Clinical Study Protocol

NCT Number: NCT03999996

Title: A Phase 3, Follow-Up Trial to Evaluate Long-Term Safety and Antibody Persistence, and the Impact of a Booster Dose of a Tetravalent Dengue Vaccine Candidate in Healthy Adolescents and Adults in Areas Non-Endemic for Dengue.

Study Number: DEN-303

Document Version and Date: Version 5.0, 22 August 2022

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 Phase 3, Follow-Up Trial to Evaluate Long-Term Safety and Antibody Persistence, and the Impact of a Booster Dose of a Tetravalent Dengue Vaccine Candidate in Healthy Adolescents and Adults in Areas Non-Endemic for Dengue

Long-Term Safety and Antibody Persistence of TDV and the Impact of a Booster Dose

Sponsor:	Takeda Vaccines, Inc. 40 Landsdowne Street Cambridge, MA 02139 USA
Trial Identifier:	DEN-303
IND Number:	014292 **EudraCT Number:** Not Applicable
Trial Vaccine Name(s):	• Investigational vaccine: tetravalent dengue vaccine candidate (TDV) comprised of a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), 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). • Placebo: saline solution.
Takeda Approval Date:	22 August 2022
Version:	Version 5.0 (supersedes Version 4.0)

1.0 ADMINISTRATIVE INFORMATION

1.1 Contacts

Issue	Contact
Serious adverse event and pregnancy reporting	IQVIA Integrated Safety Management Lifecycle Safety E-mail: TakedaDensafety@Quintiles.com Fax and telephone numbers for serious adverse event and pregnancy reporting will be provided to the site.
Medical Monitor (medical advice on conduct of protocol or compound)	Emergency medical contact information will be provided to the site.
Responsible Medical Officer (carries overall responsibility for the conduct of the trial)	Emergency medical contact information will be provided to the 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 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], MD Date
[redacted] Clinical Development and Operations
Vaccine Business Unit
Takeda Pharmaceuticals International AG

[redacted], PhD Date
[redacted]
Vaccine Business Unit
Takeda Pharmaceuticals International AG

[redacted], PhD Date
[redacted] Pharmacovigilance Benefit-Risk
Vaccine Business Unit
Takeda Pharmaceuticals International AG

[redacted], MSc Date
[redacted] Statistics
Statistical & Quantitative Sciences
Takeda Pharmaceuticals International AG

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 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 10.1.5 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 Version 5.0 Summary of Changes

This document describes the changes in reference to the protocol (Version 5.0) incorporating amendment 3.

1.3.1 Version History

Date	Version Number	Change Type	Region
04 March 2019	1.0	Not applicable	Global
09 March 2020	3.0	Substantial	Global
22 February 2021	4.0	Substantial	Global
22 August 2022	5.0	Substantial	Global

1.3.2 Summary of Changes

Amendment to Protocol Version 4.0, 22 February 2021

Rationale for the Amendment:

This amendment outlines substantial changes that will be implemented at investigational sites in Mexico to accommodate a delay in the delivery of the trial vaccines to the sites in Mexico, and administrative delays due to the COVID-19 pandemic. The booster dose, which was due to be administered at 45 months after the first vaccination in the primary vaccination series, will now be administered at 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico). All safety checks and blood draws that were scheduled to occur prior to administration of the booster dose will be conducted as planned, and all post booster trial procedures will be adjusted accordingly to ensure that any changes being made to the booster schedule do not impact subject safety or data integrity. Note that these changes only apply to investigational sites in Mexico, they will not impact sites in the United States.

When this amendment was prepared, all subjects from parent trial DEN-304 (United States) had completed their last trial visit (Visit 5) following booster administration at Visit 3 (36 months after the first vaccination in the primary vaccination series). To permit the analysis of data collected at trial sites in the United States, an interim analysis (IA) of the safety and immunogenicity data collected at trial sites in the United States has been added. No modifications to the trial are planned based on the results of this IA.

Other Modifications

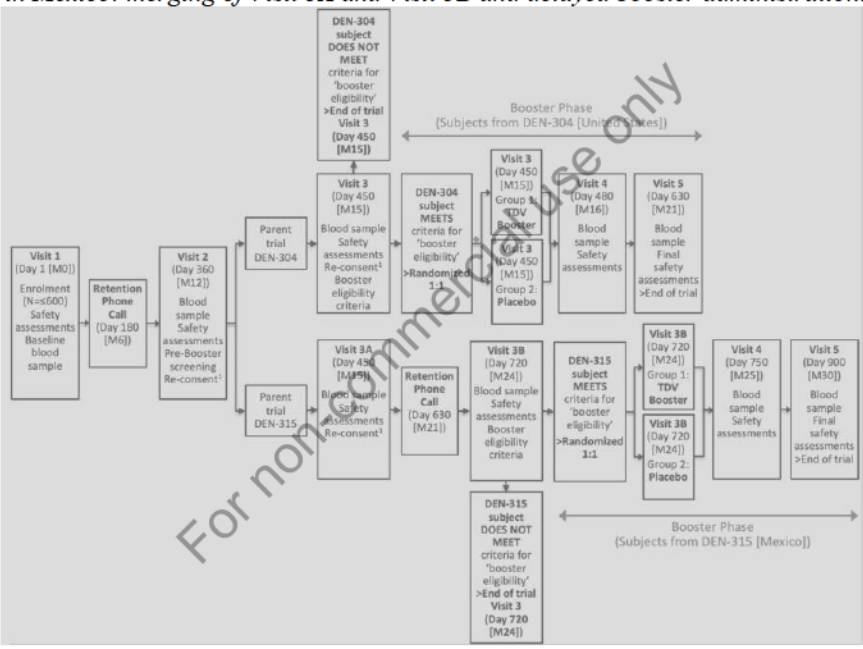
Administrative trial information and document references have been updated as necessary. Minor stylistic, grammatical, and editorial changes were included for clarification purposes only.

Details of the changes that have been made as part of this amendment are outlined below. In this section only, all new text is shown in bold italics, and any deleted text is marked using strikethrough.

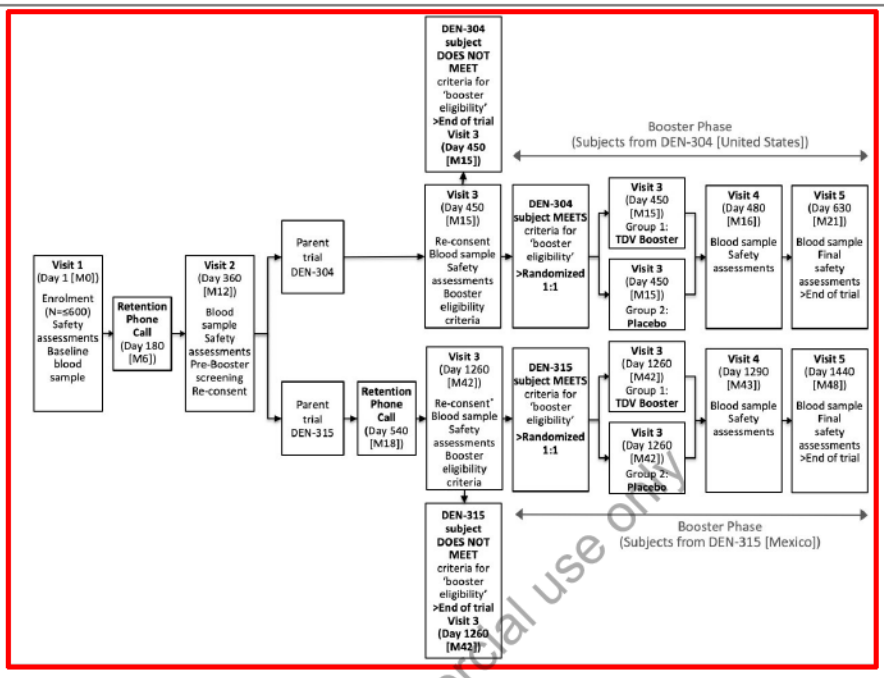
Section	Description of Change
Title Page	~~**Protocol**~~
Title Page	**Trial Vaccine Name(s):** • Investigational vaccine: tetravalent dengue vaccine candidate (TDV) comprised of a molecularly characterized, attenuated dengue serotype 2 strain (TDV-2), a dengue serotypes 2/1 recombinant ~~chimeric~~ strain (TDV-1), a dengue serotypes 2/3 recombinant ~~chimeric~~ strain (TDV-3), and a dengue serotypes 2/4 recombinant ~~chimeric~~ strain (TDV-4).
1.2	[redacted]~~, MD, PhD~~ [redacted]~~ Clinical Development Vaccines~~ [redacted]***, MD*** [redacted]***Clinical Development and Operations*** ***Vaccine Business Unit*** Takeda Pharmaceuticals International AG
1.2	[redacted], PhD [redacted] ***Vaccine Business Unit*** Takeda Pharmaceuticals International AG
1.2	[redacted]~~, PhD, MSc PV~~ [redacted]~~ Pharmacovigilance Benefit-Risk~~ [redacted]***, PhD*** [redacted]***Pharmacovigilance Benefit-Risk*** ***Vaccine Business Unit*** Takeda Pharmaceuticals International AG
1.2	[redacted]~~, MSc~~ [redacted] ~~Vaccine Statistics~~ [redacted]***, MSc*** [redacted]***Statistics*** ***Statistical & Quantitative Sciences*** Takeda Pharmaceuticals International AG
2.0	**Product Name:** ~~Tetravalent Dengue Vaccine Candidate (TDV)~~ ***Takeda's Dengue Tetravalent Vaccine (Live, Attenuated) (TDV)***
2.0	**Blinding Schema:** Open up to Visit 3~~B (Day 720 [Month 24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [Month 15]) for subjects from parent trial DEN-304 (United States)~~ and double-blind thereafter until the end of the trial
2.0	**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, ~~or~~***and*** DENV-4***)***.

Section	Description of Change
2.0	**Background and Rationale:...** A first ~~tetravalent~~***recombinant*** dengue vaccine (chimeric yellow fever virus dengue virus-tetravalent dengue vaccine [CYD-TDV]) was approved in some countries in Asia and Latin America in 2015, in Europe in 2018, and in the United States in 2019. Initial findings showed that vaccine efficacy was different between serotypes and depended on dengue pre-exposure status. Further analyses showed that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease if they were infected 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 vaccines. ***Takeda's Dengue*** **Tetravalent ~~Dengue~~ Vaccine ~~Candidate~~ *(Live, Attenuated)* (TDV) - Background:** Takeda's TDV consists of 1 molecularly characterized, attenuated dengue serotype 2 virus strain and 3 recombinant ~~chimeric~~ dengue virus strains expressing surface antigens corresponding to dengue serotypes ~~1–4~~ ***1, 3, and 4***. The dengue serotype 2 strain (TDV-2) is based upon the attenuated laboratory-derived virus 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 ~~chimeric~~, attenuated vaccine strains for dengue serotypes 1, 3 and 4 were engineered by substituting the structural genes, pre-membrane (prM) and envelope (E), of TDV-2 with the prM and E genes from the DENV virus strains, DENV-1 16007, DENV-3 16562 or DENV-4 1036, respectively. Thus, Takeda's TDV is comprised of 4 dengue virus strains: ***TDV-2 (***a molecularly characterized attenuated ~~dengue serotype 2~~ strain***)*** ~~(TDV-2)~~, a dengue serotypes 2/1 recombinant ~~chimeric~~ strain (TDV-1), a dengue serotypes 2/3 recombinant ~~chimeric~~ strain (TDV-3), and a dengue serotypes 2/4 recombinant ~~chimeric~~ strain (TDV-4). ~~Nonclinical studies carried out in mice and nonhuman primates have demonstrated an acceptable safety, immunogenicity, and efficacy profile for Takeda's TDV. Additionally, d~~***D***ata 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. ~~Ongoing and e~~***C***ompleted phase 2 clinical trials have enabled the selection of a final TDV dose (in a lyophilized formulation) and a ~~two~~***2***-dose vaccination series administered 3 months (ie, 90 days) apart by subcutaneous (SC) injection for use in the ongoing ~~pivotal~~***clinical development*** program. ~~In January 2019,~~***Results from*** the pivotal DEN-301 efficacy trial ***showed that the*** ~~met its~~ 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 >20,000 clinical trial subjects.*** All available data also showed that TDV was well tolerated with no significant safety concerns to date.
2.0	**Rationale for the Proposed Trial:...** Subjects previously enrolled in two parent trials (DEN-304 and DEN-315) will initially be invited to participate in the DEN-303 follow-up trial from 21 months after the first vaccination in the primary vaccination series in the parent trials. ~~The inclusion of subjects from other trials within the TDV program may also be considered if they meet the eligibility criteria for entry into DEN-303.~~ Antibody persistence will be assessed in all subjects for up to ~~45~~***63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315

Section	Description of Change
	(Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).
2.0	**Trial Design:...** ~~The inclusion of subjects from other trials within the TDV program may be considered if they meet the eligibility criteria for entry into DEN-303.~~ DEN-303 will include up to 600 healthy subjects aged ≥13 to ≤63 years at trial entry. To enable the assessment of a booster dose, the trial will be double-blinded, randomized, and placebo-controlled from Visit 3~~B (Day 720 [M24])~~ onwards ~~for subjects from parent trial DEN-315 (Mexico) and from Visit 3 (Day 450 [M15]) onwards for subjects from parent trial DEN-304 (United States)~~. Antibody persistence and safety will be assessed from Visit 1 ~~(Day 1 [M0])~~ through Visit 3~~B (Day 720 [M24])~~ for up to ~~45~~***63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and ~~from Visit 1 (Day 1 [M0]) through Visit 3 (Day 450 [M15])~~ for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). Further characterization of the long-term humoral and cell-mediated immune responses to Takeda's TDV will be undertaken in a subset of approximately 50 volunteers identified at enrollment (cell-mediated immunity [CMI] subset: participation is on a voluntary basis from DEN-304 only) up to Visit 5 ~~(Day 630 [M21])~~. A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) between site visits and to remind the subject/the subject's LAR of any upcoming site visits. At ~~Visit 3A (Day 450 [M15]) for subjects from parent trial DEN-315 (Mexico)/~~Visit 2 ~~(Day 360 [M12]) for subjects from parent trial DEN-304 (United States),~~ the site will discuss any information that is pertinent to the booster phase of the trial with the subject. ***A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18).*** Due to changes in the trial design ***in Mexico*** (~~protocol amendment 1, dated 09 March 2020 and protocol amendment 2, dated 22 February 2021~~***protocol amendment 3, dated 22 August 2022***), all subjects ***from parent trial DEN-315 (Mexico)*** will ~~also now~~ be asked to re-consent using a***n*** updated informed consent form ***(ICF)***~~(s)~~ or ***an*** updated informed consent and pediatric assent form~~s~~***, as applicable,*** at ~~Visit 2 (Day 360 [M12])~~***Visit 3*** ~~or at the next site visit if Visit 2 has passed~~ before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the electronic Case Report Form (eCRF). ~~An additional retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visit 3A and Visit 3B on Day 630 (M21) to maintain contact with the subject/the subject's LAR between site visits, and to remind the subject/the subject's LAR of the booster eligibility criteria and the upcoming site visit. Female subjects of childbearing age will also be reminded of pregnancy avoidance guidance and acceptable methods of contraception during this call.~~ At ~~Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/~~Visit 3 ~~(Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~, following all scheduled blood draws, all subjects will be screened for 'booster eligibility' to determine if they are eligible to go on to receive the TDV booster in the booster phase. Any subject who fails to meet the criteria for 'booster eligibility' will end the trial at Visit 3~~B~~ (~~Day 720 [M24])/Visit 3 (Day 450 [M15]), respectively~~. All eligible subjects will be randomized, using an interactive response technology at Visit 3~~B (Day 720 [M24]))/Visit 3 (Day 450 [M15]), as applicable,~~ to 1 of 2 trial groups (Group 1 and Group 2) in a 1:1 ratio stratified by parent trial and serostatus at baseline in the parent trials. Subjects allocated to Group 1 will receive the TDV booster (single dose) and

Section	Description of Change
	subjects allocated to Group 2 will receive placebo. The impact of the TDV booster on neutralizing antibody titers and seropositivity rates will be assessed at 1 month and 6 months after administration of the TDV booster or placebo. Safety assessments will continue for 6 months following TDV booster or placebo administration for subjects in Groups 1 and 2. For subjects from parent trial DEN-315 (Mexico), the duration of the current trial will be ~~24~~***42*** months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3~~B~~ and ~~30~~***48*** months for all other subjects. For subjects from parent trial DEN-304 (United States), the duration of the current trial will be 15 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 ~~(Day 450 [M15])~~ and 21 months for all other subjects. A schematic of the trial design is presented below in Figure 1.
2.0 and 6.1	*The trial design schematic has been updated to reflect the following changes to the study design in Mexico: merging of Visit 3A and Visit 3B and delayed booster administration.* 

Section	Description of Change



~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~

For all subjects, Visit 1 and Visit 2 correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3, Visit 4, and Visit 5 in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3, Visit 4, and Visit 5 in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series, respectively.

* Due to changes in the trial design ***in Mexico*** (~~protocol amendment 1, dated 09 March 2020 and protocol amendment 2, dated 22 February 2021~~***protocol amendment 3, dated 22 August 2022***), all subjects ***from parent trial DEN-315 (Mexico)*** will ~~also now~~ be asked to re-consent using a***n*** updated informed consent form~~(s)~~ ***(ICF)*** or ***an*** updated informed consent and pediatric assent form~~s~~***, as applicable,*** at ***Visit 3*** ~~Visit 2 (Day 360 [M12]) or at the next site visit if Visit 2 has passed~~ before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the eCRF.

Section	Description of Change
2.0	**Immunogenicity evaluation:** • Neutralizing antibodies (by microneutralization test 50% [MNT_{50}]) will be measured using blood samples collected from all subjects at Visit 1, Visit 2, and Visit 3~~A/3, at Visit 3B for subjects from parent trial DEN-315 (Mexico)~~, and also at Visit 4 and Visit 5 for subjects who are randomized to Groups 1 and 2. • At Visit ~~3A/~~3, a larger volume of blood will be collected from all subjects to assess exploratory markers of the long-term humoral immune response to Takeda's TDV.
2.0	**Safety evaluation:** Diary cards will be distributed to Groups 1 and 2 at Visit 3~~B for subjects from parent trial DEN-315 (Mexico)/Visit 3 for subjects from parent trial DEN-304 (United States)~~ for the recording of:
2.0	**Safety evaluation:...** • Medically attended AEs (MAAEs) will be collected, for Groups 1 and 2, following administration of the TDV booster or placebo from Visit 3~~B~~ through Visit 5 ~~for subjects from parent trial DEN-315 (Mexico)/from Visit 3 through Visit 5 for subjects from parent trial DEN-304 (United States)~~. 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.
2.0	**Primary Objectives:** • To describe antibody persistence for each of the 4 dengue serotypes for up to ~~45~~***63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). • To describe the impact of a TDV booster dose vs placebo on antibody response for each of the 4 dengue serotypes at 1 month and 6 months post administration of the TDV booster or placebo. ~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~
2.0	**Secondary Objectives:** *Immunogenicity* Antibody Persistence • To describe the overall trend in antibody decay for all 4 dengue serotypes from values obtained after the primary vaccination series in the parent trials through ~~45~~***63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and through 36 months after the first vaccination in the primary

Section	Description of Change
	vaccination series for subjects from parent trial DEN-304 (United States). Impact of a TDV Booster Dose • To describe the impact of a TDV booster on antibody response for each of the 4 dengue serotypes for up to ~~51~~***69*** months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 42 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). *Safety* • To describe the long-term safety of Takeda's TDV for up to ~~45~~***63*** months in previously vaccinated subjects from parent trial DEN-315 (Mexico) and for up to 36 months in previously vaccinated subjects from parent trial DEN-304 (United States).
2.0	**Exploratory Objectives:** ***Applicable to subjects from parent trial DEN-315 (Mexico only):*** • To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at ~~36~~***63*** months after the first vaccination in the primary vaccination series in the parent trial ***(DEN-315)***~~s and in the CMI subset (applicable to subjects from parent trial DEN-304 [United States] only) at 1 month and 6 months post booster in the current trial~~; this is inclusive of, but not restricted to, an assessment of the anti-dengue Non-Structural protein 1 (NS1) antibody response. Applicable to subjects from parent trial DEN-304 (United States only): • ***To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304), and in the CMI subset at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, an assessment of the anti-dengue NS1 antibody response.*** • To evaluate aspects of the long-term cell-mediated immune response to Takeda's TDV up to 36 months after the first vaccination in the primary vaccination series in the parent trial ***(DEN-304)*** and at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) of the long-term T cell-mediated immune response to TDV (CMI subset only).
2.0	**Subject Population:...** Planned Number of Trial Arms: A single open arm up to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ and two arms thereafter through ***the*** end of trial, randomized in a 1:1 ratio stratified by parent trial and serostatus at baseline in the parent trials
2.0	**Exclusion Criteria at Entry:...** 4. Subjects with a prolonged period of habitation (≥1 year) in a dengue endemic area within the 2 years prior to Visit 1~~ (Day 1[M0])~~.
2.0	**Booster Eligibility:** ~~Assessed at Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico) and Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States).~~ Any subject who meets any of the following criteria ***at Visit 3*** will not qualify for randomization to Group 1 or 2 to receive the TDV booster or placebo, respectively:

Section	Description of Change
2.0	**Booster Eligibility:...** 5. Known or suspected impairment/alteration of immune function, including: a) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~; use of inhaled, intranasal, or topical corticosteroids is allowed. b) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. c) Administration of immunoglobulins and/or any blood products within the 3 months prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~; consider whether applicable as an exclusion criterion or criterion for delay. d) Receipt of immunostimulants within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. e) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~.
2.0	**Booster Eligibility:...** 13. Female subjects of child-bearing potential[1] who are sexually active with men, and who have not used any of the acceptable contraceptive methods[2] for at least 2 months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. 14. Female subjects of childbearing potential who are sexually active, and who refuse to use an "acceptable contraceptive method" for up to 6 weeks after administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. In addition, they must be advised not to donate ova or breastfeed during this period. *Corresponding footnote:* [2] Hormonal contraceptives (eg, oral, injection, transdermal patch, implant, cervical ring), barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse, intrauterine device, monogamous relationship with ***a*** vasectomized partner (partner must have been vasectomized for at least ~~six~~***6*** months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. Other contraceptive methods may be considered in agreement with the sponsor and must be approved by the appropriate ethics committee.

Section	Description of Change
2.0	**Trial Vaccine and Placebo:** *Investigational vaccine* The investigational vaccine is Takeda's TDV, a tetravalent vaccine comprised of 1 molecularly characterized, attenuated dengue virus strain (TDV-2), and 3 recombinant ~~chimeric~~ dengue virus strains with potencies of not less than 3.3, 2.7, 4.0 and 4.5 $\log_{10}$ plaque forming units per dose of TDV-1, TDV-2, TDV-3, and TDV-4, respectively.
2.0	**Duration of the Trial and Subject Participation:** The trial duration for each subject will be between ~~24~~***42*** months and ~~30~~***48*** months for subjects from parent trial DEN-315 (Mexico), and between 15 months and 21 months for subjects from parent trial DEN-304 (United States).
2.0	**Criteria for Evaluation and Analyses:** ***Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.*** **Primary endpoints:** • Geometric mean titers (GMTs) of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 1 ~~(Day 1 [M0])~~, Visit 2 ~~(Day 360 [M12])~~, ***and*** Visit 3 ~~A (Day 450 [M15]), and Visit 3B (Day 720 [M24]~~; ***(***prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) ~~for subjects from parent trial DEN-315 (Mexico) and at Visit 1 (Day 1 [M0]), Visit 2 (Day 360 [M12]), and Visit 3 (Day 450 [M15]; prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) for subjects from parent trial DEN-304 (United States),~~ summarized for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials. • GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 4 ~~(Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~ and Visit 5 ~~(Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States]),~~ for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials. ~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2,~~

Section	Description of Change
	~~and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~

2.0	**Secondary endpoints**:
	Immunogenicity endpoints
	<u>Antibody Persistence</u>
	• Geometric Mean Ratio (GMR) of neutralizing antibodies for each of the 4 dengue serotypes for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials for:
	  • Visit 1 ~~(Day 1 [M0])~~ vs Visit 2 ~~(Day 360 [M12]), for all subjects~~.
	  • Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ in the current trial.
	  • Day 270 (Month 9) in the parent trials (9 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 1 ~~(Day 1 [M0])~~ and Visit 2 ~~(Day 360 [M12])~~ in the current trial~~, for all subjects~~.
	<u>Impact of a TDV Booster Dose</u>
	• GMR of neutralizing antibodies for each of the 4 dengue serotypes for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials for:
	  • Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 4 in the current trial ~~(Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~.
	  • Visit 3~~B (Day 720 [M24])~~ vs Visit 4 ~~(Day 750 [M25]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) vs Visit 4 (Day 480 [M16]) for subjects from parent trial DEN-304 (United States)~~.
	  • Visit 3~~B (Day 720 [M24])~~ vs Visit 5 ~~(Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) vs Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~.
	  • Visit 4 ~~(Day 750 [M25])~~ vs Visit 5 ~~(Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/Visit 4 (Day 480 [M16]) vs Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~.
	  • Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 5 in the current trial ~~(Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States]~~.

