Clinical Study Protocol

NCT Number: NCT06060067

Title: A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial to Investigate the Safety and Immunogenicity of a Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) Administered Subcutaneously to Healthy Subjects Aged 4 to 60 Years in India

Study Number: DEN-302

Document Version and Date: Version 2.0, 15 June 2023

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial to Investigate the Safety and Immunogenicity of a Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) Administered Subcutaneously to Healthy Subjects Aged 4 to 60 Years in India

Safety and Immunogenicity of TDV in Subjects Aged 4 to 60 Years in India

Sponsor:	Takeda Vaccines, Inc. 40 Landsdowne Street, Cambridge, MA 02139, USA
Trial Identifier:	DEN-302
IND Number:	Not applicable **EudraCT Number:** Not applicable
Investigational Medicinal Products:	Dengue Tetravalent Vaccine (Live, Attenuated) (TDV)
Takeda Approval Date:	15 June 2023
Version:	Version 2.0

1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

The list of contacts will be provided to the/each site.

1.2 Approval

REPRESENTATIVES OF TAKEDA

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

- The ethical principles that have their origin in the Declaration of Helsinki [1].
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) E6 (R2) Good Clinical Practice: Consolidated Guideline [2].
- All applicable laws and regulations, including, but not limited to those related to data privacy and clinical trial disclosure.

SIGNATURES

[redacted], MB BS, MD	Date	[redacted]	Date
[redacted]		[redacted]	
Vaccine Business Unit		Vaccine Business Unit	
Takeda Pharmaceuticals International AG		Takeda Vaccines Pte Ltd	

[redacted], PhD	Date	[redacted], MSc	Date
[redacted]		[redacted],	
Pharmacovigilance Benefit-Risk Scientist		Vaccines Statistics	
Vaccine Business Unit		Global Statistics	

INVESTIGATOR AGREEMENT

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

- The ethical principles that have their origin in the Declaration of Helsinki [1].
- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), E6 (R2) Good Clinical Practice: Consolidated Guideline [2].
- All applicable laws and regulations, including, but not limited to those related to data privacy and clinical trial disclosure.
- Regulatory requirements for reporting serious adverse events defined in Section 10.4.4 of this protocol.
- Terms outlined in the Clinical Trial 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 History and Summary of Changes

The protocol for study DEN-302 has been amended and reissued as follows:

Date	Amendment	Change Type	Region
15 June 2023	1	Non-substantial	Global
14 February 2022	Initial Protocol	Not applicable	Global

The summary of changes to the Protocol and the rationale for each change are provided below.

The primary purpose of this amendment was to update the protocol to remove the Day 30 blood sample draw to comply with a request made by Indian Central Drugs Standard Control Organisation (CDSCO) following a meeting of the Subject Expert Committee on 16 May 2023. Minor grammatical and editorial changes were made for clarification purposes only. References were also checked and updated as needed.

Section	Description of Change	Rationale for Change
1.2	Administrative updates	Document maintenance
2.0	Trial design schematic and corresponding immunogenicity evaluation text updated to remove Day 30 blood draw	CDSCO request
2.0	Trial design schematic updated to include the existing blood draw at screening	Consistency and clarity
2.0	Secondary immunogenicity objectives updated to remove Day 30 immunogenicity time point	CDSCO request
2.0	Secondary immunogenicity endpoints updated to remove Day 30 immunogenicity time point	CDSCO request
2.0	CSR now written in full in Trial Summary section	Minor editorial update
2.1	Day 30 blood draw removed from the schedule of trial procedures table and the corresponding footnote	CDSCO request

Section	Description of Change	Rationale for Change
3.2	Clinical study report now defined at first mention in the body of the document	Minor editorial update
3.3	CDSCO added to the list of abbreviations	Minor editorial update
4.1	Details of licensure added	Document maintenance
4.1	Investigator's Brochure abbreviated	Minor editorial update
5.1.2	Secondary immunogenicity objectives updated to remove Day 30 immunogenicity time point	CDSCO request
5.2.2	Secondary immunogenicity endpoints updated to remove Day 30 immunogenicity time point	CDSCO request
6.1	Figure 6.a updated to remove Day 30 blood draw	CDSCO request
6.1	Figure 6.a updated to include the blood draw at screening	Consistency and clarity
6.1	Day 30 blood draw removed from immunogenicity evaluation text	CDSCO request
8.5	Reference to study procedures manual removed.	Not needed.
8.6	Labeled storage conditions clarified	No cold chain break only applicable for TDV
9.1.6	Details pertaining to Day 30 blood draw removed and approximate total volume of blood obtained updated accordingly	CDSCO request
9.3.5	Schedule of procedures updated to reflect the removal of the Day 30 blood draw	CDSCO request
13.2	Clinical study report now defined at first mention and abbreviated here	Minor editorial update
16.0	Access dates updated and web-based references updated accordingly, where required	Document maintenance

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

2.0 TRIAL SUMMARY

Name of Sponsor: Takeda Vaccines, Inc. 40 Landsdowne Street Cambridge, MA, 02139 USA		**Product Name:** Takeda's Dengue Tetravalent Vaccine (Live, Attenuated)
Trial Title: A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial to Investigate the Safety and Immunogenicity of a Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) Administered Subcutaneously to Healthy Subjects Aged 4 to 60 Years in India		
IND No.: Not applicable	**EudraCT No.:** Not applicable	
Trial Identifier: DEN-302	**Phase:** 3	**Blinding Schema:** Double-Blind
Indication: Prevention of dengue fever of any severity due to any serotype		

Background and Rationale:

Dengue fever is caused by infection with the wild type dengue virus (DENV), a ribonucleic acid virus that occurs as 4 recognized serotypes, dengue virus serotype -1, -2, -3, and -4 (DENV-1, DENV-2, DENV-3, and DENV-4). These dengue viruses are transmitted from human to human by mosquitoes (primarily *Aedes aegypti*). The 4 dengue viruses 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.

Dengue fever is clinically defined as an acute febrile illness with 2 or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leucopenia, occurring at the same location and time as other confirmed cases of dengue fever. The most severe forms of dengue infection – dengue hemorrhagic fever (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 serotypes and these subsequent infections may increase the risk of severe disease (DHF/DSS).

Treatment of dengue fever is based solely on signs and symptoms, with fluid replacement required for hemorrhagic or shock cases. An antiviral therapy for DENV 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 unmet global public health need for a safe and effective vaccine to reduce the morbidity and mortality associated with dengue disease. Vaccine development has focused on tetravalent vaccines that provide protection against all 4 dengue serotypes simultaneously since all 4 dengue serotypes commonly co-circulate in endemic areas. A first recombinant dengue vaccine (chimeric yellow fever virus-dengue virus tetravalent dengue vaccine [CYD-TDV]) has been approved since 2015 in several Asian and Latin American countries as well as in the United States and in the European Union. Vaccine efficacy was different between serotypes and depended on dengue preexposure status. Additional analyses found that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease when they were infected with dengue virus after vaccination with CYD-TDV. In a revised Strategic Advisory Group of Experts on Immunization (SAGE) recommendation in April 2018, the SAGE concluded that for countries considering CYD-TDV vaccination as part of their dengue control program, a "pre vaccination screening strategy" would be the preferred option, in which only dengue-seropositive persons are vaccinated. Hence, there is a continued unmet public health need for safer and more efficacious dengue vaccine to reduce the morbidity and mortality associated with dengue disease.

Takeda's Dengue Tetravalent Vaccine (Live, Attenuated) – Background:

Takeda's Dengue Tetravalent Vaccine (Live, attenuated) (TDV) consists of 1 molecularly-characterized, attenuated dengue serotype 2 virus strain, plus 3 recombinant dengue virus strains expressing surface antigens corresponding to

dengue serotypes 1, 3 and 4. The dengue serotype 2 strain (TDV-2) is based upon the attenuated laboratory-derived DENV-2 virus strain, originally isolated at Mahidol University, Bangkok, Thailand and generated by 53 serial passages in primary dog kidney (PDK) cells (DENV-2 PDK-53). The recombinant strains were engineered by substituting the structural genes, premembrane (prM) and envelope (E), of TDV-2 with the prM and E genes from the DENV-1 16007, DENV-3 16562 or DENV-4 1036 virus strains, respectively. Thus, TDV comprises 4 dengue virus strains: a molecularly-characterized, attenuated TDV-2 strain, a dengue serotypes 2/1 recombinant strain (TDV-1), a dengue serotypes 2/3 recombinant strain (TDV-3), and a dengue serotypes 2/4 recombinant strain (TDV-4).

Data from completed phase 1 and phase 2 clinical trials in humans have shown satisfactory reactogenicity, safety and immunogenicity profiles for Takeda's TDV in healthy adults in non-endemic areas as well as in healthy adults and children in endemic areas in Asia and Latin America. Completed phase 2 clinical trials have enabled the selection of a final TDV dose (in a lyophilized formulation) and a 2-dose vaccination series administered 3 months (ie, 90 days) apart by subcutaneous (SC) injection for use in the ongoing clinical development program. Results from the pivotal DEN-301 efficacy trial showed that the primary endpoint was met, demonstrating that TDV was efficacious in preventing dengue fever in children and adolescents living in dengue-endemic countries. TDV has been given to >19,900 clinical trial subjects. All available data also showed that TDV was well tolerated with no significant safety concerns to date.

The current version of the Investigator's Brochure contains additional product information and a more detailed review of preclinical and clinical trials.

Rationale for the Proposed Trial:

Because of limited surveillance data and understanding of dengue fever epidemiology in India the disease burden appears to be underestimated, although emerging literature on dengue seroprevalence indicates that its endemicity in India may be as high as that of other countries in Southeast Asia. Based on the size of population, the increasing incidence of dengue fever and the consequent economic burden to the country, and its identification as an epicenter of dengue, the need for a preventive solution presents a critical unmet medical need for an important public health problem in India.

The purpose of this phase 3 trial is to generate immunogenicity and safety data in the Indian population with a view of supporting future licensure of TDV in India. Trial DEN-302 will therefore evaluate the safety and immunogenicity of TDV administered as 2 doses given 3 months apart to participants in India. A placebo has been chosen to maintain the double-blind trial design in the absence of a suitable active comparator for the age-range in this trial population.

The trial will be conducted in accordance with the protocol, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements.

Trial Design:

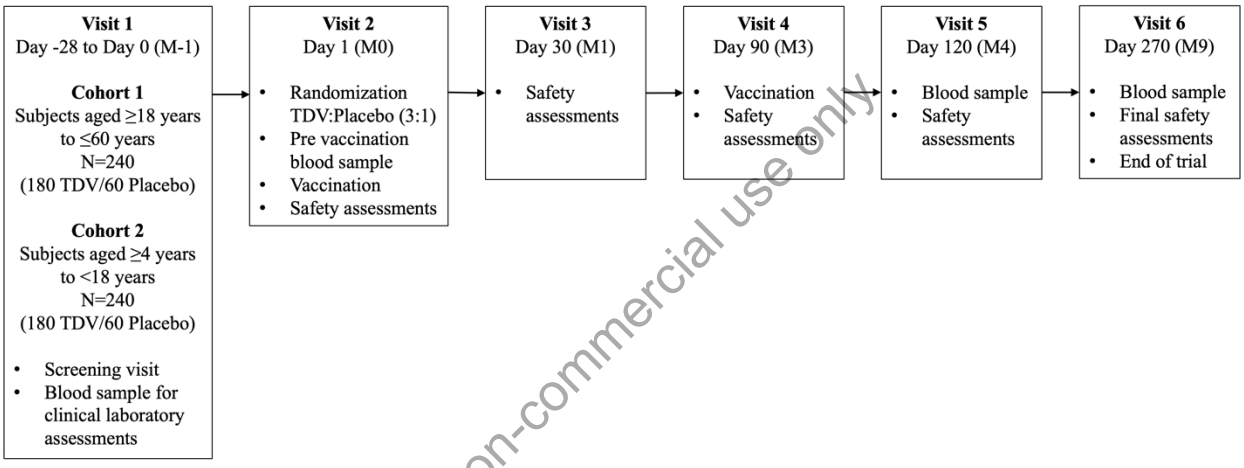
This is a phase 3, randomized, multi-site, double-blind, placebo-controlled trial in 480 healthy subjects aged ≥4 to ≤60 years living in India.

- **Cohort 1**: 240 subjects ≥18 to ≤60 years of age will be enrolled and randomly assigned (3:1) to receive either TDV (N=180) or placebo (N=60) at Day 1 (Month [M] 0) and Day 90 (M3).
- **Cohort 2**: 240 subjects ≥4 to <18 years of age will be enrolled and randomly assigned (3:1) to receive either TDV (N=180) or placebo (N=60) at Day 1 (M0) and Day 90 (M3).

Cohorts 1 and 2 will be enrolled in parallel.

Adolescents who attain the legal age of consent during or after Visit 1 (Day -28 [M -1]) will be asked to return to the investigational site to attest to the appropriate written informed consent. This may require an additional site visit.

The trial design schematic is shown below:



M: Month.

Immunogenicity evaluation (all subjects):

Neutralizing antibodies will be measured (by microneutralization test 50% [MNT_{50}]) using blood samples obtained pre vaccination at Day 1 (M0), 1 month post second vaccination at Day 120 (M4), and at the end of trial (Day 270 [M9] or earlier for early terminations).

Safety evaluation (all subjects):

Diary cards will be distributed at Day 1 (M0) and Day 90 (M3) for the recording of:

- Solicited local (injection site) adverse events (AEs) for 7 days following administration of TDV or placebo (day of vaccination + 6 days). These will include:
    - Injection site pain, injection site erythema, and injection site swelling.
- Solicited systemic AEs for 14 days following administration of TDV or placebo (day of vaccination + 13 days). These will include:
    - Subjects <6 years of age: fever, irritability/fussiness, drowsiness, and loss of appetite.
    - Subjects ≥6 years of age: asthenia, fever, headache, malaise, and myalgia.

Unsolicited AEs will be collected by interview and recorded for 28 days following administration of TDV or placebo (day of vaccination + 27 days).

All serious adverse events (SAEs), medically-attended AEs (MAAEs), AEs leading to subject withdrawal from the trial, and AEs leading to TDV or placebo discontinuation will be collected from Day 1 (M0) postvaccination

through the end of the trial. MAAEs are defined as AEs leading to an unscheduled visit to or by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria.

Primary Objectives:

Safety

- To assess the safety profile of TDV administered as 2 doses given 3 months apart in healthy adults, adolescents, and children.

Immunogenicity

- To evaluate the immunogenicity of TDV administered as 2 doses given 3 months apart in healthy adults, adolescents, and children at 1 month post second dose.

Secondary Objectives:

Immunogenicity

- To describe immunogenicity of TDV at baseline and 6 months post second TDV dose when administered as 2 doses given 3 months apart.
- To describe seropositivity (% of subjects with reciprocal neutralizing titer ≥10) at baseline, 1 month post second TDV dose, and 6 months post second TDV dose.

Subject Population:

Healthy Subjects: Yes.

Age Range: ≥4 to ≤60 years.

Planned Number of Subjects: 480 subjects in 2 cohorts of 240 subjects each (≥18 to ≤60 years of age [Cohort 1] and ≥4 to <18 years of age [Cohort 2]).

Planned Number of Trial Arms: 2 arms (TDV and placebo) for each cohort, enrolled in parallel.

Estimated Total: 480 subjects.

Key Inclusion Criteria:

Subject eligibility is determined according to the following criteria:

1. Subjects aged ≥4 to ≤60 years at the time of random assignment.
2. Male or female.
3. Subjects who are in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs), and the clinical judgment of the investigator.
4. Subjects and/or the subjects legally acceptable representative (LAR) who have signed and dated a written, informed consent/pediatric assent form, and any required privacy authorization prior to the initiation of any trial procedures, and after the nature of the trial has been explained according to local regulatory requirements.
5. Subjects who can comply with trial procedures and are available for the duration of follow-up.

