13 October 2022
EMA/862556/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Dengue Tetravalent Vaccine (Live, Attenuated) Takeda

Common name: dengue tetravalent vaccine (live, attenuated)

Procedure No. EMEA/H/W/005362/0000

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Table of contents

List of abbreviations

Term	Definition
ANOVA	Analysis of variance
BDS	Bulk drug substance
CD4+	Cluster of differentiation 4 – T-Cell co-receptor
CD8+	Cluster of differentiation 8 – T-Cell co-receptor
CDC	Centers for Disease Control and Prevention
CNS	Central nervous system
CI	Confidence interval
COVID-19	Coronavirus disease 2019
CPP	Critical process parameter
CQA	Continued process verification
CSR	Clinical study report
DCAC	Dengue Case Adjudication Committee
DENV-1/2/3/4	Dengue virus serotype (wild-type) 1/2/3/4
DHF	Dengue haemorrhagic fever
E	Envelope protein
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FAS	Full analysis set
GLP	Good Laboratory Practice
GMC	Geometric mean concentrations
GMO	Genetically modified organism
GMP	Good manufacturing practice
GMT	Geometric mean titre
HAV	Hepatitis A virus
HSA	Human serum albumin
ICS	Intracellular cytokine staining
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intramuscular
JE	Japanese encephalitis
LLOQ	Lower limit of quantification
MCB	Master cell bank
MNT_{50}	Microneutralisation test resulting in a titre reduction of at least 50%
MVS	Master virus seed
5'NCR	5 prime non-coding region
NHP	Non-human primate
NS	Nonstructural
NS1/3/5	Nonstructural protein 1/3/5
PBS	Phosphate buffered saline
PC	Product-specific in-house reference material
PCR	Polymerase chain reaction
Ph.Eur.	European Pharmacopoeia
PFS	Pre-Filled Syringe
PFU	Plaque forming units
PP	Process parameter
PPS	Per-protocol set
prM	Premembrane protein
PRNT	Plaque reduction neutralisation test
RT-PCR	Reverse transcriptase polymerase chain reaction
SAP	Statistical analysis plan
SC	Subcutaneous
TDV	Dengue Tetravalent Vaccine (Live, Attenuated), the Takeda dengue vaccine candidate also known as TAK-003
TNF—α	Tumour necrosis factor alpha
VE	Vaccine efficacy
vRNA	Viral ribonucleic acid

Term	Definition
WCB	Working cell bank
WHO	World Health Organization
WVS	Working virus seed
YF	Yellow fever
YF-17D	Yellow fever vaccine 17D

CONVENTIONS USED IN THIS DOCUMENT

Term

Serotypes and vaccine strains

Convention

The terms DENV-1, DENV-2, DENV-3, and DENV-4 are used for dengue wild type serotypes. The terms TDV-1, TDV-2, TDV-3, and TDV-4 are used for dengue vaccine strains.

Term

TDV

Convention

Dengue Tetravalent Vaccine (Live, Attenuated), the Takeda dengue vaccine candidate also known as TAK-003, is referred to as TDV in this document. TDV may also be referred to as DENVax-1, DENVax-2, DENVax-3 and DENVax-4.

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Takeda GmbH submitted on 3 March 2021 an application in accordance with Article 58 of (EC) No Regulation 726/2004 to the European Medicines Agency (EMA) for a scientific opinion in the context of cooperation with the World Health Organisation for Dengue Tetravalent Vaccine (Live, Attenuated) Takeda.

The eligibility by the World Health Organisation was agreed upon on 27 June 2019.

Dengue Tetravalent Vaccine (Live, Attenuated) Takeda will exclusively be intended for markets outside the European Union.

The applicant applied for the following indication:

"Dengue Tetravalent Vaccine (Live, Attenuated) Takeda is indicated for the prevention of dengue disease caused by any dengue virus serotype in individuals 4 years to 60 years of age.

The use of Dengue Tetravalent Vaccine (Live, Attenuated) Takeda should be in accordance with official recommendations."

1.2. Legal basis, dossier content

The legal basis for this application refers to:

This application is submitted under Article 58 of Regulation (EC) No 726/2004 and includes a complete and independent dossier, by analogy to Article 8(3) of Directive 2001/83/EC.

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Applicant's request(s) for consideration

1.3.1. Accelerated assessment

The applicant requested accelerated assessment in accordance to Article 14 (9) of Regulation (EC) No 726/2004.

1.4. PRIME

Not applicable.

1.5. Scientific advice

The applicant received the following scientific advice on the development relevant for the indication subject to the present application:

Date	**Reference**	**SAWP co-ordinators**
23 October 2014	CHMP Scientific Advice	*Jan Mueller-Berghaus, Hans Ovelgönne*
15 December 2016	CHMP Follow-up Scientific Advice	*Fernando de Andrés Trelles, Dr Hans Ovelgönne*

The Scientific advice pertained to the following *quality, non-clinical, and clinical* aspects:

- *Process validation*
- *Adventitious agent´s testing scheme*
- *Shelf-life definition and release specifications*
- *Potency of clinical trial materials*
- *Changes in manufacturing and related comparability assessment*
- *Vial and Pre-filled Syringe (PFS) Presentations for the Diluent of TDV*
- *Dual Sourcing of PFS diluent*
- *Adequacy of the preclinical package to support approval*
- *Environmental Risk Assessment (ERA)*
- *Concurrence with the Dengue MNT assay and methodology for titre calculation*
- *Agreement with Takeda's RT-PCR approach to distinguish vaccine virus-induced viraemia from a wild type infection in endemic areas*
- *Clinical vaccine viral shedding assessment*
- *Agreement with the phase 3 DEN-301 study design*
- *Clinical development plan to support benefit risk assessment in Dengue endemic and non-endemic countries and size of the safety database*
- *Agreement that Studies DEN-304, DEN-305 in non-endemic areas, assessing safety and immunogenicity, will support approval in non-endemic areas*
- *Vaccine co-administration trials*
- *Design of the Lot-to-Lot Consistency Study*

