Clinical Study Protocol

EU PAS Number: EUPAS1000000218

Title: The Association Between Prior Exposure to Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) and Dengue Hospitalization in a Pediatric and Adolescent Population: a Nested Case-Control Post-Authorization Effectiveness Study

Study Number: DEN-401

Document Version and Date: Version 2.0, 25 May 2023

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

OBSERVATIONAL POST-AUTHORIZATION EFFECTIVENESS STUDY

PROTOCOL AMENDMENT 1

Study title: The Association Between Prior Exposure to Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) and Dengue Hospitalization in a Pediatric and Adolescent Population: a Nested Case-Control Post-Authorization Effectiveness Study

Study number: DEN-401

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Ethics statement: This study will be conducted in compliance with the protocol, the Declaration of Helsinki, International Society for Pharmacoepidemiology Guidelines for Good Epidemiology Practices, European Network of Centres for Pharmacoepidemiology and Pharmacovigilance Guidelines for Methodological Standards in Pharmacoepidemiology, Good Pharmacovigilance Practices, and all applicable regulatory requirements.

Signature page

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Study number: DEN-401

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Sponsor

Name: <name and title>

Signature and date (DD/Month/YYYY)

_____/_____/_____

Investigator

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

- The ethical principles that have their origin in the International Ethical Guidelines for Biomedical Research Involving Human Study participants as set by the CIOMS.
- All applicable laws and regulations, including, without limitation, data privacy laws and regulations.
- Terms outlined in the Clinical Study Site Agreement.

Name:

Signature and date (DD/Month/YYYY)

Address:

_____/_____/_____

[Copy lines above if more than one investigator needs to sign.]

Study Information

Title	The Association Between Prior Exposure to Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) and Dengue Hospitalization in a Pediatric and Adolescent Population: a Nested Case-Control Post-Authorization Effectiveness Study
Protocol version identifier	2.0
Date of last version of protocol	25 May 2023
EU PAS register number	Not available yet
Active substance	ATC code: J07BX04, dengue virus vaccines
Medicinal product	Dengue Tetravalent Vaccine (Live, Attenuated)
Product reference	H0005362, H0005155
Procedure number	EMEA/H/W/005362/0000/ EMEA/H/C/005155/0000
Marketing authorization holder	Takeda GmbH Byk-Gulden-Strasse 2 78467 Konstanz Germany
Joint PAS	No
Research question and objectives	This study seeks to assess TDV effectiveness against hospitalized virologically confirmed dengue (VCD) in a real-world setting. Specifically, the study aims to evaluate whether serotype-specific hospitalization (in particular hospitalization due to wild type dengue virus serotype 3 or 4 [DENV-3 or DENV-4]) is increased or decreased among individuals who were dengue seronegative at the time of TDV vaccination (ie, baseline seronegative), relative to seronegative persons who were not vaccinated. **Primary Objective** • To estimate the association between completed vaccination with TDV (as part of a vaccination program) and hospitalization due to VCD, including severe dengue.

	Secondary Objective • To estimate how the association between completed vaccination with TDV (as part of a vaccination program) and subsequent hospitalization due to VCD, including severe dengue, is modified by: - The infecting dengue serotype (DENV-1, DENV-2, DENV-3, and DENV-4). - The baseline dengue serostatus (seronegative and seropositive at baseline). - The infecting dengue serotype and the baseline dengue serostatus. **Exploratory Objectives** • To estimate the association between partial vaccination with TDV (as part of a vaccination program) and hospitalization due to VCD, including severe dengue. • To describe the association between completed vaccination with TDV (as part of a vaccination program) and clinical reason for hospitalization due to VCD (observation, co-existent conditions, warning signs, or severe dengue). • To estimate any modification of the association between completed TDV vaccination (as part of a vaccination program) and hospitalization due to VCD, including severe dengue, by prior flavivirus vaccination (Japanese encephalitis and/or yellow fever).
Countries of study	Multi-country study [This study is intended to be implemented in countries that have the intention of starting a TDV vaccination program and where the study is feasible. At present it is not known which countries will start a vaccination program.]

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Marketing Authorization Holder

Marketing authorization holder	Takeda GmbH Byk-Gulden-Strasse 2 78467 Konstanz Germany
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1.0 TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

2.0 LIST OF ABBREVIATIONS

AE	adverse event
AR	adverse reaction
CDD	clinically diagnosed dengue
CYD-TDV	developed by Sanofi Pasteur and sold under the brand name Dengvaxia - a tetravalent, live attenuated, chimeric dengue vaccine in a yellow fever 17D backbone
DENV	dengue virus
DENV-1, -2, -3, -4	wild type dengue virus serotypes 1, 2, 3, 4
DHF	dengue hemorrhagic fever
DSS	dengue shock syndrome
eCRF	electronic case report form
HIV	human immunodeficiency virus
HR	hazard rate
HRR	hazard rate ratio
ICF	informed consent form
ICSR	individual case safety reports
IEC	independent ethics committee
IRB	institutional review board
JE	Japanese encephalitis
LAR	legally acceptable representative
LMP	last menstrual period
M	month
NS1	nonstructural protein 1
PI	principal investigator
PoC	point of care
RDT	rapid diagnostic test
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
SSR	special situation report
TDV	Dengue Tetravalent Vaccine (Live, Attenuated), the Takeda dengue vaccine candidate also known as TAK-003, is referred to as TDV
VCD	virologically confirmed dengue
WHO	World Health Organization
YF	yellow fever

3.0 RESPONSIBLE PARTIES

3.1 Main Authors of the Protocol

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[redacted]

[redacted] Data Science, P95

Email: [redacted]

3.2 Principal Investigators

At least one principal investigator (PI) will be appointed per country by the Ministry of Health. See Appendix A, Document 4 for details.

3.3 Co-Investigators

See Appendix A, Document 4 for details.

3.4 Contract Research Organization or Other Service Provider

See Appendix A, Document 5 for details.

4.0 ABSTRACT

Title

The Association Between Prior Exposure to Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) and Dengue Hospitalization in a Pediatric and Adolescent Population: a Nested Case-Control Post-Authorization Effectiveness Study.

Version: 2.0 – 25 May 2023

Authors: [redacted], Takeda Vaccines Business Unit; [redacted], Takeda Vaccines Business Unit; [redacted], Data Science, P95.

Rationale and Background

Dengue is considered the most common and rapidly spreading vector-borne viral disease worldwide.

Takeda Vaccines, Inc. has developed the Dengue Tetravalent Vaccine (Live, Attenuated), known as TDV. The Takeda DEN-301 Phase 3 randomized clinical trial showed overall efficacy of TDV against virologically confirmed dengue (VCD) and against VCD requiring hospitalization. However, the trial was not powered to assess TDV efficacy against VCD requiring hospitalization categorized by serotype and baseline serostatus.

Research Questions and Objectives

This study seeks to assess TDV effectiveness against hospitalized VCD in a real-world setting. Specifically, the study aims to evaluate whether serotype-specific hospitalization (in particular hospitalization due to DENV-3 or DENV-4) is increased or decreased among individuals who were dengue seronegative at the time of TDV vaccination (ie, baseline seronegative), relative to seronegative persons who were not vaccinated.

Primary Objective:

- To estimate the association between completed vaccination with TDV (as part of a vaccination program) and hospitalization due to VCD, including severe dengue.

Secondary Objective:

- To estimate how the association between completed vaccination with TDV (as part of a vaccination program) and subsequent hospitalization due to VCD, including severe dengue, is modified by:
  - The infecting dengue serotype (DENV-1, DENV-2, DENV-3, and DENV-4).
  - The baseline dengue serostatus (seronegative and seropositive at baseline).
  - The infecting dengue serotype and the baseline dengue serostatus.

Exploratory objectives:

- To estimate the overall association between partial vaccination with TDV as part of a vaccination program and hospitalization due to VCD, including severe dengue.
- To describe the association between completed vaccination with TDV as part of a vaccination program and clinical reason for hospitalization due to VCD (observation, co-existent conditions, warning signs, or severe dengue).
- To estimate any modification of the association between completed TDV vaccination as part of a vaccination program and hospitalization due to VCD, including severe dengue, by prior flavivirus vaccination (Japanese encephalitis [JE] and/or yellow fever [YF]).