Exclusion Criteria at Entry:

Any subject who meets any of the following criteria will not qualify for entry into the trial at screening (Visit 1 [Day -28 (M -1)]):

1. Subjects with any illness, or history of any illness that in the opinion of the investigator, might interfere with the results of the trial or pose an additional risk to the subject due to participation in the trial, including but not limited to:
    a. Known hypersensitivity or allergy to any of the TDV or placebo components.
    b. Abnormalities of splenic or thymic function.
    c. Bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
    d. Serious chronic or progressive disease according to judgment of the investigator (eg, neoplasm,

insulin-dependent diabetes, cardiac, hepatic or renal disease, neurologic or seizure disorder, or neuroinflammatory disease such as Guillain-Barré syndrome).

e. Known or suspected impairment/alteration of immune function, including:
   i. Chronic use of oral steroids (equivalent to 20 mg/day prednisone for ≥12 weeks / ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0) (Note: use of inhaled, intranasal, or topical corticosteroids is allowed).
   ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone for ≥12 weeks / ≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0).
   iii. Receipt of immunoglobulins and/or any blood products within 90 days prior to Day 1 (M0).
   iv. Receipt of immunostimulants within 60 days prior to Day 1 (M0).
   v. Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (M0).
   vi. Human immunodeficiency virus (HIV) infection or HIV-related disease.
   vii. Hepatitis C and/or Hepatitis B virus infection.
   viii. Genetic immunodeficiency.

2. Behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.
3. A body mass index (BMI = weight in kg/[height in meters^2]) ≥35 kg/m^2.
4. Intent to participate in another clinical trial at any time during the conduct of this trial.
5. Subject plans to receive any of the following (consider whether applicable as an exclusion criterion or as a criterion for delay of Visit 1):
   a. A licensed vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to TDV or placebo administration.
   b. A coronavirus vaccine within 14 days prior to TDV or placebo administration.
   c. A vaccine authorized for emergency use within 28 days of TDV or placebo administration.
6. Known substance or alcohol abuse within the past 2 years that may interfere with his/her ability to comply with requirements for trial participation.
7. Female subjects who are pregnant (ie, a positive or indeterminate pregnancy test) or breastfeeding.
8. Females of childbearing potential[1] who are sexually active, and who have not used any of the acceptable contraceptive methods[2] for at least 2 months prior to Day 1 (M 0).

[1] Defined as status post onset of menarche and not meeting any of the following conditions: menopausal (at least 2 years previously) 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, monogamous relationship with a 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.

9. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method up to 6 weeks after the last dose of trial vaccine (TDV or placebo). They must also be advised not to donate ova or breastfeed during this period.
10. Subjects involved in the trial conduct or their first-degree relatives.
11. Subjects identified as an employee of the investigator or trial center, with direct involvement in the proposed trial or other trials under the direction of that investigator or trial center.
12. Receipt of previous vaccination against dengue virus.
13. Previous participation in any clinical trial of a dengue candidate vaccine, except if it is known that the subject received placebo while participating in those trials.

There may be instances when individuals meet all entry criteria except 1 that relates to transient clinical circumstances eg, recent use of excluded medication(s) or vaccine(s). Under these circumstances, eligibility for trial enrolment 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.

Exclusion Criteria at Vaccination:

Any subject who successfully met the criteria for entry to this trial, but who now meets any of the following criteria will not qualify for random assignment at Day 1 (M0):

1. Subjects with febrile illness (body temperature ≥38°C [≥100.4°F]) or moderate or severe acute illness, or infection, at the time of random assignment (consider whether applicable as an exclusion criterion or as a criterion for delay of TDV or placebo administration).
2. Subjects with any new findings of illness in the interim period since Visit 1 that, in the opinion of the investigator, might interfere with the results of the trial or pose an additional risk to the subject due to participation in the trial, including but not limited to:
    a. Abnormalities of splenic or thymic function.
    b. Bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
    c. Serious chronic or progressive disease according to judgment of the investigator (eg, neoplasm, insulin-dependent diabetes, cardiac, hepatic or renal disease, neurologic or seizure disorder, or neuroinflammatory disease such as 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 for ≥12 weeks / ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0) (Note: use of inhaled, intranasal, or topical corticosteroids is allowed).
        ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone for ≥12 weeks / ≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0).
        iii. Receipt of immunoglobulins and/or any blood products within 90 days prior to Day 1 (M0) or planned TDV or placebo administration during this trial.
        iv. Receipt of immunostimulants within 60 days prior to Day 1 (M0).
        v. Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (M0).
        vi. Human immunodeficiency virus (HIV) infection or HIV-related disease.
        vii. Hepatitis C and/or Hepatitis B virus infection.
        viii. Genetic immunodeficiency.
3. Behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.

4. A BMI (weight in kg/[height in meters^2]) ≥35 kg/m^2.
5. Participation in any clinical trial with another investigational product 30 days prior to Day 1 (M0) or intent to participate in another clinical trial at any time during the conduct of this trial.
6. Subject plans to receive or has received any of the following (consider whether applicable as an exclusion criterion or as a criterion for delay of TDV or placebo administration):
    a. A licensed vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to TDV or placebo administration.
    b. A coronavirus vaccine within 14 days prior to TDV or placebo administration.
    c. A vaccine authorized for emergency use within 28 days of TDV or placebo administration.
7. Subject has been medicated with antipyretic and/or analgesic medication(s) within 24 hours prior to TDV or placebo administration (consider whether applicable as an exclusion criterion or as a criterion for delay of TDV or placebo administration).
8. Female subjects who are pregnant (ie, a positive or indeterminate pregnancy test).
9. Females of childbearing potential[3] who are sexually active, and who have not used any of the acceptable contraceptive methods[4] for at least 2 months prior to Day 1 (M0).
10. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method up to 6 weeks after the last dose of trial vaccine (TDV or placebo). They must also be advised not to donate ova or breastfeed during this period.
11. Subjects involved in the trial conduct or their first-degree relatives.
12. Subjects identified as an employee of the investigator or trial center, with direct involvement in the proposed trial or other trials under the direction of that investigator or trial center.
13. Receipt of vaccination against dengue virus.
14. Enrolment in any clinical trial of a dengue candidate vaccine (other than this trial), except if it is known that the subject has received placebo while participating in those trials.

There may be instances when individuals meet all entry criteria except for 1 that relates to transient clinical circumstances eg, body temperature elevation or recent use of excluded medication(s) or vaccine(s). Under these circumstances, eligibility for random assignment and 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.

[3] Defined as status post onset of menarche and not meeting any of the following conditions: menopausal (at least 2 years previously) bilateral tubal ligation (at least 1 year previously), bilateral oophorectomy (at least 1 year previously) or hysterectomy.

[4] 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, monogamous relationship with a 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.

Investigational Medicinal Products:

Investigational vaccine

The investigational vaccine TDV, comprises 1 molecularly-characterized, attenuated dengue virus strain (TDV-2), and 3 recombinant dengue virus strains (TDV-1, TDV-3, and TDV-4) with potencies of not less than 3.3, 2.7, 4.0 and 4.5 $\log_{10}$ plaque-forming units (PFU) per dose of TDV-1, TDV-2, TDV-3, and TDV-4, respectively. TDV is a lyophilized vaccine that will be reconstituted in diluent (37 mM sodium chloride solution) prior to administration.

Placebo

The placebo is normal saline (0.9% sodium chloride [saline] solution) for injection.

Route of administration

SC route.

Duration of the Trial and Subject Participation:

The trial duration will be approximately 270 days (9 months) for each subject.

Criteria for Evaluation and Analyses:

Primary

Safety endpoints

- Frequency and severity of solicited local (injection site) AEs for 7 days (day of vaccination + 6 subsequent days) and solicited systemic AEs for 14 days (day of vaccination + 13 subsequent days) postvaccination at Day 1 (M0) and Day 90 (M3).
- Percentage of subjects with any unsolicited AEs for 28 days postvaccination (day of vaccination + 27 subsequent days) at Day 1 (M0) and Day 90 (M3).
- Percentage of subjects with an AE leading to subject withdrawal from the trial from Day 1 (M0) postvaccination through the end of the trial.
- Percentage of subjects with an AE leading to TDV or placebo discontinuation from Day 1 (M0) postvaccination through the end of the trial.
- Percentage of subjects with an MAAE from Day 1 (M0) postvaccination through the end of the trial.
- Percentage of subjects with an SAE from Day 1 (M0) postvaccination through the end of the trial.

Immunogenicity endpoint

- Geometric mean titers (GMTs) of neutralizing antibodies (by MNT_{50}) against each of the 4 dengue virus serotypes at Day 120 (M4).

Secondary

Immunogenicity endpoints

- GMTs by MNT_{50} against each of the 4 dengue virus serotypes at Day 1 (M0) and Day 270 (M9).
- Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against each of the 4 dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).
- Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against multiple (2, 3, or 4) dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).

Statistical Considerations:

All definitions apply to both cohorts. All data will be presented overall by trial arm, and by cohort and trial arm.

Analysis sets

Safety set: All subjects who received at least 1 dose of TDV or placebo.

Full analysis set (FAS): All randomized subjects who received at least 1 dose of TDV or placebo, and for whom a valid pre dose blood sample, and at least 1 valid post dose blood sample are received for immunogenicity assessments.

Per-protocol set (PPS): All subjects in the FAS who have no major protocol violations.

The major protocol violation criteria will be defined as part of the blinded data review prior to unblinding of the subject's assignment to the TDV or placebo arm. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving the wrong trial vaccination (TDV or placebo), (3) receiving prohibited vaccinations or therapies, (4) not receiving 2 doses of TDV or placebo, or, receiving the second dose outside of the visit window, (5) not having a valid immunogenicity assessment at 1 month post second dose (Day 120 [M4]), and (6) other major protocol violations that may be identified and documented during blinded data reviews.

Analysis of demographic and other baseline characteristics

Age, gender, race, baseline serostatus, and other baseline characteristics will be summarized for all randomized subjects.

Immunogenicity analysis

For the immunogenicity endpoints (ie, GMTs of neutralizing antibodies, seropositivity rates against each of the 4 dengue serotypes, and seropositivity rates against multiple dengue serotypes), descriptive statistics and 95% CIs will be provided. Seropositivity is defined as a reciprocal neutralizing titer ≥10. Additional summaries of GMTs of neutralizing antibodies by baseline serostatus (positive or negative) will also be provided.

The primary immunogenicity analyses will be based on the PPS; additional immunogenicity analyses may be provided based on the FAS as described in the statistical analysis plan (SAP). The handling of missing data will be described in the SAP.

Potential analyses and/or table summaries may be performed to assess the effect of the coronavirus disease 2019 (COVID-19) pandemic. These may include sensitivity analyses ignoring the protocol-defined visit windows for the PPS subjects affected by COVID-19.

Safety analysis

All safety data will be summarized using the safety set.

Solicited AEs

The presence and severity (grade) of solicited local (injection site) AEs (pain, erythema, and swelling) and solicited systemic AEs (<6 years of age: fever, irritability/fussiness, drowsiness, and loss of appetite; ≥6 years of age: fever, asthenia, malaise, headache, and myalgia) will be collected via diary cards for 7 days and 14 days, respectively, following administration of each TDV or placebo dose (including the day of administration) at Day 1 (M0) and Day 90 (M3).

For each solicited AE, the number and percentage of subjects with local (injection site) and systemic AEs will be summarized and event severity for each day after each vaccination (ie, Day 1 through Day 7 for local [injection site] AEs and Day 1 through Day 14 for systemic AEs), and overall. Summaries of first onset of each event and the number of days subjects reported experiencing each event will also be provided. For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

Persistent/prolonged solicited local or systemic AEs continuing on/after Day 8 or Day 15, respectively, following each trial vaccination (TDV or placebo) will be assessed separately. Unless otherwise specified these events will not be included in the analyses/tabulations of unsolicited AEs and will have separate listings.

Unsolicited AEs

Unsolicited AEs will be assessed for 28 days following administration of each TDV or placebo dose (day of administration +27 subsequent days).

Unsolicited AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by System Organ Class (SOC) and Preferred Term (PT). AEs leading to subject withdrawal from the trial or TDV or placebo discontinuation will also be summarized.

Unsolicited AEs will be summarized as follows: by PT including events with frequency greater than a predefined frequency (the percentage will be specified in the SAP); by SOC and PT; by SOC, PT, and severity; and by SOC, PT, and relationship to TDV or placebo. Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once.

MAAEs

MAAEs will be collected from Day 1 (M0) postvaccination through the end of the trial. MAAEs will be coded using MedDRA and summarized by SOC and PT.

SAEs

SAEs will be collected from Day 1 (M0) postvaccination through the end of the trial. SAEs will be coded using MedDRA and summarized by SOC and PT.

Sample Size Justification:

The analysis of this trial is descriptive and is not based on testing formal null hypotheses. [redacted] The number of subjects receiving placebo will be 60 in each cohort (N=120 in total), as per the random assignment of 3:1 (TDV:placebo). Therefore, the total trial sample size is 480 subjects.

Interim Analysis:

In anticipation of interest by the local health authority, a blinded interim analysis of safety data will be prepared when all subjects have completed the Day 120 (M4) visit. An interim clinical study report will not be prepared. If requested, the unblinded interim analysis will be tabulated and submitted to the local health authority for review. Trial results will be reported in the final clinical study report.

Data Monitoring Committee:

A Data Monitoring Committee (DMC) will have oversight of this trial. The DMC functions at a program level and further information is available in the DMC Charter.

DEN-302 Version 2.0 (15 June 2023)

2.1 Schedule of Trial Procedures

Visit number		**V1**	**V2**	**V3**	**V4**	**V5**	**V6/ET [a]**
Day		**Day -28**	**Day 1**	**Day 30**	**Day 90**	**Day 120**	**Day 270**
Month		**M -1**	**M0**	**M1**	**M3**	**M4**	**M9**
Visit window (days)		**-28 to -1**	**0**	**-1/+7**	**-4/+7**	**-1/+7**	**-7/+14**
Signed and dated informed consent/assent [b]		X					
Assessment of eligibility criteria [c]		X	X				
Random assignment			X				
Demographic characteristics		X					
Medical history and prior medications/vaccinations [d]		X	X				
Concomitant medications/vaccinations [e]		X	X	X	X	X	X
Check criteria for delay of TDV or placebo administration			X		X		
Check contraindications to TDV or placebo administration			X		X		
Complete physical examination [f]		X	X		X		
Symptom-directed physical examination [g]				X		X	X
Vital signs [h]		X	X	X	X	X	X
Pregnancy test [i]		X	X		X		
Serum Biochemistry and Hematology/Urinalysis		X					
HIV testing and Hepatitis Panel (Hepatitis B surface antigen and anti-hepatitis C virus antibodies).		X					
Pregnancy avoidance guidance [j]		X	X	X	X	X	
Blood sample for dengue neutralizing antibodies (5 mL) [k]			X			X	X
TDV or placebo administration			X		X		
Post vaccination observation [l]			X		X		
Diary card [m]	Distribution		X		X		
	Review/collection			X		X	
Unsolicited AEs [n]			X	X	X	X	
Serious AEs, medically-attended AEs, AEs leading to TDV or placebo discontinuation and AEs leading to subject withdrawal from the trial [o]			X	X	X	X	X

AE=adverse event, ET=early termination, HIV=human immunodeficiency virus, M=month, TDV=Dengue Tetravalent Vaccine (Live, Attenuated), the Takeda dengue vaccine candidate also known as TAK-003, V=visit.
Footnotes on the next page.

Footnotes:

(a) If the subject discontinues earlier than planned from the trial, Day 270 (M9) procedures should be performed.

(b) A signed and dated informed consent, or where applicable an informed consent and assent form, must be obtained before any protocol-directed procedures are performed. Adolescents who attain the legal age of consent during or after Visit 1 (Day -28 [M -1]) will be asked to return to the investigational site to attest to the appropriate written informed consent. This may require an additional site visit.

(c) Eligibility for trial entry at Visit 1 (Day -28 [M -1]) should be assessed using the inclusion criteria, and, exclusion criteria at entry. Eligibility for random assignment at Visit 2 (Day 1 [M0]) should be assessed using the inclusion criteria, and, exclusion criteria at vaccination.