1.6. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Sol Ruiz Co-Rapporteur: Jan Mueller-Berghaus

The application was received by the EMA on	3 March 2021
Accelerated Assessment procedure was agreed-upon by CHMP on	28 May 2020

The procedure started on	25 March 2021
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	27 May 2021
The CHMP Co-Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	27 May 2021
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	1 June 2021
In accordance with Article 6(3) of Regulation (EC) No 726/2004, the CHMP Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days	
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	10 June 2021
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	24 June 2021
The applicant submitted the responses to the CHMP consolidated List of Questions on	09 September 2021
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs' Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	18 October 2021
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	28 October 2021
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	11 November 2021
The applicant submitted the responses to the CHMP List of Outstanding Issues on	20 May 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs' Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	17 June 2022
The CHMP agreed on a list of outstanding issues to be sent to the applicant on	23 June 2022
The outstanding issues were addressed by the applicant during an oral explanation before the CHMP during the meeting on	N/A
SAG was convened to address questions raised by the CHMP on The CHMP considered the views of the SAG as presented in the minutes of this meeting.	08 September 2022
The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive scientific opinion to Dengue Tetravalent Vaccine (Live, Attenuated) Takeda on	13 October 2022

Note: Timetable reverted to normal at Day 90 (please see point 2.3)

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

Dengue fever is an infectious disease caused by the dengue virus, a member of the flavivirus family. Dengue fever is a continuously global public health threat due to the increasing co-circulation of the four antigenically distinct Dengue virus serotypes (DENV 1-4) in most endemic areas. DENV infections are mostly asymptomatic but can also cause the Dengue fever acute febrile illness defined by two or more of the following manifestations:

headache, retro-orbital pain, myalgia, arthralgia, rash, haemorrhagic manifestations, and/or leucopenia.

The most severe forms of dengue infection – Dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) – are life threatening. These severe forms manifest most often with plasma leakage but may also include clinically significant bleeding; the potential result of both is decreased intravascular volume, decreased organ perfusion, and the potential for shock and death.

It is not completely understood why some people experience no disease and others severe disease, but there is strong evidence sequential infections with different DENV types separated by more than 18 months significantly increases the risk for a severe disease outcome. Primary infection with any of the 4 dengue serotypes is thought to result in decades of protection from re-infection by the same serotype but may not protect against a secondary infection by 1 or more of the other dengue serotypes and may lead to an increased risk of severe disease over the course of secondary infection.

2.1.2. Epidemiology

These dengue viruses are transmitted from human to human by mosquitoes (primarily by *Aedes aegypti* but also by *Aedes albopictus*). There are an estimated 390 million dengue infections per year worldwide, of which 100 million are symptomatic. Every year, around 500,000 cases of dengue haemorrhagic fever (DHF) require hospitalisation with an estimated death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection, with an estimated death rate of approximately 20,000 to 25,000 per year, primarily in children.

The incidence of dengue has grown dramatically around the world in recent decades. This increase has been associated with societal changes such as population growth and increasing urbanisation, particularly in tropical cities with poor waste and water management, leading to proliferation of the domestic and peri-domestic mosquito species (*Aedes aegypti* and *Aedes albopictus)*. Human migration and international trade and travel are constantly introducing new vectors and pathogens into novel geographic areas. In addition, it has been suggested that rising temperatures and global climate change may lead to the expansion of the major mosquito vectors into new areas, extension of the transmission season in areas with currently circulating dengue viruses, decrease in extrinsic incubation periods, and increase in the mosquito spp. vectorial capacity.

Before 1970, only 9 countries had experienced severe dengue epidemics. The disease is now endemic in more than 100 countries in the World Health Organization (WHO) regions of Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. The America, South-East Asia and Western Pacific regions are the most seriously affected, with Asia representing approximately 70% of the global burden of disease. Not only is the number of cases increasing as the disease spreads to areas including Europe, but outbreaks are occurring. The threat of a possible outbreak of dengue now

exists in Europe; local transmission was reported for the first time in France and Croatia in 2010 and imported cases were detected in 3 other European countries. In 2012, an outbreak of dengue on the Madeira islands of Portugal resulted in over 2000 cases and imported cases were detected in mainland Portugal and 10 other countries in Europe. Autochthonous cases are now observed on an almost annual basis in many European countries.

Among travellers returning from low- and middle-income countries, dengue is the second most diagnosed cause of fever after malaria. A secondary dengue vector in Asia, Aedes albopictus, has spread to more than 32 states in the United States (US) and more than 25 countries in the European Region.

In 2020, dengue continues to affect several countries, with reports of increases in the numbers of cases in Bangladesh, Brazil, Cook Islands, Ecuador, India, Indonesia, Maldives, Mauritania, Mayotte (Fr), Nepal, Singapore, Sri Lanka, Sudan, Thailand, Timor-Leste and Yemen. Severe dengue affects most Asian and Latin American countries and has become a leading cause of hospitalisation and death among children and adults in these regions. 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, haemorrhagic manifestations, and/or leucopenia. The most severe forms of dengue infection – DHF and dengue shock syndrome (DSS) – are life threatening. Primary infection with any of the 4 dengue serotypes is thought to result in decades of protection from re-infection by the same serotype but may not protect against a secondary infection by 1 or more of the other 3 dengue serotypes and may lead to an increased risk of severe disease over the course of secondary infection (DHF/DSS). In 2009, the WHO issued a new guideline that classifies clinical dengue as dengue (with and without warning signs) and severe dengue.