Study Design

This is a multi-country, multi-site, observational, post-authorization, hospital-based, case-control study nested within a defined cohort (nested case-control study). Vaccination status of participants hospitalized for VCD (cases) will be compared with that of participants who are not hospitalized for VCD (controls).

Population

This study will comprise pediatric and/or adolescent participants age-eligible to be vaccinated with TDV in a dengue vaccination program. The participant's parents or legally acceptable representative (LAR) must sign a written informed consent form (ICF) and agree to provide a baseline serum sample to participate in the study.

Variables

For the entire cohort, demographic data (including age and gender) and a baseline serum sample will be collected. The participant's telephone number and residential address will be collected at the site level for the entire cohort for operational reasons only. For potential cases (admitted to hospital with clinically diagnosed dengue [CDD]) and matched controls, medical and vaccination history, and dengue prevention measures employed will be collected. For potential cases only, relevant hospitalization data and a blood sample for a reverse transcription polymerase chain reaction (RT-PCR) test will be obtained.

Data Sources

Data will be collected in real-time during the period of hospital admission using a questionnaire, clinical records, and by interview. Clinical data and vaccination history will be collected by reviewing medical health records and/or vaccination record book(s). Information from controls will be collected by interview.

Study Size

A cohort of 70,000 participants is expected to result in 902 outcomes of hospitalization due to VCD caused by any serotype over a study period of 3 years in unvaccinated participants (180/902 are expected to be due to DENV-3 and 126/902 are expected to be due to DENV-4). Of the expected 902 DENV hospitalizations, 180 outcomes are expected to be in baseline seronegative participants (36/180 are expected to be due to DENV-3 and 25/180 are expected to be due DENV-4). These calculations assume that 20% of the population is baseline seronegative, a 1% annual incidence of hospitalization due to VCD, a DENV-3 prevalence of 20%, and a DENV-4 prevalence of 14%. A 50% vaccine coverage rate and a 7% annual drop-out rate from the cohort are also assumed.

Proof of concept simulation-based sample size calculations indicate that a cohort of 70,000 participants is expected to yield a power of 80% to rule out an increased risk of ≥2 for hospitalization due to DENV-3 in the vaccinated seronegative population (with a 5% Type I error).

Data Analysis

Conducted using a conditional logistic regression model with log hazard rate ratio (HRR) as the output.

Milestones

Start of data collection, Year 1 report, Year 2 report, end of data collection, and final study report.

5.0 AMENDMENTS AND UPDATES

Number	Date	Section of Study Protocol	Amendment or Update	Reason
1	25 May 2023	Study Information	Research question updated to include DENV-4; dengue serotypes (including DENV-4) added to the relevant secondary objective; and serostatus categories added to the relevant secondary objective for clarity and consistency.	[redacted]
		4.0	Research question updated to include DENV-4; dengue serotypes (including DENV-4) added to the relevant secondary objective; and serostatus categories added to the relevant secondary objective for clarity and consistency.	[redacted]
		4.0	Study size updated to include expected number of hospitalizations	[redacted]
		7.0	Outdated reference removed from Paragraph 5	Necessary update
		8.0	Research question updated to include DENV-4; dengue serotypes (including DENV-4) added to the relevant secondary objective; and serostatus categories added to the relevant secondary objective for clarity and consistency.	[redacted]
		9.1	Figure 9.a footnote updated to align with changes made to the exclusion criteria text	To ensure consistency with Section 9.3.1
		9.2.4	Additional exclusion criterion added for controls	[redacted]
		9.2.4	Exclusion criteria for cases and controls split into 2 subsections	Required layout change
		9.2.5	Correction of a typographical error	Correction
		9.2.6.3	Maximum acceptable time added for baseline blood sample	[redacted]
		9.3.1	Case and control definitions updated to reflect changes made to the exclusion criteria	Required for clarity
		9.5.1	New subsection added to include additional data for expected number of hospitalizations	[redacted]
		9.5.2	Relevant text moved to new Subsection 9.5.1 with minor edits to the existing text	[redacted]
		9.8	DENV-4 added	[redacted]
		13.0	Outdated reference removed and access dates updated	Routine update

6.0 MILESTONES

Project Milestones	**Due by**
Start of data collection	2025
Year 1 report	2027
Year 2 report	2028
End of data collection	2029
Final report of study results	2029

7.0 RATIONALE AND BACKGROUND

Disease due to dengue is caused by infection with the dengue virus (DENV), a single positive-stranded ribonucleic acid (RNA) arthropod-borne virus that occurs as 4 recognized wild type dengue serotypes: DENV-1, DENV-2, DENV-3 or DENV-4 [1]. The dengue virus belongs to the genus *Flavivirus,* family *Flaviviridae,* and is mainly transmitted from human to human by mosquitoes (primarily *Aedes aegypti*) [2].

The World Health Organization (WHO) frequently cites 50 to 100 million as the annual estimated number of symptomatic cases of dengue [3]. Modelling groups have estimated 390 million (284-528 million) total dengue infections per year, of which 96 million (67-136 million) manifest as symptomatic infections (any level of disease severity) [4]. The number of dengue cases reported to the WHO increased over 8-fold in the last two decades, from less than 505,430 cases in 2000, to over 2.4 million in 2010, and 4.2 million in 2019 [5]. Thus, dengue is an important and growing global public health threat [3,4,5].

Clinically, infection with a dengue virus can result in a range of symptoms, from subclinical illness, to debilitating but transient fever, to a life-threatening disease [6]. Clinical dengue has been classified according to the 1997 WHO guideline as dengue fever, dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) [7]. In 2009, the WHO issued new clinical guidelines that classify dengue as dengue (with and without warning signs) and severe dengue [8,9] (see Appendix B).

The first exposure of an individual to any of the 4 dengue virus serotypes is known as the primary dengue infection and is usually asymptomatic [6]. Primary infection with any of the 4 dengue virus serotypes is thought to result in life-long protection from re-infection by the same serotype but does not protect against subsequent infections by the other dengue serotypes. Rather, secondary exposure to a heterotypic dengue serotype is associated with an increased risk of severe disease, this observation has been attributed to antibody dependent enhancement [10,11]. Between 2% and 3% of secondary dengue infections may progress to shock and death [6]. Thus, dengue infections are unusual in that the risk of more severe disease is increased in those who have antibodies to a previous dengue infection. However, third and fourth infections are much less likely to result in severe disease [12,13,14].

An estimated 500,000 people with dengue require hospitalization each year [15], although hospitalization rates vary according to local standards of care and clinical practice. The appropriate and timely clinical diagnosis of dengue are important determinants of the outcomes of the hospitalization [16].

The gold standard confirmatory test for dengue infection is RT-PCR, which detects the RNA of the dengue virus [17]. However, in many dengue endemic areas, laboratory diagnostic resources are limited, and RT-PCR testing is not available for individual diagnosis at the point of care (PoC). RT-PCR testing is mostly available for research and public health purposes (virological surveillance) in public health laboratories. PoC tests, such as antibody-based rapid diagnostic tests (RDTs) for dengue, have become increasingly used to complement clinical diagnosis in routine clinical practice, as they are convenient, prompt, and relatively affordable.

However, RDTs are of variable accuracy [18] and the RDT market remains largely unregulated [19].

The detection of DENV nonstructural protein 1 (NS1) through RDTs is a useful tool to assist in the clinical management of patients [20]. [redacted]

There is no specific treatment for dengue [21]. Treatment is based solely on the early detection of warning signs and control of symptoms, with supportive measures including fluid replacement required for severe dengue cases [8]. Appropriate treatment may prevent patient progression from non-severe to severe disease. If the patient recovers, there are no sequelae in non-severe dengue [21].