(d) Collected at screening (Visit 1 [Day -28 (M -1)]) for trial entry purposes and must be rechecked at Day 1 (M0) prior to vaccination. Data should be recorded in the subject's source documents.

(e) Any other vaccinations (licensed, authorized for emergency use, or investigational, including any other dengue vaccine) and concomitant medications should be recorded in the subject's source documents at each visit.

(f) Performed pre vaccination at Day 1 (M0) and Day 90 (M3). Physical examination should include measurement of vital signs (see footnote [(h)]), and, weight and height at Day -28 (M -1) and Day 1 (M0) (body mass index [BMI] will be calculated). All physical examination findings should be documented in the subject's source documents. At Day 90 (M3), clinically significant changes from the baseline examinations, conducted at Day -28 (M -1) and Day 1 (M0), should be recorded in the subject's source documents and reported as an AE in the Case Report Form (CRF).

(g) All findings from this examination should be documented in the subject's source documents. Clinically significant changes from the baseline examinations, conducted at Day -28 (M -1) and Day 1 (M0), should be recorded in the subject's source documents and reported as an AE in the CRF. If the exam is not conducted (because of a lack of any symptoms) then the absence will be recorded as 'not needed' in the subject's source document.

(h) Performed pre vaccination at Day 1 (M0) and Day 90 (M3). Vital signs including (but not limited to) the measurement of systolic blood pressure, diastolic blood pressure, heart rate, and body temperature should be recorded at all visits.

(i) Pregnancy testing (urine) for females of childbearing potential. Results must be confirmed and documented as negative prior to each TDV or placebo dose administration. Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator; where the results of a urine pregnancy test are in doubt, a serum pregnancy test will be performed to verify the result.

(j) Females of childbearing potential who are sexually active will be provided with information on acceptable methods of contraception as part of the subject informed consent/assent process and will be asked to sign a consent form/assent form stating that they understand the requirements for avoidance of pregnancy and donation of ova. Females of childbearing potential who are sexually active will also be reminded during trial visits to adhere to acceptable contraceptive methods for up to 6 weeks after the last dose of TDV or placebo.

(k) The blood sample at Day 1 (M0) should be obtained prior to TDV or placebo administration. The blood sample obtained at Day 120 (M4) should be taken at least 28 days after the second (Day 90 [M3]) TDV or placebo vaccination.

(l) Subjects will be observed for at least 30 minutes postvaccination. Any AEs will be recorded and the injection site will be evaluated for pain, erythema, and swelling.

(m) Diary cards will be distributed to record:
1) solicited local (injection site) AEs for 7 days following TDV or placebo administration (including the day of administration). If solicited local AEs continue on/after Day 8 following each trial vaccination (TDV or placebo), record the extended information on the AE CRF.
2) solicited systemic AEs for 14 days following TDV or placebo administration (including the day of administration). If solicited systemic AEs continue on/after Day 15 following each trial vaccination (TDV or placebo), record the extended information on the AE CRF.
The investigator will categorize local and systemic events by severity (mild, moderate, or severe), and will assess causality to TDV or placebo dose administration for solicited systemic events (related or not related) only.

(n) Unsolicited AEs will be collected by interview and will be recorded for 28 days (day of vaccination + 27 days) following administration of TDV or placebo. The investigator will categorize events by severity (mild, moderate, or severe) and will assess causality (related or not related) to TDV or placebo dose administration. Adverse medical occurrences emerging during the time between signing of the informed consent or pediatric assent form at screening (Day -28 [M -1]) and administration of TDV or placebo at Day 1 (M0) will be recorded as medical history in the source documents.

(o) All serious adverse events (SAEs), medically attended AEs (MAAEs), AEs leading to subject withdrawal from the trial, and AEs leading to TDV or placebo discontinuation will be collected from Day 1 (M0) postvaccination through the end of the trial. MAAEs are defined as AEs leading to an unscheduled visit to or by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria. SAEs will be reported to the sponsor within 24 hours of the investigator becoming aware of the event.

3.0 TRIAL REFERENCE INFORMATION

3.1 Trial-Related Responsibilities

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

3.2 Principal Investigator

Selection criteria for the principal investigator(s) (PI[s]) will include significant knowledge of the trial protocol, the investigational vaccine, their expertise in the therapeutic area and the conduct of clinical research as well as trial participation. Takeda will select 1 signatory from the investigators who participate in the trial. The signatory investigator will be required to review and sign the clinical protocol. The signatory investigator will also be required to review and sign the clinical study report (CSR) and by doing so agrees that it accurately describes the results of the trial.

3.3 List of Abbreviations

AE	adverse event
BMI	body mass index
CDSCO	Central Drugs Standard Control Organisation
COVID-19	coronavirus disease 2019
CRF	case report form
CSR	clinical study report
CTM	clinical trial materials
CTRI	Clinical Trials Registry – India
CYD-TDV	Chimeric Yellow Fever Virus-Dengue virus Tetravalent Dengue Vaccine
DENV	wild type dengue virus
DENV-1, -2, -3, -4	dengue virus serotypes -1, -2, -3, -4
DHF	dengue hemorrhagic fever
DMC	Data Monitoring Committee
DSS	dengue shock syndrome
E	envelope
eCRF	electronic case report form
FAS	full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMT	geometric mean titer
HIV	human immunodeficiency virus
IB	Investigator's Brochure
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IRT	interactive response technology
LAR	Legally acceptable representative
M	month
MAAE	medically-attended adverse events
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency of United Kingdom
MNT_{50}	microneutralization test 50%
PDK	Primary dog kidney
PI	principal investigator
PMDA	Pharmaceuticals and Medical Devices Agency of Japan
PPS	per-protocol set
prM	premembrane
PT	Preferred Term

QTL	quality tolerance limits
SAE	serious adverse event
SAGE	Strategic Advisory Group of Experts on Immunization
SAP	statistical analysis plan
SC	subcutaneous
SOC	System Organ Class
SUSAR	suspected unexpected serious adverse reaction
TDV	Dengue Tetravalent Vaccine (Live, Attenuated), the Takeda dengue vaccine candidate also known as TAK-003, is referred to as TDV
TDV-1	dengue serotypes 2/1 recombinant strain
TDV-2	dengue serotype 2 strain
TDV-3	dengue serotypes 2/3 recombinant strain
TDV-4	dengue serotypes 2/4 recombinant strain
WHO	World Health Organization

3.4 Corporate Identification

TV	Takeda Vaccines, Inc.
VBU	Vaccines Business Unit

4.0 INTRODUCTION

4.1 Background

Dengue fever is caused by infection with the wild type dengue virus (DENV), a ribonucleic acid virus that occurs as 4 recognized serotypes, dengue virus serotype -1, -2, -3, and -4 (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 are endemic in Asia, Central and South America, the Caribbean, the Pacific Islands, and parts of Africa [3]. There are an estimated 390 million dengue infections per year worldwide [4]. Every year, around 500,000 cases of dengue hemorrhagic fever (DHF) require hospitalization and the estimated death rate is 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection [5].

Dengue fever is clinically defined as an acute febrile illness with 2 or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leucopenia, and occurs at the same location and time as other confirmed cases of dengue fever. The most severe forms of dengue infection – 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 serotypes and these subsequent infections may increase the risk of severe disease (DHF/DSS) [3,6-8].

Treatment of dengue fever is based solely on signs and symptoms, with fluid replacement required for hemorrhagic or shock cases. An antiviral therapy for DENV 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 unmet global public health need for a safe and effective vaccine to reduce the morbidity and mortality associated with dengue disease. Vaccine development has focused on tetravalent vaccines that provide protection against all 4 dengue serotypes simultaneously since all 4 dengue serotypes commonly co-circulate in endemic areas [9].

A first recombinant dengue vaccine (chimeric yellow fever virus-dengue virus tetravalent dengue vaccine [CYD-TDV]) has been approved since 2015 in several Asian and Latin American countries as well as in the United States and in the European Union. Vaccine efficacy was different between serotypes and depended on dengue preexposure status [10]. Additional analyses found that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease when they were infected with dengue virus after vaccination with CYD-TDV [11]. In a revised Strategic Advisory Group of Experts on Immunization (SAGE) recommendation in April 2018, the SAGE concluded that for countries considering CYD-TDV vaccination as part of their dengue control program, a "pre vaccination screening strategy" would be the preferred option, in which only dengue-seropositive persons are vaccinated [12]. Hence, there is a continued unmet public health need for safer and more efficacious dengue vaccine to reduce the morbidity and mortality associated with dengue disease.

Takeda's Dengue Tetravalent Vaccine (live, attenuated)

Takeda's Dengue Tetravalent Vaccine (Live, attenuated) (TDV) consists of 1 molecularly-characterized, attenuated dengue serotype 2 virus strain, plus 3 recombinant dengue virus strains expressing surface antigens corresponding to dengue serotypes 1, 3 and 4. The dengue serotype 2 strain (TDV-2) is based upon the attenuated laboratory-derived DENV-2 virus strain, originally isolated at Mahidol University, Bangkok, Thailand and generated by 53 serial passages in primary dog kidney (PDK) cells (DENV-2 PDK-53) [13]. The recombinant, attenuated vaccine strains for dengue serotypes 1, 3 and 4 were engineered by substituting the structural genes, premembrane (prM) and envelope (E), of TDV-2 with the prM and E genes from the DENV-1 16007, DENV-3 16562 or DENV-4 1036 virus strains, respectively. Thus, TDV is comprised of 4 dengue virus strains: a molecularly-characterized, attenuated TDV-2 strain, a dengue serotypes 2/1 recombinant strain (TDV-1), a dengue serotypes 2/3 recombinant strain (TDV-3), and a dengue serotypes 2/4 recombinant strain (TDV-4) [14].

Data from completed phase 1 and phase 2 clinical trials in humans have shown satisfactory reactogenicity, safety and immunogenicity profiles for Takeda's TDV in healthy adults in non-endemic areas as well as in healthy adults and children in endemic areas in Asia and Latin America. Completed phase 2 clinical trials have enabled the selection of a final TDV dose (lyophilized formulation) and a 2-dose vaccination series administered 3 months (ie, 90 days) apart by subcutaneous (SC) injection for use in the ongoing clinical development program. Results from the pivotal DEN-301 efficacy showed that the primary endpoint was met, demonstrating that TDV was efficacious in preventing dengue fever in children and adolescents living in dengue-endemic countries [15]. TDV has been given to >19,900 clinical trial subjects. All available data also showed that TDV was well tolerated with no significant safety concerns to date [15]. At the time of protocol finalization and approval, TDV had been approved for the prevention of dengue disease caused by any serotype in the European Union countries, the United Kingdom, Iceland, Norway, Liechtenstein, Indonesia, Thailand, Brazil, and Argentina.

The current IB contains additional product information and a more detailed review of preclinical and clinical trials [16].

4.2 Rationale for the Proposed Trial

Because of limited surveillance data and understanding of dengue fever epidemiology in India [17,18] the disease burden appears to be underestimated, although emerging literature on dengue seroprevalence indicates that its endemicity in India may be as high as that of other countries in Southeast Asia [19-21]. For example, analysis of laboratory surveillance from 2014–2017 showed that 28.4% of 211,432 adult and pediatric patients presenting with acute febrile illness were serologically confirmed for dengue fever, with seroprevalence clusters in rural as well as urban areas [20-22]. A recent systematic analysis found 38.0% laboratory-confirmed dengue infection among clinically suspected patients, and a 56.9% pooled estimate of dengue seroprevalence in the general population in India [17]. Based on the size of population, the increasing incidence of dengue fever and the consequent economic burden to the country [23],

and its identification as an epicenter of dengue [24], the need for a preventive solution presents a critical unmet medical need for an important public health problem in India.

The purpose of this phase 3 trial is to generate immunogenicity and safety data in the Indian population with a view of supporting future licensure of TDV in India. Trial DEN-302 will therefore evaluate the safety and immunogenicity of TDV administered as 2 doses given 3 months apart to participants in India. A placebo has been chosen to maintain the double-blind trial design in the absence of a suitable active comparator for the age-range in this trial population.

The trial will be conducted in accordance with the protocol, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements [2].

5.0 TRIAL OBJECTIVES AND ENDPOINTS

5.1 Objectives

The primary and secondary objectives of this trial are outlined below.

5.1.1 Primary Objectives

Safety

- To assess the safety profile of TDV administered as 2 doses given 3 months apart in healthy adults, adolescents, and children.

Immunogenicity

- To evaluate the immunogenicity of TDV administered as 2 doses given 3 months apart in healthy adults, adolescents, and children at 1 month post second dose.

5.1.2 Secondary Objectives

Immunogenicity

- To describe immunogenicity of TDV at baseline and 6 months post second TDV dose when administered as 2 doses given 3 months apart.
- To describe seropositivity (% of subjects with reciprocal neutralizing titer ≥10) at baseline, 1 month post second TDV dose, and 6 months post second TDV dose.

5.2 Endpoints

5.2.1 Primary Endpoints

Safety

- Frequency and severity of solicited local (injection site) adverse events (AEs) for 7 days (day of vaccination + 6 subsequent days) and solicited systemic AEs for 14 days (day of vaccination + 13 subsequent days) postvaccination at Day 1 (Month [M]0) and Day 90 (M3).
- Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 subsequent days) postvaccination at Day 1 (M0) and Day 90 (M3).
- Percentage of subjects with an AE leading to subject withdrawal from the trial from Day 1 (M0) postvaccination through the end of the trial.
- Percentage of subjects with an AE leading to TDV or placebo discontinuation from Day 1 (M0) postvaccination through the end of the trial.
- Percentage of subjects with a medically-attended AE (MAAE) from Day 1 (M0) postvaccination through the end of the trial.

- Percentage of subjects with a serious adverse event (SAE) from Day 1 (M0) postvaccination through the end of the trial.

Immunogenicity

- Geometric mean titers (GMTs) of neutralizing antibodies (by microneutralization test [MNT_{50}]) against each of the 4 dengue virus serotypes at Day 120 (M4).

5.2.2 Secondary Endpoints

Immunogenicity

- GMTs by MNT_{50} against each of the 4 dengue virus serotypes at Day 1 (M0) and Day 270 (M9).
- Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against each of the 4 dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).
- Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against multiple (2, 3, or 4) dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).

6.0 TRIAL DESIGN AND DESCRIPTION

6.1 Trial Design

This is a phase 3, randomized, multi-site, double-blind, placebo-controlled trial in 480 healthy subjects aged ≥4 to ≤60 years living in India.

Cohort 1: 240 subjects ≥18 to ≤60 years of age will be enrolled and randomly assigned (3:1) to receive either TDV (N=180) or placebo (N=60) at Day 1 (M0) and Day 90 (M3).

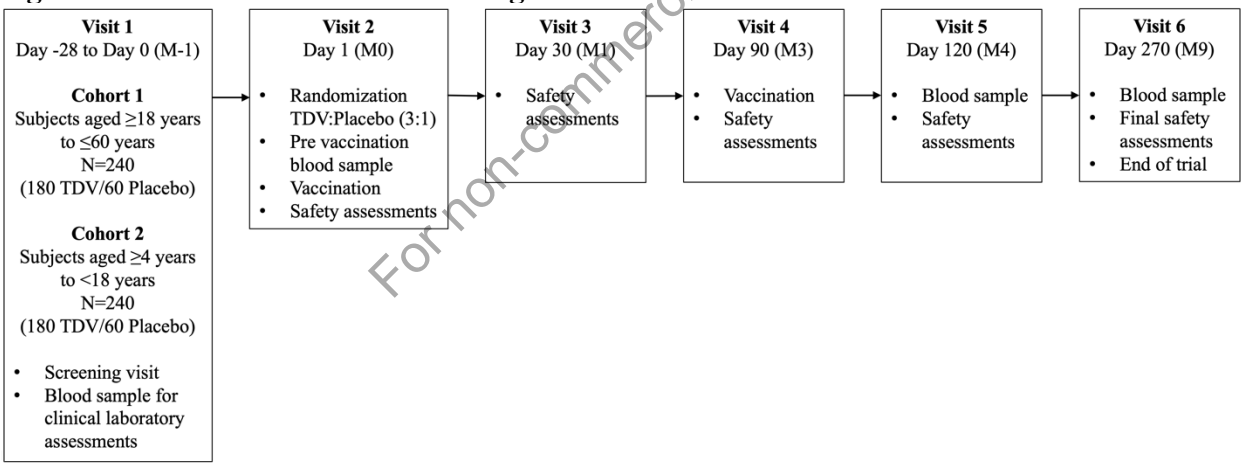
Cohort 2: 240 subjects ≥4 to <18 years of age will be enrolled and randomly assigned (3:1) to receive either TDV (N=180) or placebo (N=60) at Day 1 (M0) and Day 90 (M3).