In the absence of standardised global serotype surveillance, the most systematic data on the relative frequency of the 4 dengue serotypes may be from the placebo groups of the phase 3 dengue vaccine trials, which captured dengue serotypes for medically attended paediatric dengue cases over time across study sites in Latin America and Asia. Combining cases from the placebo groups in the Takeda phase 3 trial with up to 36 months of follow-up after vaccination (period from 2016 to 2020; conducted in The Philippines, Sri Lanka, Thailand, Brazil, Colombia, The Dominican Republic, Nicaragua, and Panama) and in the Sanofi phase 3 trials (2011 to 2014; conducted in Indonesia, Malaysia, The Philippines, Thailand, Vietnam, Brazil, Colombia, Honduras, Mexico, and Puerto Rico) results in an overall background serotype distribution of: DENV-1: 36%; DENV-2: 28%; DENV-3: 22%; DENV-4: 15%.

2.1.3. Clinical presentation and diagnosis

Clinical presentation

As stated by WHO (https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue), symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito. The World Health Organization classifies dengue into 2 major categories: dengue (with / without warning signs) and severe dengue. The sub-classification of dengue with or without warning signs is designed to help health practitioners triage patients for hospital admission, ensuring close observation, and to minimise the risk of developing the more severe dengue (see below).

Dengue

Dengue should be suspected when a high fever (40°C/104°F) is accompanied by 2 of the following symptoms during the febrile phase:

- severe headache

- pain behind the eyes
- muscle and joint pains
- nausea
- vomiting
- swollen glands
- rash.

Severe dengue

A patient enters what is called the critical phase normally about 3-7 days after illness onset. It is at this time, when the fever is dropping (below 38°C/100°F) in the patient, that warning signs associated with severe dengue can manifest. Severe dengue is a potentially fatal complication, due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment.

Warning signs that doctors should look for include:

- severe abdominal pain
- persistent vomiting
- rapid breathing
- bleeding gums
- fatigue
- restlessness
- blood in vomit.

If patients manifest these symptoms during the critical phase, close observation for the next 24–48 hours is essential so that proper medical care can be provided, to avoid complications and risk of death.

Diagnostics

Several methods can be used for diagnosis of DENV infection. These include virological tests (that directly detect elements of the virus) and serological tests, which detect human-derived immune components that are produced in response to the virus). Depending on the time of patient presentation, the application of different diagnostic methods may be more or less appropriate. Patient samples collected during the first week of illness should be tested by both serological and virological methods (reverse transcriptase polymerase chain reaction, RT-PCR).

Virological methods

The virus may be isolated from the blood during the first few days of infection. Various RT–PCR methods are available. In general, RT–PCR assays are sensitive, but they require specialised equipment and technical training for staff implementing the test, therefore they are not always available in all medical facilities. RT–PCR products from clinical samples may also be used for genotyping of the virus, allowing comparisons with virus samples from various geographical sources.

The virus may also be detected by testing for a virus-produced protein, called Nonstructural Protein 1 (NS1). There are commercially produced rapid diagnostic tests available for this; it takes only ~20 mins to determine the result, and the test does not require specialised laboratory techniques or equipment.

Serological methods

Serological methods, such as enzyme-linked immunosorbent assays (ELISA), may confirm the presence of a recent or past infection, with the detection of Immunoglobulin M (IgM) and Immunoglobulin G (IgG) anti-dengue antibodies. IgM antibodies are detectable ~1 week after infection and are highest at 2 to 4 weeks after the onset of illness. They remain detectable for about 3 months. The presence of IgM is indicative of a recent DENV infection. IgG antibody levels take longer to develop than IgM, but IgG remain in the body for years. The presence of IgG is indicative of a past infection.

2.1.4. Management

Treatment of dengue fever is based solely on the clinical signs and symptoms, with fluid replacement required for haemorrhagic or shock cases. An antiviral therapy for dengue virus infection is not available. Most of the current preventive measures that rely on mosquito control and individual protection are of limited efficacy, complex to implement, and questionable in terms of cost-effectiveness. While the malaria transmitting Anopheles mosquitoes predominantly feed during the night, the dengue transmitting Aedes mosquitoes feed predominantly at dusk and bed nets are therefore not effective. Dengue continues to spread despite the use of vector control measures. New technologies under development appear to be effective at stopping local dengue transmission, such as where mosquitoes infected with Wolbachia (which reduces a mosquito's ability to transmit human viruses) are released into the environment.

Vaccine development has assumed the need for tetravalent vaccines against all 4 serotypes to avoid any potential risk of vaccine induced immune enhancement, as has been well documented with natural (wild-type, wt) infection. A first tetravalent dengue vaccine (chimeric Yellow Fever [YF] virus-Dengue virus Tetravalent Dengue Vaccine) has been approved since 2015 in several Asian and Latin American countries as well as in the US and in the European Union (EU). This vaccine was initially authorised for use in vaccine recipients $\geqslant$9 years of age because clinical data indicated an unfavourable risk benefit profile for children <9 years of age [15]. More recent analyses found that individuals who were dengue seronegative before vaccination had a higher risk of getting severe disease and/or getting hospitalised when they were infected by dengue virus after vaccination than individuals who were already seropositive. In a revised recommendation from April 2018, the Scientific Advisory Group for Emergencies (SAGE) concluded that for countries considering vaccination as part of their dengue control programme, a "pre vaccination screening strategy" would be the preferred option, and only dengue seropositive individuals should be vaccinated.