Currently, the primary preventative measure against dengue is vector control, though the effectiveness of various vector control measures remains uncertain [8,21,22]. In 2015, CYD-TDV, developed by Sanofi Pasteur, became the first licensed vaccine against dengue [23,24]. It is a live, attenuated, recombinant tetravalent vaccine employing the attenuated YF virus 17D strain as the replication backbone. It was licensed on the basis of one Phase 2b trial [25] and two Phase 3 trials conducted in the Asia-Pacific region [26] and in Latin America [27]. Efficacy was higher in participants with pre-existing dengue neutralizing antibodies than in those who were seronegative at the time of first vaccination, in the older age cohorts than the younger ones, and against DENV-3 and DENV-4 compared to DENV-1 and DENV-2 [26].

Takeda Vaccines, Inc. has developed TDV, a tetravalent dengue vaccine which consists of 1 molecularly characterized, attenuated dengue serotype 2 virus strain and 3 recombinant dengue virus strains expressing dengue serotype 1, 3, and 4 antigens (a dengue serotype 2/1 recombinant strain, a dengue serotype 2/3 recombinant strain, and a dengue serotype 2/4 recombinant strain). The proposed dose regimen consists of 2 subcutaneous doses of 0.5 mL TDV administered at Month (M)0 and M3 [28,29].

Data from the ongoing Phase 3 DEN-301 randomized trial conducted among children and adolescents 4 to 16 years of age in endemic regions of Asia and Latin America, (at 36 months post-second dose) showed an overall vaccine efficacy of 62.0% (95% CI: 56.6-66.7) against VCD and 83.6% (95% CI: 76.8-88.4) against hospitalized VCD [30]. In the per-protocol analyses, from 30 days post second dose to 12 months, vaccine efficacy was 80.2% (95% CI: 73.3-85.3) against VCD, with 95.4% efficacy against dengue leading to hospitalization (95% CI: 88.4-98.2), and 90.4% (95% CI: 82.6-94.7) against hospitalized VCD from 30 days post second dose to 18 months [31].

[redacted]

[redacted]

The totality of data on hospitalized VCD and severe forms of dengue, along with the clinical characteristics of these cases assessed during DEN-301, did not indicate an increased risk of hospitalization or severe forms of dengue in the 36-month post vaccination follow-up period. There were some gaps in the data for efficacy against hospitalization for VCD due to DENV-3 and DENV-4 in baseline seronegative individuals. For DENV-3 the data suggested a lack of efficacy. For DENV-4 there were insufficient data to make an assessment (DEN-301 Interim CSR [36 Months]).

This post-authorization effectiveness study has been designed to close the above-mentioned gaps. Applying a nested case-control design, the serotype-specific association between TDV vaccination and VCD hospitalization by baseline serostatus will be estimated.

8.0 RESEARCH QUESTION AND OBJECTIVES

This study seeks to assess TDV effectiveness against hospitalized VCD in a real-world setting. Specifically, the study aims to evaluate whether serotype-specific hospitalization (in particular hospitalization due to DENV-3 or DENV-4) is increased or decreased among individuals who were dengue seronegative at the time of TDV vaccination (ie, baseline seronegative), relative to seronegative persons who were not vaccinated.

8.1 Primary Objective

- To estimate the association between completed vaccination with TDV (as part of a vaccination program) and hospitalization due to VCD, including severe dengue.

8.2 Secondary Objective

- To estimate how the association between completed vaccination with TDV (as part of a vaccination program) and subsequent hospitalization due to VCD, including severe dengue, is modified by:
  - The infecting dengue serotype (DENV-1, DENV-2, DENV-3, and DENV-4).
  - The baseline dengue serostatus (seronegative and seropositive at baseline).
  - The infecting dengue serotype and the baseline dengue serostatus.

8.3 Exploratory Objectives

- To estimate the overall association between partial vaccination with TDV as part of a vaccination program and hospitalization due to VCD, including severe dengue.
- To describe the association between completed vaccination with TDV as part of a vaccination program and clinical reason for hospitalization due to VCD (observation, co-existent conditions, warning signs, or severe dengue).
- To estimate any modification of the association between completed TDV vaccination as part of a vaccination program and hospitalization due to VCD, including severe dengue, by prior flavivirus vaccination (JE and/or YF).

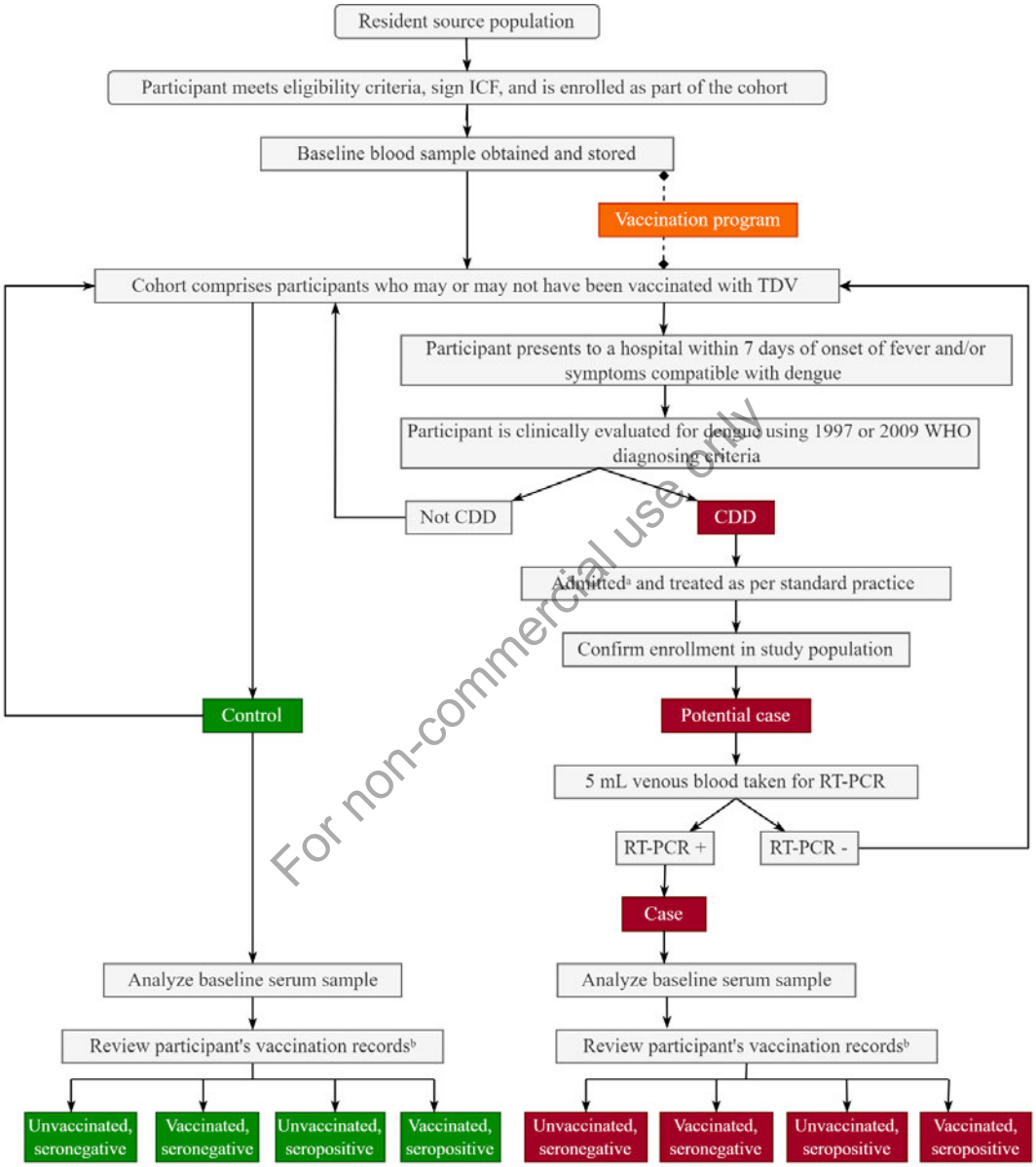
9.0 RESEARCH METHODS

9.1 Study Design

This is a multi-country, multi-site, observational, post-authorization, hospital-based, nested case-control study. Exposure (ie, vaccination) will not be allocated by a study protocol; it will be in accordance with the marketing authorization and decided by participation/non-participation in a public vaccination program. The planned study duration is 3 years, a study schematic is presented in Figure 9.a.