Cohorts 1 and 2 will be enrolled in parallel.

Adolescents who attain the legal age of consent during or after Visit 1 (Day -28 [M -1]) will be asked to return to the investigational site to attest to the appropriate written informed consent. This may require an additional site visit.

A schematic of the trial design is included as Figure 6.a. A schedule of trial procedures is provided in Section 2.1.

Figure 6.a Schematic of Trial Design



M: Month

Immunogenicity evaluation (all subjects):

Neutralizing antibodies will be measured (by MNT_{50}) using blood samples obtained pre vaccination at Day 1 (M0), 1 month post second vaccination at Day 120 (M4), and at the end of trial (Day 270 [M9] or earlier for early terminations).

Safety evaluation (all subjects):

Diary cards will be distributed at Day 1 (M0) and Day 90 (M3) for the recording of:

- Solicited local (injection site) AEs for 7 days following administration of TDV or placebo (day of vaccination + 6 days). These will include:
  - Injection site pain, injection site erythema, and injection site swelling.
- Solicited systemic AEs for 14 days following administration of TDV or placebo (day of vaccination + 13 days). These will include:
  - Subjects <6 years of age: fever, irritability/fussiness, drowsiness, and loss of appetite.
  - Subjects ≥6 years of age: asthenia, fever, headache, malaise, and myalgia.

Unsolicited AEs will be collected by interview and recorded for 28 days following administration of TDV or placebo (day of vaccination + 27 days).

All SAEs, MAAEs, AEs leading to subject withdrawal from the trial, and AEs leading to TDV or placebo discontinuation will be collected from Day 1 (M0) postvaccination through the end of the trial. MAAEs are defined as AEs leading to an unscheduled visit to or by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria.

For details on the collection and reporting of AEs see Section 10.4.

6.2 Justification for Trial Design, Dose, and Endpoints

The trial design is 2-arm, with parallel enrollment of 2 different age cohorts. The trial will be performed at multiple locations allowing an evaluation of TDV in both dengue-endemic and dengue non-endemic areas of the country and by prior exposure based on baseline serostatus. The population assessed in this trial includes both young children, adolescents, and adults as licensure sought in India applies to these age groups.

The analysis of this trial is descriptive and is not based on testing formal null hypotheses. [redacted] The number of subjects receiving placebo will be 60 in each cohort (N=120 in total), as per the random assignment of 3:1 (TDV: placebo). Therefore, the total trial sample size is 480 subjects.

The route, dose and regimen adopted for TDV in this trial correspond to the ongoing pivotal efficacy, safety, and immunogenicity trial (DEN-301; NCT02747927). A placebo has been chosen to maintain the double-blind in this trial because of the absence of a suitable active comparator for the age-range in this trial population.

The primary and secondary endpoint assessments in this protocol are consistent with those made in other TDV trials. The trial design and method for collection of solicited AEs following TDV or placebo administration are consistent with other vaccine trials.

The current IB contains additional product information and a more detailed review of the preclinical and clinical trials [16].

6.3 Planned Duration of Subject's Participation in the Trial

The trial duration will be approximately 270 days (9 months) for each subject.

6.4 Premature Termination or Suspension of Trial or Investigational Site

6.4.1 Criteria for Premature Termination or Suspension of the Trial

The trial will be completed as planned unless 1 or more of the following criteria that require temporary suspension or early termination of the trial are satisfied:

- New information or other evaluation regarding the safety or efficacy of the investigational vaccine (TDV) that indicates a change in the known risk/benefit profile, such that the risk/benefit is no longer acceptable for subjects participating in the trial.
- The Data Monitoring Committee (DMC) recommends that the trial should be suspended or terminated.
- Significant deviation from GCP that compromises the ability to achieve the primary trial objectives or compromises subject safety.
- The sponsor decides to terminate or suspend the trial.

6.4.2 Criteria for Premature Termination or Suspension of Investigational Sites

A trial 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 trial, or as otherwise permitted by the contractual agreement.

6.4.3 Procedures for Premature Termination or Suspension of the Trial 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 trial or the participation of an investigational site, a trial-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 trial suspension.

7.0 SELECTION AND DISCONTINUATION/WITHDRAWAL OF SUBJECTS

Eligibility for trial entry at Visit 1 (Day -28 [M -1]) should be assessed using the inclusion criteria, and, exclusion criteria at entry.

Eligibility for random assignment at Visit 2 (Day 1 [M0]) should be assessed using the inclusion criteria, and, exclusion criteria at vaccination.

All entry criteria, including test results, need to be confirmed prior to random assignment at Day 1 (M0).

7.1 Inclusion Criteria

Subject eligibility is determined according to the following criteria:

1. Subjects aged ≥4 to ≤60 years at the time of random assignment.
2. Male or female.
3. Subjects who are in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs), and the clinical judgment of the investigator.
4. Subjects and/or the subjects legally acceptable representative (LAR) who have signed and dated a written, informed consent/pediatric assent form, and any required privacy authorization prior to the initiation of any trial procedures, after the nature of the trial has been explained according to local regulatory requirements.
5. Subjects who can comply with trial procedures and are available for the duration of follow-up.

7.2 Exclusion Criteria at Entry

Any subject who meets any of the following criteria will not qualify for entry into the trial at screening (Visit 1 [Day -28 (M -1)]):

1. Subjects with any illness, or history of any illness that in the opinion of the investigator, might interfere with the results of the trial or pose an additional risk to the subject due to participation in the trial, including but not limited to:
    a. Known hypersensitivity or allergy to any of the TDV or placebo components.
    b. Abnormalities of splenic or thymic function.
    c. Bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
    d. Serious chronic or progressive disease according to judgment of the investigator (eg, neoplasm, insulin-dependent diabetes, cardiac, hepatic or renal disease, neurologic or seizure disorder, or neuroinflammatory disease such as Guillain-Barré syndrome).

e. Known or suspected impairment/alteration of immune function, including:

i. Chronic use of oral steroids (equivalent to 20 mg/day prednisone for ≥12 weeks / ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0) (Note: use of inhaled, intranasal, or topical corticosteroids is allowed).

ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone for ≥12 weeks / ≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0).

iii. Receipt of immunoglobulins and/or any blood products within 90 days prior to Day 1 (M0).

iv. Receipt of immunostimulants within 60 days prior to Day 1 (M0).

v. Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (M0).

vi. Human immunodeficiency virus (HIV) infection or HIV-related disease.

vii. Hepatitis C and/or Hepatitis B virus infection.

viii. Genetic immunodeficiency.

2. Behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.
3. A body mass index (BMI = weight in kg/[height in meters^2]) ≥35 kg/m^2.
4. Intent to participate in another clinical trial at any time during the conduct of this trial.
5. Subject plans to receive any of the following (consider whether applicable as an exclusion criterion or as a criterion for delay of Visit 1):
    a. A licensed vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to TDV or placebo administration.
    b. A coronavirus vaccine within 14 days prior to TDV or placebo administration.
    c. A vaccine authorized for emergency use within 28 days of TDV or placebo administration.
6. Known substance or alcohol abuse within the past 2 years that may interfere with his/her ability to comply with requirements for trial participation.
7. Female subjects who are pregnant (ie, a positive or indeterminate pregnancy test) or breastfeeding.
8. 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 (M0).
    a. Childbearing potential is defined as status post onset of menarche and not meeting any of the following conditions: menopausal (at least 2 years previously), 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.

   iv. Monogamous relationship with a vasectomized partner (partner must have been vasectomized for at least 6 months prior to Day 1 (M0). Other contraceptive methods may be considered in agreement with the sponsor and will be approved by the appropriate ethics committee.

9. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method up to 6 weeks after the last dose of trial vaccine (TDV or placebo). They must also be advised not to donate ova or breastfeed during this period.
10. Subjects involved in the trial conduct or their first-degree relatives.
11. Subjects identified as an employee of the investigator or trial center, with direct involvement in the proposed trial or other trials under the direction of that investigator or trial center.
12. Receipt of previous vaccination against dengue virus.
13. Previous participation in any clinical trial of a dengue candidate vaccine, except if it is known that the subject received placebo while participating in those trials.

There may be instances when individuals meet all entry criteria except 1 that relates to transient clinical circumstances eg, recent use of excluded medication(s) or vaccine(s). Under these circumstances, eligibility for trial enrollment 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 (Section 7.4).

7.3 Exclusion Criteria at Vaccination (Day 1 [M0])

Any subject who successfully met the criteria for entry to this trial, but who now meets any of the following criteria will not qualify for random assignment at Day 1 (M0):

1. Subjects with febrile illness (body temperature ≥38°C [≥100.4°F]) or moderate or severe acute illness, or infection, at the time of random assignment (consider whether applicable as an exclusion criterion or as a criterion for delay of TDV or placebo administration, see Section 7.4).

2. Subjects with any new findings of illness in the interim period since Visit 1 that, in the opinion of the investigator, might interfere with the results of the trial or pose an additional risk to the subject due to participation in the trial, including but not limited to:
    a. Abnormalities of splenic or thymic function.
    b. Bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
    c. Serious chronic or progressive disease according to judgment of the investigator (eg, neoplasm, insulin-dependent diabetes, cardiac, hepatic or renal disease, neurologic or seizure disorder, or neuroinflammatory disease such as 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 for ≥12 weeks / ≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0) (Note: use of inhaled, intranasal, or topical corticosteroids is allowed).
        ii. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone for ≥12 weeks / ≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (M0).
        iii. Receipt of immunoglobulins and/or any blood products within 90 days prior to Day 1 (M0) or planned TDV or placebo administration during this trial.
        iv. Receipt of immunostimulants within 60 days prior to Day 1 (M0).
        v. Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (M0).
        vi. Human immunodeficiency virus (HIV) infection or HIV-related disease.
        vii. Hepatitis C and/or Hepatitis B virus infection.
        viii. Genetic immunodeficiency.
3. Behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the trial.
4. A BMI (weight in kg/[height in meters^2]) ≥35 kg/m^2.
5. Participation in any clinical trial with another investigational product 30 days prior to Day 1 (M0) or intent to participate in another clinical trial at any time during the conduct of this trial.

6. Subject plans to receive or has received any of the following (consider whether applicable as an exclusion criterion or as a criterion for delay of TDV or placebo administration, see Section 7.4):
    a. A licensed vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to TDV or placebo administration.
    b. A coronavirus vaccine within 14 days prior to TDV or placebo administration.
    c. A vaccine authorized for emergency use within 28 days of TDV or placebo administration.
7. Subject has been medicated with antipyretic and/or analgesic medication(s) within 24 hours prior to TDV or placebo administration (consider whether applicable as an exclusion criterion or as a criterion for delay of TDV or placebo administration, Section 7.4)
8. Female subjects who are pregnant (ie, a positive or indeterminate pregnancy test).
9. 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 (M0).
    a. Childbearing potential is defined as status post onset of menarche and not meeting any of the following conditions: menopausal (at least 2 years previously), 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.
        iv. Monogamous relationship with a vasectomized partner (partner must have been vasectomized for at least 6 months prior to Day 1 [M0]).Other contraceptive methods may be considered in agreement with the sponsor and will be approved by the appropriate ethics committee.
10. Females of childbearing potential who are sexually active, and who refuse to use an acceptable contraceptive method up to 6 weeks after the last dose of trial vaccine (TDV or placebo). They must also be advised not to donate ova or breastfeed during this period.
11. Subjects involved in the trial conduct or their first-degree relatives.
12. Subjects identified as an employee of the investigator or trial center, with direct involvement in the proposed trial or other trials under the direction of that investigator or trial center.
13. Receipt of vaccination against dengue virus.

14. Enrolment in any clinical trial of a dengue candidate vaccine (other than this trial), except if it is known that the subject has received placebo while participating in those trials.

There may be instances when individuals meet all entry criteria except 1 that relates to transient clinical circumstances eg, body temperature elevation or recent use of excluded medication(s) or vaccine(s). Under these circumstances, eligibility for random assignment and 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 (Section 7.4).

7.4 Criteria for Delay of Investigational Medicinal Product Administration

After enrollment, subjects may encounter clinical circumstances that warrant a delay in the administration of TDV or placebo. These situations are listed below. In the event that a subject meets a criterion for delay of TDV or placebo administration, the subject may receive TDV or placebo (as assigned) once the window for delay has passed as long as the subject is otherwise eligible for vaccination (Section 7.1 and Section 7.3).

- Subjects with a clinically significant active infection (as assessed by the investigator) or body temperature ≥38.0°C (≥100.4°F), within 3 days of planned trial vaccination.
- Receipt of a licensed vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to TDV or placebo administration.
- Receipt of a coronavirus vaccine within 14 days prior to TDV or placebo administration.
- Receipt of any vaccine authorized for emergency use within 28 days prior to TDV or placebo administration.
- Subjects who have used antipyretics and/or analgesic medications within 24 hours prior to vaccination. The reason for their use (prophylaxis versus treatment) must be documented. TDV or placebo administration should be delayed to allow for a full 24-hours to have passed between having used antipyretics and/or analgesic medications and TDV or placebo administration.
- Receipt of blood, blood products and plasma derivatives within 2 weeks of the planned trial vaccination.

The decision to vaccinate in these situations will be taken by the investigator.

7.5 Criteria for Early Termination of a Subject's Trial Participation

For screen failure subjects, refer to Section 9.1.13.

Under some circumstances, a subject's trial participation may be terminated early. This means that no further trial procedures (including data collection) will be performed on that subject beyond the specific date of early termination of trial participation. The primary reason for early termination of the subject's trial participation should be documented using the following categories.

1. Adverse Event: The subject has experienced an AE (irrespective of being related/unrelated to TDV or placebo or trial-related procedures) that requires early termination because continued participation imposes an unacceptable risk to the subject's health and/or the subject is unwilling to continue participation because of the AE. If the subject is unwilling to continue because of the AE, then the primary reason for early termination of trial participation in this case will be 'withdrawal due to AE' and not 'withdrawal of consent', see below. Any ongoing AEs leading to early termination of trial participation should be followed up by the investigator until resolution or stabilization.
2. Lost to follow-up: The subject did not return to the clinic and at least three attempts to contact the subject were unsuccessful.
3. Withdrawal of consent: The subject (or subject's LAR) wishes to withdraw from the trial. The primary reason for early termination will be 'withdrawal of consent" if the subject withdraws from participation due to a non-medical reason (ie, reason other than AE). While the subject has no obligation to provide a reason for withdrawing consent, attempts should be made to determine the underlying reason for the withdrawal and, where possible, the primary underlying reason should be documented.
4. Study terminated by sponsor: Premature trial termination by the sponsor, a regulatory agency, the IEC/IRB, or any other authority.

   If the clinical trial is prematurely terminated by the sponsor, then the investigator is to promptly inform the trial subjects and local IEC/IRB and should assure appropriate follow up for the subjects. The primary reason for early termination in this case will be 'trial termination".
5. Death: Subject's death during trial participation.
6. Other (the specific reason should be recorded in the "Specify" field of the Case Report Form [CRF]).

7.6 Criteria for Premature Discontinuation of Investigational Medicinal Product Administration

There are also circumstances under which receipt of the second TDV or placebo vaccination could be contraindicated in this trial. These circumstances include anaphylaxis or severe hypersensitivity reactions following the first trial vaccination at Day 1 (M0). If these reactions occur, then the subject must not receive the second trial vaccination at Day 90 (M3), but instead, should be encouraged to continue trial participation to allow the collection of safety data according to protocol for the planned safety follow-up period.