Hence, considering the epidemiology of dengue, the lack of available antiviral treatments and the limitations of the vaccine, there is a continued unmet public health need for a safe and effective vaccine that will protect populations not covered by currently available vaccine option (children aged <9 years and individuals with no previous exposure to dengue virus) against dengue infection.

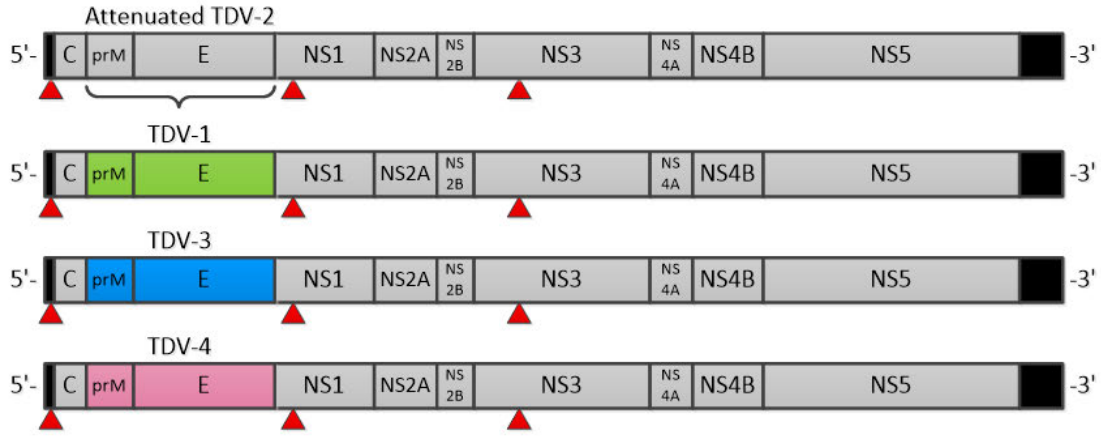
The WHO has defined and reconfirmed dengue vaccines as high priority vaccines for WHO prequalification, i.e., dengue is included in the list of High Priority Vaccines in the WHO vaccines prequalification priority list 2018-2020.

To address this unmet medical need, the applicant has developed a tetravalent vaccine that protects against dengue irrespective of baseline dengue serostatus and that can be administered to children as young as 4 years of age.

2.2. About the product

In this document, please note that "Dengue Tetravalent Vaccine (Live, Attenuated)", the Takeda dengue vaccine candidate also known as TAK-003, is referred to as TDV.

TDV comprises 4 dengue virus strains: a molecularly characterised, 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) (see Figure 1). The dengue serotype 2 vaccine strain (TDV-2) is based upon the attenuated laboratory-derived primary dog kidney (PDK) virus DENV-2 PDK-53 (parental strain: DENV-2 16681), originally isolated in Thailand in 1964. The attenuated vaccine strains for dengue serotypes 1, 3, and 4 were engineered by substituting the structural pre-membrane (prM) and envelope (E) genes of TDV-2 with the prM and E genes of the dengue virus strains, DENV-1 16007 (isolated in Thailand in 1964), DENV-3 16562 (isolated in The Philippines in 1964), or DENV-4 1036 virus (isolated in Indonesia in 1976), respectively.



Source: Module 2.4, Figure 1.a.
Abbreviations: C, capsid; E, envelope; NCR, noncoding region; NS1/3/5, nonstructural protein 1/3/5; NS2A/2B/4A/4B, nonstructural protein NS2A/2B/4A/4B; prM, pre-membrane; TDV-1/2/3/4, dengue serotype 1/2/3/4 strain.
The red triangles indicate the 3 attenuation loci present in the 5' NCR, the NS1 and NS3.

Figure 1: Diagram of the Genetic Structure of the 4 TDV Vaccine Strains

The primary mechanism of action of TDV is to replicate locally and elicit neutralising antibodies to confer protection against dengue disease caused by any of the 4 dengue virus serotypes. TDV activates multiple arms of the immune system, including binding antibodies, complement fixing antibodies, functional antibodies to dengue NS1, and cell mediated immune responses (cluster of differentiation 4 – T-Cell co-receptor [CD4+], cluster of differentiation 8 – T-Cell co-receptor [CD8+], and natural killer cells).

The Anatomical Therapeutic Chemical Classification System (ATC) code for TDV has not been assigned yet and the pharmacotherapeutic group is "Vaccines, Viral vaccines".

2.3. Type of application and aspects on development

The CHMP agreed to the applicant's request for an accelerated assessment as the product was considered to be of major public health interest. This was based on the following reasons:

To have a vaccine for the prevention of dengue disease that is safe and efficacious is a major interest from the point of view of public health, taking into account that:

- Dengue cases have increased in the past decades. Today, dengue is the most common and rapidly spreading mosquito-borne viral disease in the world.
- WHO has listed dengue as one of the ten threats to global health in 2019 and dengue is listed as High Priority Vaccine in the WHO vaccines prequalification priority list 2018-2020.
- Aggressive mosquito control efforts in endemic areas have been largely ineffective in preventing dengue outbreaks or in preventing further geographic spread of the disease.
- There are no specific treatment for dengue approved anywhere in the world. The only available dengue vaccine (CYD-TDV) has important limitations in addressing the medical need, as has been discussed above.