A cohort will be assembled from the resident source population in countries where a public vaccination program is planned. The cohort will comprise pediatric and adolescent participants eligible to be vaccinated with TDV as part of a Ministry of Health vaccination program. Getting vaccinated with TDV is not a requirement of the cohort; rather, the cohort will include both participants who later opt to receive TDV vaccination in the public program and those who decide not to.

Figure 9.a Study Design: Nested Case-Control Study of the Association Between TDV Exposure and VCD Hospitalization



Abbreviations: CDD, clinically diagnosed with dengue; ICF, informed consent form; RT-PCR, reverse transcription polymerase chain reaction; TDV, Dengue Tetravalent Vaccine (Live, Attenuated), the Takeda dengue vaccine candidate also known as TAK-003; WHO, World Health Organization.

Potential cases are defined as participants with CDD who are hospitalized and have had a blood sample taken for RT-PCR test, but whose test result is pending. Cases are defined as hospitalized study participants who meet the study inclusion and exclusion criteria for cases, have CDD, and have a confirmed positive RT-PCR test result (virologically confirmed dengue [VCD]). Controls are defined as study cohort participants who are matched to a case and meet the study inclusion and exclusion criteria for controls but have not been hospitalized with VCD on or before their case's index date.

a Admission can occur before or after CDD.

b Participants who have an unknown vaccination status, or are partially vaccinated, will only be included in sensitivity analyses.

9.2 Setting

9.2.1 Study Sites

The study will be conducted in countries where a public vaccination program with TDV will be implemented (dengue endemic areas). A feasibility assessment will be conducted to identify potential study sites and to ensure that the study design can be implemented. The feasibility assessment will look at: the availability of surveillance data for circulating DENV serotypes in the potential study area, if there will be reliable recording of TDV vaccination histories (ideally in a vaccine registry), and if the hospital-based surveillance in place will allow for the ascertainment of potential cases within the preferred 5 days from the onset of symptoms. Multiple study sites will be considered per country.

9.2.2 Study Population

This observational study will assess the real-world use of TDV based on the locally approved label/product information leaflet for TDV and national guidelines and will comprise pediatric and/or adolescent participants who are age-eligible for vaccination with TDV as part of a vaccination program. Persons meeting the eligibility criteria will be invited to participate in the study.

9.2.3 Inclusion Criteria

9.2.3.1 Cohort

To be eligible for inclusion in the study cohort, participants must meet the following inclusion criteria:

1. The participant is a child or adolescent eligible to be vaccinated with TDV as part of a vaccination program planned in the study area.
2. The participant’s family do not intend to migrate away from the cohort hospital catchment area within 3 years of his/her enrollment into the study cohort.
3. The participant's parent or LAR signs and dates a written ICF, and any required privacy authorization where applicable, prior to the initiation of any study procedures, after the nature of the study has been explained according to local regulatory requirements.
4. The participant signs and dates an age-appropriate assent form prior to the initiation of any study procedures, after the nature of the study has been explained according to local regulatory requirements.
5. The participant's parent or LAR agrees that a baseline blood sample may be taken from the participant.

9.2.3.2 Cases

To be eligible for inclusion as a case, participants must meet the following inclusion criteria:

1. The participant is part of the cohort.
2. The participant is hospitalized and clinically diagnosed with dengue.
3. The participant has a blood sample available that was taken preferably within 5 days of fever onset and/or onset of symptoms compatible with dengue.
4. The participant tested positive for dengue by RT-PCR.

9.2.3.3 Controls

To be eligible for inclusion as a matched control, participants must meet the following inclusion criteria:

1. The participant is part of the cohort.
2. The participant had not been hospitalized with VCD at any point between enrollment in the cohort and the index date (ie, when his/her matched case was hospitalized with CDD).
3. The participant is a resident in the same neighborhood as the matched case.
4. The participant's date of birth is in the same calendar year as the matched case.

9.2.4 Exclusion Criteria

9.2.4.1 Cases

To be eligible for inclusion as a case participants must not meet any of the following criteria:

1. The participant has been vaccinated with TDV, CYD-TDV, or an investigational dengue vaccine prior to cohort enrollment.
2. Contraindications as per the locally approved label/product information leaflet.

9.2.4.2 Controls

To be eligible for inclusion as a control participants must not meet any of the following criteria:

1. The participant has been vaccinated with TDV, CYD-TDV, or an investigational dengue vaccine prior to cohort enrollment.
2. Contraindications as per the locally approved label/product information leaflet.
3. The participant could not be contacted at the time of being selected as a control.

9.2.5 Study Time Period

The study period will be at least 3 years to ensure that a minimum of 239 cases of hospitalization due to DENV-3 have been identified (see Section 9.5) (collectively in vaccinated and

unvaccinated participants). Additional sites may be added and/or an extension of the study period will be considered if needed.

9.2.6 Study Procedures

The following sections describe the study procedures. A schedule of study procedures is provided in Table 9.a. For variable definitions please refer to Section 9.3.1.

Table 9.a Schedule of Study Procedures

	Contact Window (Months [M])				CDD	Case	Control [a]
	Cohort Screening	Contact 1	Contact 2	Contact 3 [b]			
	M0	M12±90 Days	M24±28 Days	M36±28 Days	Hospitalization With CDD	Hospitalization With CDD, RT-PCR +ve	Within 28 Days From Index Date [c]
Informed consent/assent	X [d]						
Obtain blood sample for RT-PCR VCD					X		
Cohort eligibility criteria assessment	X						
Record demographic data	X						
Obtain baseline blood sample	X						
Confirm contact information [e]		X	X				
Final contact with participant				X			
Capture of participants with CDD and symptom onset time					X		
Case/control eligibility criteria assessment					X		X
Record concomitant medications and medical history					X		X
Review Vaccination records						X	X
Retrieve baseline serum sample						X	X
Record febrile illness and CDD information [f]					X		
Active safety surveillance (for hospitalized CDD) [g]					X		

Abbreviations: CDD, clinically diagnosed dengue; RT-PCR, reverse transcription polymerase chain reaction; VCD, virologically confirmed dengue.

(a) A control can be randomly considered for other cases: eg, a previously identified control participant can be considered for participation as a control repeatedly, as necessary.

(b) Planned final contact with participant.

(c) Index date: the first date of hospitalization for CDD for potential cases.

(d) Prior to the participant entering the study and before any study-specific procedures are performed. Up to 28 days prior to day of enrollment.

(e) The participants phone number and residential address will be collected by the site for operational reasons only and will be updated as needed.

(f) Specifically, the febrile episode leading to hospitalization.

(g) Reporting of other suspected adverse reactions will be via spontaneous reporting, according to local legislation.

9.2.6.1 Informed Consent/Assent

The participant's parent or LAR will be asked to sign a written ICF in which they agree to their child being part of the cohort, to their child providing a blood sample which may be used for baseline (pre-vaccination program) serostatus, and to their child providing a second blood sample if their child is identified as a potential case.

Children/adolescents participating in the study will be asked to sign an assent form. Adolescents who attain the legal age of consent during or after the cohort implementation, will be asked to return to the investigational site to attest to the appropriate written informed consent. Similarly, children who attain a different level of assent during or after cohort implementation, will be asked to return to the investigational site to sign an age-appropriate assent form. This may require an additional site visit.

9.2.6.2 Demographic Data and Contact Information

Data to be collected and data sources are specified in Sections 9.3.2 and 9.4.

9.2.6.3 Baseline Blood Sample

Five milliliters of peripheral venous blood will be taken from each participant. Serum from this blood sample, termed the "baseline serum sample", will then be stored under appropriate conditions, for subsequent baseline (pre-vaccination program) serostatus determination.