In addition to criteria for early termination of a subject's participation (see Section 7.5), other situations may apply in which subjects may continue participating in the trial (eg, contributing safety data according to protocol) but TDV or placebo administration is discontinued. Even if the subject is deemed ineligible to receive further doses of TDV or placebo, all efforts should be made to continue the collection of safety data according to protocol.

In addition, the primary reason for premature discontinuation of TDV or placebo administration should be recorded in the CRF, "end of IMP administration" page, using the following categories:

1. Adverse Event: The subject has experienced an AE (irrespective of being related/unrelated to TDV or placebo or trial-related procedures) for which subsequent TDV or placebo administrations(s) impose an unacceptable risk to the subject's health, but the subject will continue trial participation for safety, or a subset of other trial procedures.
2. Lost to follow-up: The subject did not return to the clinic and at least three attempts to contact the subject were unsuccessful.
3. Withdrawal of consent: The subject (or subject's LAR) wishes to withdraw from the trial. The primary reason for early termination will be "withdrawal of consent" if the subject withdraws from participation due to a non-medical reason (ie, reason other than an AE). The reason for withdrawal, if provided, should be recorded in the CRF.
4. Study terminated by sponsor: Premature trial termination by sponsor, a regulatory agency, the IEC/IRB, or any other authority.
5. Death: Subject's death prior to next TDV or placebo administration.
6. Protocol deviation: A protocol deviation is any change, divergence, or departure from the trial design or procedures of a trial protocol. The subject may remain in the trial unless continuation in the trial jeopardizes the subject's health, safety or rights (see Section 7.5).
7. Pregnancy: Any subject who, despite the requirement for adequate contraception, becomes pregnant during the trial will not receive further TDV or placebo administrations. Pregnant subjects should, however, be asked to continue participating in the trial contributing data to the safety follow-up according to protocol. In addition, the site should maintain contact with the pregnant subject and complete a "Clinical Trial Pregnancy Form" as soon as possible. If

the subject agrees, then she 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. Data obtained from the "Clinical Trial Pregnancy Form" will be captured in the safety database.

8. Other.

Any other presenting clinical circumstances that potentially warrant a delay or are a contraindication for TDV or placebo dose administration (TDV or placebo), and that are not listed above, must be discussed with the trial medical monitor prior to a decision by the investigator.

8.0 CLINICAL TRIAL MATERIAL MANAGEMENT

This section contains information regarding TDV, placebo, and materials provided directly by the sponsor, and/or sourced by other means, that are required by the trial protocol, including important sections describing the management of clinical trial materials (CTM).

All doses should be prepared at the time of administration by the unblinded pharmacist (or designee) per the pharmacy manual. Each vial, ampoule and/or prefilled syringe and carton will contain a label that includes pertinent trial information and caution statements. The label text will be in the specific country language, depending on local requirements.

8.1 Investigational Medicinal Products

The investigational vaccine is TDV for SC administration (Section 8.2.1). TDV comprises 1 molecularly-characterized, attenuated dengue virus strain (TDV-2), and 3 recombinant dengue virus strains (TDV-1, TDV-3, and TDV-4) with potencies of not less than 3.3, 2.7, 4.0 and 4.5 $\log_{10}$ plaque-forming units (PFU) per dose of TDV-1, TDV-2, TDV-3, and TDV-4, respectively. TDV is a lyophilized vaccine that will be reconstituted in TDV diluent (37 mM sodium chloride solution) prior to administration.

The placebo is normal saline for injection (0.9% sodium chloride [saline] solution).

Details regarding the dosage form description and strengths, or composition for the extemporaneous preparation, of TDV and placebo can be found in the pharmacy manual.

8.1.1 Labeling

A clinical label will be affixed to TDV or placebo containers in accordance with local regulatory requirements.

8.1.2 Inventory and Storage

Sponsor-supplied TDV or placebo and diluent should be stored in conditions specified on the label. Refer to the package insert/label for sponsor-supplied TDV or placebo and/or diluent not manufactured by Takeda.

All CTM must be kept in an appropriate, limited-access, secure place until it is used or returned to the sponsor or designee for destruction. Receipt and dispensing of TDV or placebo must be recorded by authorized personnel at the study site. All sponsor-supplied TDV or placebo 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 TDV or placebo containers and/or diluent requires sponsor approval. Temperature excursion information can be found in the pharmacy manual.

8.1.3 Dose and Regimen

The intended schedule is a fixed 2-dose regimen, ie, 2 single doses of TDV or 2 single doses of placebo given 3 months (ie, 90 days) apart (at Day 1 [M0] and Day 90 [M3]). The same dose and regimen applies to both age cohorts (Table 8.a).

Table 8.a Sponsor-Supplied Vaccine and Placebo

Cohort	Trial Arm	Dose 1	Dose 2
1	TDV	Day 1	Day 90
	Placebo	Day 1	Day 90
2	TDV	Day 1	Day 90
	Placebo	Day 1	Day 90

8.2 Investigational Medicinal Product Assignment and Dispensing Procedures

The investigator or designee will access the interactive response technology (IRT) system at subject enrollment (Day -28 [M -1]) to obtain the subject number. This number will be used throughout the trial (see Section 9.1.1).

All trial kits (TDV/placebo) will be provided by the sponsor in a uniquely numbered carton and will be dispensed in a blinded manner by IRT. The investigator or designee will access the IRT at each dispensing visit to obtain the Vaccine Identification number for the vaccine dose (TDV or placebo).

The doses should be prepared at the time of administration by the unblinded pharmacist (or designee). The unblinded pharmacist or designee will be responsible for overseeing the administration of TDV or placebo to subjects enrolled and randomly assigned in the trial according to the procedures stipulated in this trial protocol. TDV or placebo will only be administered by unblinded personnel who are qualified to perform that function under applicable laws and regulations for this trial.

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

8.2.1 Precautions to be Observed when Administering the Investigational Medicinal Product

Prior to administration, a subject must be determined to be eligible to receive TDV or placebo (Section 7.1 and Section 7.3), and it must be clinically appropriate in the judgment of the investigator to administer TDV or placebo.

Prior to subsequent TDV or placebo dose administration, site staff must determine if the subject is eligible to receive the dose by evaluating the criteria outlined in Section 7.4, 7.5 and 7.6.

TDV or placebo must be administered subcutaneously in the deltoid region. Do not inject intramuscularly or intravascularly. Standard immunization practices are to be observed and care should be taken when administering TDV or placebo. In addition, World Health

Organization (WHO) recommendations to reduce anxiety and pain at the time of vaccination should be followed (see Appendix C, [25]). Before administration of TDV or placebo, the injection site must be disinfected with a skin disinfectant (eg, 70% alcohol). Allow the skin to dry. Refer to the pharmacy manual for details on preparation and administration of the TDV or placebo dose.

After each trial vaccination, the subject will be observed for at least 30 minutes for any reactions to the vaccination. As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following TDV or placebo dose administration. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

8.3 Randomization Code Creation and Storage

The Random assignment schedule(s) will be generated by the sponsor or designee. Random assignment information will be stored in a secure manner, accessible only to authorized personnel.

8.4 Investigational Medicinal Product Blind Maintenance

The trial blind will be maintained using IRT.

This is a double-blind trial; the investigator and subjects are blinded to TDV or placebo assignment. One or more designated pharmacists/vaccine administrators at each site will be unblinded. These personnel will be responsible for receiving and storing the trial vaccines to ensure that the trial blind is not broken. The unblinded personnel will have no role in data collection or evaluation.

8.5 Unblinding Procedure

The trial blind shall not be broken by the investigator unless information concerning TDV or placebo assignment is necessary for the medical treatment of a subject, or in cases of pregnancy if a trial subject requests it. In the event of a medical emergency or pregnancy, if possible, the medical monitor should be contacted to discuss the need for unblinding before the blind is broken.

For unblinding a subject, the blinded information can be obtained by the investigator by accessing the IRT.

The sponsor's pharmacovigilance department must be notified as soon as possible if the trial blind is broken by the investigator, and the completed SAE or pregnancy form, if applicable, must be sent within 24 hours. The date, time, and reason the blind is broken must be recorded in the source document and the same information (except the time) must also be recorded for the analysis.

If any subject is unblinded, the subject must be withdrawn from the trial, but should continue to be monitored for safety follow-up, if they/their LAR agree(s).

8.6 Accountability and Destruction of Sponsor-Supplied Investigational Medicinal Products, and other Clinical Trial Materials

The investigator or designee must ensure that sponsor-supplied TDV and placebo are used in accordance with the approved protocol and administered only to subjects randomly assigned in the trial. To document appropriate use of sponsor-supplied TDV and placebo, the investigator must maintain records of all sponsor-supplied TDV and placebo delivery to the site, site inventory, administration and use by each subject, destruction at the site or return to the sponsor or designee.

Upon receipt of sponsor-supplied TDV and placebo, the appropriate designated individual must verify the contents of the shipments against the packing list. The verifier should ensure that the quantity is correct, that TDV and placebo are received within the labeled storage conditions (ie, no cold chain break has occurred during transit for TDV), and are in good condition. If quantity and conditions are acceptable, then the investigator or designee will acknowledge receipt of the shipment.

If there are any discrepancies between the packing list and the actual product received, then the sponsor or designee must be contacted to resolve the issue. The packing list should be filed in the pharmacy investigator site file.

The appropriate designated individual must maintain 100% accountability for all sponsor-supplied TDV and placebo (and other CTM) received and administered during their entire participation in the trial. Accountability includes, but is not limited to:

- Verifying that the actual inventory matches the documented inventory.
- Verifying that the log is completed for the vaccine lot number/placebo identification number or job 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, then the sponsor must be notified immediately.

The investigator (or designated individual) must record the current inventory of all sponsor-supplied TDV and placebo 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) (TDV or placebo), expiry and/or retest date, date and amount. The log should include all required information as a separate entry for each subject to whom sponsor-supplied TDV or placebo is administered.

The investigator will be notified of any expiry date or retest date extension for TDV or placebo during the trial conduct. On expiry date notification from the sponsor or designee, the site must complete all instructions outlined in the notification, including segregation of expired CTM for return to the sponsor or designee for destruction.

All CTM will be provided by the trial site, sponsor or designee, depending upon availability. The list of CTM and source information can be found in the pharmacy manual. Prior to site closure or at appropriate intervals throughout the trial, before TDV, placebo, or CTM are returned to the sponsor or designee for destruction, a representative from the sponsor will perform CTM accountability and reconciliation. The investigator will retain a copy of the documentation regarding CTM accountability, return and/or destruction, and originals will be sent to the sponsor or designee.

9.0 TRIAL PLAN

9.1 Trial Procedures

The following sections describe the trial procedures and data to be collected. For each procedure, subjects are to be assessed by the same investigator or site personnel whenever possible. The schedule of trial procedures is located in Section 2.1. All procedures must be performed by qualified and trained staff.

9.1.1 Informed Consent and Pediatric Assent Form

The requirements of the informed consent and pediatric assent form are described in Section 15.2.

Informed consent, or, informed consent and pediatric assent must be obtained before any protocol-directed procedures are performed. Adolescents who attain the legal age of consent during or after Visit 1 (Day -28 [M -1]) will be asked to return to the investigational site to attest to the appropriate written informed consent. This may require an additional site visit.

A unique subject number will be assigned to each subject by the IRT after informed consent, or, informed consent and pediatric assent where applicable, is obtained. If all eligibility criteria for entry (Section 7.1 and Section 7.2) are fulfilled, then this subject number will be used throughout the trial. Subject numbers assigned to subjects who fail screening should not be reused (Section 9.1.13).

9.1.2 Demographics, Medical History, Prior and Concomitant Medications/Vaccinations

Demographic information to be obtained at screening (Day -28 [M -1]) will include age, date of birth (if applicable), gender, and race, as described by the subject or subject's LAR.

Medical history will be obtained at screening (Day -28 [M -1]), and, prior to vaccination at Day 1 (M0) and should be recorded in the subject's source documents. This includes, but is not limited to:

- Information that may be relevant to subject eligibility for trial entry (Sections 7.1 and 7.2) or vaccination (Sections 7.1 and 7.3) (eg, previous and ongoing illnesses and/or injuries).
- Adverse medical occurrences emerging during the time between signing of the informed consent or pediatric assent form at screening (Day -28 [M -1]) and TDV or placebo administration at Day 1 (M0) except where the adverse medical occurrence is considered to be related to a trial procedure; if the adverse medical occurrence is considered to be related to a trial procedure, then it should be recorded as an AE related to study procedure.
- Any significant conditions or diseases that have disappeared or resolved between signing of informed consent or pediatric assent form at screening (Day -28 [M -1]) and TDV or placebo administration at Day 1 (M0).

- Any medical history that contributes to the understanding of an AE that occurs during trial participation if it represents an exacerbation of an underlying disease/preexisting problem.

All history of prior medications/vaccinations will be obtained at screening (Day -28 [M -1]), and, prior to vaccination at Day 1 (M0). Concomitant medications and vaccinations (licensed, authorized for emergency use, or investigational, including any other dengue vaccine) should be recorded in the subject's source documents at all scheduled visits. This includes, but is not limited to:

- Information that may be relevant to subject eligibility for trial entry (Sections 7.1 and 7.2) or vaccination (Sections 7.1 and 7.3) (eg, prior vaccinations and concomitant medications).
- Any other medications/vaccinations (with the exception of vitamins and minerals).

9.1.3 Documentation of Trial Entry/Randomization

Only subjects who have a signed informed consent, or, informed consent and pediatric assent form and who meet all of the inclusion criteria (Section 7.1) and none of the exclusion criteria at entry (Section 7.2) are eligible for trial entry. Only those subjects who then meet none of the exclusion criteria at vaccination (Section 7.3) are eligible for random assignment and full participation in the trial.

The random assignment schedule will be created and controlled by the IRT provider. The random assignment specification will be approved by the sponsor's trial statistician, or designee.

9.1.4 Physical Examination

Physical examinations must be performed by a qualified health professional in accordance with local regulations and as listed within the Site Responsibility Delegation Log that will be provided to the site by the sponsor or designee.

A complete physical exam will be performed at screening (Day -28 [M -1]), and pre vaccination at Day 1 (M0) and Day 90 (M3), according to the investigator's standard clinical practice. The physical examination will comprise weight and height measurements (BMI will be calculated) at Day -28 (M -1) and Day 1 (M0), and the measurement of vital signs (Section 9.1.5) at all examinations. Additional physical examinations may be performed if indicated by review of the subject's medical history.

Symptom-directed physical examination may be performed if deemed necessary, by a medically qualified clinical study staff member at Day 30 (M1), Day 120 (M4), and Day 270 (M9). If a symptom-directed physical examination is not conducted (because of a lack of any symptoms) then the absence will be recorded as 'not needed' in the subject's source document.

All physical examination findings should be documented in the subject's source documents. Any clinically significant changes from the baseline examinations conducted at screening (Day -28 [M -1]) and at Day 1 (M0) pre vaccination should be recorded in the subject's source documents and reported as an AE in the CRF.

9.1.5 Vital Signs

Vital signs should be measured at each visit for each subject. These signs will include (but are not limited to) the measurement of systolic blood pressure, diastolic blood pressure, heart rate, and body temperature. All data should be recorded in the subject's source document.

9.1.6 Immunogenicity Assessments

All subjects will undergo blood sampling for serological immunogenicity testing (MNT_{50}) at Day 1 (M0, pre vaccination), Day 120 (M4), and Day 270 (M9). The blood sample obtained at Day 120 (M4) should be taken at least 28 days after the second (Day 90 [M3]) TDV or placebo vaccination. Serum will be generated and handled as described in the central laboratory manual. Immunogenicity analysis will be performed on serum samples with a validated Dengue Microneutralization Assay. MNT_{50} analysis of serum samples taken from all subjects prior to TDV or placebo dose administration at Day 1 (M0) will establish the dengue serostatus at baseline.