The recombinant dengue vaccine presented by the applicant has the potential to overcome the limitations previously discussed for existing vaccine option, since it could be used to vaccinate seronegative subjects and a broader age group including young children. There are attributes in this vaccine that justify that it is of major interest from the point of view of public health and in particular from the point of view of therapeutic innovation:

- TDV contains four live attenuated dengue serotypes (TDV-1 to TDV-4) based on a molecularly characterised, attenuated DENV-2 strain (TDV-2) providing for exposure to all DENV antigens including those that elicit a cellular immune response. The genetic structure results in an immunogenicity profile that is unique to TDV and that has the potential to not elicit an increased risk for seronegative subjects.
- There are promising safety and efficacy data already submitted by the applicant and discussed in previous sections that point to the applicant's vaccine as a solid candidate to cover the actual unmet medical need and the WHO priorities.

However, during assessment the CHMP concluded that it was no longer appropriate to pursue accelerated assessment, due to the complexity of the dossier, with quite a few issues outstanding, including Major objections, at major assessment milestone (Day 90).

2.4. Quality aspects

2.4.1. Introduction

The finished product is presented as a powder and solvent for solution for injection. The powder contains a mixture of the four serotype of live, attenuated dengue vaccine viruses as active substances:

- Dengue virus serotype 1 (live, attenuated): ≥3.3 $\log_{10}$ PFU/dose,
- Dengue virus serotype 2 (live, attenuated): ≥2.7 $\log_{10}$ PFU/dose,
- Dengue virus serotype 3 (live, attenuated): ≥4.0 $\log_{10}$ PFU/dose,
- Dengue virus serotype 4 (live, attenuated): ≥4.5 $\log_{10}$ PFU/dose.

Other ingredients (powder) are: α,α-trehalose dihydrate, poloxamer 407, human serum albumin (HSA), potassium dihydrogen phosphate, disodium hydrogen phosphate, potassium chloride, and sodium chloride. The solvent consists of sodium chloride and water for injections.

The powder's primary packaging is a borosilicate Type-I glass vial with a 13 mm neck. Vials are stoppered with dark grey bromobutyl rubber 'Type I' lyophilisation stoppers and sealed with flip-off aluminium/plastic 13 mm green, caps.

The solvent's primary packaging is glass vial (Type-I glass), with a stopper (bromobutyl rubber) and aluminium seal with purple flip-off plastic cap, or a pre-filled syringe (Type-I glass), with a plunger stopper (bromobutyl) and a tip cap (polypropylene). A Notified Body opinion is provided for the PFS.

At the time of administration, the lyophilised vaccine is reconstituted with the entire contents of the Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) diluent in either a vial or pre-filled syringe (PFS) so that upon withdraw of the reconstituted finished product a 0.5 mL dose of vaccine can be administered.

Two needles, 23 Gauge (for reconstitution) and 25 Gauge (for injection), will be supplied based on the market/country requirement and will be co-packaged along with the TDV finished product and diluent in the pre-filled syringe presentation. The needles are CE marked.

2.4.2. Active substance

2.4.2.1. General information

The active substances in tetravalent dengue vaccine (TDV) are the four serotypes of live, attenuated, dengue viruses (TDV-1, TDV-2, TDV-3, and TDV-4). Each of the four serotypes expresses the antigenic prM and Envelope protein (E) from each of the four serotypes and share a common attenuated dengue-2 genetic backbone. Each virus serotype is produced and released as individual bulk active substance.

Whole genome sequencing and molecular genetics techniques have demonstrated that the mutations identified by red triangles in Figure 1 (above), are necessary and sufficient for attenuation of DENV-2, lie outside the structural genes in the 5' non-coding region (5'NCR) at nucleotide 57, and are within NS1 at amino acid position 53 and NS3 at amino acid position 250 (Table 1).

Table 1: Key Attenuating Mutations in Takeda's Dengue Vaccine Viruses

Location of Attenuating Mutation	Nucleotide Change	Amino Acid Change
5' noncoding region	57C to T	Not applicable
Nonstructural Protein 1 (NS1)	2579G to A	53Gly to Asp
Nonstructural Protein 3 (NS3)	5270A to T	250Glu to Val

The serotype 2 strain is based upon the attenuated laboratory-derived virus, DEN-2 PDK-53, originally isolated at Mahidol University, Bangkok, Thailand. The recombinant genetically modified organism (GMO) TDV strains TDV-1, TDV-3 and TDV-4 express the surface antigens of the DENV-1, DENV-3 or DENV-4 viruses, respectively, and retain the genetic alterations responsible for the attenuation of the TDV-2 strain.

2.4.2.2. Manufacture, process controls and characterisation

The active substance is manufactured and released by IDT Biologika GmbH, Germany. Good Manufacturing Practice (GMP) compliance has been documented for all sites involved in the production and testing of the active substance.

2.4.2.3. Description of manufacturing process and process controls

The active substance manufacturing process has been adequately described. It follows an appropriate approach for live attenuated viral vaccines, including cell culture expansion, viral culture, clarification, purification, stabilisation, and final filling and storage of the active substance.

The active substance manufacturing process is divided into upstream (Module 1) and downstream (Module 2) operations, which have been adequately described. Module 1 describes the cell expansion steps. Module 2 describes inoculation and virus harvest through purification and storage of bulk active substance.

The manufacturing process of TDV-1 and TDV-2 is identical, while the manufacturing processes for TDV-3 and TDV-4 differ downstream from the harvest and TDV-4 is produced at a larger scale than the other serotypes. In-process controls and critical parameters are listed and presented in the flowchart included in the dossier. No reprocessing options are considered for this manufacturing process. The provided information is considered acceptable.

