study.
18. An explanation of whom to contact for answers to pertinent questions about the research (investigator), subject's rights, and IRB/IEC and whom to contact in the event of a research-related injury to the subject.

19. A statement that participation is voluntary, that refusal to participate will involve no penalty or loss of benefits to which the subject otherwise is entitled, and that the subject/subject's parent(s)/LAR may discontinue participation of their child/ward at any time without penalty or loss of benefits to which the subject is otherwise entitled.
20. The consequences of a subject's decision to withdraw from the research and procedures for orderly termination of participation by the subject.
21. A statement that the subject/the subject's parent(s)/LAR will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the study.
22. A statement that results of pharmacogenomic analysis will not be disclosed to an individual, unless prevailing laws require the sponsor to do so.
23. The foreseeable circumstances or reasons under which the subject's participation in the study may be terminated.
24. A written subject authorization (either contained within the informed consent form or provided as a separate document) describing to the subject/subject's parent(s)/LAR the contemplated and permissible uses and disclosures of the subject's personal information (including personal health information) for purposes of conducting the study. The subject authorization must contain the following statements regarding the uses and disclosures of the subject's personal information:
    a) that personal information (including personal health information) may be processed by or transferred to other parties in other countries for clinical research and safety reporting purposes, including, without limitation, to the following: (1) Takeda, its affiliates, and licensing partners; (2) business partners assisting Takeda, its affiliates, and licensing partners; (3) regulatory agencies and other health authorities; and (4) IRBs/IECs;
    b) it is possible that personal information (including personal health information) may be processed and transferred to countries that do not have data protection laws that offer subjects the same level of protection as the data protection laws within this country; however, Takeda will make every effort to keep your personal information confidential, and your name will not be disclosed outside the clinic unless required by law;
    c) that personal information (including personal health information) may be added to Takeda's research databases for purposes of developing a better understanding of the safety and effectiveness of the investigational vaccine(s), studying other therapies for patients, developing a better understanding of disease, and improving the efficiency of future clinical studies;
    d) that subjects agree not to restrict the use and disclosure of their personal information (including personal health information) upon withdrawal from the study to the extent that the restricted use or disclosure of such information may impact the scientific integrity of the research; and

e) that the subject's identity will remain confidential in the event that study results are published.

25. Female subjects of childbearing potential (eg, non-sterilized, premenopausal female subjects) who are sexually active must use adequate contraception (as defined in the informed consent) from 2 months prior to Day 1 (Month 0) up to 6 weeks post second vaccination. Pregnancy tests will be performed at Screening, prior to vaccination at Day 1 (Month 0), and prior to vaccination at Day 90 (Month 3). Additional pregnancy tests may be performed during the study if deemed necessary by the investigator. Female of childbearing potential who are sexually active will be reminded during study visits to adhere to acceptable contraceptive methods (see Section 7.2) up to 6 weeks post second vaccination. If a subject is found to be pregnant during the study, no further vaccine doses will be administered.
26. A statement that clinical study information from this study will be publicly disclosed in a publicly accessible website, such as ClinicalTrials.gov.

Appendix D Serology Plan

Table 16.a Serology Plan

Timing	Blood volume	Assessments
Screening or Day 1 (Month 0)	Approximately 3 mL	Dengue IgG indirect ELISA
Day 1 (Month 0), Day 30 (Month 1), Day 90 (Month 3), Day 120 (Month 4), Day 270 (Month 9), and annually for 3 years post second vaccination	20 mL	Cellular immune responses
Day 14 (In subjects >10 years of age)		
Day 1 (Month 0), Day 90 (Month 3), Day 120 (Month 4), Day 270 (Month 9), and annually for 3 years post second vaccination	5 mL	Dengue neutralizing antibodies (MNT)
Day 30 (Month 1)	2.5 mL	
Acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever)	4 mL	RT-PCR, NS1 ELISA

ELISA=enzyme-linked immunosorbent assay, IgG=immunoglobulin G, MNT=microneutralization test, RT-PCR= reverse transcriptase polymerase chain reaction
Note: Blood sampling at Screening or pre-first vaccination at Day 1 (Month 0).
Note: The IgG indirect ELISA is to be performed locally as per local laboratory requirements. The blood sample volume may be different as per local laboratory requirements.
Note: Samples at Day 1 (Month 0) and Day 90 (Month 3) should be collected prior to vaccination.
Note: In subjects ≤10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 3 mL/kg or 50 mL in total (whichever is lower) within 8 weeks. In subjects >10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 5 mL/kg within 8 weeks [16].
Note: Samples will be sent to the central laboratory, as applicable. RT-PCR and NS1 ELISA results from the central laboratory will not be available for real time case management. Additional blood samples may be taken for diagnosis and clinical management of the subject as per standard medical practice.