Section	Description of Change
2.0	**Secondary endpoints**:... Safety endpoints • Frequency and severity of solicited local (injection site) reactions for 7 days (day of vaccination + 6 days), and solicited systemic AEs for 14 days (day of vaccination + 13 days) following administration of the TDV booster or placebo at Visit 3~~B (Day 750 [M25]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ by trial group. • Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 days) following administration of the TDV booster or placebo at Visit 3~~B (Day 750 [M25]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ by trial group. • Percentage of subjects with any MAAEs following administration of the TDV booster or placebo from Visit 3~~B (Day 750 [M25])~~ through Visit 5 ~~(Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/from Visit 3 (Day 450 [M15]) through Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States),~~ by trial group. • Percentage of subjects with any SAEs from Visit 1 ~~(Day 1 [M0])~~ through Visit 3~~B (Day 750 [M25]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ prior to administration of the TDV booster or placebo. • Percentage of subjects with any SAEs ***following administration of the TDV booster or placebo from Visit 3 through Visit 5 by trial group*** ~~from Visit 3B (Day 750 [M25]) after administration of the TDV booster or placebo through Visit 5 (Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/from Visit 3 (Day 450 [M15]), after administration of the TDV booster or placebo through Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States), by trial group~~.
2.0	**Exploratory endpoints:** ***Applicable to subjects from parent trial DEN-315 (Mexico only):*** • The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3~~A/3 (Day 450 [M15]) and from subjects in the CMI subset (applicable to subjects from parent trial DEN-304 [United States] only) at Visit 4 (Day 480 [M16]) and Visit 5 (Day 630 [M21])~~. Additional exploratory techniques may be added as the field evolves. Applicable to subjects from parent trial DEN-304 (United States only): • ***The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3, and from subjects in the CMI subset at Visit 4 and Visit 5. Additional exploratory techniques may be added as the field evolves.*** • The assessment of the long-term cell-mediated response to TDV will include, but is not restricted to, the frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 PBMC) of IFN-γ ELISpot responses to TDV using blood samples collected from subjects in the CMI subset at Visit 1 ~~(Day 1 [M0])~~, Visit 2 ~~(Day 360 [M12])~~,

Section	Description of Change
	Visit 3 ~~(Day 450 [M15])~~, Visit 4 ~~(Day 480 [M16])~~, and Visit 5 ~~(Day 630 [M21])~~. Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥ 50 spots per 10^6 PBMC. Additional exploratory techniques may be added as the field evolves (CMI subset only). ~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~
2.0	**Statistical Considerations:** The following analysis sets are defined for this trial: ***All Screened: All subjects who agreed to participate in the current trial.*** ***All Screened-Booster: All subjects who agreed to participate in the current trial and who were screened for 'booster eligibility' to determine if they were eligible to go on to be randomized to Group 1 or Group 2.*** ***Randomized Set-Booster: All subjects randomized at Visit 3 regardless of whether they received the trial vaccination in the current trial. Subjects in this set will be summarized according to randomized treatment.***
2.0	**Statistical Considerations:...** **Full Analysis Set (FAS):** All subjects who received at least one dose of Takeda's TDV in the parent trials and for whom there is at least one valid follow-up measurement up to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/up to Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ for immunogenicity assessments in the current trial. **Full Analysis Set-Booster (FAS-B):** All subjects who received at least one dose of Takeda's TDV in the parent trials, the TDV booster or placebo in the current trial, and for whom there is at least one valid follow-up measurement after administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ for immunogenicity assessments in the current trial. **Per Protocol Set (PPS):** All subjects from the FAS who received two doses of Takeda's TDV in the parent trials with no new major protocol violations prior to administration of the booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ that could potentially confound the primary endpoints in the current trial. **Per Protocol Set-Booster (PPS-B):** All subjects from the FAS-B who received two doses of Takeda's TDV in the parent trials with no new major protocol violations after administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315~~

Section	Description of Change
	~~(Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ that could potentially confound the primary endpoints in the current trial. The major protocol violation criterion will be defined as part of a data review prior to analysis. The categories of new major protocol violations include: (1) not meeting selected entry criteria, (2) receiving the wrong trial vaccination at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15] for subjects from parent trial DEN-304 (United States);~~ *(*subjects randomized to Groups 1 and 2 only), (3) receiving prohibited vaccinations or therapies (subjects randomized to Groups 1 and 2 only), (4) performing Visit 4 inadmissibly outside the visit window (subjects randomized to Groups 1 and 2 only), and (5) other major protocol violations that may be identified during data reviews. ***Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.***
2.0	**Statistical Considerations:...** <u>Immunogenicity evaluation</u> For the primary and secondary immunogenicity endpoints (ie, GMTs of neutralizing antibodies and seropositivity rates for each of the 4 dengue serotypes and multiple [2, 3 or 4]), descriptive statistics and 95% CIs will be provided for each applicable visit ***(Visit 1, Visit 2, Visit 3, Visit 4, and Visit 5 in the current trial*** ~~(Visit 1 [Day 1 (M0)], Visit 2 [Day 360 (M12)], Visit 3A [Day 450 (M15)], Visit 3B [Day 720 (M24)], Visit 4 [Day 750 (M25)], and Visit 5 [Day 900 (M30)] in the current trial for subjects from parent trial DEN-315 [Mexico]/Visit 1 [Day 1 (M0)], Visit 2 [Day 360 (M12)], Visit 3 [Day 450 (M15)], Visit 4 [Day 480 (M16)], and Visit 5 [Day 630 (M21)] in the current trial for subjects from parent trial DEN-304 [United States]~~, and for Day 120 [Month 4] and Day 270 [Month 9] in both the parent trials [which corresponds to 4 months and 9 months after the first vaccination in the primary vaccination series in the parent trials, respectively]).
2.0	<u>Immunogenicity evaluation...</u> Further details on the statistical analysis including exploratory endpoints will be provided in the ***statistical analysis plan (***SAP***).***
2.0	**Statistical Considerations:...** *Unsolicited AEs* Unsolicited AEs will be summarized by trial group for 28 days following administration of the TDV booster or placebo (day of vaccination + 27 days). Unsolicited AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by Preferred Term (PT) and System Organ Class (SOC) for each trial group. AEs leading to trial ~~or vaccine~~ withdrawal will be summarized up to Visit 3~~B (Day 720 [M24] –~~ *(*pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5 ~~(Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/up to Visit 3 (Day 450 [M15] – pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~.

Section	Description of Change
	Unsolicited AEs will be tabulated at each of the following levels: overall summary (subjects with at least 1 AE), and by SOC and PT. In addition, unsolicited AEs will be summarized as follows: by PT including events with frequency greater than a pre-defined frequency (the percentage will be specified in the SAP~~Statistical Analysis Plan~~); by SOC and PT; by SOC, PT, and severity; and by SOC, PT, and relationship to the trial vaccine (TDV booster or placebo). Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once. *MAAEs* MAAEs will be collected for subjects randomized to Group 1 and Group 2 and will be presented by trial group from Visit 3~~B (Day 720 [M24])~~ through Visit 5 (~~Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/from Visit 3 (Day 450 [M15]) through Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~. MAAEs will be coded using MedDRA and summarized by SOC and PT for each trial group. *SAEs* SAEs will be collected throughout the trial. SAEs will be coded using MedDRA and summarized by PT and SOC up to Visit 3~~B (Day 720 [M24])~~ ***(pre-vaccination)*** and thereafter (post-vaccination) by trial group up to Visit 5~~ (Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/up to Visit 3 (Day 450 [M15], pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~. ~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~
2.0	**Interim Analysis:** ~~No interim analyses are planned.~~***Due to significant delays (of >2 years) to booster administration for subjects from parent trial DEN-315 (Mexico), an interim analysis (IA) of the safety and immunogenicity data collected at trial sites in the United States is planned when all subjects from parent trial DEN-304 (United States) have completed their last trial visit (Visit 5 [Day 630 (M21)]). This IA will be performed in an unblinded manner and will include the necessary steps to ensure that no database modifications are made after unblinding for subjects at trial sites in the United States. Unblinding of subjects from parent trial DEN-315 (Mexico) will occur after all subjects at trial sites in Mexico have completed their last trial visit (Visit 5 [Day 1440 (M48)]) and the trial database has been locked. No modifications to the trial are planned based on the results of this IA. An interim CSR of data from parent trial DEN-304 (United States) will not be prepared; all trial results will be reported in the final CSR. More details regarding the IA will be provided in the SAP.***

Section	Description of Change
2.1	*Table headers have been updated to reflect merging of Visit 3A and Visit 3B at sites in Mexico; delays to Visit 3, Visit 4, and Visit 5 at sites in Mexico; and an extension of the Visit 3 visit window in Mexico.*

2.1 Schedule of Trial Procedures

Visit By parent trial (DEN-304/DEN-315)	1 (Day 1[M0]/ Day 1[M0])	2 [a] (Day 360 [M12]/ Day 360 [M12])	3 [b] (Day 450 [M15]/ ~~Day 450 [M15]~~*Day 1260 [M42]*)	3A [b]	3B [b] ~~(NA/ Day 720 [M24])~~	4 [c] (Day 480 [M16]/ ~~Day 750 [M25]~~ *Day 1290 [M43]*)	5 [c] (Day 630 [M21]/ ~~Day 900 [M30]~~ *Day 1440 [M48]*)
Visit Window (Days)	-15/+75 (Linked to Parent Day 1)	-45/+45 (Linked to Visit 1)	-45/+45 (Linked to Visit 1)	-120/+120 (Linked to Visit 1)	~~-45/+90 (Linked to Visit 1)~~	-1/+7 (Linked to Visit 3)	-0/+60 (Linked to Visit 3)
Applicable to subjects from parent trial	DEN-304 DEN-315	DEN-304 DEN-315	DEN-304	DEN-315	~~DEN-315~~	DEN-304 DEN-315	DEN-304 DEN-315

2.1	*Table columns updated to reflect merging of Visit 3A and Visit 3B in Mexico.*

Visit By parent trial (DEN-304/DEN-315)	3 [b] (Day 450 [M15]/ ~~Day 450 [M15]~~*Day 1260 [M42]*)	3A [b]	3B [b] ~~(NA/ Day 720 [M24])~~
Visit Window (Days)	-45/+45 (Linked to Visit 1)	-120/+120 (Linked to Visit 1)	~~-45/+90 (Linked to Visit 1)~~
Applicable to subjects from parent trial	DEN-304	DEN-315	~~DEN-315~~
Site visit	X	~~X~~	~~X~~
Phone contact			
Signed Informed Consent /Pediatric Assent Form [c]	X	~~X~~	~~X~~
Eligibility criteria	X [g]		~~X~~ [g]
Randomization [h]	X		~~X~~
Demographics			
Pregnancy test [i]	X		~~X~~
Medical history update	X [c]		~~X~~ [c]
Prior medication update	X [c]		~~X~~ [c]
Concomitant medication update [k]	X [c]		~~X~~ [c]
Concomitant vaccination update [l]	X	~~X~~	~~X~~
Complete physical examination [m]	X[c]		~~X~~ [c]
Targeted physical examination [n]			
Vital signs [o]	~~X~~	~~X~~	~~X~~
Review of systems	~~X~~ [o]		~~X~~ [c]
Pregnancy avoidance guidance [p]	X [c]	~~X~~	~~X~~ [c]
Investigational product [q] administered by SC injection	X [c]		~~X~~ [c]

Visit By parent trial (DEN-304/DEN-315)		3 [b] (Day 450 [M15]/ ~~Day 450 [M15]~~*Day 1260 [M42]*)	3A [b]	3B [b] ~~(NA/ Day 720 [M24])~~
Visit Window (Days)		-45/+45 (Linked to Visit 1)	-120/+120 (Linked to Visit 1)	~~-45/+90 (Linked to Visit 1)~~
Applicable to subjects from parent trial		DEN-304	DEN-315	~~DEN-315~~
Injection site evaluation [r]		X [c]		~~X~~ [c]
Diary card [s]	Distribution	X [c]		~~X~~ [c]
	Review/collection of solicited local reactions and systemic AEs	X [c]		~~X~~ [c]
Unsolicited AEs [t]		X [c]		~~X~~ [c]
MAAEs [u]		X [c]		~~X~~ [c]
SAEs and AEs leading to subject discontinuation or withdrawal [v]		X	~~X~~	X
Criteria for delay of blood sampling		X	~~X~~	~~X~~ [e]
Blood draw (10 mL) [w]		X	~~X~~	~~X~~ [e]
Blood draw (40 mL) [w]		X	~~X~~	
Additional blood draw (10 mL CMI subset only, n=50) [x]				
Additional blood draw (40 mL CMI subset only, n=50) [x]		X		

2.1	*Mark removed for "Signed Informed Consent/Pediatric Assent Form" at Week 2 as re-consent will take place at Visit 3 for subjects from parent trial DEN-315 (Mexico).*

Visit By parent trial (DEN-304/DEN-315)	1 (Day 1[M0]/ Day 1[M0])	2 [a] (Day 360 [M12]/ Day 360 [M12])
Visit Window (Days)	-15/+75 (Linked to Parent Day 1)	-45/+45 (Linked to Visit 1)
Applicable to subjects from parent trial	DEN-304 DEN-315	DEN-304 DEN-315
Site visit	X	X
Phone contact	X [d]	X [d]
Signed Informed Consent /Pediatric Assent Form [c]	X	~~X~~

Section	Description of Change
2.1	~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~ ***Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.*** ~~AE=Adverse Event; BMI=Body Mass Index; CMI=Cell-Mediated Immunity; M=Month; MAAE=Medically Attended Adverse Event; NA=Not Applicable; SAE=Serious Adverse Event; SC=Subcutaneous~~ ***AE: adverse event; CMI: cell-mediated immunity; ICF: informed consent form M: Month, MAAE; medically attended adverse event; NA: not applicable; SAE: serious adverse event; SC: subcutaneous.***
2.1	(a) ~~At Visit 3A, subjects from parent trial DEN-315 (Mexico) will be reminded of any issues relating to administration of/or eligibility for the upcoming booster dose at Visit 3B.~~ At Visit 2, subjects ~~from parent trial DEN-304 (United States)~~ will be reminded of any issues relating to administration of/or eligibility for the upcoming booster dose at Visit 3. This includes, but is not limited to, the collection of any SAEs and any AEs leading to subject discontinuation or withdrawal, a review of prohibited therapies, pregnancy avoidance guidance and information on acceptable methods of contraception (for female subjects of childbearing age). (b) ***Visit 3 will be delayed until Day 1260 (M42) for subjects from parent trial DEN-315 (Mexico).*** ~~Booster administration and all booster related procedures will occur at Visit 3B for subjects from parent trial DEN-315 (Mexico) and at Visit 3 for subjects from parent trial DEN-304 (United States). For subjects from parent trial DEN-315 (Mexico), Visit 3 is split into Visit 3A and Visit 3B to accommodate delayed booster administration.~~ (c) Applicable only to subjects randomized to Group 1 or Group 2 at ~~Visit 3B/~~Visit 3. (d) A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) and to remind the subject/the subject's LAR about the upcoming site visit. A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on ~~Day 630 (M21)~~ ***Day 540 (M18)*** ~~to maintain contact with the subject/the subject's LAR between site visits, and to remind the subject/the subject's LAR of the booster eligibility criteria and~~

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	~~the upcoming site visit. Female subjects of childbearing age will also be reminded of pregnancy avoidance guidance and acceptable methods of contraception during this call~~.
	(e) Prior to the subject entering into the trial and before any protocol-directed procedures are performed; up to 28 days prior to the day of enrollment. Adolescents from parent trial DEN-315 (Mexico) who become 18 years of age during the course of the trial will be asked to return to the investigational site for an additional site visit to provide the appropriate written informed consent. This should be done as soon as possible after their 18th birthday. ***Due to changes in the trial design in Mexico (protocol amendment 3, dated 22 August 2022), all subjects from parent trial DEN-315 (Mexico) will now be asked to re-consent using an updated ICF or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed.*** ~~Due to changes in the trial design (protocol amendment 1, dated 09 March 2020 and protocol amendment 2, dated 22 February 2021), all subjects will now be asked to re-consent using a updated informed consent form(s) or updated informed consent and pediatric assent forms at Visit 2 (Day 360 [M12]) or at the next site visit if Visit 2 has passed before any further protocol-directed procedures are performed.~~ An oral summary of any major changes that have been made to the study will be provided to the subject and the subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the electronic Case Report Form (eCRF).
	(f) After informed consent has been obtained, eligibility of the subject will be assessed by review of inclusion/exclusion criteria for trial entry at Visit 1.
	(g) A review of the 'booster eligibility' will be performed prior to randomization at Visit 3~~B for subjects from parent trial DEN-315 (Mexico)/at Visit 3 for subjects from parent trial DEN-304 (United States) to determine if the subject is eligible to go on to receive the TDV booster in the booster phase of the trial~~.
	(h) Stratified by parent trial and serostatus at baseline in the parent trials.
	(i) For female subjects of childbearing potential, pregnancy testing (urine) will be performed at Visit 3~~B for subjects from parent trial DEN-315 (Mexico)/Visit 3 for subjects from parent trial DEN-304 (United States)~~. Results must be confirmed and documented as negative prior to administration of the TDV booster or placebo at Visit 3~~B/Visit 3, as applicable~~. 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) Any relevant information collected during the parent trials will be accessed via the database and updated as necessary throughout the trial conduct.
	(k) All medications from 1 month (minimum 28 days) prior to administration of the TDV booster or placebo at Visit 3~~B/Visit 3, as applicable,~~ and up to 1 month (minimum 28 days) thereafter~~,~~*;* steroids and immunostimulants within 60 days prior to Visit 3~~B/Visit 3, as applicable,~~*;* immunoglobulins and blood products within 3 months prior to Visit 3~~B/Visit 3, as applicable,~~*;* and immunosuppressive therapy within 6 months prior to Visit 3~~B/Visit 3, as applicable~~.
	(l) Any inactivated vaccines administered ≤14 days ~~prior to blood sample collection and TDV booster/placebo administration at Visit 3B/Visit 3, as applicable,~~ and any live attenuated vaccines administered ≤28 days prior to blood sample collection or TDV booster/placebo administration at Visit 3~~B/Visit 3, as applicable~~.

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	(m) Physical examination including measurement of weight and height – ~~BMI~~***body mass index*** will be calculated automatically. (n) Subjects may undergo a targeted symptom-directed physical examination. Clinically significant changes from the baseline examination (Visit 1) should be recorded in the subject's source documents and eCRF. (o) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature. (p) 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 at Visit 1 stating that they understand the requirements for avoidance of pregnancy and donation of ova. ~~For subjects from parent trial DEN-315 (Mexico), further guidance with respect to the avoidance of pregnancy will be provided to all female subjects of childbearing potential at Visit 3A and to all female subjects of childbearing potential who are randomized to Group 1 or 2 at Visit 3B, and at Visit 4. For subjects from parent trial DEN-304 (United States),~~ ~~f~~***F***urther guidance with respect to the avoidance of pregnancy will be provided to all female subjects of childbearing potential at Visit 2 and to all female subjects of childbearing potential who are randomized to Group 1 or 2 at Visit 3, and at Visit 4. Females of childbearing potential, who are randomized to Group 1 or 2 and are sexually active, will also be reminded to adhere to acceptable contraceptive methods for up to 6 weeks after TDV booster or placebo administration. (q) Subjects who meet the criteria for 'booster eligibility' and are subsequently randomized to Group 1 or Group 2 at Visit 3~~B for subjects from parent trial DEN-315 (Mexico)/Visit 3 for subjects from parent trial DEN-304 (United States),~~ ***will*** receive the TDV booster or placebo, respectively. (r) Injection site pain, erythema, and swelling assessed by trial staff for 30 minutes post-vaccination. (s) Diary cards (paper or electronic) will be distributed to subjects in Groups 1 and 2 at Visit 3~~B/Visit 3, as applicable~~, for the collection of 1) solicited local (injection site) reactions for 7 days (day of vaccination + 6 subsequent days) following TDV booster or placebo administration, and 2) solicited systemic AEs for 14 days (day of vaccination + 13 subsequent days) following TDV booster or placebo administration. The investigator will ~~categorize events by severity (mild, moderate or severe) and will~~ assess causality of solicited systemic AEs to vaccine administration (related or not related). (t) Unsolicited AEs will be collected by interview and recorded for Groups 1 and 2 for 28 days (day of vaccination + 27 subsequent days) following TDV booster or placebo administration. The investigator will categorize each event by severity (mild, moderate or severe) and will assess causality to trial vaccine administration (related or not related). If solicited local (injection site) reactions and systemic AEs continue on Day 8 and Day 15 (after administration of the TDV booster or placebo), respectively, record the full duration of the event on the "Adverse Event" eCRF. (u) MAAEs will be collected for Groups 1 and 2 from Visit 3~~B/Visit 3, as applicable,~~ through Visit 5. (v) Any SAEs and any AEs leading to subject discontinuation or withdrawal will be collected for all subjects for the trial duration. (w) At Visit 3~~A/3~~, a 10 mL blood sample and 40 mL blood sample will be taken from all subjects. For those subjects ~~from parent trial DEN-304 (United States)~~ who are randomized to Groups 1 and 2 at Visit 3, all blood samples should be taken prior to administration of the

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	TDV booster dose or placebo. ~~An additional 10 mL blood sample will also be collected prior to administration of the TDV booster dose or placebo at Visit 3B from subjects from parent trial DEN-315 (Mexico) randomized to Groups 1 and 2 at Visit 3B.~~ The blood samples taken at Visit 4 should be taken at least 29 days (-1, +7 days) after administration of the TDV booster or placebo at ~~either Visit 3B/~~Visit 3. ***(x) One additional 40 mL blood sample will be collected from a subset of 50 subjects (CMI subset; from DEN-304 only) for exploratory immunogenicity analyses from Visit 1 through Visit 5. An additional 10 mL blood sample will also be collected from subjects in the CMI subset at Visit 4 and Visit 5 for further exploratory immunogenicity analyses.***
3.3	IA interim analysis TDV-1 dengue serotypes 2/1 recombinant ~~chimeric~~ strain TDV-3 dengue serotypes 2/3 recombinant ~~chimeric~~ strain TDV-4 dengue serotypes 2/4 recombinant ~~chimeric~~ strain
3.4	*Section deleted* ~~**3.4 Corporate Idetification**~~ ~~TV Takeda Vaccines, Inc.~~
4.1	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~~***and*** DENV-4).
4.1	A first ~~tetravalent~~***recombinant*** dengue vaccine (chimeric yellow fever virus dengue virus-tetravalent dengue vaccine [CYD-TDV]) was approved in some countries in Asia and Latin America in 2015, in Europe in 2018, and in the United States in 2019 [10]. Initial findings showed that vaccine efficacy was different between serotypes and depended on dengue pre-exposure status [11]. Further analyses showed that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease if they were infected after vaccination with CYD-TDV [12]. ***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 vaccines.
4.1	***Takeda's Dengue*** **Tetravalent ~~Dengue~~ Vaccine ~~Candidate~~ *(Live, Attenuated)* (TDV) - Background:** Takeda's TDV consists of 1 molecularly characterized, attenuated dengue serotype 2 virus strain and 3 recombinant ~~chimeric~~ dengue virus strains expressing surface antigens corresponding to dengue serotypes ~~1–4~~ ***1, 3, and 4***.
4.1	**Takeda's Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) - Background:...** The recombinant ~~chimeric~~, attenuated vaccine strains for dengue serotypes 1, 3 and 4 were engineered by substituting the structural genes, pre-membrane (prM) and envelope (E), of TDV-2 with the prM and E genes from the DENV virus strains, DENV-1 16007, DENV-3 16562 or DENV-4 1036, respectively [14]. Thus, Takeda's TDV is comprised of 4 dengue virus strains: ***TDV-2 (***a molecularly characterized attenuated ~~dengue serotype 2~~ strain***)*** ~~(TDV-2)~~, a dengue serotypes 2/1 recombinant ~~chimeric~~ strain (TDV-1), a dengue serotypes 2/3 recombinant ~~chimeric~~ strain (TDV-3), and a dengue serotypes 2/4 recombinant ~~chimeric~~ strain (TDV-4).