Control of materials

The list of raw materials used in the manufacture of the Takeda's live attenuated dengue tetravalent vaccine active substances is described in detail. Compendial raw materials are tested in accordance with the corresponding monograph, while specifications (including test methods) for non-compendial raw materials are presented. Foetal bovine serum, human serum albumin and porcine trypsin are the only biological materials used for active substance manufacture. These raw materials are properly controlled and the Vero master cell bank (MCB) and working cell bank (WCB), as well as each dengue serotype pre-Master virus seed (MVS), working virus seed (WVS) and bulk active substance have been tested negative for adventitious agents. Refer to section on adventitious agents for further details.

Source, History and Generation of the Cell Substrate

The applicant has implemented a two-tiered cell banking system in which the Vero MCB was manufactured, characterised in compliance with ICH Q5D, and was subsequently expanded to produce the first WCB for use in routine active substance manufacturing process.

The generation of the Vero MCB and WCB is well described. The number of passages is particularly well taken into consideration. An extensive characterisation of MCB and WCB was performed including analysis of sterility, morphology, identity, test for extraneous viral agents, and *in vivo* tumorigenicity test.

Source, History and Generation of the Viral Vaccine Strain

A description for the attenuated TDV-2 as well as the recombinant TDV-1, TDV-3 and TDV-4 viruses and their generation is provided and considered adequate. The generation of the MVS and WVS for each serotype is well described.

Extensive characterisation of virus seed banks for all serotypes was performed including analysis of: 1) genetic characterisation; 2) phenotypic characterisation; and 3) test for adventitious agents.

Genetic stability studies for the three attenuating mutations were performed and demonstrated that only the 5'NC-57-T mutation show a propensity to revert during passage. However, the percentage reversion at the 5'NC-57 locus among the four MVS stocks is considered acceptable.

In conclusion, the MCB, WCB, MVS and WVS are suitable for use in pharmaceutical production of the Takeda's live attenuated dengue tetravalent vaccine active substances.

Control of critical steps and intermediates

Critical steps have been identified through a series of risk assessments (PFMEA), process validation and characterisation of the process after each validation exercise.

All process parameters identified as critical (CPPs) and quality attributes identified as critical (CQAs) must remain within their pre-determined acceptance criteria/specifications. All results that fall outside of their respective pre-determined acceptance criteria are identified as deviations or Out of specification (OOS) and will be investigated with regards to root cause and impact to the process and product, and appropriate corrective and preventive actions will be implemented as required.

The control approach is deemed acceptable. The test methods are relevant for this type of production. The acceptance criteria are well described and acceptable.

Process validation

The applicant has undertaken a detailed process validation including manufacture of consecutive lots for the general as well as the serotype specific processes.

Results are presented for all manufacturing steps and processes and have been shown to be within the proposed acceptance criteria. These results support the reproducibility of the different manufacturing processes used for manufacturing of active substance for each serotype.

Manufacturing process development

As a result of process development efforts, changes have been implemented from phase 1 manufacturing process, referred to as Process 1, to the commercial process, referred to as Process 3b (post-PPQ) (for TDV-1, TDV-2, and TDV-3) and Process 4 (for the manufacture of TDV-4). While the general process steps for the manufacture of all four serotypes are the same, there are differences in the process and process scale across serotypes (for the active substance component) with the largest concentration in the finished product (TDV-4), requiring an increased scale of production to maintain product supply.

In order to demonstrate comparability between the active substances from the different processes, the applicant performed the following comparability studies:

- TDV-1: comparability for Process 3a to Process 3b and for Process 3b to Commercial (Process 3b Post-PPQ) process.

- TDV-2: comparability for Process 3a to Process 3b and for Process 3b to Commercial (Process 3b Post-PPQ) process.

- TDV-3: comparability for Process 3a to Process 3b and for Process 3b to Commercial (Process 3b Post-PPQ) process.

- TDV-4: comparability for Process 3a to Process 3b and for Process 3b to Process 4.

The active substance comparability study encompasses the conformity to the release testing specifications, the stability along the product shelf-life and the active substance comparison based on a battery of selected tests for appropriate attributes including potency, pH, impurities, identity, appearance, genomic RNA content, and attenuating genotype. All batches complied with the release and stability specifications. Overall, all batches showed comparable results for the battery of selected tests.

The comparability exercise is focused on process 3a, 3b and 4 as batches produced with these manufacturing processes were used for the efficacy study as well as the lot to lot consistency study.

In conclusion, these studies showed that the quality of Takeda's live attenuated dengue virus vaccine active substances manufactured by the commercial processes proposed by the applicant is highly comparable to the material used for Phase 3 clinical trials even though it is not possible to assess the comparability with active substance lots produced with earlier processes.

2.4.2.4. Characterisation

The structure, biophysical characteristics and potency of the TDV viruses were investigated and a summary of the studies and conclusions was provided. The following attributes of TDV virus were evaluated:

- Structure, by genotypic and phenotypic evaluation
- Potency
- Non-structural protein characteristics
- Virus maturation

Particular attention was given to the structure of the viral particles. The genotypic evaluation seeks to ensure that the correct genetic sequences are retained in the four viral serotypes that are part of the TDV, with a low frequency of mutation and without losing the important attenuating mutations. It was found that for each serotype there were consistent numbers of intact and infectious viral particles.