The maximum volume of blood taken at any single visit will be approximately 5 mL, and the approximate total volume of blood obtained from each subject for immunogenicity assessments in the trial will be 15 mL.

All blood samples must be obtained in accordance with acceptable laboratory procedures.

9.1.7 Processing, Labeling and Storage of Biological Samples

All blood and urine samples will be processed, labeled, and stored according to the laboratory manual or other appropriate guidelines provided to the site (Section 9.4).

9.1.8 Safety Assessments

Safety assessments will include collection and recording of solicited local (injection site) and systemic AEs, unsolicited AEs, SAEs, AEs leading to subject withdrawal from the trial, AEs leading to TDV or placebo discontinuation, pregnancies and MAAEs. Refer to Section 10.1 for safety definitions. Details on collection and reporting of AEs are in Section 10.4.

9.1.9 Pregnancy Testing

A urine pregnancy test must be performed for female subjects of childbearing potential at screening (Day -28 [M -1]), and, prior to administering TDV or placebo at Day 1 (M0) and at Day 90 (M3).

Results of all pregnancy tests must be confirmed and documented as negative prior to the administration of any TDV or placebo dose.

Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator. Where the results of a urine pregnancy test are in doubt, a serum pregnancy test will be performed to verify the result.

9.1.10 Contraception and Pregnancy Avoidance Procedure

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 stating that they understand the requirements for avoidance of pregnancy and donation of ova (post menarchal subjects) for up to 6 weeks after the last dose of TDV or placebo. During the course of the trial, subjects will receive continued guidance with respect to the avoidance of pregnancy as part of the trial procedures (Section 2.1). The investigator or designee should explain pertinent aspects of contraception and pregnancy avoidance in the trial in an age-appropriate manner to pediatric subjects in accordance with local regulations.

Refer to Section 7.2, exclusion criterion at entry 8 for definitions.

9.1.11 Pregnancy

To ensure subject safety and the safety of the unborn child, each pregnancy in a subject having received TDV or placebo must be reported to the sponsor promptly, within 24 hours of the site learning of its occurrence.

If the subject becomes pregnant during the trial, she will not receive any further doses of TDV or placebo.

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 trial has ended. Any pregnancy occurring following TDV or placebo administration should be reported immediately, using a pregnancy notification form, to the contact listed in the investigator site file.

Should the pregnancy occur after blinded administration of TDV or placebo, the investigator must inform the subject of their right to receive information concerning the trial vaccine they were administered. If the subject chooses to receive the unblinded information, the individual blind should be broken by the investigator and procedures must be followed as described in Section 8.5.

Any SAE that occurred during pregnancy should be reported throughout the trial as per timelines and procedures described in Section 10.4.4.

9.1.12 Procedures for Clinical Laboratory Assessments

Blood and urine samples will be collected from each subject in accordance with acceptable laboratory procedures as part of the eligibility assessment at screening (Day -28 [M -1]). Details of blood sampling and processing procedures and required safety monitoring during sample collection will be given in the laboratory manual.

Clinical laboratory assessments to be performed at screening (Day -28 [M -1]) are shown in Table 9.a. Clinical laboratory testing will be performed at a central laboratory located in India. The results of all clinical laboratory tests will be reviewed by the PI or designee.

Table 9.a Clinical Safety Laboratory Tests

Urine	**Blood Chemistry**
Bilirubin	Alanine aminotransferase (ALT)
Blood	Albumin
Glucose	Total bilirubin
Ketones	Aspartate aminotransferase (AST)
Leukocytes	Total Protein
Nitrite	Creatinine
pH	Blood glucose (fasting or non-fasting)
Protein	
Specific Gravity	
Hematology	**Other Safety Variables**
Red blood cells	human immunodeficiency virus (HIV) test
White blood cells	
Hemoglobin	Hepatitis panel (including Hepatitis B surface antigen and anti-hepatitis C virus antibodies)
Hematocrit	

9.1.13 Documentation of Subjects who are not Randomized

Investigators must account for all subjects who sign an informed consent form or who have a signed pediatric assent form. If the subject is found ineligible at screening (Day -28 [M -1]) or at Visit 1 (Day 1 [M1]), then the primary reason for non-randomization should be recorded in the CRF using the following categories:

- Screen failure (did not meet ≥1 inclusion criteria or did meet ≥1 exclusion criteria).
- Withdrawal of consent.
- Study terminated by the sponsor.
- Site terminated by the sponsor.

Subject numbers assigned to subjects who fail screening should not be re-used.

9.2 Monitoring Subject Compliance

The investigator or designee records all injections of TDV or placebo given to the subject in their respective source documents and CRF.

9.3 Schedule of Observations and Procedures

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

When a site visit cannot be conducted because of exceptional circumstances (eg, pandemic), alternative methods of contact (eg, telephone contact) will be implemented for subjects who are still under monitoring for safety reporting. Furthermore, if, owing to exceptional circumstances, the site anticipates difficulties with scheduling site visit(s) for administration of scheduled dose(s) of vaccine, then the trial medical monitor should be contacted at the earliest opportunity for further guidance and advice.

In the event of a conflict of interpretation between the procedures/instructions outlined below and those stated in Sections 2.0 and 2.1, the investigator or designee should, in the first instance, attempt to seek advice from the medical monitor for further clarification to avoid protocol deviation where possible.

9.3.1 Screening Procedures (Day -28 [M -1])

- An informed consent form, or, informed consent and pediatric assent form must be signed and dated before performing any trial procedure (see Section 9.1.1).
- Assessment of eligibility criteria for trial entry (Section 7.1 and Section 7.2).
- Demographic characteristics (Section 9.1.2).
- Medical history and prior medications and vaccinations (Section 9.1.2).
- Concomitant medications and vaccinations (Section 9.1.2).
- Perform complete physical examination including weight and height measurements (BMI will be calculated) (Section 9.1.4), and vital signs (Section 9.1.5).
- Obtain a blood sample by aseptic venipuncture (5 mL) for clinical laboratory assessments (Section 9.1.12).
- Obtain a urine sample for urinalysis (Section 9.1.12).
- Pregnancy test for women of childbearing potential (Section 9.1.9) and provision of pregnancy avoidance guidance (Section 9.1.10).
- Schedule the next site visit or other trial activity with the subject or the subject's LAR.

9.3.2 Pre-Vaccination Procedures (Day 1 [M0] and Day 90 [M3])

9.3.2.1 Pre-vaccination procedures to be performed at Day 1 (M0) only

- Assessment of eligibility for vaccination (Section 7.1 and Section 7.3).

- Medical history and prior medications and vaccinations update (Section 9.1.2).
- Note, any adverse medical occurrences emerging during the time between signing of informed consent or pediatric assent form at screening (Day -28 [M -1]) and administration of TDV or placebo at Day 1 (M0) will be recorded as medical history in the source documents.
- Random assignment (Section 9.1.3).
- Obtain a blood sample by aseptic venipuncture (5 mL) for immunogenicity assessments (Section 9.1.6).

9.3.2.2 Pre-vaccination procedures to be performed at Day 1 (M0) and Day 90 (M3)

- Check concomitant medications and vaccinations (Section 9.1.2).
- Check criteria for delay of TDV or placebo administration (Section 7.4).
- Check contraindications to TDV or placebo administration (Section 7.6).
- Perform complete physical examination (Section 9.1.4), including vital signs (Section 9.1.5).
- Pregnancy test for women of childbearing potential (Section 9.1.9) and provision of pregnancy avoidance guidance (Section 9.1.10).

9.3.3 Vaccination Procedures (Day 1 [M0] and Day 90 [M3])

After confirming eligibility for vaccination and following random assignment of the subject (Day 1 [M0]):

- Administer TDV or placebo as assigned, according to the procedures described in Section 8.2.
- At the clinic visit where the second dose of TDV or placebo is to be administered (Day 90 [M3]):
- Confirm that the subject does not meet any criteria for premature discontinuation of additional TDV or placebo dose administration, as described in Section 7.4.
- Administer TDV or placebo as assigned, according to the procedures described in Section 8.2.

9.3.4 Post Vaccination Procedures (Day 1 [M0] and Day 90 [M3])

The following postvaccination procedures will be performed at Day 1 (M0) and Day 90 (M3):

- Observe subject for at least 30 minutes after vaccination, evaluate the injection site for pain, erythema, and swelling, and record any AEs.
- Collect and record unsolicited AEs by interview (Section 10.4).

- Collect and report any SAEs, MAAEs, AEs leading to subject withdrawal from the trial, and AEs leading to TDV or placebo discontinuation (Section 10.4).
- Distribute diary card.
- Training of the subject or the subject's LAR on how to measure solicited local (injection site) AEs and body temperature, solicited systemic symptoms, how to complete the diary card, and how often to complete the diary card. Training should be directed at the individual(s) who will perform the measurements of solicited local (injection site) AEs and those who will enter the information into the diary card. This individual may or may not be the subject or the subject's LAR, but if a person other than the subject or the subject's LAR enters information into the diary card, this person's identity must be documented in the source and this person must receive training on the diary card. Training of the subject or the subject's LAR on how to measure an injection site AE reaction and how to take their temperature, as well as how to record the information in the diary card, should be performed while the subject is under observation after vaccination.

Diary card instructions must include the following:

- The individual(s) who will enter the information into the diary card must understand that timely completion of the diary card on a daily basis is a critical component of trial participation. This individual should also be instructed to write clearly and to complete the diary card in pen, if applicable. Any corrections to the diary card that are performed by the individual(s) completing the diary card should include a single strikethrough line with a brief explanation for any change and be initialed and dated.

Please note:

Diary cards will be the only source document allowed for remote recording of solicited local (injection site) and systemic AEs (including body temperature measurements) by each subject or the subject's LAR. The following additional rules apply to the documentation of safety information in the diary card:

- The diary card should be reviewed with the subject or the subject's LAR.
- No corrections or additions to the diary card will be allowed after it is reviewed with the investigator/designee.
- Any data that are identified as implausible or incorrect, and confirmed by the subject and/or the subject's LAR to be a transcription error, should be corrected by the subject or the subject's LAR on the diary card (the correction should include a single strikethrough line and should be initialed and dated by the subject and/or the subject's LAR).
- Any blank or illegible fields on the diary card not otherwise corrected as above will be missing in the CRF.
- The site must enter all readable entries on the diary card into the CRF.

- Any newly described solicited safety information should be added to the diary card by the subject, initialed, and dated. Any new unsolicited safety information would be recorded in the subject source document as a verbally reported event and therefore captured as an AE and recorded in the AE CRF.
- Starting on the day of vaccination, the subject or the subject's LAR will check for specific types of events: solicited local (injection site) AEs, any specific generalized symptoms (solicited systemic AEs), body temperature (preferably by the oral route), any other symptoms or change in the subject's health status, and any medications taken (excluding vitamins and minerals). These solicited AEs and body temperature will be recorded in the diary. Assessments should preferably be made in the evening. However, if the subject feels unusually hot or cold during the day, the subject and/or the subject's LAR should check their temperature. If the subject has fever, the highest body temperature observed on that day should be recorded on the diary card. Temperature measurements are to be performed using the thermometer provided the site, and preferably by the oral route.
- The measurements of solicited local (injection site) AEs (erythema and swelling) are to be performed using the ruler provided by the trial site. Pain will be assessed using an intensity scale (Table 10.a and Table 10.b).
- The collection on the diary card of solicited local (injection site) AEs will continue for a total of 7 days (including day of vaccination) following TDV or placebo dose administration. Solicited systemic AEs will be collected on the diary card for a total of 14 days (including day of vaccination) following TDV or placebo dose administration. The collection of unsolicited AEs and medications will continue for 28 days following TDV or placebo administration by interview.

After TDV or placebo administration, the subject will be observed for at least 30 minutes including observation for unsolicited AEs, solicited local (injection site) AEs, and body temperature measurement. Information should be recorded in the CRF. The investigator or delegate will take the opportunity to remind the subject or the subject's LAR how to measure solicited local (injection site) AEs (pain, swelling and erythema) and body temperature as part of this observation period. All safety data will be collected in the subject's source documents.

The site staff should schedule the next trial activity reminder call or visit.

The subject or the subject's LAR will receive a written reminder of the next planned trial activity. The subject or the subject's LAR will be reminded to complete the diary card daily, 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. All contact details will be provided to the subject.

9.3.5 Site Visits after Vaccination (Day 30 [M1] and Day 120 [M4])

Site visits that do NOT include TDV or placebo administration will be performed at Day 30 (M1) and Day 120 (M4). At these site visits, the investigator or designee will:

- Check concomitant medications and vaccinations (Section 9.1.2).
- Check vital signs (Section 9.1.5).
- Perform symptom-directed physical examination if warranted (Section 9.1.4).
- Provision of pregnancy avoidance guidance (Section 9.1.10).
- Review the diary card together with the subject or subject's LAR. The healthcare professional reviewing these data will discuss the AEs (if any) reported by the subject and will determine if any additional diagnoses and/or AEs are present and/or if concomitant medications have been used. This information must be entered in the subject's CRF.
- Check for prolonged solicited local (injection site) and systemic AEs.
- Collect and record unsolicited AEs by interview (Section 10.4).
- Collect and report any SAEs, MAAEs, AEs leading to subject withdrawal from the trial, and AEs leading to TDV or placebo discontinuation (Section 10.4).
- At Day 120 (M4) only, obtain a blood sample by aseptic venipuncture (5 mL) for immunogenicity assessments (Section 9.1.6).
- Schedule the next site visit or other trial activity with the subject or the subject's LAR.

The subject or the subject's LAR will receive a written reminder of the next planned trial activity. The subject or the subject's LAR 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.

9.3.6 Final (End of Trial) Visit (Day 270 [M9])

The final (end of trial) visit will be performed at Day 270 (M9). The following activities will be performed:

- Check concomitant medications and vaccinations (Section 9.1.2).
- Check vital signs (Section 9.1.5).
- Perform symptom-directed physical examination if warranted (Section 9.1.4).
- Obtain a blood sample by aseptic venipuncture (5 mL) for immunogenicity assessments (Section 9.1.6).
- Record SAEs, MAAEs, and AEs leading to subject withdrawal from the trial, if applicable.

The investigator must complete the End of Trial CRF page for all subjects receiving TDV or placebo.

9.3.7 Early Termination Visit Procedures

If a subject terminates earlier, then (s)he should be invited to the site and the final (end of trial) visit procedures (Section 9.3.6) will be performed.

9.3.8 Post-Trial Care

No post trial care will be provided.

9.4 Biological Sample Retention and Destruction

In this trial, blood samples for immune response testing will be obtained as described in Section 9.1.6. After blood draw and serum processing, the serum samples will be preserved and retained at a central laboratory 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 a system in place to protect the subjects' personal information to ensure optimal confidentiality and defined standard processes for sample and data collection, storage, analysis, and destruction.

Serum samples will be used for the analyses defined in this protocol, but can also, with permission from the subject or subject's LAR, be used to assess, improve or develop tests related to flaviviruses or TDV that will allow more reliable measurement of the response to the investigational vaccine.

Urine samples obtained at the screening visit will be used only for urinalysis (Section 9.1.12), and residual samples will be discarded by the central laboratory according to applicable local authority procedures for disposition of biological samples.

10.0 ADVERSE EVENTS

10.1 Definitions

10.1.1 Adverse Events

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered TDV or placebo; it does not necessarily have to have a causal relationship with TDV or placebo 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 TDV or placebo whether or not it is considered related to TDV or placebo.

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 Solicited Adverse Events

The occurrence of selected indicators of safety will be measured/collected for 7 days for solicited local (injection site) AEs and 14 days for solicited systemic AEs following administration of TDV or placebo (including the day of administration), and will be recorded on the "Local and Systemic Reactions" CRF page as applicable and as listed in Table 10.a for children <6 years and adults in Table 10.b. The intensity of solicited safety parameters will also be assessed as described for children <6 years in Table 10.a, and adults in Table 10.b.