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4.1	**Takeda's Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) - Background:...** ~~Nonclinical studies carried out in mice and nonhuman primates have demonstrated an acceptable safety, immunogenicity, and efficacy profile for Takeda's TDV. Additionally, d~~***D***ata 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. ~~Ongoing and c~~***C***ompleted phase 2 clinical trials have enabled the selection of a final TDV dose (in a lyophilized formulation) and a ~~two~~***2***-dose vaccination series administered 3 months (ie, 90 days) apart by subcutaneous (SC) injection for use in the ongoing ~~pivotal~~***clinical development*** program. ~~In January 2019,~~***Results from*** the pivotal DEN-301 efficacy trial ***showed that the*** ~~met its~~ 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 >20,000 clinical trial subjects.*** All available data also showed that TDV was well tolerated with no significant safety concerns to date [~~15~~***16***].
4.2	Subjects previously enrolled in two parent trials (DEN-304 and DEN-315) will initially be invited to participate in the DEN-303 follow-up trial from 21 months after the first vaccination in the primary vaccination series in the parent trials. ~~The inclusion of subjects from other trials within the TDV program may also be considered if they meet the eligibility criteria for entry into DEN-303.~~ Antibody persistence will be assessed in all subjects for up to ~~45~~***63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).
5.1.1	• To describe antibody persistence for each of the 4 dengue serotypes for up to ~~45~~***63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).
5.1.2	<u>Antibody Persistence</u> • To describe the overall trend in antibody decay for all 4 dengue serotypes from values obtained after the primary vaccination series in the parent trials through ~~45~~***63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and through 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). <u>Impact of a TDV Booster Dose</u> • To describe the impact of a TDV booster on antibody response for each of the 4 dengue serotypes for up to ~~51~~***69*** months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 42 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). *Safety* • To describe the long-term safety of Takeda's TDV for up to ~~45~~***63*** months in previously vaccinated subjects from parent trial DEN-315 (Mexico) and for up to 36 months in previously vaccinated subjects from parent trial DEN-304 (United States).
5.1.3	***Applicable to subjects from parent trial DEN-315 (Mexico only):*** • To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at ~~36~~***63*** months after the first vaccination in the primary vaccination series in the parent trial ***(DEN-315)***~~s and in the cell-mediated immunity (CMI) subset (applicable to~~

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	~~subjects from parent trial DEN-304 [United States] only) at 1 month and 6 months post booster in the current trial~~; this is inclusive of, but not restricted to, an assessment of the anti-dengue Non-Structural protein 1 (NS1) antibody response. Applicable to subjects from parent trial DEN-304 (United States only): • ***To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304), and in the cell-mediated immunity (CMI) subset at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, an assessment of the anti-dengue NS1 antibody response.*** • To evaluate aspects of the long-term cell-mediated immune response to Takeda's TDV up to 36 months after the first vaccination in the primary vaccination series in the parent trial ***(DEN-304)*** and at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) of the long-term T cell-mediated immune response to TDV (CMI subset only).
5.2	***Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.***
5.2.1	• Geometric mean titers (GMTs) of neutralizing antibodies (by microneutralization test 50% [MNT_{50}]) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 1~~ (Day 1 [M0])~~, Visit 2 ~~(Day 360 [M12])~~, ***and*** Visit 3~~A (Day 450 [M15]), and Visit 3B (Day 720 [M24]~~; ***(***prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) ~~for subjects from parent trial DEN-315 (Mexico) and at Visit 1 (Day 1 [M0]), Visit 2 (Day 360 [M12]), and Visit 3 (Day 450 [M15]; prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) for subjects from parent trial DEN-304 (United States),~~ summarized for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials. • GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 4 ~~(Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~ and Visit 5 ~~(Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States]),~~ for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials. ~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and~~

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	~~Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months, and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~
5.2.2	**Immunogenicity Endpoints:** *Antibody Persistence* • Geometric Mean Ratio (GMR) of neutralizing antibodies for each of the 4 dengue serotypes for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials for: • Visit 1 ~~(Day 1 [M0])~~ vs Visit 2 ~~(Day 360 [M12]), for all subjects~~. • Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ in the current trial. • Day 270 (Month 9) in the parent trials (9 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 1 ~~(Day 1 [M0])~~ and Visit 2 ~~(Day 360 [M12])~~ in the current trial~~, for all subjects~~.
5.2.2	*Impact of a TDV Booster Dose...* • Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 4 in the current trial ~~(Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~. • Visit 3~~B (Day 720 [M24])~~ vs Visit 4 ~~(Day 750 [M25]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) vs Visit 4 (Day 480 [M16]) for subjects from parent trial DEN-304 (United States)~~. • Visit 3~~B (Day 720 [M24])~~ vs Visit 5 ~~(Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) vs Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~. • Visit 4 (~~Day 750 [M25])~~ vs Visit 5 ~~(Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/Visit 4 (Day 480 [M16]) vs Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~. • Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 5 in the current trial ~~(Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States])~~.
5.2.2	**Safety Endpoints:** • Frequency and severity of solicited local (injection site) reactions for 7 days (day of vaccination + 6 days), and solicited systemic AEs for 14 days (day of vaccination + 13 days) following administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ by trial group.

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	• Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 days) following administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ by trial group. • Percentage of subjects with any medically attended adverse events (MAAEs) following administration of the TDV booster or placebo from Visit 3~~B (Day 720 [M24])~~ through Visit 5 ~~(Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/from Visit 3 (Day 450 [M15]) through Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States),~~ by trial group. • Percentage of subjects with any serious adverse events (SAEs) from Visit 1 ~~(Day 1 [M0])~~ through Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ prior to administration of the TDV booster or placebo. • Percentage of subjects with any SAEs ***following administration of the TDV booster or placebo from Visit 3 through Visit 5 by trial group*** ~~from Visit 3B (Day 720 [M24]) after administration of the TDV booster or placebo through Visit 5 (Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/from Visit 3 (Day 450 [M15]), after administration of the TDV booster or placebo through Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States), by trial group~~. ~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months, and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~
5.2.3	***Applicable to subjects from parent trial DEN-315 (Mexico only):*** • The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3~~A/3 (Day 450 [M15]) and from subjects in the CMI subset (applicable to subjects from parent trial DEN-304 [United States] only) at Visit 4 (Day 480 [M16]) and Visit 5 (Day 630 [M21])~~. Additional exploratory techniques may be added as the field evolves. Applicable to subjects from parent trial DEN-304 (United States only): • ***The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples***

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	collected from all subjects at Visit 3, and from subjects in the CMI subset at Visit 4 and Visit 5. Additional exploratory techniques may be added as the field evolves. • The assessment of the long-term cell-mediated response to TDV will include, but is not restricted to, the frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 PBMC) of IFN-γ ELISpot responses to TDV using blood samples collected from subjects in the CMI subset at Visit 1~~ (Day 1 [M0])~~, Visit 2~~ (Day 360 [M12])~~, Visit 3~~ (Day 450 [M15])~~, Visit 4~~ (Day 480 [M16])~~, and Visit 5~~ (Day 630 [M21])~~. Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥ 50 spots per 10^6 PBMC. Additional exploratory techniques may be added as the field evolves (CMI subset only). ~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months, and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~
6.1	~~The inclusion of subjects from other trials within the TDV program may be considered if they meet the eligibility criteria for entry into DEN-303.~~ DEN-303 will include up to 600 healthy subjects aged ≥13 to ≤63 years at trial entry. To enable the assessment of a booster dose, the trial will be double-blinded, randomized, and placebo-controlled from Visit 3~~B (Day 720 [M24])~~ onwards~~ for subjects from parent trial DEN-315 (Mexico) and from Visit 3 (Day 450 [M15]) onwards for subjects from parent trial DEN-304 (United States)~~. Antibody persistence and safety will be assessed from Visit 1 ~~(Day 1 [M0])~~ through Visit 3~~B (Day 720 [M24]) for up to 45~~***; up to 63*** months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and ~~from Visit 1 (Day 1 [M0]) through Visit 3 (Day 450 [M15]) for~~ up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). Further characterization of the long-term humoral and cell-mediated immune responses to Takeda's TDV will be undertaken in a subset of approximately 50 volunteers identified at enrollment (CMI subset: participation is on a voluntary basis from DEN-304 only) up to Visit 5~~ (Day 630 [M21])~~. A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) between site visits and to remind the subject/the subject's LAR of any upcoming site visits. At ~~Visit 3A (Day 450 [M15]) for subjects from parent trial DEN-315 (Mexico)~~/Visit 2 ~~(Day 360 [M12]) for subjects from parent trial DEN-304 (United States)~~ the site will discuss any information that is pertinent to the booster phase of the trial with the subject. ***A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18).*** ~~An additional retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visit 3A and Visit 3B on Day 630 (M21) to maintain contact with the subject/the subject's LAR between site visits, and to remind the subject/the subject's LAR of the booster eligibility criteria and the upcoming site visit. Female subjects of childbearing age~~

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	~~will also be reminded of pregnancy avoidance guidance and acceptable methods of contraception during this call.~~ At Visit 3~~B (Day 1260 [M42]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~, following all scheduled blood draws, all subjects will be screened for 'booster eligibility' to determine if they are eligible to go on to receive the TDV booster in the booster phase. Any subject who fails to meet the criteria for 'booster eligibility' will end the trial at Visit 3~~B (Day 1260 [M42]) or Visit 3 (Day 450 [M15]), respectively~~. All eligible subjects will be randomized~~,~~ using an interactive response technology (IRT) at Visit 3~~B (Day 1260 [M42])/Visit 3 (Day 450 [M15]), as applicable,~~ to 1 of 2 trial groups (Group 1 and Group 2) in a 1:1 ratio stratified by parent trial and serostatus at baseline in the parent trials.
6.1	Immunogenicity evaluation: • Neutralizing antibodies (by MNT_{50}) will be measured using blood samples collected from all subjects at Visit 1, Visit 2, and Visit 3~~A/3, at Visit 3B for subjects from parent trial DEN-315 (Mexico)~~, and ~~also~~ at Visit 4 and Visit 5 for subjects who are randomized to Groups 1 and 2. • At Visit 3~~A/3~~, a larger volume of blood will be collected from all subjects to assess exploratory markers of the long-term humoral immune response to Takeda's TDV.
6.1	Safety evaluation: • Diary cards will be distributed to Groups 1 and 2 at Visit 3~~B for subjects from parent trial DEN-315 (Mexico)/Visit 3 for subjects from parent trial DEN-304 (United States)~~ for the recording of:
6.1	Safety evaluation:... • MAAEs will be collected, for Groups 1 and 2, following administration of the TDV booster or placebo from Visit 3~~B~~ through Visit 5~~ for subjects from parent trial DEN-315 (Mexico)/from Visit 3 through Visit 5 for subjects from parent trial DEN-304 (United States)~~. 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.
6.1	Summary: For subjects from parent trial DEN-315 (Mexico), the duration of the current trial will be ~~24~~***42*** months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3~~B~~ (inclusive of ***3***~~-4~~ site visits~~, and~~ 3 blood draws) and ~~30~~***48*** months for all other subjects (***inclusive of 5***~~6~~ site visits~~, and 56~~ blood draws). For subjects from parent trial DEN-304 (United States), the trial duration will be 15 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 (inclusive of 3 site visits, 3 blood draws) and 21 months for all other subjects (inclusive of 5 site visits,~~-4~~ ***5*** blood draws).

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6.2	~~Ongoing and c~~*C*ompleted phase 2 trials have enabled the selection of the final TDV formulation, administered by SC injection, that is used in this study and has been taken forward in Takeda's pivotal dengue program. The current version of the IB provides additional information and a more detailed review of non-clinical studies and clinical trials. In order to maintain the double-blind design, following administration of the TDV booster to Group 1 at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~, a placebo (saline solution for injection) will be administered to Group 2. Justification of the sample size (up to 300 subjects per Group) is included in Section 13.3 and the rationale for the proposed trial is given in Section 4.2. The timing of the primary and secondary endpoints assessing antibody persistence aims to provide data on persistence of the immune response for ~~≥3~~***>2.5*** years (~~3~~***2.75*** years for subjects from parent trial DEN-304 and ~~3.75~~***5*** years for subjects from parent trial DEN-315) following completion of the primary vaccination series.
6.3	For subjects from parent trial DEN-315 (Mexico), the duration of the current trial will be ~~24~~***42*** months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3~~B (Day 720 [M24]~~) and ~~30~~***48*** months for all other subjects (inclusive of booster administration at Visit 3~~B [Day 720 (M24)~~] and follow-up through Visit 5~~ [Day 900 (M30)]~~). For subjects from parent trial DEN-304 (United States), the trial duration will be 15 months for those subjects who fail to meet the criteria for 'booster eligibility' at Visit 3~~ (Day 450 [M15])~~ and 21 months for all other subjects (inclusive of booster administration at Visit 3 ~~[Day 450 (M15)]~~ and follow-up through Visit 5 ~~[Day 630 (M21)]~~).
7.2	**Exclusion Criteria...** 4. Subjects with a prolonged period of habitation (≥1 year) in a dengue endemic area within the 2 years prior to Visit 1~~ (Day 1 [M0])~~.
7.3	**Booster Eligibility** Eligibility, including test results, must ~~to~~ be confirmed ***at Visit 3*** prior to randomization. ~~Assessed at Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico) and Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States).~~ Any subject who meets any of the following criteria ***at Visit 3*** will not qualify for randomization to Group 1 or 2 to receive the TDV booster or placebo, respectively:
7.3	**Booster Eligibility...** 5. Known or suspected impairment/alteration of immune function, including: a) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~; use of inhaled, intranasal, or topical corticosteroids is allowed. b) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. c) Administration of immunoglobulins and/or any blood products within the 3 months prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~; consider whether applicable as an

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	exclusion criterion or criterion for delay. d) Receipt of immunostimulants within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. e) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~.
7.3	13. Female subjects of child-bearing potential who are sexually active with men, and who have not used any of the acceptable contraceptive methods for at least 2 months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. a) Of "childbearing potential" is defined as status post-onset of menarche and not meeting any of the following conditions: menopausal for at least 2 years, status after bilateral tubal ligation for at least 1 year, status after bilateral oophorectomy, or status after hysterectomy. b) "Acceptable birth control methods" are defined as one or more of the following: I. Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring). II. Barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse. III. Intrauterine device (IUD). IV. Monogamous relationship with ***a*** vasectomized partner. Partner must have been vasectomized for at least ~~six~~*6* months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. 14. Female subjects of childbearing potential who are sexually active, and who refuse to use an "acceptable contraceptive method" for up to 6 weeks after administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. In addition, they must be advised not to donate ova or breastfeed during this period.
8.1	The investigational vaccine is Takeda's TDV, a tetravalent vaccine comprised of 1 molecularly characterized, attenuated dengue virus strain (TDV-2), and 3 recombinant ~~chimeric~~ 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 per dose of TDV-1, TDV-2, TDV-3, and TDV-4, respectively.
8.1.1	The sponsor will supply study sites with TDV, TDV diluent, and placebo packaged into single dose dispensing cartons. The cartons will be labeled in a***n un***blinded fashion ~~that~~***and*** will contain pertinent trial information and caution statements in local languages. ~~TDV or placebo will be dispensed in a blinded manner by IRT.~~ ***Receiving, storage, accountability, and dispensing of unblinded trial vaccines should only be performed by unblinded personnel (see Sections 8.2 and 8.4) to ensure that the trial blind is not broken***. Further details can be found in the Pharmacy Manual.

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8.1.3	***The*** ~~T~~trial vaccine doses that will be provided to each trial group at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ are presented in Table 8.a.
8.1.3	**Table 8.a Sponsor-Supplied Vaccines and Placebo** **Group** / **Description** / **Timing** Group 1 ~~(parent trial DEN-315 [Mexico])~~ / TDV, SC / Visit 3[a]~~B (Day 720 [M24])~~ Group 2 ~~(parent trial DEN-315 [Mexico])~~ / Placebo, SC / Visit 3[a]~~B (Day 720 [M24])~~ ~~Group 1 (parent trial DEN-304 [United States])~~ / ~~TDV, SC~~ / ~~Visit 3 (Day 450 [M15])~~ ~~Group 2 (parent trial DEN-304 [United States])~~ / ~~Placebo, SC~~ / ~~Visit 3 (Day 450 [M15])~~ M: Month; SC: subcutaneous. [a] ***Day 1260 (M42) for subjects from parent trial DEN-315 (Mexico) and Day 450 (M15) for subjects from parent trial DEN-304 (United States).***
8.2	The designee will use IRT again at randomization ~~(Visit 3B [Day 720 (M24)] for subjects from parent trial DEN-315 [Mexico]/Visit 3 [Day 450 (M15)] for subjects from parent trial DEN-304 [United States])~~ on the day of TDV booster or placebo administration ***(Visit 3)*** to provide the vaccination identification number for the vaccine dose. ***The trial vaccine (TDV booster/placebo) will be administered only by unblinded personnel who are qualified to perform that function under applicable laws and regulations for that specific trial.*** The ***blinded*** investigator or designee will be responsible for overseeing the administration of the trial vaccine (TDV booster/placebo) to subjects enrolled in the trial according to the procedures stipulated in this trial protocol. ~~The trial vaccine (TDV booster/placebo) will be administered only by unblinded personnel who are qualified to perform that function under applicable laws and regulations for that specific trial.~~
8.2.1	Prior to TDV booster or placebo vaccination, a subject must be determined to be eligible and it must be deemed clinically appropriate in the judgment of the investigator to administer the booster dose. Eligibility for trial entry is evaluated according to the inclusion and exclusion criteria for entry outlined in this protocol (Section 7.1 and Section 7.2). Prior to TDV booster or placebo administration at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~, site staff must determine if the subject is eligible to receive the TDV booster dose by evaluating the criteria for 'booster eligibility' outlined in Section 7.3. Standard immunization practices are to be observed and care should be taken to administer the injection by the SC route. In addition, WHO recommendations to reduce anxiety and pain at the time of vaccination should be followed [~~16~~***17***].
8.4	The trial will be conducted in a double-blind manner from Visit 3~~B (Day 720 [M24]) onwards for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) onwards for subjects from parent trial DEN-304 (United States)~~, meaning that from this point onwards the subject, those responsible for the evaluation of any trial endpoint, and the sponsor will all be unaware of whether the TDV booster or placebo was administered.
8.5	The trial vaccine blind, which will be in place from randomization at Visit 3~~ (Day 450 [M15])~~, shall not be broken by the investigator unless information concerning the TDV booster or placebo is necessary for the medical treatment of a subject, or in cases of pregnancy if a trial subject requests it.
9.0	~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons,~~

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	~~administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~ ***Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.***
9.1.1	A subset of 50 volunteers (DEN-304 only) will be assigned to the CMI subset at trial entry to permit a more detailed characterization of the long-term humoral and cell-mediated immune response to Takeda's TDV ~~(~~up to Visit 5~~:~~ ~~[Day 900 (M30)] for subjects from parent trial DEN-315 [Mexico] and [Day 630 (M21)] for subjects from parent trial DEN-304 [United States]~~). Due to changes in the trial design ***in Mexico*** (~~protocol amendment 1, dated 09 March 2020 and protocol amendment 2, dated 22 February 2021~~***protocol amendment 3, dated 22 August 2022***), all subjects ***from parent trial DEN-315 (Mexico)*** will now be asked to re-consent using a***n*** updated informed consent form~~(s)~~ ***(ICF)*** or ***an*** updated informed consent and pediatric assent form~~s~~***, as applicable,*** at ~~Visit 2 (Day 360 [M12])~~***Visit 3*** ~~or at the next site visit if Visit 2 has passed~~ before any further protocol-directed procedures are performed.
9.1.2	Demographic information to be obtained at Visit 1 ~~(Day 1 [M0])~~ will include age/date of birth, sex, race, and ethnicity as described by the subject or subject's LAR. A medical history update will be collected at enrollment (Visit 1~~ [Day 1 (M0)]~~) and after randomization in subjects that are eligible for TDV booster or placebo ***administration*** at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~.
9.1.2	Medical history (including corresponding medication) to be obtained at Visit 1~~ (Day 1 [M0])~~ will include any significant conditions or diseases that have disappeared or resolved at or prior to signing of informed consent or pediatric assent form. Medical history update (including corresponding medication) to be obtained prior to booster administration at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ will include any significant conditions or diseases that have disappeared or resolved between Visit 1 ~~(Day 1 [M0])~~ and Visit 3~~B (Day 720 [M24]/Visit 3 (Day 450 [M15]), as applicable~~.

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9.1.2	All medications, vaccines and blood products taken or received by the subjects are to be assessed and collected as Prior or Concomitant Medications and recorded in the subject's source document and eCRF as follows: a) Medications: from 1 month (minimum 28 days) prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ and up to 1 month (minimum 28 days) thereafter. b) Vaccines: from 1 month (minimum 28 days) prior to any blood sample collection or administration of the TDV booster/placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ and up to 1 month (minimum 28 days) thereafter. c) Blood products and immunoglobulins: within 3 months prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. d) Steroids and immunostimulants: within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. e) Immunosuppressive therapies: within 6 months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~.
9.1.2	Prohibited therapies (Refer to Sections 7.2 and 7.3): a) Previous and planned vaccination (during the trial conduct), against any flavivirus including dengue (other than Takeda's TDV), YF, JE viruses or tick-borne encephalitis. b) Any other vaccines within the 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to any blood sample collection or TDV booster/placebo administration at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. c) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~; use of inhaled, intranasal, or topical corticosteroids is allowed. d) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. e) Administration of immunoglobulins and/or any blood products within the 3 months prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. f) Receipt of immunostimulants within 60 days prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~.

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	g) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~.
9.1.3	Only subjects who meet the criteria for 'booster eligibility' at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ are eligible for randomization into the booster phase.
9.1.3	If the subject is ineligible for randomization at Visit 3,~~B (Day 720 [M24])/Visit 3 (Day 450 [M15]), as applicable,~~ they will end the trial at this point and the investigator should record the primary reason for non-randomization in the subject's source documents and eCRF.
9.1.4	A complete physical exam will be performed in all subjects at Visit 1 ~~(Day 1 [M0])~~ and in subjects randomized to Groups 1 and 2 at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ according to the investigator's standard practice.
9.1.4	Subjects randomized to Groups 1 and 2 will also undergo a targeted symptom-directed physical examination at Visit 4 ~~(Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~ and Visit 5~~ (Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States])~~. Any clinically significant changes from the baseline examination performed at Visit 1 ~~(Day 1 [M0])~~ should be recorded in the subject's source documents and eCRF.
9.1.5	~~For subjects from parent trial DEN-315 (Mexico), vital signs will be measured in all subjects at Visit 1 (Day 1 [M0]), Visit 2 (Day 360 [M12]), Visit 3A (Day 450 [M15]), and Visit 3B (Day 720 [M24]), and in those subjects randomized to Groups 1 and 2 at Visit 4 (Day 750 [M25]) and Visit 5 (Day 900 [M30]). For subjects from parent trial DEN-304 (United States),~~ ~~v~~*Vital* signs will be measured in all subjects at Visit 1~~ (Day 1 [M0])~~, Visit 2~~ (Day 360 [M12])~~, and Visit 3~~ (Day 450 [M15])~~, and in those subjects randomized to Groups 1 and 2 at Visit 4 ~~(Day 480 [M16]~~ and Visit 5~~ (Day 630 [M21])~~. These will include (but are not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature.
9.1.6	~~For subjects from parent trial DEN-315 (Mexico), b~~***B***lood samples for immunogenicity assessments will be collected from all subjects (10 mL) at Visit 1~~ (Day 1 [M0])~~, Visit 2~~ (Day 360 [M12])~~, ~~Visit 3A (Day 450 [M15],~~ and Visit 3~~B (Day 720 [M24])~~, and from those subjects randomized to Groups 1 and 2 at Visit 4 ~~(Day 750 [M25])~~ and Visit 5~~ (Day 900 [M30]). For subjects from parent trial DEN-304 (United States), blood samples for immunogenicity assessments will be collected from all subjects (10 mL) at Visit 1 (Day 1 [M0]), Visit 2 (Day 360 [M12]), and Visit 3 (Day 450 [M15] and from those subjects randomized to Groups 1 and 2 at Visit 4 (Day 480 [M16]) and Visit 5 (Day 630 [M21]).~~ A larger blood sample (40 mL) will also be collected from all subjects to assess exploratory markers of the long-term humoral immune response to Takeda's TDV at Visit 3~~A/3 (Day 450 [M15]),~~ prior to TDV booster or placebo administration for subjects randomized to Groups 1 and 2. Applicable only to subjects from parent trial DEN-304 [United States]: Additional blood samples (***40***-50 mL) will be collected from subjects in the CMI subset to allow further characterization of the long-term humoral and cell-mediated immune responses to Takeda's TDV at Visit 1~~ (Day 1 [M0])~~, Visit 2 ~~(Day 360 [M12])~~ and Visit 3, ~~(Day 450 [M15])~~ and in subjects randomized to Groups 1 and 2 at Visit 4~~ (Day 480 [M16])~~ and Visit 5~~ (Day 630 [M21])~~.