In addition, a phenotypic evaluation was undertaken using different techniques including visualisation of the viral particles differentiating their maturation state by their smooth, bumpy or spiky structure (the smooth particles are the mature, infective ones). Viral attenuation studies were carried out in mosquito cells to demonstrate the retention of the attenuating mutations in the TDV (these assays include plaque size and in vitro growth on mosquito cells).

Other characteristics studied were the potency, measurement of levels of non-structural protein 1 (NS1), a diagnostic immunogenic target of Dengue infection (this protein is identical in the four components of the vaccine, as all share the DEN-2 NS1 backbone), and virus maturation was investigated (presence of prM signifies an immature/partially immature particle), showing varied results between serotypes, but consistent for each of them.

The impurities analysed are all relevant process-related impurities and were present in product used in clinical studies.

The absence of adventitious agents has been confirmed.

2.4.2.5. Specification

An appropriate set of release and stability specifications with tests, methods and acceptance criteria were provided for each monovalent active substance to adequately control the release of active substance and ensure quality through the end of shelf-life. The specification includes physico-chemical, identity, potency, purity and safety tests. The specifications are the same for all serotypes, with the only difference being potency. In addition, safety specifications were provided for control cells.

Appearance, bioburden, potency and pH are tests performed on stability samples. Potency specifications are serotype specific. The setting of specifications was informed by data obtained during development and stability studies. The applicant proposed to change the specification for Host Cell DNA. This is considered acceptable. These specifications are endorsed, however, Takeda has committed to some improvements to the specifications and/or acceptance criteria.

Analytical methods

Analytical procedures used for release and stability testing of TDV-1 to TDV-4 bulk drug substance (BDS) have been adequately described and are considered acceptable.

TDV potency (active substance and finished product) is measured by a validated in vitro cell based assay. This assay is based on measuring the number of infectious particles that form plaques on a culture plate and allows identification of the virus serotype. Potency is reported as $\log_{10}$ plaque forming units (PFU) /mL. Compendial methods were verified to be suitable for use, while non-compendial test methods developed specifically for TDV have been validated taking into account ICH Q2(R1).

Reference materials

There is no compendial or international reference standard for TDV, therefore the applicant established in-house reference standards as positive control for the potency and identity methods for both BDS and finished product release testing and stability testing.

The current reference standard is a tetravalent finished product batch and it is used for release and stability testing of active substance and finished product. The dossier provides the list of tests performed for qualification of the existing reference standards and also includes the plan for qualification of all future primary/working reference standards.

Batch analysis

Release testing data have been presented for a sufficient number of lots representative of the commercial process.

Along the history of this vaccine development, the specifications have changed (some tests have been abandoned or replaced by others, new tests have been added and some acceptance criteria have been modified), but in all cases the BDS lots meet the specifications set at the time.

Container closure

The TDV BDS is stored in a sterile container. The container is described in sufficient detail and complies with relevant European guidance. The use of this container is considered acceptable.

The compatibility of the container closure system for all 4 serotypes has been confirmed by stability studies undertaken with relevant containers. Extractables and leachables testing and results were provided.

2.4.2.6. Stability

To support the proposed shelf life, data are presented for serotype TDV-1 and TDV-2 active substance manufactured with validated commercial Process. Results indicate that specifications are met through the proposed shelf-life at the long-term storage condition. Data support the shelf life claim and the consistency between batches.

For serotype TDV-3, the shelf-life claim is supported by primary validation lots manufactured using the validated commercial process, and meeting the specifications when stored at the long-term conditions. Data with stress storage conditions and from additional batches manufactured with the same process and with the previous process, support the shelf life claim and the consistency between batches.

In the case of serotype TDV-4, the shelf-life claim is supported by data meeting the specifications for primary validation batches stored at the long-term conditions. Supporting data came from stress

conditions and from additional batches manufactured with the validated commercial manufacturing and stored at the long-term and stress conditions.

In addition, the applicant indicates that as more stability data becomes available, shelf-life extensions will be evaluated for the active substances. The potency declines under accelerated/stress conditions for all serotypes.

The stability protocols for each serotype and storage condition are presented. The stability protocols and the shelf-life claims for the different active substance serotypes of Takeda Dengue vaccine are supported.

The provided stability data is considered sufficient to support the claimed shelf life of the TDV-1, TDV-2, TDV-3 and TDV-4 active substances.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

Takeda's Dengue Tetravalent Vaccine (TDV) is a sterile lyophilised compact cake that is reconstituted prior to injection. At the time of administration, vials are reconstituted with diluent (supplied as 37 mM sodium chloride solution) so that a 0.5 mL dose of vaccine can be withdrawn and administered.

TDV finished product contains four monovalent bulk drug substances (BDS) (TDV-1, TDV-2, TDV-3 and TDV-4) and multiple excipients. It is presented as an off-white lyophilised powder in a Type I, 2 mL glass vial. The active substance contains approximately 15% (w/v) trehalose (dihydrate basis), 1% (w/v) Pluronic F127, and 0.1% (v/v) HSA in a phosphate buffered saline (PBS) matrix. Upon reconstitution with the diluent (37 mM sodium chloride), the finished product forms a clear solution, essentially free of foreign particulate. Each finished product vial provides an extractable 0.5 mL dose of reconstituted vaccine.