This sample will be taken as close as possible to (but within a maximum of 6 months prior to) the start of the public vaccination program in the country of study conduct. This will be carried out in coordination with the Ministry of Health and timing will be stated by country and/or region in the study procedures manual as applicable.

9.2.6.4 Confirm Contact Information

Participants in the cohort will be contacted annually (eg, by phone) to confirm that they have not migrated outside of the catchment area of the study hospital. The participants phone number and residential address will be collected by the site for operational reasons only and will be updated as needed.

9.2.6.5 Case Ascertainment

As per standard practice, participants presenting to a study hospital with febrile illness and/or symptoms compatible with dengue will be clinically evaluated for dengue. Participants diagnosed with CDD who require hospitalization will be classified as potential cases.

Hospitalization of cohort participants will be ascertained through hospital-based active surveillance conducted by dedicated study personnel. Hospitals will be selected to ensure their catchment population matches that of the assembled cohort.

Hospitalized patients clinically diagnosed with dengue who meet the eligibility criteria (see Sections 9.2.3 and 9.2.4) will be enrolled as potential cases in the study. All potential cases will receive the standard of care according to prevailing medical practice in that area. In addition to

blood sampling conducted as per standard practice, an additional 5 mL venous blood sample will be taken for confirmatory dengue testing by RT-PCR. If the RT-PCR test result is positive, then the potential case will be designated as a confirmed case. If negative, the participant will remain eligible to be considered as a potential case or control in the future.

All participants hospitalized with CDD will be considered as potential cases even if they are more than 5 days after symptom onset when detection of the virus will be lower.

9.2.6.6 Control Ascertainment

For each case, the aim is to select 10 matched controls. Controls will be identified from participants in the cohort who have not been hospitalized for CDD at any point between the date of the screening (whether they are vaccinated or not) and the date of hospitalization (ie, the index date) of the matched case. Controls may later become cases in the study if they are subsequently hospitalized with CDD and test positive for dengue by RT-PCR test.

Each control will be matched to the case by age and neighborhood to help control for socioeconomic status and risk of dengue infection. Age-matching of controls will be based on the same birth year as the case. Neighborhood will be based on the area of residence of the case (as defined in the procedures manual). The identification of controls will be performed using a random selection process detailed in the study procedures manual.

Controls will be matched to potential cases within 28 days from the date of hospitalization for CDD (the index date) and will be included in the analysis if the RT-PCR test result for the matched potential case is positive.

9.2.6.7 Laboratory Confirmation of Dengue Infection

It is anticipated that in most settings, RT-PCR testing will not be possible at the admitting hospital laboratory. Under such circumstances, the serum of each study participant who is hospitalized with CDD will be stored under the appropriate conditions prior to transport to a central/reference laboratory. At the central/reference laboratory, dengue virus and serotype will be confirmed by RT-PCR test. Serum sample storage and laboratory confirmation procedures will be defined in a study-specific laboratory manual.

9.2.6.8 Baseline Serostatus Determination

If a cohort participant is identified as a case or control, the respective baseline serum sample will be analyzed for baseline serostatus. Details regarding the process of baseline serostatus determination will be described in the study procedures manual.

9.2.6.9 Vaccination Status Ascertainment

The vaccination record from the clinic where the study participant previously received vaccinations (if any), as well as other sources of vaccination history, such as vaccine registries or medical records will be sought.

A valid TDV vaccination record for the purpose of this study will be defined in the study procedures manual. The precise mode of vaccine record ascertainment will be specified in the study procedures manual.

Study participants who do not have a valid vaccination record will be considered to be of "unknown vaccination status". Participants with unknown vaccination status will be evaluated in sensitivity analyses but will not be considered in the primary analysis. For vaccination status definitions see Section 9.3.1.

9.3 Variables

9.3.1 Operational Variable Definitions

Vaccination Status

The exposure of interest in this study is vaccination history with TDV as part of a public vaccination program.

A participant is considered:

- Fully vaccinated/completed vaccination: If he/she has received a 2-dose series of TDV according to national recommendations at least 30 days before developing fever and/or symptoms compatible with dengue.
- Partially vaccinated: If he/she has only received 1 dose of TDV, or the second dose was given less than 30 days before developing fever and/or symptoms compatible with dengue.
- Unvaccinated: If he/she has not received TDV at all.
- Unknown vaccination status: If TDV vaccination documentation is missing.

Hospitalization: admission to a hospital, including an emergency room, triage tent or equivalent setting with a minimum duration of 24 hours stay (the detailed definition of hospitalization will be described in the study procedures manual).

CDD: participants clinically diagnosed with dengue using the 1997 or 2009 WHO case definition for dengue (see Appendix B).

VCD: positive for dengue by RT-PCR test.

Severe dengue: participants who meet the criteria described in Appendix B, Figure B-2.

DHF: participants who meet the criteria described in Appendix B, Figure B-2.

DSS: participants who are diagnosed with DHF and have signs of circulatory failure, as described in Appendix B.

Potential case: participants with CDD who are hospitalized and had a blood sample taken for RT-PCR test, but whose test result is pending.

Index dates: the first date of hospitalization for CDD for potential cases.

Case: hospitalized study participant who meets the study inclusion and exclusion criteria for cases (Sections 9.2.3.2 and 9.2.4.1), and has CDD, and is confirmed to have a positive RT-PCR test result (ie, VCD).

Control: study cohort participant who is matched to a case (Section 9.2.6.6) and meets the study inclusion and exclusion criteria for controls (Sections 9.2.3.3 and 9.2.4.2) but is not hospitalized with VCD on or before their case's index date.

9.3.2 Data Recorded

For the entire cohort, age, gender, and the baseline serum sample will be collected as analysis relevant data. The participant's phone number and residence address will be collected by the site for the entire cohort for operational reasons only.

For participants hospitalized with CDD, clinical data such as time of onset of symptoms and time at which the blood sample for RT-PCR was taken will be collected.

For potential cases and matched controls, the following will be collected:

- Length of time living at current address.
- Medical history, concomitant medications, and underlying conditions that are contraindicated for TDV vaccination as stated in the locally approved label/product information leaflet.
- Vaccination history (including JE and YF vaccines):
  - Date(s) of TDV vaccination doses (if applicable).
- Dengue prevention measures employed (details of specific measures will be provided in the study procedures manual).

For cases only, the following will be also recorded:

- Date of first medical attendance for current illness.
- Date of hospital admission.
- Clinical reason for hospitalization due to CDD (eg, observation, co-existent conditions, warning signs, or severe dengue).
- Hospitalization treatment and outcome.
- Date of blood sample for RT-PCR test.
- Symptoms and signs of CDD.
- Date of onset of fever and/or symptoms compatible for dengue.
- Disease severity (ie, DHF, severe dengue, dengue with warning signs).

9.4 Data Sources

Cohort data will be collected in real-time during the period of hospital admission using a questionnaire, clinical records, the opinion of the treating physician, and by interviewing the participant's parents or LAR. Information from the controls will be collected by interview.

Clinical data and vaccination history will be collected by reviewing medical health records and/or vaccination record books.

9.5 Study Size

9.5.1 Expected Number of Outcomes in the Unvaccinated Study Population

It was assumed that 50% of the study cohort are vaccinated, and that 20% of the vaccine-eligible population were seronegative at the time of vaccination. [redacted] Based on these assumptions and a planned total sample size of 70,000, 902 outcomes of hospitalization due to VCD (180/902 due to DENV-3 and 126/902 due to DENV-4) are expected to be ascertained over a study period of 3 years in unvaccinated participants in DEN-401 when assuming an annual cohort drop-out rate of 7%. Of the expected 902 DENV hospitalizations, 180 outcomes (36/180 due to DENV-3 and 25/180 due DENV-4) are expected to be in baseline seronegative participants. In the placebo arm of Trial DEN-301, there were 3 baseline seronegative subjects hospitalized for VCD due to DENV-3, and 1 due to DENV-4 at 54 months post second dose (DEN-301 54 month CSR [Part 1, Part 2, Part 3]).