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	~~For subjects from parent trial DEN-315 (Mexico), the maximum volume of blood taken at any single visit is approximately 50 mL. The total volume of blood collected for the trial duration is approximately 80-100 mL.~~ ~~For subjects from parent trial DEN-304 (United States), t~~*T*he maximum volume of blood taken at any single visit is approximately 90 mL for subjects in the CMI subset and approximately 50 mL for all other subjects. The total volume of blood collected for the trial duration is approximately 190-310 mL for subjects in the CMI subset and approximately 70-90 mL for all other subjects.
9.1.8	Safety assessments will include the collection and recording of solicited local (injection site) and systemic AEs, unsolicited AEs, AEs (serious and non-serious), pregnancies, and MAAEs from Visit 3~~B (Day 720 [M24])~~ onwards for subjects ~~from parent trial DEN-315 (Mexico)/from Visit 3 (Day 450 [M15]) onwards for subjects from parent trial DEN-304 (United States)~~ randomized to Groups 1 and 2 following administration of the booster dose or placebo, respectively.
9.1.9	For female subjects of childbearing potential, pregnancy testing (urine) will be performed prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. Results must be confirmed and documented as negative prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24])/Visit 3 (Day 450 [M15]), as applicable~~.
9.1.9	Female subjects of child-bearing potential who are sexually active with men, are advised to use an acceptable contraceptive method for at least 2 months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~. 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 at Visit 1 ~~(Day 1 [M0])~~ stating that they understand the requirements for avoidance of pregnancy and donation of ova. Further guidance with respect to the avoidance of pregnancy will be provided to all subjects of childbearing potential at ~~Visit 3A (Day 450 [M15]) for subjects from parent trial DEN-315 (Mexico)/~~Visit 2*,* ~~(Day 360 [M12]) for subjects from parent trial DEN-304 (United States)~~ and to all subjects of childbearing potential randomized to Group 1 or 2 at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ as part of the trial procedures (Section 2.1). Females of childbearing potential~~,~~ who are randomized to Group 1 or 2 and are sexually active, must also be reminded at Visit 3~~B (Day 720 [M24])/Visit 3 (Day 450 [M15])~~ and at Visit 4 ~~(Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~ to adhere to acceptable contraceptive methods for up to 6 weeks after TDV booster or placebo administration; they will also be advised not to donate ova or breastfeed during this period.
9.1.9	(d) Monogamous relationship with ***a*** vasectomized partner. Partner must have been vasectomized for at least ~~six~~***6*** months prior to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~.
9.1.11	**9.1.11 Documentation of Subjects Who Are Not Considered Eligible for Trial Entry (Visit 1 ~~[Day 1 (M0)]~~) or Randomization (Visit 3~~B [Day 720 (M24)] for Subjects from Parent Trial DEN-315 [Mexico]/Visit 3 [Day 450 (M15)] for Subjects from Parent Trial DEN-304 [United States]~~)** Investigators must account for all subjects who sign an informed consent or who have a signed pediatric assent form. If the subject is not eligible for trial entry at Visit 1 ~~(Day 1 [M0])~~ or for randomization to receive the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects~~

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	~~from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~, the investigator should complete the eCRF accordingly. The primary reason for denial of trial entry (at Visit 1~~ [Day 1 (M0)]~~) or non-randomization (at Visit 3~~B [Day 720 (M24)]/Visit 3 [Day 450 (M15)]~~) is ***to be*** recorded in the eCRF using the following categories:
9.3.1	**9.3.1 Site Visits Prior to TDV Booster or Placebo Administration (Visit 1~~ [Day 1 (M0)]~~ and Visit 2~~ [Day 360 (M12)] – All Subjects, and Visit 3A [Day 450 (M15)] – Applicable to Subjects from Parent Trial DEN-315 [Mexico] Only~~)** Site visits that occur prior to administration of the TDV booster or placebo and do not include a vaccination will be performed at Visit 1 ~~(Day 1 [M0])~~ and Visit 2~~ (Day 360 [M12])~~. Additional procedures to be performed at Visit 1~~ (Day 1 [M0])~~:
9.3.1	Procedures to be performed at Visit 1 ~~(Day 1 [M0])~~ and Visit 2 ~~(Day 360 [M12])~~ include: 1. Before performing any trial procedure, an ~~informed consent form~~***ICF*** or informed consent and pediatric assent form must be signed. Refer to Section 9.1.1.
9.3.1	~~Additional processes to be completed at Visit 2 (Day 360 [M12]):~~ ~~1. Before performing any further protocol-directed procedures, all sites should ask subjects to re-consent using the updated informed consent form(s) or updated informed consent and pediatric assent forms corresponding to protocol amendment 1, dated 09 March 2020 and protocol amendment 2, dated 22 February 2021. An oral summary of any major changes that have been made to the study should be provided to the subject and the subject's LAR where applicable in addition to the ICF/Assent Form. Upon completion, this process should be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the electronic Case Report Form (eCRF). If Visit 2 has passed, re-consent will occur at the next site Visit. Refer to Section 9.1.1.~~ ~~Procedures to be performed at Visit 3A (Day 450 [M15]) – subjects from parent trial DEN-315 (Mexico) only, include:~~ ~~1. Before performing any further protocol-directed procedures, all sites should check that the subject has re-consented using the updated informed consent form(s) or updated informed consent and pediatric assent forms corresponding to protocol amendment 1, dated 09 March 2020 and protocol amendment 2, dated 22 February 2021. Refer to Section 9.1.1 for details.~~ ~~2. Concomitant vaccination update.~~ ~~3. Check vital signs. Refer to Section 9.1.5.~~ ~~4. Collect and record any SAEs and any AEs leading subject discontinuation or withdrawal. Refer to Sections 10.4.4 and 10.4.1.~~ ~~5. Check that the subject does not meet any of the criteria for delay of blood sampling. Refer to Section 7.4.~~ ~~6. Collect a blood sample from all subjects (one additional 40 mL blood sample should be also collected from subjects in the CMI subset). Blood should be taken from subjects using an aseptic venipuncture technique for serological immunogenicity testing. Refer to Sections 9.1.6 and 9.1.7.~~
9.3.2	**9.3.2 Pre-Vaccination Procedures (Visit 3~~B [Day 720 (M24)] for Subjects from Parent Trial DEN-315 [Mexico]/Visit 3 [Day 450 (M15)] for Subjects from Parent Trial DEN-304 [United States]~~)** Before performing any further protocol-directed procedures, all sites ***in Mexico*** should check that the subject has re-consented using the updated ~~informed consent form(s)~~***ICF*** or ***an*** updated

Section	Description of Change
	informed consent and pediatric assent form~~s~~, ***as applicable,*** corresponding to ~~protocol amendment 1, dated 09 March 2020 and protocol amendment 2, dated 22 February 2021~~***protocol amendment 3, dated 22 August 2022***. Refer to Section 9.1.1 for details. Prior to administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ the following procedures will be undertaken:
9.3.2	5. Review the criteria for 'booster eligibility'. Refer to Section 7.3. a. Any subject who fails to meet the criteria for 'booster eligibility' will end the trial following blood sample collection(s) at Visit 3~~B (Day 720 [M24])/Visit 3 (Day 450 [M15]), as applicable~~. Refer to Section 9.3.7.
9.3.3	**9.3.3 Vaccination Procedures (Visit 3~~B [Day 720 (M24)] for Subjects from Parent Trial DEN-315 [Mexico]/Visit 3 [Day 450 (M15)] for Subjects from Parent Trial DEN-304 [United States]~~)** Vaccination procedures will only be performed for subjects who meet the criteria for 'booster eligibility' (Section 7.3) at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ and are randomized to Groups 1 (TDV) and 2 (placebo):
9.3.4	**9.3.4 Post-Vaccination Procedures (Visit 3~~B [Day 720 (M24)] for Subjects from Parent Trial DEN-315 [Mexico]/Visit 3 [Day 450 (M15)] for Subjects from Parent Trial DEN-304 [United States]~~)** The following post-vaccination procedures will be performed at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~:
9.3.4	4. The site should schedule the next trial visit (Visit 4~~: Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States]~~) with the subject or subject's LAR.
9.3.4	The subject or the subject's LAR will receive a written reminder of the next planned trial activity (Visit 4~~: Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States]~~).
9.3.5	**9.3.5 Site Visits After Vaccination (Visit 4~~: Day 750 [M25] for Subjects from Parent Trial DEN-315 [Mexico]/Day 480 [M16] for Subjects from Parent Trial DEN-304 [United States]~~, and Visit 5~~: Day 900 [M30] for Subjects from Parent Trial DEN-315 [Mexico]/Day 630 [M21] for Subjects from Parent Trial DEN-304 [United States]~~)** A site visit will be performed after the administration of the TDV booster or placebo at Visit 4 ~~(Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~. This visit should occur at least 29 days after TDV booster or placebo administration. A site visit will also be performed at Visit 5~~ (Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States])~~. The following procedures will be performed at Visit 4~~ (Day 750 [M25] for subjects from parent trial DEN-315 [Mexico]/Day 480 [M16] for subjects from parent trial DEN-304 [United States])~~:
9.3.5	8. The site should schedule the next site visit or other trial activity with the subject or the subject's LAR (Visit 5~~): Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States]~~.

Section	Description of Change
	The subject or the subject's LAR will receive a written reminder of the next planned trial activity (Visit 5~~: Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States]~~). 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. The following procedures will be performed at Visit 5~~ (Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States])~~:
9.3.6	A~~n additional~~ ***second*** retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on ~~Day 630 (M21)~~***Day 540 (M18)*** ~~to maintain contact with the subject/the subject's LAR between site visits, and to remind the subject/the subject's LAR of the booster eligibility criteria and the upcoming site visit. Female subjects of childbearing age will also be reminded of pregnancy avoidance guidance and acceptable methods of contraception during this call.~~ Telephone contacts will also be made for those subjects who are still under monitoring for safety reporting when a site visit cannot be carried out due to ***exceptional circumstances such as*** the COVID-19 pandemic.
9.3.7	For subjects who fail to meet the criteria for 'booster eligibility' at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~, the final (end of trial) visit will be performed at Visit 3~~B/Visit 3 (Day 720 [M24]/Day 450 [M15]), respectively~~. For those subjects who meet the criteria for 'booster eligibility' and are randomized to Group 1 or Group 2, the final (end of trial) visit will be performed at Visit 5~~ (Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States])~~.
10.0	~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~ ***Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.***

Section	Description of Change
10.1.2	*Footnote:* **Table 10.a Solicited Local (Injection Site) Reactions and Systemic AEs** (a) Fever is defined as body temperature greater than or equal to 38°C (100.4°F) regardless of method taken [~~17~~***18***].
10.1.2	*Footnote:* **Table 10.b Solicited safety parameters** (b) Fever is defined as ***body temperature*** greater than or equal to 38°C (100.4°F) regardless of method taken [~~17~~***18***].
10.4.1	AEs leading to discontinuation ~~(~~from the trial ~~or from the vaccination regimen)~~ are collected throughout the trial and will be summarized up to Visit 3~~B (Day 720 [M24] –~~ ***(***pre-vaccination) ~~for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15] – pre-vaccination) for subjects from parent trial DEN-304 (United States)~~ and separately thereafter (post-vaccination) by trial group up to Visit 5~~ (Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States])~~.
10.4.2	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 the TDV booster or placebo dose at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States),~~ (inclusive of the day of administration), and will be recorded on the "Local and Systemic AEs" eCRF~~,~~ as applicable.
10.4.3	MAAEs will be collected by close monitoring of Groups 1 and 2, following administration of the TDV booster or placebo from Visit 3~~B (Day 720 [M24] for subjects from parent trial DEN-315 [Mexico])/from Visit 3 (Day 450 [M15] for subjects from parent trial DEN-304 [United States])~~ through Visit 5~~ (Day 900 [M30] for subjects from parent trial DEN-315 [Mexico]/Day 630 [M21] for subjects from parent trial DEN-304 [United States]~~.
10.4.4	Collection of SAEs will commence from the time that the subject is enrolled in the trial (Visit 1 ~~[Day 1 (M0)]~~). Routine collection of SAEs will continue until the end of the trial (Visit 3~~B [Day 750 (M25)]~~ for subjects who fail to meet the criteria for 'booster eligibility' and Visit 5 ~~[Day 900 (M30)],~~ for all other subjects ~~from parent trial DEN-315 [Mexico]/Visit 3 [Day 450 (M15)] for subjects who fail to meet the criteria for 'booster eligibility' and Visit 5 [Day 630 (M21)], for all other subjects from parent trial DEN-304 [United States]~~).
12.1	When a site visit cannot be carried out due to ***exceptional circumstances such as*** the COVID-19 pandemic, telephone contacts will be made for subjects who are still under monitoring for safety reporting. Refer also to Section 14.1.
13.0	~~Note that the timing of Visit 1, Visit 2, and Visit 3A/3 will be the same for all subjects (Day 1 [M0], Day 360 [M12], and Day 450 [M15], respectively), but for operational reasons, administration of the booster dose will be delayed until Visit 3B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico). In subjects who receive the TDV booster or placebo, Visit 4 and Visit 5 will occur at 1 month and 6 months post booster, respectively: Day 750 (M25) and Day 900 (M30) for subjects from parent trial DEN-315 (Mexico)/Day 480 (M16) and Day 630 (M21) for subjects from parent trial DEN-304 (United States). For all subjects, Visit 1, Visit 2, and Visit 3A/3 correspond to 21 months, 33 months, and 36 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-315 (Mexico), Visit 3B, Visit 4, and Visit 5 in this trial correspond to 45 months, 46 months and 51 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 4, and Visit 5 in this trial correspond to 37 months and 42 months after the first vaccination in the primary vaccination series, respectively.~~

Section	Description of Change
	Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.
13.1	A statistical analysis plan (SAP) will be prepared and finalized prior to unblinding of subject~~'~~s' trial arm assignment. This document will provide further details regarding the definition of analysis variables and analysis methodology to address all trial objectives. ~~A b~~*B*linded data review*s* will be conducted prior to the unblinding of subjects' trial arm assignment. ~~This~~*These* review*s* will assess the accuracy and completeness of the trial database~~,~~ *and* subject evaluability.
13.1.1	***All Screened: All subjects who agreed to participate in the current trial.*** ***All Screened-Booster: All subjects who agreed to participate in the current trial and who were screened for 'booster eligibility' to determine if they were eligible to go on to be randomized to Group 1 or Group 2.*** ***Randomized Set-Booster: All subjects randomized at Visit 3 regardless of whether they received the trial vaccination in the current trial. Subjects in this set will be summarized according to randomized treatment.***
13.1.1	**Full Analysis Set (FAS):** All subjects who received at least one dose of Takeda's TDV in the parent trials and for whom there is at least one valid follow-up measurement up to Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/up to Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ for immunogenicity assessments in the current trial. **Full Analysis Set-Booster (FAS-B):** All subjects who received at least one dose of Takeda's TDV in the parent trials, the TDV booster or placebo in the current trial, and for whom there is at least one valid follow-up measurement after administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ for immunogenicity assessments in the current trial. **Per Protocol Set (PPS):** All subjects from the FAS who received two doses of Takeda's TDV in the parent trials with no new major protocol violations prior to administration of the booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ that could potentially confound the primary endpoints in the current trial. **Per Protocol Set-Booster (PPS-B):** All subjects from the FAS-B who received two doses of Takeda's TDV in the parent trials with no new major protocol violations after administration of the TDV booster or placebo at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15]) for subjects from parent trial DEN-304 (United States)~~ that could potentially confound the primary endpoints in the current trial.

Section	Description of Change
	The major protocol violation criterion will be defined as part of a data review prior to analysis. The categories of new major protocol violations include: (1) not meeting selected entry criteria, (2) receiving the wrong trial vaccination at Visit 3~~B (Day 720 [M24]) for subjects from parent trial DEN-315 (Mexico)/Visit 3 (Day 450 [M15] for subjects from parent trial DEN-304 (United States); (~~subjects randomized to Groups 1 and 2 only),
13.1.3	For the primary and secondary immunogenicity endpoints (ie, GMTs of neutralizing antibodies and seropositivity rates for each of the 4 dengue serotypes and multiple [2, 3 or 4]), descriptive statistics and 95% CIs will be provided for each applicable visit (Visit 1~~ [Day 1 (M0)]~~, Visit 2 ~~[Day 360 (M12)]~~, ~~Visit 3A [Day 450 (M15)],~~ Visit 3~~B [Day 720 (M24)]~~, Visit 4~~ [Day 750 (M25)]~~, and Visit 5 ~~[Day 900 (M30)]~~ in the current trial ~~for subjects from parent trial DEN-315 [Mexico]/Visit 1 [Day 1 (M0)], Visit 2 [Day 360 (M12)], Visit 3 [Day 450 (M15)], Visit 4 [Day 480 (M16)], and Visit 5 [Day 630 (M21)] in the current trial for subjects from parent trial DEN-304 [United States]~~, and for Day 120 [Month 4] and Day 270 [Month 9] in ~~both~~ ***the*** parent trials [which corresponds to 4 months and 9 months after the first vaccination in the primary vaccination series in the parent trials, respectively]).
13.1.4	*Unsolicited AEs...* AEs leading to trial ~~or vaccine~~ withdrawal will be summarized up to Visit 3~~B (Day 720 [M24] – pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5 (Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/up to Visit 3 (Day 450 [M15] – pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~. Unsolicited AEs will be tabulated at each of the following levels: overall summary (subjects with at least 1 AE), and by SOC and PT. In addition, unsolicited AEs will be summarized as follows: by PT including events with frequency greater than a pre-defined frequency (the percentage will be specified in the ~~Statistical Analysis Plan~~***SAP***); by SOC and PT; by SOC, PT, and severity; and by SOC, PT, and relationship to the trial vaccine (TDV booster or placebo).
13.1.4	*MAAEs* MAAEs will be collected for subjects randomized to Group 1 and Group 2 and will be presented by trial group from Visit 3~~B (Day 720 [M24])~~ through Visit 5~~ (Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/from Visit 3 (Day 450 [M15]) through Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~. MAAEs will be coded using MedDRA and summarized by SOC and PT for each trial group.
13.1.4	*SAEs* SAEs will be collected throughout the trial. SAEs will be coded using MedDRA and summarized by PT and SOC up to Visit 3~~B (Day 720 [M24])~~ ***(pre-vaccination)*** and thereafter (post-vaccination) by trial group up to Visit 5~~ (Day 900 [M30]) for subjects from parent trial DEN-315 (Mexico)/up to Visit 3 (Day 450 [M15] – pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5 (Day 630 [M21]) for subjects from parent trial DEN-304 (United States)~~.
13.2	~~No interim analyses are planned.~~ ***Due to significant delays (of >2 years) to booster administration for subjects from parent trial DEN-315 (Mexico), an interim analysis (IA) of the safety and immunogenicity data collected at trial sites in the United States is planned when all subjects from parent trial DEN-304 (United States) have completed their last trial visit (Visit 5 [Day 630 (M21)]). This IA will be performed in an unblinded manner and will include the necessary steps to ensure that no database modifications are made after unblinding for subjects at trial sites in the United***

Section	Description of Change
	States. Unblinding of subjects from parent trial DEN-315 (Mexico) will occur after all subjects at trial sites in Mexico have completed their last trial visit (Visit 5 [Day 1440 (M48)]) and the trial database has been locked. No modifications to the trial are planned based on the results of this IA. An interim CSR of data from parent trial DEN-304 (United States) will not be prepared; all trial results will be reported in the final CSR. More details regarding the IA will be provided in the SAP.
14.1	In the event a monitor cannot visit the site in a timely manner due to ***exceptional circumstances such as*** the COVID-19 pandemic, alternative monitoring approaches such as remote source data verification or telephone contact may be used to ensure data quality and integrity and to maintain subject safety.
15.4.1	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***/or*** EudraCT, according to local requirements, before trial initiation.
15.4.2	In line with EC Regulation N° 1901/2006 [~~1~~***19***], the sponsor will submit a summary of the results of a pediatric trial within ~~six~~***6*** months of completion and irrespective of whether it is part of a Pediatric Investigational Plan (completed or not yet completed) or not, or whether it is intended for submission later on as part of a variation, extension or new stand-alone marketing authorization application or not.
16.0	5. World Health Organization. Dengue and severe dengue. Fact Sheet. ~~2020~~***2022***. (Available at: http://www.who.int/mediacentre/factsheets/fs117/en/) (accessed ~~22 February 2021~~***29 July 2022***). 7. World Health Organization. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control***, 2nd Edition.*** ~~Geneva~~ 1997. ~~2nd Edition~~. (Available at: ~~http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/~~***https://apps.who.int/iris/handle/10665/41988***) (accessed ~~22 February 2021~~***29 July 2022***). 8. World Health Organization. Dengue guidelines for diagnosis, treatment, prevention and control***: new edition.*** 2009. (Available at: ~~http://www.who.int/tdr/publications/documents/dengue-diagnosis.pdf~~***https://apps.who.int/iris/handle/10665/44188***) (accessed ~~22 February 2021~~***29 July 2022***). 15. ~~Biswal S, Reynales H, Saez-Llorens X, Lopez P, Borja-Tabora C, Kosalaraksa P, et al. Efficacy of a Tetravalent Dengue Vaccine in Healthy Children and Adolescents. N Engl J Med. 2019;381(21):2009-19.~~***Biswal S, Borja-Tabora C, Martinez Vargas L, Velásquez H, Theresa Alera M, Sierra V, et al; TIDES study group. Efficacy of a tetravalent dengue vaccine in healthy children aged 4-16 years: a randomised, placebo-controlled, phase 3 trial. Lancet. 2020 May 2;395(10234):1423-33.*** ***16. Patel SS, Rauscher M, Kudela M, Pang H. Clinical Safety Experience of TAK-003 for Dengue Fever: a new Tetravalent Live Attenuated Vaccine Candidate. Clin Infect Dis. 2022 May 26:ciac418.*** ~~16~~***17***. World Health Organization. Reducing pain at the time of vaccination: WHO position paper - September 2015. Wkly Epidemiol Rec. 2015;90(39):505-10. ~~17~~***18***. Kohl KS, Marcy SM, Blum M, Connell Jones M, Dagan R, Hansen J, et al. Fever after immunization: current concepts and improved future scientific understanding. Clin Infect Dis. 2004;39(3):389-94.

Section	Description of Change
	~~18~~*19*.EC Regulation N° 1901/2006 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 December 2006 on medicinal products for pediatric use and amending Regulation (EEC) No 1768/92, Directive 2001/20/EC, Directive 2001/83/EC and Regulation (EC) No 726/2004.

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2.0 TRIAL SUMMARY

<table>
<tr><td colspan="2">Name of Sponsor(s):
Takeda Vaccines, Inc.
40 Landsdowne Street, Cambridge, MA 02139, USA</td><td>Product Name:
Takeda's Dengue Tetravalent Vaccine (Live, Attenuated) (TDV)</td></tr>
<tr><td colspan="3">Trial Title: A Phase 3, Follow-Up Trial to Evaluate Long-Term Safety and Antibody Persistence, and the Impact of a Booster Dose of a Tetravalent Dengue Vaccine Candidate in Healthy Adolescents and Adults in Areas Non-Endemic for Dengue</td></tr>
<tr><td colspan="2">IND No.: 014292</td><td>EudraCT No.: Not applicable</td></tr>
<tr><td>Trial Identifier: DEN-303</td><td>Phase: 3</td><td>Blinding Schema: Open up to Visit 3 and double-blind thereafter until the end of the trial</td></tr>
<tr><td colspan="3">Indication: Prevention of dengue fever of any severity due to any serotype</td></tr>
<tr><td colspan="3">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, which is more than 3 times the previous World Health Organization (WHO) estimate of 50 to 100 million cases. Every year, around 500,000 cases of dengue hemorrhagic fever (DHF) require hospitalization with an estimated annual death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection.

Dengue fever is clinically defined as an acute febrile illness with 2 or more of the following manifestations: headache, retro-orbital pain, myalgia, arthralgia, rash, hemorrhagic manifestations, or leucopenia, and occurring 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 one of the 4 dengue serotypes is thought to result in life-long protection from re-infection by the same serotype, but it does not protect against a secondary infection by one of the other 3 dengue serotypes and may lead to an increased risk of severe disease (DHF/DSS) upon infection with one of the other 3 dengue serotypes.

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]) was approved in some countries in Asia and Latin America in 2015, in Europe in 2018, and in the United States in 2019. Initial findings showed that vaccine efficacy was different between serotypes and depended on dengue pre-exposure status. Further analyses showed that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease if they were infected 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 vaccines.</td></tr>
</table>

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

Takeda's TDV consists of 1 molecularly characterized, attenuated dengue serotype 2 virus strain and 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 virus 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, attenuated vaccine strains for dengue serotypes 1, 3 and 4 were engineered by substituting the structural genes, pre-membrane (prM) and envelope (E), of TDV-2 with the prM and E genes from the DENV virus strains, DENV-1 16007, DENV-3 16562 or DENV-4 1036, respectively. Thus, Takeda's TDV is comprised of 4 dengue virus strains: TDV-2 (a molecularly characterized attenuated 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 >20,000 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 pre-clinical and clinical trials.

Rationale for the Proposed Trial:

This phase 3 trial will capture long-term antibody persistence and safety data in healthy subjects in areas non-endemic for dengue who have previously received a primary TDV vaccination. It will then go on to assess the immunogenicity and safety of a TDV booster dose in this population.

Subjects previously enrolled in two parent trials (DEN-304 and DEN-315) will initially be invited to participate in the DEN-303 follow-up trial from 21 months after the first vaccination in the primary vaccination series in the parent trials. Antibody persistence will be assessed in all subjects for up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). The impact of a TDV booster versus placebo on the immune response will be assessed at 1 month and 6 months after booster administration in all eligible subjects randomized to receive the TDV booster or placebo (in a 1:1 ratio). Safety assessments will continue for the duration of the trial (up to 6 months post booster administration).

The safety and immunogenicity data collected in this trial will be of importance not only to individuals travelling to endemic regions but also for the significant number of residents with no prior history of dengue infection who live in endemic and semi-endemic areas. The introduction of a TDV booster in a non-endemic setting will begin the assessment of whether a booster is likely to be of benefit in this 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.