Any solicited local or systemic AE observed as continuing on/after Day 8 and Day 15, respectively, following each trial vaccination will be recorded as an AE on the AE CRF for follow-up. For these persistent/prolonged solicited AEs the end date will be captured on the AE CRF to permit a separate analysis from the unsolicited AEs (see Section 10.4.2).

Table 10.a Solicited Safety Parameters for Infant/toddler/child (<6 years)

Adverse Event	Intensity grade	Intensity
Pain at injection site	0	None
	1	Mild: Minor reaction to touch
	2	Moderate: Cries/protests on touch
	3	Severe: Cries when limb is moved/spontaneously painful
Erythema at injection site [(a)]	0	<10 mm
	1	Mild: ≥10 – ≤20 mm
	2	Moderate: >20 – ≤40 mm
	3	Severe: >40 mm
Swelling at injection site [(a)]	0	<10 mm
	1	Mild: ≥10 – ≤20 mm
	2	Moderate: >20 – ≤40 mm
	3	Severe: >40 mm
Drowsiness	0	Behavior as usual
	1	Mild: Drowsiness easily tolerated
	2	Moderate: Drowsiness that interferes with normal activity
	3	Severe: Drowsiness that prevents normal activity
Irritability/fussiness	0	Behavior as usual
	1	Mild: Crying more than usual/no effect on normal activity
	2	Moderate: Crying more than usual/interferes with normal activity
	3	Severe: Crying that cannot be comforted/prevents normal activity
Loss of appetite	0	Appetite as usual
	1	Mild: Eating less than usual/no effect on normal activity
	2	Moderate: Eating less than usual/interferes with normal activity
	3	Severe: Not eating at all
Fever [(b)]	Record body temperature in °C/°F	

(a) Subjects are to record greatest surface diameter in mm on the diary card.
(b) Fever is defined as body temperature ≥38°C (≥100.4°F) regardless of method used [26].

Table 10.b Solicited Safety Parameters for Child/adolescent/adult (≥ 6 years old)

Adverse Event	Intensity grade	Intensity
Pain at injection site	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity with or without treatment
	3	Severe: Prevents daily activity with or without treatment
Erythema at injection site [(a)]	0	<25 mm
	1	Mild: ≥25 – ≤50 mm
	2	Moderate: >50 – ≤100 mm
	3	Severe: >100 mm
Swelling at injection site [(a)]	0	<25 mm
	1	Mild: ≥25 – ≤50 mm
	2	Moderate: >50 – ≤100 mm
	3	Severe: >100 mm
Headache	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity with or without treatment
	3	Severe: Prevents normal activity with or without treatment
Asthenia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Malaise	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Myalgia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Fever [(b)]	Record body temperature in °C/°F	

(a) Subjects are to record greatest surface diameter in mm on the diary card.
(b) Fever is defined as body temperature ≥38°C (≥100.4°F) regardless of method used [26].

10.1.3 Adverse Events of Special Interest

Not applicable. No AEs of special interest have been identified for this trial.

10.1.4 Medically-Attended Adverse Events

MAAEs are defined as AEs leading to an unscheduled visit to or by a healthcare professional including visits to an emergency department, but not fulfilling seriousness criteria.

10.1.5 Serious Adverse Events

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 Adverse Events

Relationship (causality) to TDV or placebo will also be assessed by the investigator. The relationship of each AE to TDV or placebo, including solicited systemic AEs (solicited local AEs are considered as related by default) will be assessed using the following categories:

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

10.2.1 Relationship to Trial Procedures

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

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

10.2.2 Outcome of Adverse Events

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 Adverse Events

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 TDV or placebo 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 administration of TDV or placebo, 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, 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/pediatric assent form 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.

Trial procedures:

- Adverse occurrences related to trial procedures after signing of informed consent/pediatric assent form are considered as AEs and should be reported as AEs.

10.4 Procedures

10.4.1 Collection and Reporting of Adverse Events

All AEs, whether considered related to TDV or placebo 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 autopsy report should be supplied, if possible. All findings must be reported on an AE CRF and on the SAE form*, if necessary (see Section 10.4.4). All findings in subjects experiencing AEs must also be documented in the subject's source documents. Any unsolicited AEs will be collected for 28 days (day of vaccination + 27 days) following administration of TDV or placebo during site visits via interview. AEs leading to TDV or placebo discontinuation or subject withdrawal from the trial are collected from Day 1 (M0) postvaccination through the end of the trial. Even if the subject is deemed ineligible to receive further TDV or placebo doses, all efforts should be made to continue the collection of safety data according to protocol.

The following information will be documented for each event:

- Reported term for the AE.
- Start and end date, duration.
- Serious (Y/N).
- Severity.

- Investigator's opinion of the causality (relationship) between the event and administration of TDV or placebo ("related" or "not related").
- Investigator's opinion of the causality (relationship) to trial procedure(s), including the details of the suspected procedure.
- Action taken with TDV or placebo.
- Outcome of event.

**SAE reporting will be done by electronic CRF (eCRF). If the eCRF system is unavailable, a paper sponsor SAE form/paper CRF should be completed and the event must be entered into the eCRF once access is restored.*

10.4.2 Collection and Reporting of Solicited Adverse Events

The occurrence of selected indicators of safety will be collected on diary cards by the subjects for 7 days (solicited local [injection site] AEs) and 14 days (solicited systemic AEs), following administration of each TDV or placebo dose (including the day of administration) and will be recorded on the "Local and Systemic AEs" CRF, as applicable. These will be summarized in the final report under the category "solicited AEs" to differentiate them from unsolicited AEs. Any solicited local (injection site) or systemic AE observed as continuing on/after Day 8 and Day 15, respectively, following each TDV or placebo dose administration will be additionally recorded as an AE on the AE CRF for follow-up. For these persistent/prolonged solicited AEs, the end date will be recorded on the AE CRF to permit a separate analysis from the unsolicited AEs.

Any solicited AE that meets any of the following criteria must be entered as an AE on the AE CRF page.

- Solicited local (injection site) or systemic AEs that lead the subject to withdraw from the trial.
- Solicited local (injection site) or systemic AEs that lead to the subject being withdrawn from the trial by the investigator.
- Solicited local (injection site) and systemic AEs that otherwise meet the definition of an SAE (see Section 10.1.2).

10.4.3 Collection and Reporting of Medically-Attended Adverse Events

MAAEs will be collected by close monitoring from Day 1 (M0) postvaccination through the end of the trial. MAAEs need to be reported to the sponsor as soon as possible after the investigator becoming aware of the event.

MAAEs must be recorded as an AE on the AE CRF page. MAAEs will be summarized separately at the end of the trial.

10.4.4 Collection and Reporting of Serious Adverse Events

Collection of SAEs will commence from the time that the subject is administered TDV or placebo at Day 1 (M0). Routine collection of SAEs will continue until the end of the trial (Day 270 [M9]).

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.
- Causality assessment.
- Protocol number.
- Subject identification number.
- Investigator's name.
- For open label trials: name of investigational medicinal product.

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

10.5 Follow-up Procedures

10.5.1 Adverse Events

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 trial, whichever occurs first.

10.5.2 Serious Adverse Events

If information not available at the time of the first report becomes available later, the investigator should complete a follow-up SAE form or provide other written documentation immediately. Copies of any relevant data from the hospital notes (eg, laboratory tests, discharge summary, postmortem results) should be sent to the sponsor after redaction for privacy.

All SAEs should be followed up until resolution, 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, Investigational Review Boards or Independent Ethics Committees, and Regulatory Authorities

The sponsor or designee will be responsible for the reporting of all suspected unexpected serious adverse reactions (SUSAR) and any other SAEs to regulatory authorities, investigators and IEC/IRB, as applicable, in accordance with national regulations in the countries where the trial is conducted. Relative to the first awareness of the event by/or further provision to the sponsor or 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 vaccine or that would be sufficient to consider changes in TDV or placebo administration or in the overall conduct of the trial. The investigational site will also forward a copy of all expedited reports to their IEC/IRB in accordance with national regulations.

10.5.4 Post-Trial Events

Any SAE that occurs outside of the protocol-specified observation period or after the end of the trial but is considered to be caused by TDV or placebo must be reported to the sponsor. These SAEs will be processed by the sponsor's pharmacovigilance department. Instructions for how to submit these SAEs will be provided in a handout (eg, the SAE form) in the investigator site file.

11.0 TRIAL-SPECIFIC REQUIREMENTS

11.1 Trial-Specific Committees

11.1.1 Data Monitoring Committee

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

12.0 DATA HANDLING AND RECORD KEEPING

The full details of procedures for data handling will be documented in the data management plan. AEs, medical history, and concurrent medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Drugs will be coded using the WHO Drug Dictionary [27].

12.1 CRFs (Electronic and Paper)

Completed CRFs are required for each subject for whom signed informed consent/pediatric assent form has been obtained.

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

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 designee[s]) and will be answered by the site.

Corrections to CRFs 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. Reasons for significant corrections should additionally be included.

The PI must review the CRFs for completeness and accuracy and must sign and date the appropriate CRFs as indicated. Furthermore, the investigator must retain full responsibility for the accuracy and authenticity of all data entered on the CRFs.

CRFs will be reviewed for completeness and acceptability at the trial site by trial monitors during periodic visits or if needed, by remote source data verification or telephone contact (Section 14.1). The sponsor or designee will be permitted to review the subject's medical and hospital records pertinent to the trial to ensure accuracy of the CRFs. The completed CRFs 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 Appendix A and those documents that include (but are not limited to) the trial-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 or informed consent and pediatric assent forms, subject authorization forms regarding the use of personal health information (if separate from the informed consent or pediatric assent forms), copies of all paper CRFs and query responses/electronic copy of CRFs, including the audit trail,

and detailed records of vaccine disposition to enable evaluations or audits from regulatory authorities, the sponsor or designee. 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 [2]. In addition, ICH E6 Section 4.9.5 states that the trial records should be retained until an amount of time specified by applicable regulatory requirements [2], or for a time specified in the clinical trial 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

All definitions apply to both cohorts. All data will be presented overall by trial arm, and by cohort and trial arm.

13.1 Statistical and Analytical Plans

A statistical analysis plan (SAP) will be prepared and finalized prior to the database lock and unblinding of subject's group assignment. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all trial objectives.

A blinded data review will be conducted prior to unblinding of subject's group assignment. This review will assess the accuracy and completeness of the trial database, subject evaluability, and appropriateness of the planned statistical methods.

13.1.1 Analysis Sets

Safety set: All subjects who received at least 1 dose of TDV or placebo.

Full analysis set (FAS): All randomized subjects who received at least 1 dose of TDV or placebo, and for whom a valid pre dose blood sample, and at least 1 valid post dose blood sample are received for immunogenicity assessments.

Per-protocol set (PPS): All subjects in the FAS who have no major protocol violations.

The major protocol violation criteria will be defined as part of the blinded data review prior to unblinding of the subject's assignment to the TDV or placebo arm. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving the wrong trial vaccination (TDV or placebo), (3) receiving prohibited vaccinations or therapies, (4) not receiving 2 doses of TDV or placebo, or, receiving the second dose outside of the visit window, (5) not having a valid immunogenicity assessment at 1 month post second dose (Day 120 [M4]), and (6) other major protocol violations that may be identified and documented during blinded data reviews.

13.1.2 Analysis of Demographic Characteristics and Other Baseline Characteristics

Age, gender, race, baseline serostatus, and other baseline characteristics will be summarized for all randomized subjects.

13.1.3 Immunogenicity analysis

For the immunogenicity endpoints (ie, GMTs of neutralizing antibodies, seropositivity rates against each of the 4 dengue serotypes, and seropositivity rates against multiple dengue serotypes), descriptive statistics and 95% CIs will be provided for each applicable visit. Seropositivity is defined as a reciprocal neutralizing titer ≥10. Additional summaries of GMTs of neutralizing antibodies by baseline serostatus (positive or negative) will also be provided.

The primary immunogenicity analyses will be based on the PPS; additional immunogenicity analyses may be provided based on the FAS as described in the SAP.

Potential analyses and/or table summaries may be performed to assess the effect of the coronavirus disease 2019 (COVID-19) pandemic. These may include sensitivity analyses ignoring the protocol-defined visit windows for the PPS subjects affected by COVID-19.

The handling of missing data will be described in the SAP.

13.1.4 Safety Analysis

All safety data will be summarized using the safety set.

Solicited AEs

The presence and severity (grade) of solicited local (injection site) AEs (pain, erythema, and swelling) and solicited systemic AEs (<6 years of age: fever, irritability/fussiness, drowsiness, and loss of appetite; ≥6 years of age: fever, asthenia, malaise, headache, and myalgia) will be collected via diary cards for 7 days and 14 days, respectively, following administration of each TDV or placebo dose (including the day of administration) at Day 1 (M0) and Day 90 (M3).

For each solicited AE, the number and percentage of subjects with local (injection site) and systemic AEs will be summarized and event severity for each day after vaccination (ie, Day 1 through Day 7 for local [injection site] AEs and Day 1 through Day 14 for systemic AEs), and overall. Summaries of first onset of each event and the number of days subjects reported experiencing each event will also be provided. For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

Persistent/prolonged solicited local or systemic AEs continuing on/after Day 8 or Day 15, respectively, following each trial vaccination will be assessed separately. Unless otherwise specified these events will not be included in the analyses/tabulations of unsolicited AEs and will have separate listings.

Unsolicited AEs

Unsolicited AEs will be assessed for 28 days following administration of each TDV or placebo dose (day of administration +27 subsequent days).

Unsolicited AEs will be coded using MedDRA and summarized by System Organ Class (SOC) and Preferred Term (PT). AEs leading to subject withdrawal from the trial or TDV or placebo discontinuation will also be summarized.

Unsolicited AEs will be summarized as follows: by PT including events with frequency greater than a predefined frequency (the percentage will be specified in the SAP); by SOC and PT; by SOC, PT, and severity; and by SOC, PT, and relationship to TDV or placebo. Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once.

MAAEs

MAAEs will be collected from Day 1 (M0) postvaccination through the end of the trial. MAAEs will be coded using MedDRA and summarized by SOC and PT.

SAEs

SAEs will be collected from Day 1 (M0) postvaccination through the end of the trial. SAEs will be coded using MedDRA and summarized by SOC and PT.

13.2 Interim Analysis and Criteria for Early Termination

In anticipation of interest by the local health authority, a blinded interim analysis of safety data will be prepared when all subjects have completed the Day 120 (M4) visit. An interim CSR will not be prepared. If requested, the unblinded interim analysis will be tabulated and submitted to the local health authority for review. Trial results will be reported in the final CSR.

13.3 Determination of Sample Size

The analysis of this trial is descriptive and is not based on testing formal null hypotheses. [redacted] The number of subjects receiving placebo will be 60 in each cohort (N=120 in total), as per the random assignment of 3:1 (TDV: placebo). Therefore, the total trial sample size is 480 subjects.

14.0 QUALITY CONTROL AND QUALITY ASSURANCE

14.1 Trial-Site Monitoring Visits

Monitoring visits to the trial site will be made periodically during the trial to ensure that all aspects of the protocol are followed. Source documents will be reviewed for verification of data recorded on the CRFs. Source documents are defined as original documents, data, and records. The investigator and institution guarantee access to source documents by the sponsor or designee (clinical research organization) and by the IRB or IEC.

All aspects of the trial and its documentation will be subject to review by the sponsor or designee (as long as blinding is not jeopardized), including but not limited to the Investigator Site File, trial vaccination (TDV and placebo) records, subject medical records, informed consent/pediatric assent form documentation, documentation of subject authorization to use personal health information (if separate from the informed consent/pediatric assent forms), and review of CRFs and associated source documents. It is important that the investigator and other trial personnel are available during the monitoring visits and that sufficient time is devoted to the process.

In the event a monitor cannot visit the site in a timely manner due to exceptional circumstances (eg, pandemic), alternative monitoring approaches such as remote source data verification or telephone contact may be used to ensure data quality and integrity and maintain patient safety. Alternative monitoring approaches should be used only where allowed by the local Health Authority and when approved by the IRB/IEC.