9.5.2 Simulation-Based Sample Size Calculations for DENV-3 (Vaccinated and Unvaccinated)

As proof of concept, simulation-based sample size calculations [32] were performed to determine the minimal total cohort size (N) expected to rule out an increased risk of ≥2 for hospitalization due to DENV-3 in the vaccinated seronegative population.

For these calculations, the null hypothesis was a HRR of 2.0 for hospitalization due to DENV-3 in the vaccinated versus unvaccinated seronegative population. Sample size calculations were powered to reject the null hypothesis when the true HRR for hospitalization due to DENV-3 in the seronegative population was assumed to be 1.0 with a Type I error of 5%.

The simulation model was set-up based on the assumptions outlined in Section 9.5.1, and assuming an annual cohort drop-out rate of 7%, to mimic a nested case-control study in which cases and controls were drawn from a cohort over a 3-year follow-up period.

The dengue hospitalization rate was considered to be lower in the vaccinated versus unvaccinated population. [redacted]

[redacted]

When a case occurred, 10 controls were sampled using incidence density sampling [33,34]. On average, sampling resulted in 2 seronegative controls per seronegative case (assuming 80% of the cohort were initially seropositive). The simulated cases and controls were then used to estimate the HR and corresponding 95% CI of dengue hospitalizations for the vaccinated versus unvaccinated population, controlled for serostatus, by applying the conditional logistic regression approach of Thomas (1977) [35].

For each set of assumptions, 1000 case-control studies were simulated, yielding 1000 estimated HRs and 95% CIs. The power (1 – Type II error) was calculated for a 5% significance level (Type I error) as the proportion of the 1000 simulations for which the lower (1-α) % confidence limit was >1.

The simulations showed that assuming a true HRR of 1.0 (half-width of the 95% CI of the HRR: 0.52), a study population of 70,000 is expected to result in 1085 hospitalizations in 3 years (vaccinated and unvaccinated), inclusive of 239 hospitalizations due to DENV-3 (73/239 in baseline seronegative participants). This would give 80% power to rule out that the risk of hospitalization with DENV-3 in the vaccinated baseline seronegative population is <2 (with a 5% Type I error).

Variations to the incidence and prevalence assumptions may result in adjustments to enrollment as there is no way to guarantee a fixed number of events. To ensure that the study population does not deviate substantially from these assumptions, the sponsor will monitor the baseline serostatus of cases and controls, the incidence of hospitalization, and the prevalence of DENV-3 among cases and controls. These parameters will be reported in the planned Year 1 and Year 2 reports and study centers will be added to increase the cohort size if needed.

9.6 Data Management and Analysis

A statistical analysis plan (SAP) will be developed ensuring its consistency with the final study protocol. The SAP will be finalized and approved prior to enrolment of the first study participant. Any amendment(s) to the SAP will be finalized and approved prior to the study database lock. Any changes made to the planned analysis after database lock will be explained and justified in the study report.

Study participants will be described by their characteristics at inclusion into the study. The association between vaccination status and characteristics at inclusion into the study will be assessed. Since this is a multi-country and multi-site study and significant differences in the epidemiology and severity of dengue exist across different regions of the world, a descriptive analysis per site will be conducted. Local uptake of TDV will also be described.

The relationship between TDV and hospitalization due to VCD will be assessed using the conditional logistic regression approach of Thomas (1977) [35]. Strata will consist of one case

and 10 corresponding controls matched based on age and neighborhood. The outcome will be occurrence of hospitalization due to VCD (either hospitalizations due to any serotype or serotype-specific), the main predictors of interest are vaccination status (for the estimation of the overall vaccine effectiveness), serostatus at baseline, and the interaction effect between vaccination status and baseline serostatus (for the estimation of vaccine effectiveness by serostatus). Other predictors related to potential confounders may also be included. The output of the regression model will be log HRR and the associated 95% CI. The handling of missing data will be described in the SAP. The SAP will also provide further details regarding the analysis methodology to address all trial objectives.

All analysis will be conducted using SAS Version 9.2 or higher. Full details of procedures for data handling (inclusive of data collection, retrieval, and preparation) will be documented in the study data management plan.

9.7 Quality Control

Monitoring of study sites will be performed as per the monitoring plan. If potential quality risks are identified, on-site visits may be performed to verify that the study is being carried out according to the protocol. Alternative monitoring approaches such as remote source data verification or telephone contact may also be used to ensure data quality and integrity. Alternative monitoring approaches will only be used only where permitted by the local Health Authority and the institutional review board (IRB)/independent ethics committee (IEC).

Representatives of the sponsor's quality assurance unit/monitoring team and competent regulatory authorities must be permitted to inspect all study-related documents and other materials at the site, including the Investigator Site File, the completed electronic case report form (eCRF) and the participants' original medical records. Audits may be conducted at any time during or after the study to ensure the validity and integrity of the study data.

The final study report, all source data, hard copies of the signed ICFs/assent forms, electronic information and documentation used for data collection and processing will be kept in secured storage areas for at least five years after the final study report, or according to each country's retention policies.

9.8 Limitations of the Research Methods

The protocol assumes Takeda has been granted marketing authorization for TDV irrespective of previous dengue infection at the time of vaccination by the regulatory authority. The protocol is intended to be generic as it is unknown which countries will include the vaccine in a public vaccination program after marketing authorization at present. The protocol can only be implemented in the context of a population-based vaccination program and therefore requires the collaboration of National Public Health Authorities. The protocol may need to be amended following discussions with National Public Health Authorities. Takeda also intends to collaborate with local reference laboratories and clinical investigators to implement their feedback into the protocol by means of an amendment if necessary.

Potential bias could be introduced by variabilities in local medical practice and diagnostic criteria of dengue and dengue severity; diagnostic tests; and clinical decision-making being influenced by knowledge of prior TDV vaccination. However, the strategy for the match control should ensure potential biased is balanced among vaccinated and unvaccinated.

The estimation for association by serostatus and serotype will require a minimum number of events in each analysis category. The cohort sample size has been calculated based on expected rates of hospitalization for VCD, vaccination coverage, proportion of participants with no previous exposure to dengue, and prevalence of the dengue serotypes circulating during the study period. The observed incidence of events by serostatus and serotype category will be evaluated annually. The focus will be on seronegative participants subsequently hospitalized for VCD with DENV-3 and DENV-4. If the observed number of cases is fewer than expected, then additional study locations or extending the duration of follow-up at the sites included will be considered.

10.0 PROTECTION OF HUMAN SUBJECTS

Takeda and its collaborators respect the participants' rights to privacy and will ensure the confidentiality of their medical information in accordance with applicable laws and regulations. Information from the reference laboratories may become available several months after being obtained (therefore, with no clinical relevance), and data analysis will be completed after the case management of the study participant. For these reasons, it is not planned to provide results of this observational study to participating families.

All demographic, clinical, epidemiologic, and laboratory data for each sample will be anonymized at the study laboratory and assigned a participant code. This information will then be entered into a database which will be indexed by study identification code number. This database will be password protected and will remain within the study site secure network under lock and key. Only information that is pertinent to local health authorities will be shared with them if required. Data transferred from all sites will be treated with the same level of confidentiality.

All official protocol files and documents (protocol, ICFs/assent forms, data, IRB minutes, and approvals) will be kept at the study sites. All data collection tools, and ICF/assent forms will be kept at the study sites with restricted access. Only authorized personnel will have access. Coded data will not have any personal identifiers such as name or government identification numbers. No data with identifiers will be released. This is an observational study, and the likelihood of a public health issue is minimal.

Participants will not be randomized to receive any specific therapy or undergo clinical intervention. No medical monitor is required.

10.1 Regulatory and Ethical Compliance

This study will be conducted according to the protocol and in compliance with all local laws and regulatory requirements.