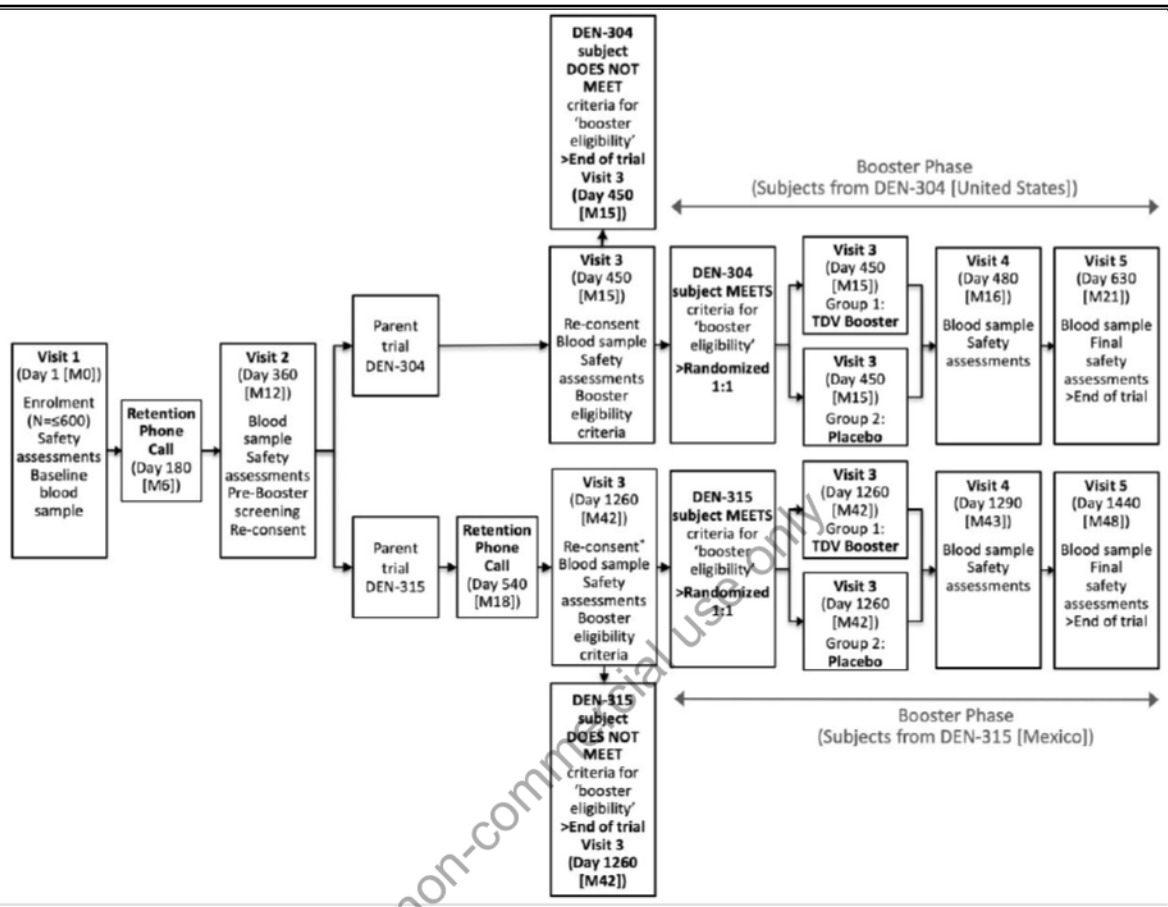
Trial Design:

This is a phase 3 follow-up trial that will evaluate the long-term antibody persistence and safety of Takeda's TDV in healthy adolescents and adults in areas non-endemic for dengue, in addition to assessing the impact of a booster dose in this population. Subjects who previously received TDV in two parent trials, DEN-304 and DEN-315, will be invited to participate in this follow-up trial. DEN-303 will include up to 600 healthy subjects aged ≥13 to ≤63 years at trial entry. To enable the assessment of a booster dose, the trial will be double-blinded, randomized, and placebo-controlled from Visit 3 onwards.

Antibody persistence and safety will be assessed from Visit 1 through Visit 3 for up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). Further characterization of the long-term humoral and cell-mediated immune responses to Takeda's TDV will be undertaken in a subset of approximately 50 volunteers identified at enrollment (cell-mediated immunity [CMI] subset: participation is on a voluntary basis from DEN-304 only) up to Visit 5. A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) between site visits and to remind the subject/the subject's LAR of any upcoming site visits. At Visit 2 the site will discuss any information that is pertinent to the booster phase of the trial with the subject. A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18). Due to changes in the trial design in Mexico (protocol amendment 3, dated 22 August 2022), all subjects from parent trial DEN-315 (Mexico) will be asked to re-consent using an updated informed consent form (ICF) or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the electronic Case Report Form (eCRF).

At Visit 3, following all scheduled blood draws, all subjects will be screened for 'booster eligibility' to determine if they are eligible to go on to receive the TDV booster in the booster phase. Any subject who fails to meet the criteria for 'booster eligibility' will end the trial at Visit 3. All eligible subjects will be randomized, using an interactive response technology at Visit 3 to 1 of 2 trial groups (Group 1 and Group 2) in a 1:1 ratio stratified by parent trial and serostatus at baseline in the parent trials. Subjects allocated to Group 1 will receive the TDV booster (single dose) and subjects allocated to Group 2 will receive placebo. The impact of the TDV booster on neutralizing antibody titers and seropositivity rates will be assessed at 1 month and 6 months after administration of the TDV booster or placebo. Safety assessments will continue for 6 months following TDV booster or placebo administration for subjects in Groups 1 and 2.

For subjects from parent trial DEN-315 (Mexico), the duration of the current trial will be 42 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 and 48 months for all other subjects. For subjects from parent trial DEN-304 (United States), the duration of the current trial will be 15 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 and 21 months for all other subjects. A schematic of the trial design is presented below in Figure 1.



For all subjects, Visit 1 and Visit 2 correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3, Visit 4, and Visit 5 in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3, Visit 4, and Visit 5 in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series, respectively.

* Due to changes in the trial design in Mexico, (Protocol Amendment 3, dated 22 August 2022), all subjects from parent trial DEN-315 (Mexico) will be asked to re-consent using an updated informed consent form (ICF) or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the eCRF.

Immunogenicity Evaluation:

- Neutralizing antibodies (by microneutralization test 50% [MNT_{50}]) will be measured using blood samples collected from all subjects at Visit 1, Visit 2, and Visit 3, and also at Visit 4 and Visit 5 for subjects who are randomized to Groups 1 and 2.
- At Visit 3, a larger volume of blood will be collected from all subjects to assess exploratory markers of the long-term humoral immune response to Takeda's TDV.
- Additional blood samples will be collected from subjects in the CMI subset to allow further characterization of the long-term humoral and cell-mediated immune response to Takeda's TDV at Visit 1, Visit 2, and Visit 3, and in subjects randomized to Groups 1 and 2 at Visit 4 and Visit 5 – applicable to subjects from parent trial DEN-304 (United States only).

Safety Evaluation:

- Diary cards will be distributed to Groups 1 and 2 at Visit 3 for the recording of:
  - Solicited local (injection site) reactions for 7 days (day of vaccination + 6 days) following administration of the TDV booster or placebo. These include: injection site pain, injection site erythema, and injection site swelling.
  - Solicited systemic adverse events (AEs) for 14 days (day of vaccination + 13 days) following administration of the TDV booster or placebo. These include: fever, headache, asthenia, malaise, and myalgia.
- Unsolicited AEs will be collected by interview and recorded for 28 days (day of vaccination + 27 days) following administration of the TDV booster or placebo.
- All serious adverse events (SAEs) and any AEs leading to subject discontinuation and withdrawal will be collected for the trial duration for all subjects.
- Medically attended AEs (MAAEs) will be collected, for Groups 1 and 2, following administration of the TDV booster or placebo from Visit 3 through Visit 5. 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 subjects in Groups 1 and 2 the final safety assessments will be performed approximately 6 months after administration of the TDV booster or placebo at Visit 5.

Data collection will be by electronic Case Report Form (eCRF).

Primary Objectives:

- To describe antibody persistence for each of the 4 dengue serotypes for up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).
- To describe the impact of a TDV booster dose vs placebo on antibody response for each of the 4 dengue serotypes at 1 month and 6 months post administration of the TDV booster or placebo.

Secondary Objectives:

Immunogenicity

Antibody Persistence

- To describe the overall trend in antibody decay for all 4 dengue serotypes from values obtained after the primary vaccination series in the parent trials through 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and through 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).

Impact of a TDV Booster Dose

- To describe the impact of a TDV booster on antibody response for each of the 4 dengue serotypes for up to 69 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 42 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).

Safety

- To describe the long-term safety of Takeda's TDV for up to 63 months in previously vaccinated subjects from parent trial DEN-315 (Mexico) and for up to 36 months in previously vaccinated subjects from parent trial DEN-304 (United States).
- To assess safety for 6 months following administration of the TDV booster or placebo in Groups 1 and 2, respectively.

Exploratory Objectives:

Applicable to subjects from parent trial DEN-315 (Mexico only):

- To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 63 months after the first vaccination in the primary vaccination series in the parent trial (DEN-315); this is inclusive of, but not restricted to, an assessment of the anti-dengue Non-Structural protein 1 (NS1) antibody response.

Applicable to subjects from parent trial DEN-304 (United States only):

- To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304), and in the CMI subset at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, an assessment of the anti-dengue NS1 antibody response.
- To evaluate aspects of the long-term cell-mediated immune response to Takeda's TDV up to 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304) and at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) of the long-term T cell-mediated immune response to TDV (CMI subset only).

Subject Population:

Healthy Subjects: yes

Age Range: ≥13 to ≤63 years

Planned Number of Subjects: up to 600

Planned Number of Trial Arms: A single open arm up to Visit 3 and two arms thereafter through the end of trial, randomized in a 1:1 ratio stratified by parent trial and serostatus at baseline in the parent trials

Inclusion Criteria at Entry:

1. The subject is aged ≥13 and ≤63 years at entry into the current trial.
2. Male or female subjects (irrespective of serostatus at baseline in the parent trials) who received at least one dose of Takeda's TDV in the parent trials and have data from at least one blood draw post-vaccination.
3. Subjects who are in good health at the time of entry into this trial as determined by medical history, physical examination (including vital signs), and the clinical judgment of the investigator.
4. The subject/the subject's LAR signs and dates 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. Assent is obtained from the subject where required.
5. Subjects who can comply with trial procedures and are available for the duration of the follow-up.

Exclusion Criteria at Entry:

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

1. Subjects with 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.
2. Subjects involved in the trial conduct or their first-degree relatives.
3. Subjects that, in the opinion of the investigator, are not medically eligible to provide blood samples.
4. Subjects with a prolonged period of habitation (≥1 year) in a dengue endemic area within the 2 years prior to Visit 1.
5. Previous and planned vaccination (during the trial conduct), against any flavivirus including dengue (other than Takeda's TDV), yellow fever (YF), Japanese encephalitis (JE) viruses or tick-borne encephalitis.
6. Participation in any clinical trial is allowed, on condition that no investigational product is administered within 30 days prior to blood sampling in the current trial.
7. Subjects with any illness, or a history of any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.

Booster Eligibility:

Any subject who meets any of the following criteria at Visit 3 will not qualify for randomization to Group 1 or 2 to receive the TDV booster or placebo, respectively:

1. Subjects for whom baseline serostatus is not defined in the parent trials.
2. Subjects with a known hypersensitivity or allergy to any of the trial vaccine components (including excipients).
3. Subjects with any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (eg, Guillain-Barré syndrome).
4. Subjects with any illness, or a history of any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.
5. Known or suspected impairment/alteration of immune function, including:
    a) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3; use of inhaled, intranasal, or topical corticosteroids is allowed.
    b) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3.
    c) Administration of immunoglobulins and/or any blood products within the 3 months prior to administration of the TDV booster or placebo at Visit 3; consider whether applicable as an exclusion criterion or criterion for delay.
    d) Receipt of immunostimulants within 60 days prior to Visit 3.
    e) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Visit 3.
    f) Known human immunodeficiency virus (HIV) infection or HIV-related disease.
    g) Hepatitis C virus infection.
    h) Genetic immunodeficiency.
6. Abnormalities of splenic or thymic function.
7. Subjects with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
8. Subjects with any serious chronic or progressive disease according to the judgment of the investigator (eg, neoplasm, hematologic malignancies, insulin dependent diabetes, cardiac, renal, or hepatic disease).
9. Subjects with body mass index (BMI) greater than or equal to 35 kg/m^2 (= weight in kg/[height in $meters^2$]).
10. Subjects who have received any other vaccines within the 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to TDV booster or placebo administration.
11. Subjects with a history of substance or alcohol abuse within the past 2 years.

12. Female subjects who are pregnant or breastfeeding.
13. Female subjects of child-bearing potential[1] who are sexually active with men, and who have not used any of the acceptable contraceptive methods[2] for at least 2 months prior to Visit 3.
14. Female subjects of childbearing potential who are sexually active, and who refuse to use an "acceptable contraceptive method" for up to 6 weeks after administration of the TDV booster or placebo at Visit 3. In addition, they must be advised not to donate ova or breastfeed during this period.
15. Any positive or indeterminate pregnancy test.
16. Subjects with history of current or previous infection with a flavivirus such as dengue, Zika, YF, JE, West Nile fever, tick-borne encephalitis or Murray Valley encephalitis and subjects with a prolonged period of habitation (≥1 year) in a dengue endemic area during trial conduct.

There may be instances when subjects meet all the criteria for 'booster eligibility' except one that relates to transient clinical circumstances (eg, body temperature elevation or recent use of excluded medications[s] or vaccine[s]). Under these circumstances, eligibility for the TDV booster/placebo may be considered if the appropriate window for delay has passed, if 'booster eligibility' has been rechecked, and if the subject is confirmed to be eligible.

Trial Vaccine and Placebo:

Investigational vaccine

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

Placebo

The placebo is normal saline for injection (0.9% saline).

Route of administration

SC route

Duration of the Trial and Subject Participation:

The trial duration for each subject will be between 42 months and 48 months for subjects from parent trial DEN-315 (Mexico), and between 15 months and 21 months for subjects from parent trial DEN-304 (United States).

Criteria for Evaluation and Analyses:

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

[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 contraceptives (eg, oral, injection, transdermal patch, implant, cervical ring), barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse, intrauterine device, monogamous relationship with a vasectomized partner (partner must have been vasectomized for at least 6 months prior to Visit 3. Other contraceptive methods may be considered in agreement with the sponsor and must be approved by the appropriate ethics committee.

Primary endpoints:

- Geometric mean titers (GMTs) of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 1, Visit 2, and Visit 3 (prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) summarized for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials.
- GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 4 and Visit 5 for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials.

Secondary endpoints:

Immunogenicity endpoints

Antibody Persistence

- Geometric Mean Ratio (GMR) of neutralizing antibodies for each of the 4 dengue serotypes for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials for:
  - Visit 1 vs Visit 2.
  - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 3 in the current trial.
  - Day 270 (Month 9) in the parent trials (9 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 1 and Visit 2 in the current trial.

Impact of a TDV Booster Dose

- GMR of neutralizing antibodies for each of the 4 dengue serotypes for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials for:
  - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 4 in the current trial.
  - Visit 3 vs Visit 4.
  - Visit 3 vs Visit 5.
  - Visit 4 vs Visit 5.
  - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 5 in the current trial.

Safety endpoints

- Frequency and severity of solicited local (injection site) reactions for 7 days (day of vaccination + 6 days), and solicited systemic AEs for 14 days (day of vaccination + 13 days) following administration of the TDV booster or placebo at Visit 3 by trial group.
- Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 days) following administration of the TDV booster or placebo at Visit 3 by trial group.
- Percentage of subjects with any MAAEs following administration of the TDV booster or placebo from Visit 3 through Visit 5 by trial group.
- Percentage of subjects with any SAEs from Visit 1 through Visit 3 prior to administration of the TDV booster or placebo.
- Percentage of subjects with any SAEs following administration of the TDV booster or placebo from Visit 3 through Visit 5 by trial group.

Exploratory endpoints:

Applicable to subjects from parent trial DEN-315 (Mexico only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3. Additional exploratory techniques may be added as the field evolves.

Applicable to subjects from parent trial DEN-304 (United States only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3, and from subjects in the CMI subset at Visit 4 and Visit 5. Additional exploratory techniques may be added as the field evolves.
- The assessment of the long-term cell-mediated response to TDV will include, but is not restricted to, the frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 PBMC) of IFN-γ ELISpot responses to TDV using blood samples collected from subjects in the CMI subset at Visit 1, Visit 2, Visit 3, Visit 4, and Visit 5. Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥50 spots per 10^6 PBMC. Additional exploratory techniques may be added as the field evolves (CMI subset only).

Statistical Considerations:

The following analysis sets are defined for this trial:

All Screened: All subjects who agreed to participate in the current trial.

All Screened-Booster: All subjects who agreed to participate in the current trial and who were screened for 'booster eligibility' to determine if they were eligible to go on to be randomized to Group 1 or Group 2.

Randomized Set-Booster: All subjects randomized at Visit 3 regardless of whether they received the trial vaccination in the current trial. Subjects in this set will be summarized according to randomized treatment.

Safety Set (SAF): All subjects who agreed to participate in the current trial and who received at least one dose of Takeda's TDV in the parent trials.

Safety Set-Booster (SAF-B): All subjects who received at least one dose of Takeda's TDV in the parent trials and who received the TDV booster or placebo in the current trial.

Full Analysis Set (FAS): All subjects who received at least one dose of Takeda's TDV in the parent trials and for whom there is at least one valid follow-up measurement up to Visit 3 for immunogenicity assessments in the current trial.

Full Analysis Set-Booster (FAS-B): All subjects who received at least one dose of Takeda's TDV in the parent trials, the TDV booster or placebo in the current trial, and for whom there is at least one valid follow-up measurement after administration of the TDV booster or placebo at Visit 3 for immunogenicity assessments in the current trial.

Per Protocol Set (PPS): All subjects from the FAS who received two doses of Takeda's TDV in the parent trials with no new major protocol violations prior to administration of the booster or placebo at Visit 3 that could potentially confound the primary endpoints in the current trial.

Per Protocol Set-Booster (PPS-B): All subjects from the FAS-B who received two doses of Takeda's TDV in the parent trials with no new major protocol violations after administration of the TDV booster or placebo at Visit 3 that could potentially confound the primary endpoints in the current trial.

The major protocol violation criterion will be defined as part of a data review prior to analysis. The categories of new major protocol violations include: (1) not meeting selected entry criteria, (2) receiving the wrong trial vaccination at Visit 3 (subjects randomized to Groups 1 and 2 only), (3) receiving prohibited vaccinations or

therapies (subjects randomized to Groups 1 and 2 only), (4) performing Visit 4 inadmissibly outside the visit window (subjects randomized to Groups 1 and 2 only), and (5) other major protocol violations that may be identified during data reviews.

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

Analysis of demographics and other Baseline characteristics

Age, gender, race, and other baseline characteristics (including age at informed consent in the parent trial) will be summarized descriptively for all enrolled subjects upon entry into the current trial.

Immunogenicity evaluation

For the primary and secondary immunogenicity endpoints (ie, GMTs of neutralizing antibodies and seropositivity rates for each of the 4 dengue serotypes and multiple [2, 3 or 4]), descriptive statistics and 95% CIs will be provided for each applicable visit (Visit 1, Visit 2, Visit 3, Visit 4, and Visit 5 in the current trial, and for Day 120 [Month 4] and Day 270 [Month 9] in both the parent trials [which corresponds to 4 months and 9 months after the first vaccination in the primary vaccination series in the parent trials, respectively]).

For the visit comparisons GMRs will be summarized descriptively, including 95% CIs.

Seropositivity is defined as a reciprocal neutralizing titer ≥10. Other immunogenicity measurements obtained at baseline and any post-vaccination visits in the parent trials may be accessed from databases to contribute to designated summaries of immunogenicity endpoints over time following vaccination.

The primary immunogenicity analyses will be based on the PPS/PPS-B; sensitivity analyses may be provided based on the FAS/FAS-B.

Similar descriptive analyses as for the primary immunogenicity endpoint will be provided for the exploratory endpoints for each applicable assay at all relevant time points, based on the PPS/PPS-B. Supportive analyses based on the FAS/FAS-B may also be provided for selected endpoints.

Further details on the statistical analysis including exploratory endpoints will be provided in the statistical analysis plan (SAP).

Safety evaluation

All summaries of safety data will be based on subjects in the SAF/SAF-B.

Solicited AEs

Presence and severity (Grade) of solicited local reactions (injection site pain, injection site erythema and injection site swelling) and solicited systemic AEs (fever, asthenia, malaise, headache, and myalgia) will be collected by diary card for 7 days and 14 days, respectively, following administration of the TDV booster or placebo.

For each solicited AE, the number and percentage of subjects with local (injection site) reactions and systemic AEs will be summarized by trial group and event severity for each day following administration of the TDV booster or placebo (Day 1 through Day 7 for local [injection site] reactions 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 reactions or systemic AEs continuing on Day 8 and Day 15 (after administration of the TDV booster or placebo), respectively, will be assessed separately. Unless otherwise

specified these reactions/AEs will not be included in the analyses/tabulations of unsolicited AEs and will have separate listings.

Unsolicited AEs

Unsolicited AEs will be summarized by trial group for 28 days following administration of the TDV booster or placebo (day of vaccination + 27 days). Unsolicited AEs will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by Preferred Term (PT) and System Organ Class (SOC) for each trial group. AEs leading to trial withdrawal will be summarized up to Visit 3 (pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5.

Unsolicited AEs will be tabulated at each of the following levels: overall summary (subjects with at least 1 AE), and by SOC and PT. In addition, unsolicited AEs will be summarized as follows: by PT including events with frequency greater than a pre-defined 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 the trial vaccine (TDV booster or placebo). Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once.

MAAEs

MAAEs will be collected for subjects randomized to Group 1 and Group 2 and will be presented by trial group from Visit 3 through Visit 5. MAAEs will be coded using MedDRA and summarized by SOC and PT for each trial group.

SAEs

SAEs will be collected throughout the trial. SAEs will be coded using MedDRA and summarized by PT and SOC up to Visit 3 (pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5.

Sample Size Justification:

This trial is designed to be descriptive and is not based on testing formal null hypotheses. Therefore, the sample size was not determined based on formal statistical power calculations. The number of subjects is considered to be sufficient for the evaluation of the objectives of the trial.

Interim Analysis:

Due to significant delays (of >2 years) to booster administration for subjects from parent trial DEN-315 (Mexico), an interim analysis (IA) of the safety and immunogenicity data collected at trial sites in the United States is planned when all subjects from parent trial DEN-304 (United States) have completed their last trial visit (Visit 5 [Day 630 (M21)]). This IA will be performed in an unblinded manner and will include the necessary steps to ensure that no database modifications are made after unblinding for subjects at trial sites in the United States. Unblinding of subjects from parent trial DEN-315 (Mexico) will occur after all subjects at trial sites in Mexico have completed their last trial visit (Visit 5 [Day 1440 (M48)]) and the trial database has been locked. No modifications to the trial are planned based on the results of this IA. An interim CSR of data from parent trial DEN-304 (United States) will not be prepared; all trial results will be reported in the final CSR. More details regarding the IA will be provided in the SAP.

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-303 Version 5.0 (22 August 2022)

2.1 Schedule of Trial Procedures

Visit By Parent Trial (DEN-304/DEN-315)	1 (Day 1[M0]/ Day 1[M0])	2 [a] (Day 360 [M12]/ Day 360 [M12])	3 [b] (Day 450 [M15]/ Day 1260 [M42])		4 [c] (Day 480 [M16]/ Day 1290 [M43])	5 [c] (Day 630 [M21]/ Day 1440 [M48])
Visit Window (Days)	-15/+75 (Linked to Parent Day 1)	-45/+45 (Linked to Visit 1)	-45/+45 (Linked to Visit 1)	-120/+120 (Linked to Visit 1)	-1/+7 (Linked to Visit 3)	-0/+60 (Linked to Visit 3)
Applicable to Subjects From Parent Trial	DEN-304 DEN-315	DEN-304 DEN-315	DEN-304	DEN-315	DEN-304 DEN-315	DEN-304 DEN-315
Site visit	X	X	X		X	X
Phone contact	X [d]	X [d]				
Signed Informed Consent/ Pediatric Assent Form [e]	X		X			
Eligibility criteria	X [f]		X [g]			
Randomization [h]			X			
Demographics	X					
Pregnancy test [i]			X			
Medical history update	X [j]		X [c]			
Prior medication update	X [j]		X [c]			
Concomitant medication update [k]		X	X [c]		X	
Concomitant vaccination update [l]	X	X	X		X	X
Complete physical examination [m]	X		X [c]			
Targeted physical examination [n]					X	X
Vital signs [o]	X	X	X		X	X
Review of systems			X [c]			X
Pregnancy avoidance guidance [p]		X	X [c]		X	
Investigational product [q] administered by SC injection			X [c]			
Injection site evaluation [r]			X [c]			

Visit By Parent Trial (DEN-304/DEN-315)		1 (Day 1[M0]/ Day 1[M0])	2 [a] (Day 360 [M12]/ Day 360 [M12])	3 [b] (Day 450 [M15]/ Day 1260 [M42])		4 [c] (Day 480 [M16]/ Day 1290 [M43])	5 [c] (Day 630 [M21]/ Day 1440 [M48])
Visit Window (Days)		-15/+75 (Linked to Parent Day 1)	-45/+45 (Linked to Visit 1)	-45/+45 (Linked to Visit 1)	-120/+120 (Linked to Visit 1)	-1/+7 (Linked to Visit 3)	-0/+60 (Linked to Visit 3)
Applicable to Subjects From Parent Trial		DEN-304 DEN-315	DEN-304 DEN-315	DEN-304	DEN-315	DEN-304 DEN-315	DEN-304 DEN-315
Diary card [s]	Distribution				X [c]		
	Review/collection of solicited local reactions and systemic AEs				X [c]	X	
Unsolicited AEs [t]					X [c]	X	
MAAEs [u]					X [c]	X	X
SAEs and AEs leading to subject discontinuation or withdrawal [v]		X	X	X		X	X
Criteria for delay of blood sampling		X	X	X		X	X
Blood draw (10 mL) [w]		X	X	X		X	X
Blood draw (40 mL) [w]				X			
Additional blood draw (10 mL CMI subset only, n = 50) [x]						X	X
Additional blood draw (40 mL CMI subset only, n = 50) [x]		X	X	X		X	X

Abbreviations: AE, adverse event; CMI, cell-mediated immunity; M, Month; MAAE, medically attended adverse event; NA, not applicable; SAE, serious adverse event; SC, subcutaneous.