14.2 Protocol Deviations

The investigator should not deviate from the protocol, except where necessary to eliminate an immediate hazard to trial 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 trial site also may be subject to quality assurance audits by the sponsor or designee. In this circumstance, the sponsor-designated auditor will contact the site in advance to arrange an auditing visit. In the event the auditor or inspector cannot visit a site due to exceptional circumstances (eg, pandemic), alternative approaches such as remote audit/inspection may be used. 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 trial. In addition, there is the possibility that this trial may be inspected by regulatory agencies, including those of foreign governments (eg, the Food and Drug Administration [FDA], the Medicines and Healthcare Products Regulatory Agency of United Kingdom [MHRA], the Pharmaceuticals and Medical Devices Agency of Japan [PMDA]). If the trial 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 trial documents as described in Section 14.1.

14.4 Trial Risk Management

The ICH E6 addendum (R2) guidance encourages a risk-based approach to the management of clinical trials and includes requirements for risk control and risk reporting. Before initiation of the trial, Takeda or designee will establish quality tolerance limits (QTL) taking into consideration the medical and statistical characteristics of the variables and the statistical design of the trial. This process will be performed according to Takeda internal procedures.

At the end of the trial, the quality management approach implemented will be described in the CSR. If applicable, the CSR will summarize important deviations from the predefined QTL and the remedial actions taken.

15.0 ETHICAL ASPECTS OF THE TRIAL

This trial will be conducted with the highest respect for the trial subjects according to the protocol, the ethical principles that have their origin in the Declaration of Helsinki [1], and the ICH Harmonized Tripartite Guideline for GCP E6 (R2) [2]. Each investigator will conduct the trial 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 Institutional Review Board and/or Independent Ethics Committee Approval

IRBs and IECs must be constituted according to the applicable local requirements of each participating region. The sponsor or 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 trial, written notification regarding his or her abstinence from voting must also be obtained.

The sponsor or designee will supply relevant documents for submission to the respective IRB or IEC for the protocol's review and approval. This protocol, the IB, a copy of the informed consent/pediatric 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/pediatric assent form must be obtained and submitted to the sponsor or designee before commencement of the trial (ie, before shipment of TDV and placebo or trial specific screening activity). The IRB or IEC approval must refer to the trial by exact protocol title, number, and version date; identify versions of other documents (eg, informed consent/pediatric 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 trial. Until the site receives approval 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/pediatric assent form, recruitment materials intended for viewing by subjects, local safety reporting requirements, reports and updates regarding the ongoing review of the trial 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 designee.

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

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

Written consent documents will embody the elements of informed consent/pediatric assent 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/pediatric assent form, subject authorization form (if applicable), and subject information sheet describe the planned and permitted uses, transfers, and disclosures of the subject's personal and personal health information for the purpose of conducting the trial. The informed consent/pediatric assent form and the subject information sheet further explain the nature of the trial, its objectives, and potential risks and benefits, as well as the date informed consent/pediatric assent is given. The informed consent/pediatric assent form will detail the requirements of the subject and the fact that the subject is free to withdraw/the subject's LAR is free to withdraw their child at any time without giving a reason and without prejudice to the subject's further medical care.

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

The informed consent/pediatric assent form, subject authorization form (if applicable), and subject information sheet must be written in a language fully comprehensible to the prospective subject/subject's LAR. It is the responsibility of the investigator to explain the detailed elements of the informed consent/pediatric assent form, subject authorization form (if applicable), and subject information sheet (if applicable) to the subject/subject'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 LAR may provide such consent for the subject in accordance with applicable laws and regulations (eg, pediatric assent form).

The subject/the subject's LAR must be given ample opportunity to: (1) inquire about details of the trial and (2) decide whether or not to (allow the child to) participate in the trial. If the subject, or the subject's LAR, determines he or she/their child will participate in the trial, then the informed consent/pediatric assent form and subject authorization form (if applicable) must be signed and dated by the subject/the subject's LAR, at the time of consent and prior to the subject entering into the trial. The subject or the subject'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/pediatric assent form and subject authorization (if applicable) at the time of consent and prior to the subject entering into the trial; 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/pediatric assent form, subject authorization form (if applicable), and subject information sheet will be stored in the investigator's site file. The investigator must document the date the subject/subject's LAR signs the informed

consent/pediatric assent form in the subject's medical record and CRF. Copies of the signed informed consent/pediatric assent form, the signed subject authorization form (if applicable), and subject information sheet (if applicable) shall be given to the subject.

Re-consent, re-affirmation of consent: The investigator should assess the need to re-consent/re-affirmation of consent in situations wherein there has been substantial changes to the trial design or the subject's condition since the original consent. The process should comply with relevant local regulations.

Example: Where there is a likelihood that pediatric subjects reach adulthood while the trial is still in progress, the consent process has to be re-evaluated. The necessity to re-consent or re-affirm has to be described here as well as in the informed consent form.

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

15.3 Subject Confidentiality

The sponsor and designee affirm and uphold the principle of the subject's right to protection against invasion of privacy. Throughout this trial, a subject's source data will only be linked to the sponsor's clinical trial 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 designee, representatives from any regulatory authority (eg, FDA, MHRA, PMDA), the sponsor's designated auditors, and the appropriate IECs/IRBs to review the subject's original medical records (source data or documents), including, but not limited to, laboratory test result reports, electrocardiogram reports, admission and discharge summaries for hospital admissions occurring during a subject's trial participation, and autopsy reports. Access to a subject's original medical records requires the specific authorization of the subject/subject's LAR as part of the informed consent/pediatric assent form 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 CRF).

15.4 Clinical Trial Registration, Publication and Disclosure Policy

15.4.1 Clinical Trial Registration

In order to ensure that information on clinical trials reaches the public in a timely manner and to comply with applicable law, regulation and guidance, the sponsor will, as a minimum register all clinical trials conducted in subjects that it sponsors anywhere in the world, on publicly accessible websites such as ClinicalTrials.gov and EudraCT, and according to local requirements in India on Clinical Trial Registry- India (CTRI), before trial initiation [28]. The sponsor contact information, along with the investigator's city, country, and recruiting status will be registered and available for public viewing.

15.4.2 Clinical Trial Results Disclosure

Takeda clinical trial disclosure policy aims to comply with the clinical trial data disclosure requirements of all relevant regions. The sponsor will post the results of this clinical trial regardless of outcome, on publicly accessible websites such as ClinicalTrials.gov and/or EudraCT and/or CTRI, as required by applicable laws and/or regulations.

Completion of trial corresponds to the date on which the final subject was examined or received an intervention for the purpose of final collection of data (usually corresponds to Last Subject Last Visit).

15.4.3 Publication of Trial Results

The results of this trial are expected to be published in a peer-reviewed scientific journal. Publication of trial results will follow Takeda publication policies, applicable international standards and guidelines for good publication practice, applicable laws, and/or regulations.

15.5 Insurance and Compensation for Injury

Each subject in the trial 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 trial insurance against the risk of injury to clinical trial subjects. Refer to the clinical trial 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

1. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2 13;310(20):2191-4.
2. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH harmonized guideline. Integrated Addendum to ICH E6 (R2): Guideline for Good Clinical Practice E6.
3. World Health Organization. Dengue and severe dengue. Fact Sheet. 2023. Available at: http://www.who.int/mediacentre/factsheets/fs117/en/ (accessed 12 June 2023).
4. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature 2013;496(446):504-7.
5. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6(8):e1760.
6. Guzman M, Kouri G. Dengue: an update. Lancet Infect Dis 2002;2(1):33-42.
7. World Health Organization. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control, 2nd Edn. World Health Organization, Geneva, Switzerland; 1997. Available at: https://apps.who.int/iris/handle/10665/41988 (accessed 12 June 2023).
8. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control: new edition. 2009. Available at: https://apps.who.int/iris/handle/10665/44188 (accessed 12 June 2023).
9. Halstead S, Deen J. The future of dengue vaccines. Lancet 2002;360(9341):1243-5.
10. Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med 2015;373(13):1195-206.
11. Sridhar S, Luedtke A, Langevin E, Zhu M, Bonaparte M, Machabert T *et al*. Effect of Dengue Serostatus on Dengue Vaccine Safety and Efficacy. N Engl J Med. 2018;379(4):327-340.
12. The SAGE Working Group on Dengue Vaccines and WHO Secretariat. Background Paper on Dengue Vaccines; 18 April 2018. Available at: https://terrance.who.int/mediacentre/data/sage/SAGE_Docs_Ppt_April2018/5_session_dengue/April2018_session5_BGpaper_dengue-vaccines.pdf (accessed 12 June 2023).
13. Yoksan S, Bhamarapravati N, Halstead SB. Dengue virus vaccine development: study on biological markers of uncloned dengue 1-4 viruses serially passaged in primary kidney cells. In: TD St. George, BH Kay and J Blok (ed.), Arbovirus research in Australia. Proceedings of the 4th Symposium. CSIRO/QIMR, Brisbane, Australia, 1986: 35-8.

14. Huang CYH, Butrapet S, Tsuchiya KR, Bhamarapravati N, Gubler DJ, Kinney RM. Dengue 2 PDK-53 virus as a chimeric carrier for tetravalent dengue vaccine development. J Virol 2003; 77:11436-47.

15. Biswal S, Reynales H, Saez-Llorens X, Lopez P, Borja-Tabora C, Kosalaraksa P *et al*; TIDES Study Group. Efficacy of a Tetravalent Dengue Vaccine in Healthy Children and Adolescents. N Engl J Med. 2019;381(21):2009-2019.

16. Dengue Tetravalent Vaccine (Live, Attenuated) Investigator's Brochure – current version.

17. Ganeshkumar P, Murhekar MV, Poornima V, Saravanakumar V, Sukumaran K, Anandaselvasankar A et al. Dengue Infection in India: A Systematic Review and Meta-analysis. PLoS Negl Trop Dis. 2018;12(7):e0006618.

18. Pilot E, Nittas V, Murthy GVS. The Organization, Implementation, and Functioning of Dengue Surveillance in India-A Systematic Scoping Review. Int J Environ Res Public Health. 2019;24;16(4). pii: E661.

19. Dengue Epidemiology. Available at: https://www.denguevirusnet.com/epidemiology/28-dengue-epidemiology.html (accessed 12 June 2023).

20. Murhekar M, Joshua V, Kanagasabai K, Shete V, Ravi M, Ramachandran R *et al*. Epidemiology of Dengue Fever in India, Based on Laboratory Surveillance Data, 2014-2017. Int J Infect Dis. 2019;84S:S10-S14.

21. Rose W, Sindhu KN, Abraham AM, Kang G, John J. Incidence of Dengue Illness Among Children in an Urban Setting in South India: A Population-based Study. Int J Infect Dis. 2019;84S:S15-S18.

22. Shah PS, Alagarasu K, Karad S, Deoshatwar A, Jadhav SM, Raut T *et al*. Seroprevalence and Incidence of Primary Dengue Infections Among Children in a Rural Region of Maharashtra, Western India. BMC Infect Dis. 2019;19(1):296.

23. Hariharan D, Das MK, Shepard DS, Arora NK. Economic Burden of Dengue Illness in India from 2013 to 2016: A Systematic Analysis. Int J Infect Dis. 2019;84S:S68-S73.

24. Stanaway JD, Shepard DS, Undurraga EA, Halasa YA, Coffeng LE, Brady OJ *et al*. The Global Burden of Dengue: an Analysis from the Global Burden of Disease Study 2013. Lancet Infect Dis. 2016;16(6):712-723.

25. World Health Organization. Reducing pain at the time of vaccination: WHO position paper. September 2015-Recommendations. Vaccine. 2016, 34(32):3629-30.

26. Kohl KS, Marcy M, Blum M, Jones MC, Dagan R, Hansen J. et al. Fever after immunization: current concepts and improved future scientific understanding. Clin Infect Dis 2004;39:389-94.

27. WHO Collaborating Centre for International Drug Monitoring. WHO drug dictionary. Uppsala: WHO Collaborating Centre for International Drug Monitoring. 1992. Available at: https://who-umc.org/whodrug/whodrug-global/ (accessed 12 June 2023).

28. Clinical Trial Registry- India. Available at: www.ctri.nic.in (accessed 12 June 2023).

APPENDIX A RESPONSIBILITIES OF THE INVESTIGATOR

Clinical research trials 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 trial in accordance with the protocol.
2. Personally conduct or supervise the staff that will assist in the protocol.
3. Ensure that trial related procedures, including trial 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 trial are informed of these obligations.
5. Secure prior approval of the trial and any changes by an appropriate IRB/IEC that conforms to 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 trial to the IRB/IEC, and issue a final report within 3 months of trial completion.
7. Ensure that requirements for informed consent/pediatric assent, as outlined in ICH and local regulations, are met.
8. Obtain valid informed consent/pediatric assent from the LAR of each subject/each subject who participates in the trial, and document the date of consent in the subject's medical chart. Valid informed consent/pediatric assent form is the most current version approved by the IRB/IEC. Each informed consent/pediatric assent 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 trial. If an informed consent/pediatric assent 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 LAR.
9. Prepare and maintain adequate case histories of all persons entered into the trial, including CRFs, 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 (if designated as Signatory/Coordinating Investigator).

APPENDIX B INVESTIGATOR CONSENT TO USE OF PERSONAL INFORMATION

Takeda will collect and retain personal information of the 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 trial and/or other clinical studies.
- Management, monitoring, inspection, and audit of the trial.
- Analysis, review, and verification of the trial results.
- Safety reporting and pharmacovigilance relating to the trial.
- Preparation and submission of regulatory filings, correspondence, and communications to regulatory agencies relating to the trial.
- 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 trial.
- Self-inspection and internal audit within Takeda, its affiliates, and licensing partners.
- Archiving and audit of trial records.
- Posting investigator site contact information, trial details and results on publicly accessible clinical trial registries, databases, and websites.

The 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 the investigator's own country. The 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 REDUCING ANXIETY AND PAIN AT VACCINATION

WHO recommendations to reduce anxiety and pain at the time of vaccination:

General measures	(i) Health-care personnel carrying out vaccination should be calm, collaborative and well informed; they should use neutral words (e.g. "here I go" rather than "here comes the sting") and avoid language that increases anxiety, promotes distrust and/or is falsely reassuring or dishonest (e.g. phrases such as "it will only hurt for a second"). (ii) Proper positioning of the vaccine recipient should be ensured. Holding by the caregiver is recommended for infants and young children, and sitting upright for older populations. Lying down may be preferred for those with a history of fainting.
Specific additional measures	For children: (i) The caregiver should be present throughout and after the vaccination procedure. (ii) For children <6 years of age, distractions to divert attention away from pain to something more pleasant (eg, with toys, video, music, or conversation with an adult) are recommended. For adults: (i) Distractions using breathing interventions (slight coughing that does not lead to moving of the fixed arm or breath-holding) are recommended for adults. For adolescents: (i) Distraction is not effective, and there are no additional evidence-based, age-specific recommendations available for this group beyond the general measures for all age groups.
Not recommended	(i) Topical anesthetics, although effective, are not recommended for systematic use by national programmes globally because of high costs, lack of availability, and the additional time required for their application. (ii) Warming the vaccine (eg, by rubbing it between the hands). (iii) Manual stimulation of the injection site (eg, by rubbing or pinching). (iv) Administration of oral analgesics (eg, acetaminophen, ibuprofen) before or at the time of vaccination. If pain occurs later, during the days after vaccination, oral analgesics can then be given to mitigate pain and/or fever linked to delayed reactogenicity.

Adapted from the 2015 WHO position paper [25].

Signature Page for DEN-302 Protocol Amendment 1, Version 2.0, 15 June 2023

Title:

Approval	[redacted] Clinical 21-Jun-2023 09:27:56 GMT+0000
Approval	[redacted] Statistics 21-Jun-2023 11:43:42 GMT+0000
Approval	[redacted] Clinical 21-Jun-2023 11:56:11 GMT+0000
Approval	[redacted] Pharmacovigilance 22-Jun-2023 07:27:12 GMT+0000

Document Number: TAK-003-12179 v2.0