10.2 Informed Consent and Ethical Approval

The study will follow the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use and the International Ethical Guidelines for Epidemiological studies prepared by the Council for International Organizations of Medical Sciences [36]. The study will be submitted to and approved by the ethical review committee and any other relevant review board/committee in each country.

All parents/LARs/participants will receive an explanation of the study procedures and will be asked to read and sign an ICF/assent form, as appropriate. The assent form will complement the ICF signed by the parent/LAR and will be obtained to manifest that the child agrees to participate in the study. Participation will be voluntary.

Adolescents who attain the legal age of consent during or after cohort implementation, will be asked to return to the investigational site to attest to the appropriate written informed consent. Similarly, children who attain a different level of assent during or after cohort implementation, will be asked to return to the investigational site to sign an age-appropriate assent form. This may require an additional site visit.

11.0 MANAGEMENT AND REPORTING OF ADVERSE EVENTS

11.1 Definitions

11.1.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a subject/participant administered a medicinal product and which does not necessarily have to have a causal relationship with this product/vaccine. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, a new disease or worsening in severity or frequency of a concomitant disease, temporally associated with the use of a medicinal product, whether or not the event is considered causally related to the use of the product/vaccine.

Although abnormal laboratory values are typically not considered AEs, the following considerations may result in an abnormal laboratory value being considered an AE:

- A laboratory test result that meets the criteria for a serious adverse event (SAE).
- A laboratory test result that requires the subject/participant to receive specific corrective therapy.
- A laboratory abnormality that leads to discontinuation of therapy.
- A laboratory abnormality that the health care provider considers to be clinically significant.

11.1.2 SAEs

An SAE is any untoward medical occurrence that at any dose:

- Results in death. Note that death is an outcome of an event. The event(s) causing death should be recorded.
- In the view of the health care provider, places the subject/participant at immediate risk of death (a life-threatening event); however, this does not include an event that, had it occurred in a more severe form, might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Results in a congenital anomaly/birth defect.
- An SAE may also be any other medically important event that, in the opinion of the healthcare provider, may jeopardize the subject/participant or may require intervention to prevent one of the other outcomes listed in the definition above (examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or convulsions occurring at home that do not require an inpatient hospitalization).

11.1.3 Adverse Reactions

An adverse reaction (AR) is an AE for which there is at least a reasonable suspicion of a causal relationship between an AE and a suspected medicinal product.

11.1.4 Product Quality Complaint

A product quality complaint is any written, electronic, or oral communication that alleges deficiencies related to the identity, quality, durability, reliability, safety, strength, purity, effectiveness, or performance of a product or device and combination product after it is released for distribution.

11.1.5 Special Situation Reports

A special situation report (SSR) includes any of the following events:

- Pregnancy: Any case in which a pregnant participant is exposed to TDV, or in which a female participant becomes pregnant after exposure to TDV.
- Breastfeeding: Infant exposure from breast milk.
- Overdose: All information of any accidental or intentional overdose.
- Drug abuse, misuse, or medication error: All information on medicinal product abuse, misuse, or medication error (potential or actual).
- Suspected transmission of an infectious agent: Suspected (in the sense of confirmed or potential) transmission of an infectious agent by a medicinal product.
- Lack of efficacy of Takeda Product.
- Accidental/Occupational exposure.
- Use outside the terms of the marketing authorization, also known as “off-label”.
- Use of falsified medicinal product.
- Use of counterfeit medicinal product.
- Drug-drug interactions and drug-food interactions.
- Inadvertent or accidental exposure with or without an AE.
- Unintended benefit.

A SSR should be reported even if there is no associated AE.

11.1.6 Relationship of an AE to Studied Medicinal Product

For all VCD cases (RT-PCR-confirmed as defined in Section 9.3.1) dengue illness is considered as causally related to previous TDV exposure by default. This also applies for potential cases (as defined in Section 9.3.1) as long as RT-PCR confirmation is pending. Causal relationship has to be reconsidered by the investigator in case of a negative RT-PCR result, as per the definitions

provided below. The investigator is also required to provide a causality assessment for non-dengue AEs that occur during the dengue hospitalization as described below.

The investigator is required to provide an assessment of the relationship of an AE to the studied vaccine, based on the consideration of all available information about the event, including temporal relationship to vaccine administration, recognized association with vaccine product/class, pharmacological plausibility, and alternative etiology (eg, underlying illness, concurrent conditions, concomitant treatments).

- Related (Yes): An AE that follows a reasonable temporal sequence from administration of the medication, vaccine or device (including the course after withdrawal of the medication), and for which a causal relationship is at least a reasonable possibility, ie, the relationship cannot be ruled out, although factors other than the medication, vaccine or device, such as underlying diseases, complications, concomitant drugs and concurrent treatments, may also have contributed.
- Not related (No): An AE that does not follow a reasonable temporal sequence from administration of the medication, vaccine, or device and/or that can reasonably be explained by other factors, such as underlying disease, complications, concomitant drugs and concurrent treatments.

11.2 Scope of Adverse Event Collection

Within the scope of this study, only AEs for potential cases and cases are actively collected. This includes any dengue- and non-dengue events during or relevant to the participant's hospitalization.

The Investigators should report any other suspected adverse reactions, in accordance with their local national regulatory requirements, direct to <PV post marketing contact details> or via the national pharmacovigilance reporting system. These will be treated as spontaneous reports independently of the study.

11.3 Collection and Recording of Adverse Events, SSRs and Product Quality Issues

Collection of AEs is limited to the occurrence of Potential Cases and Cases (as defined in Section 9.3.1) and starts with the date of hospitalization until resolution or final outcome. For Potential Cases and Cases, the investigator should notify Takeda within 1 working day of becoming aware of a fatal or life-threatening SAE, within 4 calendar days for other SAEs, and within 7 calendar days for all other events/issues. This is typically achieved by the investigator completing the adverse event report pages of an eCRF or by submitting an AE Report Form to Takeda.

The Investigator may be contacted by Takeda to obtain additional information on the event or for data clarification. The investigator shall make best effort to obtain the requested additional information and will notify Takeda within 1 working day of obtaining the additional information for a fatal or life-threatening SAE, within 4 calendar days for other SAEs, and within 7 calendar days for all other events/issues.

11.4 Reporting of Adverse Reactions to Regulatory Agencies and IRB/IEC

Hospitalized cases of dengue fever related to the study's main objectives (ie, hospitalization for VCD, including severe dengue), will not be submitted as individual case safety reports (ICSRs) [37]. Potential cases and cases as defined in Section 9.3.1 will not be submitted as ICSRs but will be described in the study report.

The investigator is responsible for reporting ARs to the IRB/IEC and/or regulatory authorities, if required by national law or regulation, within the timelines required by such law or regulation. The investigator shall maintain records of all such submissions. Takeda is responsible for reporting serious and non-serious ARs suspected of being related to Takeda products/vaccines and which are addressed to Takeda to regulatory authorities.

12.0 PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

12.1 Study Registration

The study will comply with all applicable laws and regulations regarding clinical study registration. Registration will occur on publicly accessible websites prior to the start of the study (defined as first study participant in the cohort following completion of the informed consent/assent process) and in accordance with the national and local laws and regulations.

12.2 Results Disclosure

The results of this study will be published on publicly accessible websites according to applicable laws and regulations.