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

(a) At Visit 2, subjects will be reminded of any issues relating to administration of/or eligibility for the upcoming booster dose at Visit 3. This includes, but is not limited to, the collection of any SAEs and any AEs leading to subject discontinuation or withdrawal, a review of prohibited therapies, pregnancy avoidance guidance and information on acceptable methods of contraception (for female subjects of childbearing age).

(b) Visit 3 will be delayed until Day 1260 (M42) for subjects from parent trial DEN-315 (Mexico).

(c) Applicable only to subjects randomized to Group 1 or Group 2 at Visit 3.

(d) A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) and to remind the subject/the subject's LAR about the upcoming site visit. A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18).

(e) Prior to the subject entering into the trial and before any protocol-directed procedures are performed; up to 28 days prior to the day of enrollment. Adolescents from parent trial DEN-315 (Mexico) who become 18 years of age during the course of the trial will be asked to return to the investigational site for an additional site visit to provide the appropriate written informed consent. This should be done as soon as possible after their 18th birthday. Due to changes in the trial design in Mexico (Protocol Amendment 3, dated 22 August 2022), all subjects from parent trial DEN-315 (Mexico) will now be asked to re-consent using an updated ICF or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and the subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the electronic Case Report Form (eCRF).

(f) After informed consent has been obtained, eligibility of the subject will be assessed by review of inclusion/exclusion criteria for trial entry at Visit 1.

(g) A review of the 'booster eligibility' will be performed prior to randomization at Visit 3.

(h) Stratified by parent trial and serostatus at baseline in the parent trials.

(i) For female subjects of childbearing potential, pregnancy testing (urine) will be performed at Visit 3. Results must be confirmed and documented as negative prior to administration of the TDV booster or placebo at Visit 3. 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) Any relevant information collected during the parent trials will be accessed via the database and updated as necessary throughout the trial conduct.

(k) All medications from 1 month (minimum 28 days) prior to administration of the TDV booster or placebo at Visit 3 and up to 1 month (minimum 28 days) thereafter; steroids and immunostimulants within 60 days prior to Visit 3; immunoglobulins and blood products within 3 months prior to Visit 3; and immunosuppressive therapy within 6 months prior to Visit 3.

(l) Any inactivated vaccines administered ≤14 days and any live attenuated vaccines administered ≤28 days prior to blood sample collection or TDV booster/placebo administration at Visit 3.

(m) Physical examination including measurement of weight and height – body mass index will be calculated automatically.

(n) Subjects may undergo a targeted symptom-directed physical examination. Clinically significant changes from the baseline examination (Visit 1) should be recorded in the subject's source documents and eCRF.

(o) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature.

(p) 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 at Visit 1 stating that they understand the requirements for avoidance of pregnancy and donation of ova. Further guidance with respect to the avoidance of pregnancy will be provided to all female subjects of childbearing potential at Visit 2 and to all female subjects of childbearing potential who are randomized to Group 1 or 2 at Visit 3, and at Visit 4. Females of childbearing potential, who are randomized to Group 1 or 2 and are sexually active, will also be reminded to adhere to acceptable contraceptive methods for up to 6 weeks after TDV booster or placebo administration.

(q) Subjects who meet the criteria for 'booster eligibility' and are subsequently randomized to Group 1 or Group 2 at Visit 3 will receive the TDV booster or placebo, respectively.

(r) Injection site pain, erythema, and swelling assessed by trial staff for 30 minutes post-vaccination.

(s) Diary cards (paper or electronic) will be distributed to subjects in Groups 1 and 2 at Visit 3 for the collection of 1) solicited local (injection site) reactions for 7 days (day of vaccination + 6 subsequent days) following TDV booster or placebo administration, and 2) solicited systemic AEs for 14 days (day of vaccination + 13 subsequent days) following TDV booster or placebo administration. The investigator will assess causality of solicited systemic AEs to vaccine administration (related or not related).

(t) Unsolicited AEs will be collected by interview and recorded for Groups 1 and 2 for 28 days (day of vaccination + 27 subsequent days) following TDV booster or placebo administration. The investigator will categorize each event by severity (mild, moderate or severe) and will assess causality to trial vaccine administration (related or not related). If solicited local (injection site) reactions and systemic AEs continue on Day 8 and Day 15 (after administration of the TDV booster or placebo), respectively, record the full duration of the event on the "Adverse Event" eCRF.

(u) MAAEs will be collected for Groups 1 and 2 from Visit 3 through Visit 5.

(v) Any SAEs and any AEs leading to subject discontinuation or withdrawal will be collected for all subjects for the trial duration.

(w) At Visit 3, a 10 mL blood sample and 40 mL blood sample will be taken from all subjects. For those subjects who are randomized to Groups 1 and 2 at Visit 3, all blood samples should be taken prior to administration of the TDV booster dose or placebo. The blood samples taken at Visit 4 should be taken at least 29 days (-1, +7 days) after administration of the TDV booster or placebo at Visit 3.

(x) One additional 40 mL blood sample will be collected from a subset of 50 subjects (CMI subset; from DEN-304 only) for exploratory immunogenicity analyses from Visit 1 through Visit 5. An additional 10 mL blood sample will also be collected from subjects in the CMI subset at Visit 4 and Visit 5 for further exploratory immunogenicity analyses.

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 investigators (PIs) 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 one signatory from the investigators who participate in the study. 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

Term	Definition
AE	adverse event
BMI	body mass index
CD	cluster of differentiation
CMI	cell-mediated immunity
CSR	clinical study report
CYD-TDV	Chimeric Yellow fever virus Dengue virus-Tetravalent Dengue Vaccine
DENV	wild type dengue virus
DENV-1, -2, -3, -4	dengue virus serotype 1, 2, 3, and 4
DHF	dengue hemorrhagic fever
DMC	Data Monitoring Committee
DSS	dengue shock syndrome
E	envelope
eCRF	electronic case report form
FAS	full analysis set
FAS-B	full analysis set – after TDV booster administration
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMR	geometric mean ratio
GMT	geometric mean titer
HIV	human immunodeficiency virus
IA	interim analysis
IB	Investigator's Brochure
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IFN-γ ELISpot	interferon-gamma enzyme-linked immunospot
IND	Investigational New Drug
IRB	Institutional Review Board
IRT	interactive response technology
IUD	intrauterine device
JE	Japanese encephalitis
LAR	legally acceptable representative

Term	Definition
M0, 6, 12, 15, 16, 21	Month 0, 6, 12, 15, 16, 21
MAAE	medically attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency of United Kingdom
MNT_{50}	microneutralization test 50%
NS1	non-structural protein 1
PBMC	peripheral blood mononuclear cells
PDK	primary dog kidney
PI	principal investigator
PMDA	Pharmaceuticals and Medical Devices Agency of Japan
PPS	per-protocol analysis set
PPS-B	per-protocol analysis set – after TDV booster administration
prM	pre-membrane
PT	Preferred Term
QTL	quality tolerance limit
SAE	serious adverse event
SAF	safety set
SAF-B	safety set – after TDV booster administration
SAGE	Strategic Advisory Group of Experts on Immunization
SAP	statistical analysis plan
SC	subcutaneous
SFC	spot forming cells
SOC	System Organ Class
SOP	standard operating procedures
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
YF	yellow fever

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, 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, which is more than 3 times the previous World Health Organization (WHO) estimate of 50 to 100 million cases. Every year, around 500,000 cases of dengue hemorrhagic fever (DHF) require hospitalization with an estimated annual death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection [3-6].

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 occurring 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 one of the 4 dengue serotypes is thought to result in life-long protection from re-infection by the same serotype, but it does not protect against a secondary infection by one of the other 3 dengue serotypes and may lead to an increased risk of severe disease (DHF/DSS) upon infection with one of the other 3 dengue serotypes [4,5,7,8].

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 [3-9]. A first recombinant dengue vaccine (chimeric yellow fever virus dengue virus-tetravalent dengue vaccine [CYD-TDV]) was approved in some countries in Asia and Latin America in 2015, in Europe in 2018, and in the United States in 2019 [10]. Initial findings showed that vaccine efficacy was different between serotypes and depended on dengue pre-exposure status [11]. Further analyses showed that people who had not been infected by dengue virus before vaccination had a higher risk of getting severe disease if they were infected after vaccination with CYD-TDV [12]. 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 vaccines.

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

Takeda's TDV consists of 1 molecularly characterized, attenuated dengue serotype 2 virus strain and 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 virus 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, pre-membrane (prM) and envelope (E), of TDV-2 with the prM and E genes from the DENV virus strains, DENV-1 16007, DENV-3 16562 or DENV-4 1036, respectively [14]. Thus, Takeda's TDV is comprised of 4 dengue virus strains: TDV-2 (a molecularly characterized attenuated 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 [15]. TDV has been given to >20,000 clinical trial subjects. All available data also showed that TDV was well tolerated with no significant safety concerns to date [16].

The current version of the Investigator's Brochure (IB) contains additional product information and a more detailed review of pre-clinical and clinical trials.

4.2 Rationale for the Proposed Trial

This phase 3 trial will capture long-term antibody persistence and safety data in healthy subjects in areas non-endemic for dengue who have previously received a primary TDV vaccination. It will then go on to assess the immunogenicity and safety of a TDV booster dose in this population.

Subjects previously enrolled in two parent trials (DEN-304 and DEN-315) will initially be invited to participate in the DEN-303 follow-up trial from 21 months after the first vaccination in the primary vaccination series in the parent trials. Antibody persistence will be assessed in all subjects for up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). The impact of a TDV booster versus placebo on the immune response will be assessed at 1 month and 6 months after booster administration in all eligible subjects randomized to receive the TDV

booster or placebo (in a 1:1 ratio). Safety assessments will continue for the duration of the trial (up to 6 months post booster administration).

The safety and immunogenicity data collected in this trial will be of importance not only to individuals travelling to endemic regions but also for the significant number of residents with no prior history of dengue infection who live in endemic and semi-endemic areas. The introduction of a TDV booster in a non-endemic setting will begin the assessment of whether a booster is likely to be of benefit in this 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 [2].

5.0 TRIAL OBJECTIVES AND ENDPOINTS

5.1 Objectives

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

5.1.1 Primary Objectives

- To describe antibody persistence for each of the 4 dengue serotypes for up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).
- To describe the impact of a TDV booster dose vs placebo on antibody response for each of the 4 dengue serotypes at 1 month and 6 months post administration of the TDV booster or placebo.

5.1.2 Secondary Objectives

Immunogenicity

Antibody Persistence

- To describe the overall trend in antibody decay for all 4 dengue serotypes from values obtained after the primary vaccination series in the parent trials through 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and through 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).

Impact of a TDV Booster Dose

- To describe the impact of a TDV booster on antibody response for each of the 4 dengue serotypes for up to 69 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 42 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).

Safety

- To describe the long-term safety of Takeda's TDV for up to 63 months in previously vaccinated subjects from parent trial DEN-315 (Mexico) and for up to 36 months in previously vaccinated subjects from parent trial DEN-304 (United States).
- To assess safety for 6 months following administration of the TDV booster or placebo in Groups 1 and 2, respectively.

5.1.3 Exploratory Objectives

Applicable to subjects from parent trial DEN-315 (Mexico only):

- To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 63 months after the first vaccination in the primary vaccination series in the parent trial (DEN-315); this is inclusive of, but not restricted to, an assessment of the anti-dengue Non-Structural Protein 1 (NS1) antibody response.

Applicable to subjects from parent trial DEN-304 (United States only):

- To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304), and in the cell-mediated immunity (CMI) subset at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, an assessment of the anti-dengue NS1 antibody response.
- To evaluate aspects of the long-term cell-mediated immune response to Takeda's TDV up to 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304) and at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) of the long-term T cell-mediated immune response to TDV (CMI subset only).

5.2 Endpoints

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

5.2.1 Primary Endpoints

- Geometric mean titers (GMTs) of neutralizing antibodies (by microneutralization test 50% [MNT_{50}]) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 1, Visit 2, and Visit 3 (prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) summarized for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials.
- GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 4 and Visit 5 for subjects randomized to

Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials.

5.2.2 Secondary Endpoints

Immunogenicity Endpoints:

Antibody Persistence

- Geometric Mean Ratio (GMR) of neutralizing antibodies for each of the 4 dengue serotypes for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials for:
  - Visit 1 versus Visit 2.
  - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) versus Visit 3 in the current trial.
  - Day 270 (Month 9) in the parent trials (9 months after the first vaccination in the primary vaccination series in the parent trials) versus Visit 1 and Visit 2 in the current trial.

Impact of a TDV Booster Dose

- GMR of neutralizing antibodies for each of the 4 dengue serotypes for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials for:
  - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) versus Visit 4 in the current trial.
  - Visit 3 versus Visit 4.
  - Visit 3 versus Visit 5.
  - Visit 4 versus Visit 5.
  - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) versus Visit 5 in the current trial.

Safety Endpoints:

- Frequency and severity of solicited local (injection site) reactions for 7 days (day of vaccination + 6 days), and solicited systemic AEs for 14 days (day of vaccination + 13 days) following administration of the TDV booster or placebo at Visit 3 by trial group.
- Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 days) following administration of the TDV booster or placebo at Visit 3 by trial group.
- Percentage of subjects with any medically attended adverse events (MAAEs) following administration of the TDV booster or placebo from Visit 3 through Visit 5 by trial group.

- Percentage of subjects with any serious adverse events (SAEs) from Visit 1 through Visit 3 prior to administration of the TDV booster or placebo.
- Percentage of subjects with any SAEs following administration of the TDV booster or placebo from Visit 3 through Visit 5 by trial group.

5.2.3 Exploratory Endpoints

Applicable to subjects from parent trial DEN-315 (Mexico only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3. Additional exploratory techniques may be added as the field evolves.

Applicable to subjects from parent trial DEN-304 (United States only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3, and from subjects in the CMI subset at Visit 4 and Visit 5. Additional exploratory techniques may be added as the field evolves.
- The assessment of the long-term cell-mediated response to TDV will include, but is not restricted to, the frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 PBMC) of IFN-γ ELISpot responses to TDV using blood samples collected from subjects in the CMI subset at Visit 1, Visit 2, Visit 3, Visit 4, and Visit 5. Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥ 50 spots per 10^6 PBMC. Additional exploratory techniques may be added as the field evolves (CMI subset only).

6.0 TRIAL DESIGN AND DESCRIPTION

6.1 Trial Design

This is a phase 3 follow-up trial that will evaluate the long-term antibody persistence and safety of Takeda's TDV in healthy adolescents and adults in areas non-endemic for dengue, in addition to assessing the impact of a booster dose in this population. Subjects who previously received TDV in two parent trials, DEN-304 and DEN-315, will be invited to participate in this follow-up trial. DEN-303 will include up to 600 healthy subjects aged ≥13 to ≤63 years at trial entry. To enable the assessment of a booster dose, the trial will be double-blinded, randomized, and placebo-controlled from Visit 3 onwards.

Antibody persistence and safety will be assessed from Visit 1 through Visit 3; up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). Further characterization of the long-term humoral and cell-mediated immune responses to Takeda's TDV will be undertaken in a subset of approximately 50 volunteers identified at enrollment (CMI subset: participation is on a voluntary basis from DEN-304 only) up to Visit 5. A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) between site visits and to remind the subject/the subject's LAR of any upcoming site visits. At Visit 2 the site will discuss any information that is pertinent to the booster phase of the trial with the subject. A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18).

At Visit 3, following all scheduled blood draws, all subjects will be screened for 'booster eligibility' to determine if they are eligible to go on to receive the TDV booster in the booster phase. Any subject who fails to meet the criteria for 'booster eligibility' will end the trial at Visit 3. All eligible subjects will be randomized using an interactive response technology (IRT) at Visit 3 to 1 of 2 trial groups (Group 1 and Group 2) in a 1:1 ratio stratified by parent trial and serostatus at baseline in the parent trials. Subjects allocated to Group 1 will receive the TDV booster (single dose) and subjects allocated to Group 2 will receive placebo. The impact of the TDV booster on neutralizing antibody titers and seropositivity rates will be assessed at 1 month and 6 months after administration of the TDV booster or placebo. Safety assessments will continue for 6 months following TDV booster or placebo administration for subjects in Groups 1 and 2.

Immunogenicity evaluation:

- Neutralizing antibodies (by MNT_{50}) will be measured using blood samples collected from all subjects at Visit 1, Visit 2, and Visit 3, and at Visit 4 and Visit 5 for subjects who are randomized to Groups 1 and 2.
- At Visit 3, a larger volume of blood will be collected from all subjects to assess exploratory markers of the long-term humoral immune response to Takeda's TDV.

- Applicable to subjects from parent trial DEN-304 (United States only): Additional blood samples will be collected from subjects in the CMI subset to allow further characterization of the long-term humoral and cell-mediated immune response to Takeda's TDV at Visit 1, Visit 2, and Visit 3, and in subjects randomized to Groups 1 and 2 at Visit 4 and Visit 5.

Safety evaluation:

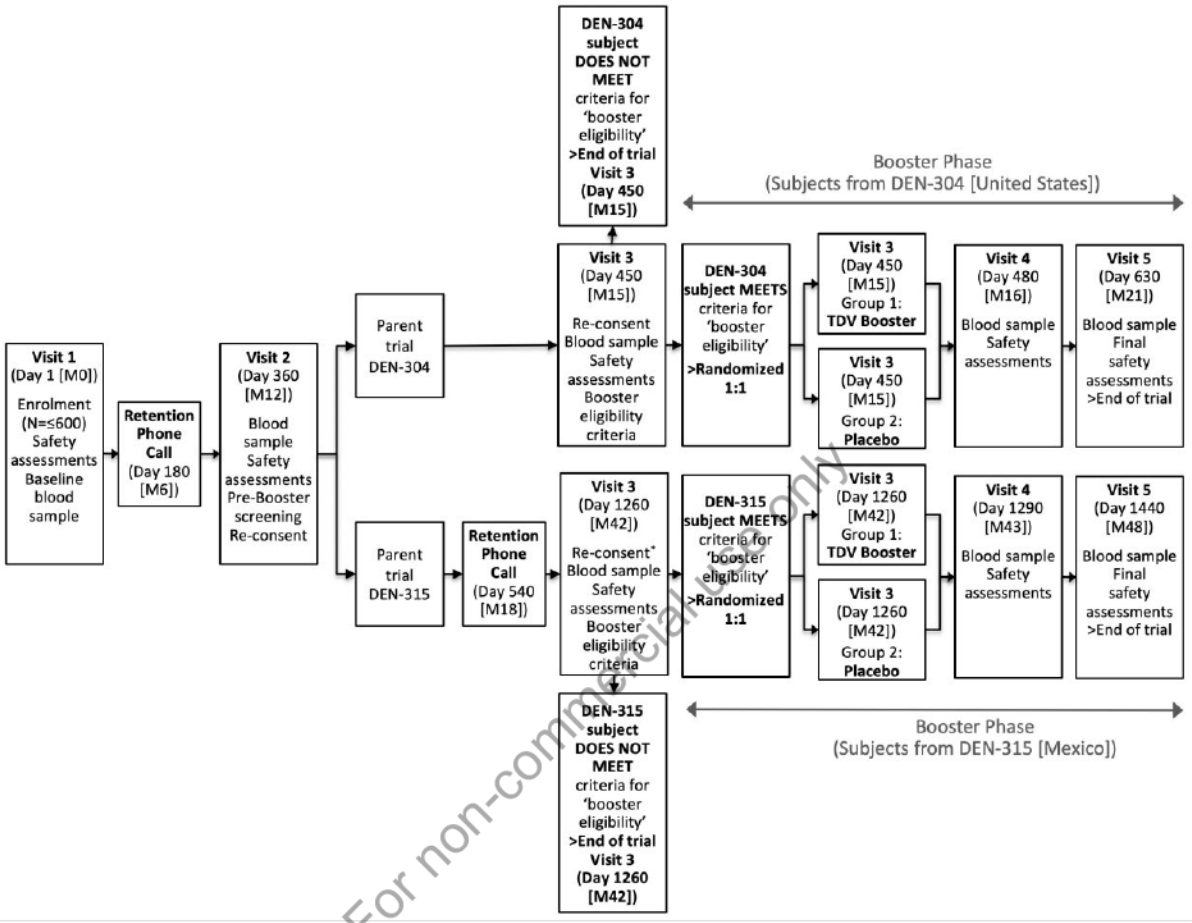
- Diary cards will be distributed to Groups 1 and 2 at Visit 3 for the recording of:
  - Solicited local (injection site) reactions for 7 days (day of vaccination + 6 days) following administration of the TDV booster or placebo. These include: injection site pain, injection site erythema, and injection site swelling.
  - Solicited systemic adverse events (AEs) for 14 days (day of vaccination + 13 days) following administration of the TDV booster or placebo. These include: fever, headache, asthenia, malaise, and myalgia.
- Unsolicited AEs will be collected by interview and recorded for 28 days (day of vaccination + 27 days) following administration of the TDV booster or placebo.
- All SAEs and any AEs leading to subject discontinuation and withdrawal will be collected for the trial duration for all subjects.
- MAAEs will be collected, for Groups 1 and 2, following administration of the TDV booster or placebo from Visit 3 through Visit 5. 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 subjects in Groups 1 and 2 the final safety assessments will be performed approximately 6 months after administration of the TDV booster or placebo at Visit 5.

Data collection will be by electronic Case Report Form (eCRF).

Summary:

For subjects from parent trial DEN-315 (Mexico), the duration of the current trial will be 42 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 (inclusive of 3 site visits, 3 blood draws) and 48 months for all other subjects (inclusive of 5 site visits, 5 blood draws). For subjects from parent trial DEN-304 (United States), the trial duration will be 15 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 (inclusive of 3 site visits, 3 blood draws) and 21 months for all other subjects (inclusive of 5 site visits, 5 blood draws). 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



For all subjects, Visit 1 and Visit 2 correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3, Visit 4, and Visit 5 in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3, Visit 4, and Visit 5 in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series, respectively.

* Due to changes in the trial design in Mexico (Protocol Amendment 3, dated 22 August 2022), all subjects from parent trial DEN-315 will be asked to re-consent using an updated informed consent form (ICF) or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the eCRF.

6.2 Justification for Trial Design, Dose, and Endpoints

The study population in this trial consists of healthy adolescents and adults in areas non-endemic for dengue to permit the long-term follow-up of safety and immunogenicity, and the assessment of a TDV booster in previously vaccinated subjects. The trial design and the collection of

solicited reactions, symptoms and AEs following TDV booster or placebo administration are consistent with other vaccine evaluation trials. As the trial will be conducted in areas non-endemic for dengue, a 6-month follow-up period after administration of the booster dose is considered adequate.

The CMI subset will be restricted to subjects from DEN-304 as these trial sites are known to have the infastructure in place that is required to collect, process, store, and ship PBMC samples according to the Standard Operating Procedures (SOP).

Completed phase 2 trials have enabled the selection of the final TDV formulation, administered by SC injection, that is used in this study and has been taken forward in Takeda's pivotal dengue program. The current version of the IB provides additional information and a more detailed review of nonclinical studies and clinical trials.

In order to maintain the double-blind design, following administration of the TDV booster to Group 1 at Visit 3, a placebo (saline solution for injection) will be administered to Group 2. Justification of the sample size (up to 300 subjects per group) is included in Section 13.3 and the rationale for the proposed trial is given in Section 4.2.

The timing of the primary and secondary endpoints assessing antibody persistence aims to provide data on persistence of the immune response for >2.5 years (2.75 years for subjects from parent trial DEN-304 and 5 years for subjects from parent trial DEN-315) following completion of the primary vaccination series. The timing of primary and secondary endpoints following administration of the TDV booster will allow a fair comparison with titer values obtained in the parent trials and provide data on adequate timing for booster administration after a 2 dose schedule. Dengue neutralizing antibodies have generally been accepted as the immune response endpoint for dengue vaccine trials and will be the primary variable measured in this trial.

6.3 Planned Duration of Subject's Expected Participation in the Entire Trial

For subjects from parent trial DEN-315 (Mexico), the duration of the current trial will be 42 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 and 48 months for all other subjects (inclusive of booster administration at Visit 3 and follow-up through Visit 5). For subjects from parent trial DEN-304 (United States), the trial duration will be 15 months for those subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 and 21 months for all other subjects (inclusive of booster administration at Visit 3 and follow-up through Visit 5).

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 one 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 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

All entry criteria, including test results, need to be confirmed prior to trial entrance.

7.1 Inclusion Criteria

Subject eligibility at trial entry is determined according to the following criteria:

1. The subject is aged ≥13 and ≤63 years at entry into the current trial.
2. Male or female subjects (irrespective of serostatus at baseline in the parent trials) who received at least one dose of Takeda's TDV in the parent trials and have data from at least one blood draw post-vaccination.
3. Subjects who are in good health at the time of entry into this trial as determined by medical history, physical examination (including vital signs), and the clinical judgment of the investigator.
4. The subject/the subject's LAR signs and dates 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. Assent is obtained from the subject where required.
5. Subjects who can comply with trial procedures and are available for the duration of the follow-up.

7.2 Exclusion Criteria

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

1. Subjects with 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.
2. Subjects involved in the trial conduct or their first-degree relatives.
3. Subjects that, in the opinion of the investigator, are not medically eligible to provide blood samples.
4. Subjects with a prolonged period of habitation (≥1 year) in a dengue endemic area within the 2 years prior to Visit 1.
5. Previous and planned vaccination (during the trial conduct), against any flavivirus including dengue (other than Takeda's TDV), yellow fever (YF), Japanese encephalitis (JE) viruses or tick-borne encephalitis.
6. Participation in any clinical trial is allowed, on condition that no investigational product is administered within 30 days prior to blood sampling in the current trial.
7. Subjects with any illness, or a history of any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.

There may be instances when individuals meet all entry criteria except one that relates to transient clinical circumstances (eg, body temperature elevation or recent use of excluded medication[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.

7.3 Booster Eligibility

Eligibility, including test results, must be confirmed prior to randomization.

Any subject who meets any of the following criteria at Visit 3 will not qualify for randomization to Group 1 or 2 to receive the TDV booster or placebo, respectively:

1. Subjects for whom baseline serostatus is not defined in the parent trials.
2. Subjects with a known hypersensitivity or allergy to any of the trial vaccine components (including excipients).
3. Subjects with any history of progressive or severe neurologic disorder, seizure disorder or neuro-inflammatory disease (eg, Guillain-Barré syndrome).
4. Subjects with any illness, or a history of any illness that, in the opinion of the investigator, might interfere with the results of the trial or pose additional risk to the subjects due to participation in the trial.
5. Known or suspected impairment/alteration of immune function, including:
    a) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3; use of inhaled, intranasal, or topical corticosteroids is allowed.
    b) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3.
    c) Administration of immunoglobulins and/or any blood products within the 3 months prior to administration of the TDV booster or placebo at Visit 3; consider whether applicable as an exclusion criterion or criterion for delay.
    d) Receipt of immunostimulants within 60 days prior to Visit 3.
    e) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Visit 3.
    f) Known human immunodeficiency virus (HIV) infection or HIV-related disease.
    g) Hepatitis C virus infection.
    h) Genetic immunodeficiency.
6. Abnormalities of splenic or thymic function.

7. Subjects with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
8. Subjects with any serious chronic or progressive disease according to the judgment of the investigator (eg, neoplasm, hematologic malignancies, insulin dependent diabetes, cardiac, renal, or hepatic disease).
9. Subjects with body mass index (BMI) greater than or equal to 35 kg/m^2 (= weight in kg/[height in $meters^2$]).
10. Subjects who have received any other vaccines within the 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to TDV booster or placebo administration.
11. Subjects with a history of substance or alcohol abuse within the past 2 years.
12. Female subjects who are pregnant or breastfeeding.
13. Female subjects of child-bearing potential who are sexually active with men, and who have not used any of the acceptable contraceptive methods for at least 2 months prior to Visit 3.
    a) Of "childbearing potential" is defined as status post-onset of menarche and not meeting any of the following conditions: menopausal for at least 2 years, status after bilateral tubal ligation for at least 1 year, status after bilateral oophorectomy, or status after hysterectomy.
    b) "Acceptable birth control methods" are defined as one or more of the following:
        I. Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring).
        II. Barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse.
        III. Intrauterine device (IUD).
        IV. Monogamous relationship with a vasectomized partner. Partner must have been vasectomized for at least 6 months prior to Visit 3.
14. Female subjects of childbearing potential who are sexually active, and who refuse to use an "acceptable contraceptive method" for up to 6 weeks after administration of the TDV booster or placebo at Visit 3. In addition, they must be advised not to donate ova or breastfeed during this period.
15. Any positive or indeterminate pregnancy test (Section 9.1.10).
16. Subjects with history of current or previous infection with a flavivirus such as dengue, Zika, YF, JE, West Nile fever, tick-borne encephalitis or Murray Valley encephalitis and subjects with a prolonged period of habitation (≥1 year) in a dengue endemic area during trial conduct.

There may be instances when subjects meet all the criteria for 'booster eligibility' except one 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 the TDV booster/placebo may be considered if the appropriate window for delay has passed, if 'booster eligibility' has been rechecked, and if the subject is confirmed to be eligible.

7.4 Criteria for Delay of Trial Vaccine or Placebo Administration or Blood Sampling

After enrollment, subjects may encounter clinical circumstances that warrant a delay in blood sampling or the administration of trial vaccine (TDV booster/placebo). These situations are listed below. In the event that a subject meets a criterion for delayed blood sample collection or trial vaccine (TDV booster/placebo) administration, the subject may have blood drawn or receive the trial vaccine (TDV booster/placebo) once the window for delay has passed as long as the subject is otherwise eligible for trial participation.

- Subjects who received any other vaccines within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to planned trial vaccine (TDV booster/placebo) administration or blood sampling.
- Subjects participating in any clinical trial where another investigational product is administered within 30 days prior to any blood sample collection.
- 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 vaccine (TDV booster/placebo) administration. This does not apply to blood sample collection.
- Subjects who have received blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation in the past 3 months prior to administration of the trial vaccine (TDV booster/placebo). This does not apply to blood sample collection.
- 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 booster 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 booster or placebo administration. This does not apply to blood sample collection.

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

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. 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.

For screen failure subjects, refer to Section 9.1.11.

1. Adverse Event: The subject has experienced an AE (irrespective of being related/unrelated to the trial vaccine [TDV booster/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, 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). The reason for withdrawal, if provided, should be recorded in the eCRF.
4. 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, 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. Subject's death during trial participation.
6. Other (the specific reason should be recorded in the "Specify" field of the eCRF).

7.6 Criteria for Premature Discontinuation of Trial Vaccine Administration

Early termination of a subject's trial participation will by default prevent the subject from receiving further doses of trial vaccine, as the subject will no longer be participating in the trial. 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 trial vaccine administration is discontinued. Even if the subject is deemed ineligible to receive further doses of trial vaccine, all efforts should be made to continue the collection of safety data according to protocol.

In addition, the primary reason for premature discontinuation of trial vaccine administration should be recorded in the eCRF "end of trial vaccine administration" page) using the following categories.

1. Adverse Event: The subject has experienced an AE (irrespective of being related/unrelated to the trial vaccine or trial-related procedures) for which subsequent trial vaccine administrations 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 3 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). The reason for withdrawal, if provided, should be recorded in the eCRF.
4. Premature trial termination by sponsor, a regulatory agency, the IEC/IRB, or any other authority.

   If the clinical trial is prematurely terminated by the sponsor, 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. Subject´s death during trial participation.
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 trial vaccine 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. The subject should be followed-up until the birth of the child, or spontaneous or voluntary termination; when pregnancy outcome information becomes available, the information should be captured using the same form. Data obtained from the "Clinical Trial Pregnancy Form" will be captured in the safety database.
8. Other (the specific reason should be recorded in the "Specify" field of the eCRF).

8.0 CLINICAL TRIAL MATERIAL MANAGEMENT

This section contains information regarding all trial vaccines, 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 material.

8.1 Trial Vaccine, Placebo and Materials

The investigational vaccine is Takeda's TDV, a tetravalent vaccine comprised of 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 per dose of TDV-1, TDV-2, TDV-3, and TDV-4, respectively. Takeda's 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% saline).

Details regarding the dosage form description and strengths, or composition for the extemporaneous preparation, of the trial vaccine and placebo can be found in the Pharmacy Manual or in the referenced compounding manual when applicable. Trial vaccine will be packaged to support enrollment and replacement of subjects as required.

8.1.1 Dosage Form, Manufacturing, Packaging, and Labeling

TDV Kits (TDV and TDV Diluent):

Manufacturing of monovalent bulk vaccine substances of Takeda's TDV, mixing of the four TDV vaccine substances, filling into vials, and lyophilization of the TDV is done at IDT Biologika GmbH, Germany.

Lyophilized TDV is presented in a single-dose 2 mL glass vial with a dark grey butyl rubber stopper and flip-off aluminum/plastic over seal.

TDV diluent (37 mM NaCl solution) is a clear, colorless solution provided in a single-use pre-filled glass syringe and is used to reconstitute the lyophilized TDV to deliver a 0.5 mL dose.

The doses should be prepared at the time of administration by the unblinded pharmacist (or administrator) as per the Pharmacy Manual.

Placebo:

Normal saline for injection (0.9% sodium chloride solution) will be used as placebo. The placebo is presented as single dose units for 0.5 mL dosing.

The sponsor will supply study sites with TDV, TDV diluent, and placebo packaged into single dose dispensing cartons. The cartons will be labeled in an unblinded fashion and will contain pertinent trial information and caution statements in local languages. Receiving, storage, accountability, and dispensing of unblinded trial vaccines should only be performed by

unblinded personnel (see Sections 8.2 and 8.4) to ensure that the trial blind is not broken. Further details can be found in the Pharmacy Manual.

8.1.2 Storage

The trial vaccine (TDV/placebo) and TDV diluent will be shipped as per the Pharmacy Manual. From receipt and prior to use, the trial vaccine, placebo, and diluent must be stored as per the label and Pharmacy Manual.

All clinical trial material must be kept in an appropriate, limited-access, secure place until it is used or returned to the sponsor or designee for destruction. All sponsor-supplied trial vaccines and 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 vaccines, TDV diluent, and placebo requires sponsor approval.

8.1.3 Dose and Regimen

The trial vaccine doses that will be provided to each trial group at Visit 3 are presented in Table 8.a.

The 0.5 mL trial vaccine doses will be prepared and administered by the unblinded pharmacist or vaccine administrator according to the instructions in the Pharmacy Manual or as per sponsor instructions.

TDV and placebo will be administered by the SC route.

Table 8.a Sponsor-Supplied Vaccines and Placebo

Group	Description	Timing
Group 1	TDV, SC	Visit 3 [(a)]
Group 2	Placebo, SC	Visit 3 [(a)]

Abbreviations: M, Month; SC, subcutaneous.

(a) Day 1260 (M42) for subjects from parent trial DEN-315 (Mexico) and Day 450 (M15) for subjects from parent trial DEN-304 (United States).

8.2 Trial Vaccine Assignment and Dispensing Procedures

The vaccine to be used will be identifiable by a unique identification number and managed by IRT. Refer to Section 8.6. for accountability of sponsor-supplied vaccines.

The investigator or designee will use IRT at subject enrollment to obtain the subject number. This number will be used throughout the trial.

The designee will use IRT again at randomization on the day of TDV booster or placebo administration (Visit 3) to provide the vaccination identification number for the vaccine dose.

The trial vaccine (TDV booster/placebo) will be administered only by unblinded personnel who are qualified to perform that function under applicable laws and regulations for that specific trial. The blinded investigator or designee will be responsible for overseeing the administration of the trial vaccine (TDV booster/placebo) to subjects enrolled in the trial according to the procedures stipulated in this trial protocol.

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

8.2.1 Precautions to Be Observed When Administering the TDV Booster or Placebo

Prior to TDV booster or placebo vaccination, a subject must be determined to be eligible and it must be deemed clinically appropriate in the judgment of the investigator to administer the booster dose. Eligibility for trial entry is evaluated according to the inclusion and exclusion criteria for entry outlined in this protocol (Section 7.1 and Section 7.2). Prior to TDV booster or placebo administration at Visit 3, site staff must determine if the subject is eligible to receive the TDV booster dose by evaluating the criteria for 'booster eligibility' outlined in Section 7.3.

Standard immunization practices are to be observed and care should be taken to administer the injection by the SC route. In addition, WHO recommendations to reduce anxiety and pain at the time of vaccination should be followed [17]. Before administering the trial vaccine (TDV booster/placebo), the vaccination site must be disinfected with a skin disinfectant (eg, 70% alcohol). Allow the skin to dry. DO NOT inject intravascularly. Refer to the Pharmacy Manual for details on preparation and administration of trial vaccination (TDV booster/placebo).

After administration of the trial vaccination or placebo, the subject will be observed for at least 30 minutes for severe reactions. As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccination. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available; these rescue medications will not be supplied by the sponsor.

8.3 Randomization Code Creation and Storage

Randomization personnel of the sponsor or designee will generate the randomization schedule. Randomization information will be stored in a secured area, accessible only by authorized personnel.

8.4 Trial Vaccine Blind Maintenance

The trial will be conducted in a double-blind manner from Visit 3, meaning that from this point onwards the subject, those responsible for the evaluation of any trial endpoint, and the sponsor will all be unaware of whether the TDV booster or placebo was administered. The investigational vaccine blind will be maintained using IRT. The subjects, data collectors (eg, investigator), and data evaluators (eg, trial statisticians) are blinded. One or more designated pharmacists or vaccine administrators at the site will be unblinded. These unblinded personnel

will be responsible for receiving and storing the trial vaccines to ensure that the trial blind is not broken. They will also be responsible for the trial vaccine accountability. The unblinded personnel will have no role in data collection or evaluation.

All care must be taken to ensure that the unblinded reports and documents are shared only with unblinded personnel and properly stored in a secured area, accessible only by authorized personnel.

8.5 Unblinding Procedure

The trial vaccine blind, which will be in place from randomization at Visit 3, shall not be broken by the investigator unless information concerning the TDV booster or placebo 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 before the trial vaccine blind is broken to discuss the need for unblinding.

For unblinding a subject, the trial vaccine blind 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 vaccine 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 be recorded on the eCRF.

If any subject is unblinded, the subject must be withdrawn from the trial and their data no longer evaluated. Subjects should continue to be monitored for safety follow-up.

8.6 Accountability and Destruction of Sponsor-Supplied Trial Vaccine, Placebo, and Other Clinical Trial Materials

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

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

If there are any discrepancies between the packing list and the actual product received, the sponsor or designee must be contacted to resolve the issue. The packing list should be filed in the Pharmacy Investigator Site File by a qualified investigator designee.

The investigator (or designated individual) at the site must maintain 100% accountability for all sponsor-supplied trial vaccines, placebo, and other clinical trial material 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 or placebo ID used to prepare each dose.
- Verifying that all trial vaccine kits used are documented accurately on the log.
- Verifying that required fields are completed accurately and legibly.

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

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

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

9.0 TRIAL PLAN

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

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 or informed consent and pediatric assent form are described in Section 15.2.

Informed consent or informed consent and pediatric assent must be obtained prior to the subject entering into the trial, and before any protocol-directed procedures are performed. Adolescents who become 18 years of age during the course of the trial will be asked to return to the investigational site for an additional site visit to provide the appropriate written informed consent. This should be done as soon as possible (but no later than 2 month) after their 18th birthday.

A unique subject number will be assigned to each subject by the IRT after informed consent or informed consent and pediatric assent is obtained. If all eligibility criteria for entry are fulfilled, this subject number will be used throughout the trial. Subject numbers assigned to subjects who fail screening at trial entry should not be reused (Section 9.1.11).

A subset of 50 volunteers (DEN-304 only) will be assigned to the CMI subset at trial entry to permit a more detailed characterization of the long-term humoral and cell-mediated immune response to Takeda's TDV up to Visit 5.

Due to changes in the trial design in Mexico (protocol amendment 3, dated 22 August 2022), all subjects from parent trial DEN-315 (Mexico) will now be asked to re-consent using an updated informed consent form (ICF) or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and the subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the eCRF.

9.1.2 Demographics, Medical History and Prior Medications

Demographic information to be obtained at Visit 1 will include age/date of birth, sex, race, and ethnicity as described by the subject or subject's LAR.

A medical history update will be collected at enrollment (Visit 1) and after randomization in subjects that are eligible for TDV booster or placebo administration at Visit 3. This may include but is not limited to any medical history that may be relevant to subject eligibility for trial participation and TDV booster or placebo administration, such as prior vaccinations, concomitant medications, and previous and ongoing illnesses and/or injuries. Relevant medical history can also include 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.

Medical history (including corresponding medication) to be obtained at Visit 1 will include any significant conditions or diseases that have disappeared or resolved at or prior to signing of informed consent or pediatric assent form. Medical history update (including corresponding medication) to be obtained prior to booster administration at Visit 3 will include any significant conditions or diseases that have disappeared or resolved between Visit 1 and Visit 3. Any relevant information collected during the parent trials will be accessed via the database and updated as necessary throughout the trial conduct.

Adverse medical occurrences emerging during the time between signing of informed consent or pediatric assent form and the first administration of trial vaccine (TDV booster/placebo) will be recorded in the medical history eCRF page. If such an adverse medical occurrence is assessed as related to a trial procedure this should be recorded as an AE related to study procedure in the eCRF.

All medications, vaccines and blood products taken or received by the subjects are to be assessed and collected as Prior or Concomitant Medications and recorded in the subject's source document and eCRF as follows:

a) Medications: from 1 month (minimum 28 days) prior to administration of the TDV booster or placebo at Visit 3 and up to 1 month (minimum 28 days) thereafter.

b) Vaccines: from 1 month (minimum 28 days) prior to any blood sample collection or administration of the TDV booster/placebo at Visit 3 and up to 1 month (minimum 28 days) thereafter.

c) Blood products and immunoglobulins: within 3 months prior to administration of the TDV booster or placebo at Visit 3.

d) Steroids and immunostimulants: within 60 days prior to Visit 3.

e) Immunosuppressive therapies: within 6 months prior to Visit 3.

The use of antipyretics and/or analgesic medications within 24 hours prior to vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be documented in the subject's source document and eCRF.

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

Prohibited therapies (Refer to Sections 7.2 and 7.3):

a) Previous and planned vaccination (during the trial conduct), against any flavivirus including dengue (other than Takeda's TDV), YF, JE viruses or tick-borne encephalitis.

b) Any other vaccines within the 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to any blood sample collection or TDV booster/placebo administration at Visit 3.

c) Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3; use of inhaled, intranasal, or topical corticosteroids is allowed.

d) Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Visit 3.

e) Administration of immunoglobulins and/or any blood products within the 3 months prior to administration of the TDV booster or placebo at Visit 3.

f) Receipt of immunostimulants within 60 days prior to Visit 3.

g) Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Visit 3.

h) Participation in any clinical trial where another investigational product is administered within 30 days prior to any blood sample collection.

These data must be written in the source documents.

9.1.3 Documentation of Trial Entrance and Randomization

Only subjects who have a signed informed consent or informed consent and pediatric assent form, and meet all of the inclusion criteria at entry and none of the exclusion criteria at entry are eligible for entry into this trial. Only subjects who meet the criteria for 'booster eligibility' at Visit 3 are eligible for randomization into the booster phase. Randomization to one of two groups in a 1:1 ratio will be stratified by parent trial and serostatus at baseline in the parent trials. The randomization schedule will be created and controlled by the IRT provider using the randomization specification approved by the sponsor's trial statistician, or designee.

If the subject is ineligible for randomization at Visit 3, they will end the trial at this point and the investigator should record the primary reason for non-randomization in the subject's source documents and eCRF.

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. A complete physical exam will be performed in all subjects at Visit 1 and in subjects randomized to Groups 1 and 2 at Visit 3 according to the investigator's standard practice. A complete physical examination includes but is not limited to: auscultation of heart and lungs, palpation of the abdomen, inspection of extremities (including skin over intended vaccination site[s]), a check of general appearance and the measurement of weight and height; BMI will be calculated automatically. Additional physical examinations may be performed if indicated by review of the subject's medical history. Clinically significant findings should be documented in the subject's source document and eCRF.

Subjects randomized to Groups 1 and 2 will also undergo a targeted symptom-directed physical examination at Visit 4 and Visit 5. Any clinically significant changes from the baseline examination performed at Visit 1 should be recorded in the subject's source documents and eCRF.

9.1.5 Vital Signs

Vital signs will be measured in all subjects at Visit 1, Visit 2, and Visit 3, and in those subjects randomized to Groups 1 and 2 at Visit 4 and Visit 5. These will include (but are not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature.

9.1.6 Immunogenicity Assessments

Blood samples for immunogenicity assessments will be collected from all subjects (10 mL) at Visit 1, Visit 2, and Visit 3, and from those subjects randomized to Groups 1 and 2 at Visit 4 and Visit 5.

A larger blood sample (40 mL) will also be collected from all subjects to assess exploratory markers of the long-term humoral immune response to Takeda's TDV at Visit 3 prior to TDV booster or placebo administration for subjects randomized to Groups 1 and 2.

Applicable only to subjects from parent trial DEN-304 (United States): Additional blood samples (40-50 mL) will be collected from subjects in the CMI subset to allow further characterization of the long-term humoral and cell-mediated immune responses to Takeda's TDV at Visit 1, Visit 2 and Visit 3, and in subjects randomized to Groups 1 and 2 at Visit 4 and Visit 5.

The maximum volume of blood taken at any single visit is approximately 90 mL for subjects in the CMI subset and approximately 50 mL for all other subjects. The total volume of blood collected for the trial duration is approximately 190 to 310 mL for subjects in the CMI subset and approximately 70 to 90 mL for all other subjects.

Blood should be taken from subjects using an aseptic venipuncture technique for serological immunogenicity testing. All samples must be collected in accordance with acceptable laboratory procedures.

9.1.7 Processing, Labeling and Storage of Biological Samples

All blood samples will be processed, labeled and stored according to the Laboratory Manual or other appropriate guidelines provided to the site.

PBMCs will be collected, processed, labeled and stored according to trial site Standard Operating Procedures (SOP). Refer to the SOP for detailed instructions.

9.1.8 Safety Assessments

Safety assessments will include the collection and recording of solicited local (injection site) and systemic AEs, unsolicited AEs, AEs (serious and non-serious), pregnancies, and MAAEs from Visit 3 onwards for subjects randomized to Groups 1 and 2 following administration of the booster dose or placebo, respectively.

Any SAEs and any AEs leading to subject discontinuation and withdrawal will be collected for the entire trial duration for all subjects.

Refer to Section 10.1 for safety definitions and Section 10.4 for details on the collection and reporting of AEs.

9.1.9 Contraception and Pregnancy Avoidance Procedure

For female subjects of childbearing potential, pregnancy testing (urine) will be performed prior to administration of the TDV booster or placebo at Visit 3. Results must be confirmed and documented as negative prior to administration of the TDV booster or placebo at Visit 3. 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.

Female subjects of child-bearing potential who are sexually active with men, are advised to use an acceptable contraceptive method for at least 2 months prior to Visit 3. 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 at Visit 1 stating that they understand the requirements for avoidance of pregnancy and donation of ova. Further guidance with respect to the avoidance of pregnancy will be provided to all subjects of childbearing potential at Visit 2, and to all subjects of childbearing potential randomized to Group 1 or 2 at Visit 3 as part of the trial procedures (Section 2.1). Females of childbearing potential who are randomized to Group 1 or 2 and are sexually active, must also be reminded at Visit 3 and at Visit 4 to adhere to acceptable contraceptive methods for up to 6 weeks after TDV booster or placebo administration; they will also be advised not to donate ova or breastfeed during this period.

Acceptable birth control methods are defined as one or more of the following:

a) Hormonal contraceptive (such as oral, injection, transdermal patch, implant, cervical ring).

b) Barrier method (condom with spermicide or diaphragm with spermicide) every time during intercourse.

c) IUD.

d) Monogamous relationship with a vasectomized partner. Partner must have been vasectomized for at least 6 months prior to Visit 3.

For further details refer to Section 7.3.

9.1.10 Pregnancy

To ensure subject safety and the safety of the unborn child, each pregnancy in a subject having received the TDV booster or placebo must be reported to the sponsor within 24 hours of the site learning of its occurrence. 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 administration of the TDV booster or placebo should be reported immediately, using a pregnancy notification form, to the contact listed in the Investigator Site File.

Should the pregnancy occur after administration of the blinded TDV booster dose or placebo, the investigator must inform the subject of their right to receive information concerning the TDV booster or placebo 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.

9.1.11 Documentation of Subjects Who Are Not Considered Eligible for Trial Entry (Visit 1) or Randomization (Visit 3)

Investigators must account for all subjects who sign an informed consent or who have a signed pediatric assent form. If the subject is not eligible for trial entry at Visit 1 or for randomization to receive the TDV booster or placebo at Visit 3, the investigator should complete the eCRF accordingly.

The primary reason for denial of trial entry (at Visit 1) is to be recorded in the eCRF using the following categories:

a) Screen failure (did not meet one or more inclusion criteria at trial entry or did meet one or more exclusion criteria at trial entry)

b) Failed to meet the criteria for 'booster eligibility' (met one of more of the criteria for 'booster eligibility')

c) Withdrawal by subject

d) Site terminated by sponsor

e) Trial terminated by the sponsor

f) Other (specify reason)

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

9.2 Monitoring Subject Compliance

The investigator records all injections of trial vaccine (TDV booster/placebo) given to the subject in the subject's source document and the eCRF.

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).

9.3.1 Site Visits Prior to TDV Booster or Placebo Administration (Visit 1 and Visit 2)

Site visits that occur prior to administration of the TDV booster or placebo and do not include a vaccination will be performed at Visit 1 and Visit 2.

Additional procedures to be performed at Visit 1:

1. Before performing any trial procedure, an ICF or informed consent and pediatric assent form must be signed. Refer to Section 9.1.1.
2. Check inclusion and exclusion criteria for entry. Refer to Sections 7.1 and 7.2.
3. Collect demographic data, medical history update, and prior medication update. Refer to Section 9.1.2.
4. Perform a complete physical examination. Refer to Section 9.1.4.