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[redacted]

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APPENDICES

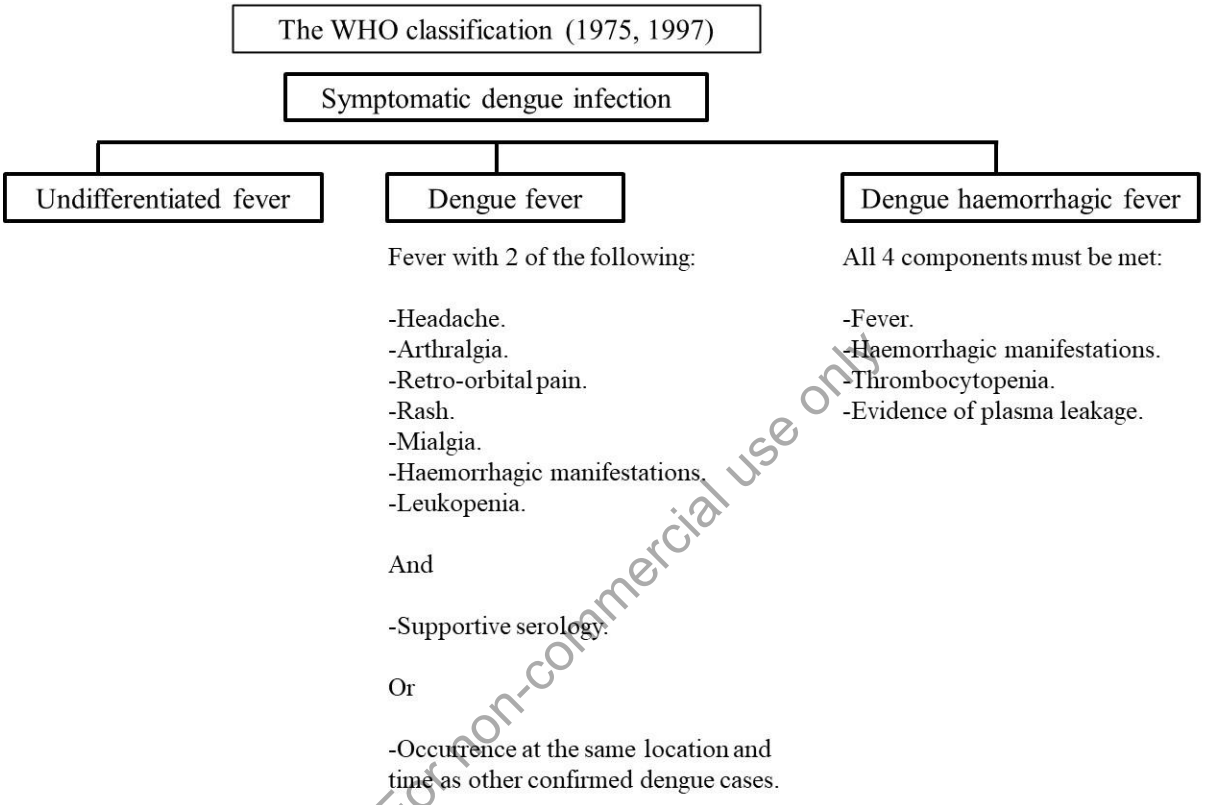
Appendix A List of Stand-Alone Documents

Documents available upon request.

Number	Document Reference Number	Title
1	None	Informed consent/Assent form
2	None	Study procedures manual
3	None	Laboratory manual
4	None	List of Investigators and Co-Investigators
5	None	List of Contract Research Organizations and Other Service Providers

Appendix B WHO Criteria for Dengue Case Classification

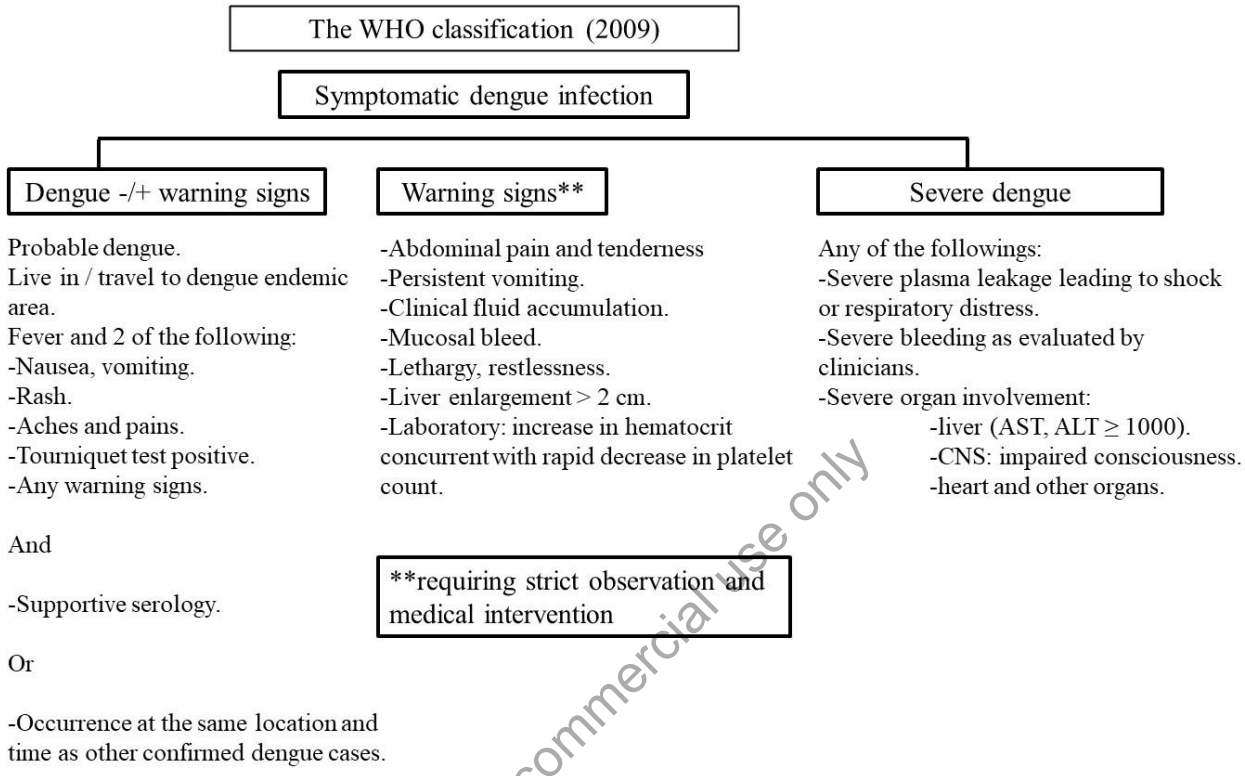
Figure B-1 Dengue Classification According to the WHO Guidelines Issued in 1975 and Updated in 1997



Abbreviations: WHO, World Health Organization.

1997 WHO dengue classification: dengue is classified as (1) undifferentiated fever, (2) dengue fever (DF), and (3) dengue hemorrhagic fever (DHF). In addition to fever and at least 2 clinical findings, diagnosis of DF requires epidemiological or laboratory evidence supporting a dengue virus infection. To meet a case definition of DHF, all 4 criteria are required: (1) fever, (2) hemorrhagic manifestations, (3) thrombocytopenia (platelet count, ≤100,000 platelets/mm^3), and (4) evidence of plasma leakage. Diagnosis of DHF does not require laboratory support. Additionally, patients with DHF can progress to dengue shock syndrome (DSS) if they also have evidence of circulatory failure: rapid, weak pulse with narrow pulse pressure or hypotension, and have clammy skin and restlessness [11].

Figure B-2 Dengue Classification According to the WHO Guidelines Issued in 2009



Abbreviations: ALT, Alanine Transaminase; AST, Aspartate Aminotransferase; CNS, Central Nervous System; WHO, World Health Organization.

2009 WHO dengue classification: dengue is classified as dengue with or without warning signs and severe dengue. Diagnosis of dengue requires the presence of fever and at least 2 clinical findings or any warning signs. Epidemiological or laboratory evidence is required to make the diagnosis. Severe dengue is defined as dengue with any of the following: (1) severe plasma leakage leading to shock or respiratory distress, (2) severe hemorrhage, or (3) any organ failure [8].

Appendix C [redacted]

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Appendix D [redacted]

[redacted]

Signature Page for DEN-401 Protocol Amendment 1, Version 2.0, 25 May 2023

Title:

Approval	[redacted] Pharmacovigilance 29-May-2023 11:46:48 GMT+0000
Approval	[redacted] Pharmacovigilance 30-May-2023 07:31:30 GMT+0000
Approval	[redacted] Clinical 30-May-2023 10:31:06 GMT+0000

Document Number: TAK-003-07766 v4.0