Procedures to be performed at Visit 1 and Visit 2 include:

1. Concomitant vaccination update.
2. Check vital signs. Refer to Section 9.1.5.
3. Collect and record any SAEs and any AEs leading subject discontinuation or withdrawal. Refer to Sections 10.4.4 and 10.4.1.
4. Check that the subject does not meet any of the criteria for delay of blood sampling. Refer to Section 7.4.
5. Collect a blood sample from all subjects (one additional 40 mL blood sample should be also collected from subjects in the CMI subset). Blood should be taken from subjects using an aseptic venipuncture technique for serological immunogenicity testing. Refer to Sections 9.1.6 and 9.1.7.

9.3.2 Pre-Vaccination Procedures (Visit 3)

Before performing any further protocol-directed procedures, all sites in Mexico should check that the subject has re-consented using the updated ICF or an updated informed consent and pediatric assent form, as applicable, corresponding to protocol amendment 3, dated 22 August 2022. Refer to Section 9.1.1 for details.

Prior to administration of the TDV booster or placebo at Visit 3 the following procedures will be undertaken:

1. Concomitant vaccination update.
2. Check vital signs. Refer to Section 9.1.5.
3. Collect and record any SAEs and any AEs leading to subject discontinuation or withdrawal. Refer to Sections 10.4.4 and 10.4.1.
4. Perform pregnancy testing in female subjects of childbearing age. Refer to Section 9.1.10.
5. Review the criteria for 'booster eligibility'. Refer to Section 7.3.
   a. Any subject who fails to meet the criteria for 'booster eligibility' will end the trial following blood sample collection(s) at Visit 3. Refer to Section 9.3.7.
   b. Subjects who meet the criteria for 'booster eligibility' will proceed to randomization.
6. Randomization. Refer to Section 9.1.3.
7. Collect medical history, prior medication and concomitant medications update. Refer to Section 9.1.2
8. Perform a complete physical examination. Refer to Section 9.1.4.
9. Review of systems: review of systems is a structured interview that queries the subject OR the subject's LAR as to any complaints the subject has experienced across each organ system.
10. Check that the subject does not meet any of the criteria for delay of blood sampling. Refer to Section 7.4.
11. Collect blood samples from all subjects (one 10 mL blood sample and one 40 mL from all subjects, plus one additional 40 mL blood sample from subjects in the CMI subset). Blood should be taken from subjects using an aseptic venipuncture technique for serological immunogenicity testing. Refer to Sections 9.1.6 and 9.1.7.

9.3.3 Vaccination Procedures (Visit 3)

Vaccination procedures will only be performed for subjects who meet the criteria for 'booster eligibility' (Section 7.3) at Visit 3 and are randomized to Groups 1 (TDV) and 2 (placebo):

1. Check that the subject does not meet any of the criteria for delay, early termination, or premature discontinuation of the trial vaccine (Sections 7.4, 7.5, and 7.6).
2. Administer the trial vaccine (TDV booster/placebo) according to the procedures described in Sections 8.1.3 and 8.2.

9.3.4 Post-Vaccination Procedures (Visit 3)

The following post-vaccination procedures will be performed at Visit 3:

1. Observation for at least 30 minutes (refer to Section 8.2.1) including unsolicited AEs, solicited local (injection site) reactions, and body temperature measurement. Information should be recorded in the eCRF. All safety data will be collected in the subject's source documents.
2. Distribution of the diary card.
3. Careful training of the subject or the subject's LAR on how to measure solicited local (injection site) reactions and body temperature, 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) reactions 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 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. 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 collection of solicited local (injection site) reactions and systemic AEs (including body temperature measurements). The following additional rules apply to the documentation of safety information collected by 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 is 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 eCRF.
- The site must enter all readable entries on the diary card into the eCRF.
- 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's source document as a verbally reported event and therefore captured as an AE and recorded in the AE eCRF.
- Starting on the day of vaccination, the subject or the subject's LAR will check for specific types of events at the injection site (solicited local [injection site] reactions), any specific generalized symptoms (solicited systemic AEs), body temperature (any route may be used; where possible the same route should consistently be used for all temperature measurements), 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 take place in the evening at day's end.
- Body temperature measurement is to be performed using the thermometer provided by the site. 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 a fever, the highest body temperature observed that day should be recorded on the diary card.
- The measurements of solicited local (injection site) reactions are to be performed using the ruler provided by the site.
- The collection of solicited local (injection site) reactions and solicited systemic AEs (including body temperature) on the diary card will continue for a total of 7 days and 14 days, respectively, following trial vaccine (TDV booster/placebo) administration. The collection of unsolicited AEs and medications on the diary card will continue for 28 days following trial vaccine (TDV booster/placebo) administration by interview.

4. The site should schedule the next trial visit (Visit 4) with the subject or subject's LAR.
5. Provide female subjects of childbearing age with pregnancy avoidance guidance and information on acceptable methods of contraception. Refer to Sections 9.1.9 and 9.1.10.
6. Collect and record unsolicited AEs. Refer to Section 10.4.1.

7. Collect and record MAAEs. Refer to Section 10.4.3.
8. Collect and record any post-vaccination SAEs. Refer to Section 10.4.4.

The subject or the subject's LAR will receive a written reminder of the next planned trial activity (Visit 4). The subject or the subject's LAR will be reminded to complete the diary card daily and 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 (Visit 4 and Visit 5)

A site visit will be performed after the administration of the TDV booster or placebo at Visit 4. This visit should occur at least 29 days after TDV booster or placebo administration. A site visit will also be performed at Visit 5.

The following procedures will be performed at Visit 4:

1. A review of the diary card with the subject or subject's LAR to:
    - Collect and record solicited local (injection site) and systemic AEs. Refer to Section 10.4.2.
    - Collect and record persistent/prolonged solicited local (injection site) and systemic AEs. Refer to Section 10.4.2
    - Collect and record unsolicited AEs. Refer to Section 10.4.1.
    - Collect and record MAAEs. Refer to Section 10.4.3.
    - Collect and record any SAEs and any AEs leading subject discontinuation or withdrawal. Refer to Sections 10.4.4 and 10.4.1.

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. For further details see Section 9.3.4.

2. Update concomitant medications and vaccinations. Refer to Section 9.1.2
3. Perform a symptom-directed physical examination. Refer to Section 9.1.4.
4. Check vital signs. Refer to Section 9.1.5.
5. Provide female subjects of childbearing age with pregnancy avoidance guidance and information on acceptable methods of contraception. Refer to Sections 9.1.9 and 9.1.10.
6. Check that the subject does not meet any of the criteria for delay of blood sampling. Refer to Section 7.4.
7. Collect a 10 mL blood sample from all subjects randomized to Groups 1 and 2. Collect an additional 10 mL blood sample and one 40 mL from subjects in the CMI subset. Blood

should be taken from subjects using an aseptic venipuncture technique for serological immunogenicity testing. Refer to Sections 9.1.6 and 9.1.7.

8. The site should schedule the next site visit or other trial activity with the subject or the subject's LAR (Visit 5).

The subject or the subject's LAR will receive a written reminder of the next planned trial activity (Visit 5). 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.

The following procedures will be performed at Visit 5:

1. Concomitant vaccination update.
2. Check vital signs. Refer to Section 9.1.5.
3. Check that the subject does not meet any of the criteria for delay of blood sampling. Refer to Section 7.4.
4. Collect a 10 mL blood sample from all subjects randomized to Groups 1 and 2. Collect an additional 10 mL blood sample and one 40 mL from subjects in the CMI subset. Blood should be taken from subjects using an aseptic venipuncture technique for serological immunogenicity testing. Refer to Sections 9.1.6 and 9.1.7.
5. Proceed to end of trial procedures (Section 9.3.7).

9.3.6 Phone Contact – Reminder Call (Day 180 [M6])

A reminder phone call will be performed as a retention strategy between Visit 1 and Visit 2 on Day 180 (M6). The purpose of this call is to maintain contact with the subject/the subject's LAR and to remind the subject/the subject's LAR about any upcoming site visits. If the subject/the subject's LAR wishes to describe safety information, this information should only be collected by a trained healthcare professional at the site, and the safety data described must be written down in source documents. The subject/the subject's LAR should be reminded to write the information down in the diary card and to contact the site via the telephone number provided in the informed consent/pediatric assent form to discuss medical questions. A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18).

Telephone contacts will also be made for those subjects who are still under monitoring for safety reporting when a site visit cannot be carried out due to exceptional circumstances such as the COVID-19 pandemic.

9.3.7 Final (End of Trial) Visit

For subjects who fail to meet the criteria for 'booster eligibility' at Visit 3, the final (end of trial) visit will be performed at Visit 3. For those subjects who meet the criteria for 'booster eligibility' and are randomized to Group 1 or Group 2, the final (end of trial) visit will be performed at

Visit 5. If a subject terminates earlier, the final (end of trial) visit procedures should be performed at their last trial visit, if possible. For all subjects receiving trial vaccine (TDV booster/placebo), the investigator must complete the End of Trial eCRF page, check vital signs (refer to Section 9.1.5), collect and record MAAEs (refer to Section 10.4.3), collect and record any SAEs and any AEs leading subject discontinuation or withdrawal (refer to Sections 10.4.4 and 10.4.1), and perform a review of systems (a structured interview that queries the subject OR the subject's LAR as to any complaints the subject has experienced across each organ system).

9.3.8 Post-Trial Care

No post-trial care will be provided.

9.4 Biological Sample Retention and Destruction

In this trial, specimens for immune response testing will be collected 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 that was contracted by the sponsor for this purpose for up to but not longer than 20 years or as required by applicable law. The sponsor has put into place a system to protect the subjects' personal information to ensure optimal confidentiality and defined standard processes for sample and data collection, storage, analysis, and destruction.

Serum 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 the disease or the investigational vaccine that will allow more reliable measurement of the response to the investigational vaccine.

10.0 ADVERSE EVENTS

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

10.1 Definitions

10.1.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence in a clinical investigation subject administered a trial vaccine or placebo (inclusive of the TDV booster, placebo or TDV administered as part of the primary vaccination series in the parent trials); it does not necessarily have to have a causal relationship with trial vaccine administration.

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

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

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

10.1.2 Solicited Adverse Events

The occurrence of selected indicators of safety will be measured/collected for 7 days (solicited local [injection site] AEs) and 14 days (solicited systemic AEs) following administration of the trial vaccine dose (including the day of administration) and will be recorded on the "Local and Systemic Reactions" eCRF page as applicable and as listed in Table 10.a.

Any solicited local or systemic AE observed as continuing on Day 8 and Day 15, respectively, following administration of the trial vaccination or placebo, will be recorded as an AE on the Adverse Event eCRF for follow-up. For these persistent/prolonged solicited AEs the end date

will be captured on the Adverse Event eCRF to permit a separate analysis from the unsolicited AEs (see Section 10.4.2).

Table 10.a Solicited Local (Injection Site) Reactions and Systemic AEs

Local (injection site) reactions:	Pain
	Erythema
	Swelling
Systemic AEs:	Fever [(a)]
	Headache
	Asthenia
	Malaise
	Myalgia

(a) Fever is defined as body temperature greater than or equal to 38°C (100.4°F) regardless of method taken [18].

The intensity of solicited safety parameters will be assessed as described in Table 10.b.

Table 10.b Solicited Safety Parameters

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

Table 10.b Solicited Safety Parameters (continued)

Adverse Event	Intensity Grade	Intensity
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 greater than or equal to 38°C (100.4°F) regardless of method taken [18].

10.1.3 Adverse Events of Special Interest

Not applicable.

10.1.4 Medically-Attended Adverse Events

Medically attended AEs (MAAE) 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 (SAEs)

An SAE is defined as any untoward medical occurrence that:

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

10.2 Causality of AEs

Relationship (causality) to the trial vaccine and placebo will also be assessed by the investigator. The relationship of each AE to the trial vaccine and 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 the trial vaccine and the AE (without determining the extent of probability); there is a reasonable possibility that the trial vaccine contributed to the AE.
Not Related:	There is no suspicion that there is a relationship between the trial vaccine and the AE; there are other more likely causes and administration of the trial vaccine 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 trial vaccine discontinuation or a change in concomitant medication.
- Be considered unfavorable by the investigator for any reason.

Diagnoses versus 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 the trial vaccine 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 AEs

All AEs, whether considered related to the use of the TDV booster 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 eCRF and on the SAE form[1], 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 the TDV booster or placebo during site visits via interview. AEs leading to discontinuation from the trial are collected throughout the trial and will be summarized up to Visit 3 (pre-vaccination) and separately thereafter (post-vaccination) by trial group up to Visit 5.

The following information will be documented for each event:

- Reported term for the AE
- Start and end date
- Serious (Y/N)
- Severity
- Investigator's opinion of the causality (relationship) between the event and administration of trial vaccine(s) or placebo ("related" or "not related")
- Investigator's opinion of the causality (relationship) to trial procedure(s), including the details of the suspected procedure
- Outcome of event

10.4.2 Collection and Reporting of Solicited AEs

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 the TDV booster or placebo dose at Visit 3 (inclusive of the day of administration), and will be recorded on the "Local and Systemic AEs" eCRF 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 Day 8 and Day 15, respectively, following the trial vaccination or placebo, will be additionally recorded as an AE on the Adverse Event eCRF for follow-up. For these persistent/prolonged solicited AEs, the end date will be captured on the Adverse Event eCRF 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 eCRF page.

1. Solicited local (injection site) reactions or systemic AEs that lead the subject to withdraw from the trial.

[1] SAE reporting will be done by 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.

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

10.4.3 Collection and Reporting of MAAEs

MAAEs will be collected by close monitoring of Groups 1 and 2, following administration of the TDV booster or placebo from Visit 3 through Visit 5. 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 AEs on the AE eCRF page. MAAEs will be summarized separately at the end of the trial.

10.4.4 Collection and Reporting of SAEs

Collection of SAEs will commence from the time that the subject is enrolled in the trial (Visit 1). Routine collection of SAEs will continue until the end of the trial (Visit 3 for subjects who fail to meet the criteria for 'booster eligibility' and Visit 5 for all other subjects).

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:

1. A short description of the event (indicating whether the event occurred pre- or post-vaccination) and the reason why the event is categorized as serious
2. Causality assessment
3. Protocol number
4. Subject identification number
5. Investigator's name

The SAE form should be transmitted within 24 hours 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.

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 IRBs or IECs, 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 the trial vaccine or placebo administration or in the overall conduct of the trial. The investigational site also will also forward a copy of all expedited reports to their IRB or IEC 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 the trial vaccine 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 in the Investigator Site File.

11.0 TRIAL-SPECIFIC REQUIREMENT(S)

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.

12.1 CRFs (Electronic)

Completed eCRFs are required for each subject who provides a signed informed consent/ pediatric assent form.

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

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 eCRFs are recorded in an audit trail that captures the old information, the new information, identification of the person making the correction, the date the correction was made, and the reason for the change. Reasons for significant corrections should additionally be included.

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

eCRFs will be reviewed for completeness and acceptability at the trial site during periodic visits by trial monitors. 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 eCRFs. The completed eCRFs are the sole property of the sponsor and should not be made available in any form to third parties, except for authorized representatives of appropriate governmental health or regulatory authorities, without written permission of the sponsor.

When a site visit cannot be carried out due to exceptional circumstances such as the COVID-19 pandemic, telephone contacts will be made for subjects who are still under monitoring for safety reporting. Refer also to Section 14.1.

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

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

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

13.1 Statistical and Analytical Plans

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

Blinded data reviews will be conducted prior to the unblinding of subjects' trial arm assignment. These reviews will assess the accuracy and completeness of the trial database and subject evaluability.

13.1.1 Analysis Sets

The following analysis sets are defined for this trial:

All Screened: All subjects who agreed to participate in the current trial.

All Screened-Booster: All subjects who agreed to participate in the current trial and who were screened for 'booster eligibility' to determine if they were eligible to go on to be randomized to Group 1 or Group 2.

Randomized Set-Booster: All subjects randomized at Visit 3 regardless of whether they received the trial vaccination in the current trial. Subjects in this set will be summarized according to randomized treatment.

Safety Set (SAF): All subjects who agreed to participate in the current trial and who received at least one dose of Takeda's TDV in the parent trials.

Safety Set-Booster (SAF-B): All subjects who received at least one dose of Takeda's TDV in the parent trials and who received the TDV booster or placebo in the current trial.

Full Analysis Set (FAS): All subjects who received at least one dose of Takeda's TDV in the parent trials and for whom there is at least one valid follow-up measurement up to Visit 3 for immunogenicity assessments in the current trial.

Full Analysis Set-Booster (FAS-B): All subjects who received at least one dose of Takeda's TDV in the parent trials, the TDV booster or placebo in the current trial, and for whom there is at least one valid follow-up measurement after administration of the TDV booster or placebo at Visit 3 for immunogenicity assessments in the current trial.

Per Protocol Set (PPS): All subjects from the FAS who received two doses of Takeda's TDV in the parent trials with no new major protocol violations prior to administration of the booster or placebo at Visit 3 that could potentially confound the primary endpoints in the current trial.

Per Protocol Set-Booster (PPS-B): All subjects from the FAS-B who received two doses of Takeda's TDV in the parent trials with no new major protocol violations after administration of the TDV booster or placebo at Visit 3 that could potentially confound the primary endpoints in the current trial.

The major protocol violation criterion will be defined as part of a data review prior to analysis. The categories of new major protocol violations include: (1) not meeting selected entry criteria, (2) receiving the wrong trial vaccination at Visit 3 (subjects randomized to Groups 1 and 2 only), (3) receiving prohibited vaccinations or therapies (subjects randomized to Groups 1 and 2 only), (4) performing Visit 4 inadmissibly outside the visit window (subjects randomized to Groups 1 and 2 only), and (5) other major protocol violations that may be identified during data reviews.

13.1.2 Analysis of Demographics and Other Baseline Characteristics

Age, gender, race, and other baseline characteristics (including age at informed consent in the parent trial) will be summarized descriptively for all enrolled subjects upon entry into the current trial.

13.1.3 Immunogenicity Analysis

For the primary and secondary immunogenicity endpoints (ie, GMTs of neutralizing antibodies and seropositivity rates for each of the 4 dengue serotypes and multiple [2, 3 or 4]), descriptive statistics and 95% CIs will be provided for each applicable visit (Visit 1, Visit 2, Visit 3, Visit 4, and Visit 5 in the current trial, and for Day 120 [Month 4] and Day 270 [Month 9] in the parent trials [which corresponds to 4 months and 9 months after the first vaccination in the primary vaccination series in the parent trials, respectively]).

For the visit comparisons GMRs will be summarized descriptively, including 95% CIs.

Seropositivity is defined as a reciprocal neutralizing titer ≥10. Other immunogenicity measurements obtained at baseline and any post-vaccination visits in the parent trials may be accessed from databases to contribute to designated summaries of immunogenicity endpoints over time following vaccination.

The primary immunogenicity analyses will be based on the PPS/PPS-B; sensitivity analyses may be provided based on the FAS/FAS-B.

Similar descriptive analyses as for the primary immunogenicity endpoint will be provided for the exploratory endpoints for each applicable assay at all relevant time points, based on the PPS/PPS-B. Supportive analyses based on the FAS/FAS-B may also be provided for selected endpoints.

Further details on the statistical analysis including exploratory endpoints will be provided in the SAP.

13.1.4 Safety Analysis

All summaries of safety data will be based on subjects in the SAF/SAF-B.

Solicited AEs

Presence and severity (Grade) of solicited local reactions (injection site pain, injection site erythema and injection site swelling) and solicited systemic AEs (fever, asthenia, malaise, headache and myalgia) will be collected by diary card for 7 days and 14 days, respectively, following administration of the TDV booster or placebo.

For each solicited AE, the number and percentage of subjects with local (injection site) reactions and systemic AEs will be summarized by trial group and event severity for each day following administration of the TDV booster or placebo (Day 1 through Day 7 for local [injection site] reactions 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 (injection site) reactions or systemic AEs continuing on Day 8 and Day 15 (after administration of the TDV booster or placebo), respectively, will be assessed separately. Unless otherwise specified these reactions/AEs will not be included in the analyses/tabulations of unsolicited AEs and will have separate listings.

Unsolicited AEs

Unsolicited AEs will be summarized by trial group for 28 days following administration of the TDV booster or placebo (day of vaccination + 27 days). Unsolicited AEs will be coded using the latest version of MedDRA and summarized by Preferred Term (PT) and System Organ Class (SOC) for each trial group. AEs leading to trial withdrawal will be summarized up to Visit 3 (pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5.

Unsolicited AEs will be tabulated at each of the following levels: overall summary (subjects with at least 1 AE), and by SOC and PT. In addition, unsolicited AEs will be summarized as follows: by PT including events with frequency greater than a pre-defined 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 the trial vaccine (TDV booster or placebo). Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once.

MAAEs

MAAEs will be collected for subjects randomized to Group 1 and Group 2 and will be presented by trial group from Visit 3 through Visit 5. MAAEs will be coded using MedDRA and summarized by SOC and PT for each trial group.

SAEs

SAEs will be collected throughout the trial. SAEs will be coded using MedDRA and summarized by PT and SOC up to Visit 3 (pre-vaccination) and thereafter (post-vaccination) by trial group up to Visit 5.

13.2 Interim Analysis and Criteria for Early Termination

Due to significant delays (of >2 years) to booster administration for subjects from parent trial DEN-315 (Mexico), an interim analysis (IA) of the safety and immunogenicity data collected at trial sites in the United States is planned when all subjects from parent trial DEN-304 (United States) have completed their last trial visit (Visit 5 [Day 630 (M21)]). This IA will be performed in an unblinded manner and will include the necessary steps to ensure that no database modifications are made after unblinding for subjects at trial sites in the United States. Unblinding of subjects from parent trial DEN-315 (Mexico) will occur after all subjects at trial sites in Mexico have completed their last trial visit (Visit 5 [Day 1440 (M48)]) and the trial database has been locked. No modifications to the trial are planned based on the results of this IA. An interim CSR of data from parent trial DEN-304 (United States) will not be prepared; all trial results will be reported in the final CSR. More details regarding the IA will be provided in the SAP.

13.3 Determination of Sample Size

This trial is designed to be descriptive and is not based on testing formal null hypotheses. Therefore, the sample size was not determined based on formal statistical power calculations. The number of subjects is considered to be sufficient for the evaluation of the objectives of the trial.

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 eCRFs. Source documents are defined as original documents, data, and records. The investigator and institution guarantee access to source documents by the sponsor or designee (contract 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 vaccine 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 eCRFs 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 such as the COVID-19 pandemic, alternative monitoring approaches such as remote source data verification or telephone contact may be used to ensure data quality and integrity and to maintain subject safety. Alternative monitoring approaches should be used only where allowed by the local Health Authority and when approved by the IRB/IEC. During remote monitoring, the monitor should focus on trial activities that are essential to the safety of trial subjects and/or data reliability.

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. 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 [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 state, federal, and 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. Those US sites unwilling to provide names and titles of all members due to privacy and conflict of interest concerns should instead provide a Federal Wide Assurance Number or comparable number assigned by the Department of Health and Human Services.

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 IRBs or IECs 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 the trial vaccine(s)/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 notification no protocol activities, including screening may occur.

Sites must adhere to all requirements stipulated by their respective IRB or IEC. This may include notification to the IRB or IEC regarding protocol amendments, updates to the informed consent/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 [1] and the ICH Guidelines [2] 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/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.

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 subject's status of 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 ICF.

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/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/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 eCRF. 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.

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 eCRF, 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 IRBs and IECs 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 eCRF).

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/or EudraCT, according to local requirements, before trial initiation. 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

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

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, 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).

In case the deadline for results disclosure cannot be met, an application for extension with scientific justification will be initiated.

In line with EC Regulation N°1901/2006 [19], the sponsor will submit a summary of the results of a pediatric trial within 6 months of completion and irrespective of whether it is part of a Pediatric Investigational Plan (completed or not yet completed) or not, or whether it is intended for submission later on as part of a variation, extension or new stand-alone marketing authorization application or not.

15.4.3 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 Study Site Agreement regarding the sponsor's policy on subject compensation and treatment for injury. If the investigator has questions regarding this policy, he or she should contact the sponsor or sponsor's designee.

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Appendix A Responsibilities of the Investigator

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

The investigator agrees to assume the following responsibilities:

1. Conduct the 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 21 Code of Federal Regulations (CFR) Part 56, ICH, and local regulatory requirements.
6. Ensure that the IRB/IEC will be responsible for initial review, continuing review, and approval of the protocol. Promptly report to the IRB/IEC all changes in research activity and all anticipated risks to subjects. Make at least yearly reports on the progress of the 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 21 Code of Federal Regulations (CFR) Part 50, 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 eCRFs, hospital records, laboratory results, etc, and maintain these data for a minimum of 2 years following notification by the sponsor that all investigations have been discontinued or that the regulatory authority has approved the marketing application. The investigator should contact and receive written approval from the sponsor before disposing of any such documents.
10. Allow possible inspection and copying by the regulatory authority of GCP-specified essential documents.

11. Maintain current records of the receipt, administration, and disposition of sponsor-supplied vaccines, and return all unused sponsor-supplied vaccines to the sponsor.
12. Report AEs to the sponsor promptly. In the event of an SAE, notify the sponsor within 24 hours.
13. Review and provide a signature as approval of the content of the CSR.

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.

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

Please note that this consent will not cover investigator personnel.

Signature Page for DEN-303 Protocol Amendment 3, Version 5.0, 22 August 2022

Title:

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