The applicant has provided a comprehensive description of the pharmaceutical development. Due to the labile nature of the live viruses, modifications in the formulation were implemented throughout the development of TDV finished product with the aim to optimise and maintain the activity of the virus to ensure potency and stability throughout the product shelf-life. Early development studies were conducted to determine a combination of pharmaceutically accepted excipients that would provide better stability of the vaccine candidate. Four major processes can be identified in the development: *Process 1* represents early formulations, *Process 2* represents the introduction of the lyophilisation process, *Process 3* represents the scale-up to commercial scale, *Process 4* is the commercial manufacturing process. The relationship of each step of the manufacturing stages with regard to titre loss and variability was studied and these data were used to develop the formulation strategy.

The physicochemical and biological properties of the formulation are monitored routinely as part of product release testing.

Product and process knowledge in combination with quality risk management (ICH Q9), supported the process controls that are in place to ensure delivery of TDV finished product commercial material which consistently meets the required finished product specifications. The applicant has satisfactorily presented the quality characteristics that the product should have to ensure the desired quality taking into account safety and efficacy. The applicant has also presented comparability exercises for the different process changes implemented during late manufacturing development. This is acceptable.

The applicant has applied Quality by Design principles in the development of the finished product and their manufacturing process. However, no design spaces were claimed for the manufacturing process of the finished product.

Finished product manufactured, in most early development through commercial phase, has been filled into 2 mL Type I glass containers closed with grey butyl rubber lyophilisation stoppers. The selected vial was chosen because it is highly resistant to hydrolysis. Compatibility of the finished product with the contact materials it is exposed to during dose preparation has also been presented.

Overall, the level of information presented by the applicant for the pharmaceutical development of TDV finished product is deemed appropriate.

2.4.3.2. Manufacture of the product and process controls

TDV finished product is manufactured, tested, packaged and released for commercial distribution at IDT Biologika GmbH (Dessau-Rosslau, Germany) and Takeda GmbH (Singen, Germany). A valid proof of GMP compliance has been provided.

The finished product manufacturing process is designed to provide sterility assurance through process and facility controls. The manufacture of finished product includes strict controls surrounding the amount of each serotype to ensure the target potency of each batch, the buffer content to ensure appropriate composition of the formulated product and the duration of each operational unit to manage the loss in viral titre during manufacturing processing. The volume of each serotype used in the formulation varies based on the release active substance potency, considering the minimum manufacturing release specifications for the potency of each serotype within the final formulated finished product.

The criticality of process steps is assessed through a risk assessment which considers the development data. Based on validation exercises performed to date and process characterisation data, parameters in specific manufacturing steps are critical because of the potential impact on the product critical quality attributes (CQA). The risk mitigation measures include accurate daily calibration of equipment (where applicable), specified ranges of operation within the batch record as well as second person verification. The failure to comply with the critical process parameters specifications/ranges will lead to an investigation and may lead to rejection of the batch. In general, relevant process parameters quality attributes have been properly described in the process description with justified set points or ranges.

Released bulk finished product vials are shipped to the labelling and packaging facility for long-term storage. The vials are labelled and packed in appropriate presentations depending on the intended market for distribution. Finished product vials are placed in shipping cases and transferred to long-term storage prior to being transported to distribution centre with validated shipping lane and transport mode. This approach is deemed acceptable.

A description of the process validation strategy has been provided. Process performance qualification (PPQ) was performed to demonstrate manufacturing process control and robustness across different batches and process (i.e., a process validation series). Each PPQ series covered a minimum of three consecutive manufacturing runs. Performance of three batches for each of the PPQ campaigns was evaluated for inter-batch consistency and reproducibility. Three batches meeting the definition of consecutiveness were completed for each PPQ campaign. Consecutive batches are defined as a series that does not include a batch assigned the outcome of Fail. The process validation is presented clearly and the validation strategy is properly justified. Upon completion of each validation, the process was

evaluated and summarised in a report. Overall, the information provided by the applicant in the manufacture section is considered adequate.

2.4.3.3. Product specification

The TDV finished product is appropriately controlled by release and end of shelf-life specifications. The specification includes physico-chemical, identity, potency, purity and safety tests. Some of the tests are in compliance with pharmacopoeial methods and therefore analytical validation data were not provided, and this is considered acceptable.

For analytical procedures not described in pharmacopoeias descriptions of the analytical methods and relevant analytical validation results were provided.

TDV finished product is filled into glass vials and lyophilised as part of the manufacturing of the finished product. No new impurities are expected to be contributed by the finished process manufacturing steps.

The potential presence of elemental impurities in the finished product has been assessed on a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities.

A risk assessment for nitrosamines presence has been presented. The risk evaluation performed by Takeda have determined that there is no risk for the presence of N-nitrosamines in DTV finished product. The assessment is deemed approvable.

The justification of the specifications proposed for TDV finished product at release and stability are in general considered acceptable.

The rationale for the control strategy for potency during finished product manufacturing is aimed to assure that the labile active substances will consistently meet the manufacturing release specifications and thus maintain the expected end of shelf-life potency following 18 months of storage at 2°C-8°C. The potency specifications considered information from nonclinical and clinical studies, method performance, product stability and serotype specific decay rates, while ensuring immunogenicity of the product even at lower levels of potency. This is endorsed.

Overall, the level of information presented by the applicant is deemed appropriate.

Analytical methods

For analytical procedures not described in pharmacopoeias, description of the analytical methods is provided and a summary of its validation has been provided, stating that the validations were executed according to ICH guidelines.

As for the active substance specification, a potency assay is performed at release of the TDV finished product. The assay is based on measuring the number of infectious particles that form plaques on a culture plate and allows identification of the virus serotype. Potency is reported as $\log_{10}$ plaque forming units (PFU) per dose.

Batch analysis

The proposed specifications were met on the batches analysed. Batch to batch consistency has also been demonstrated across all batches.

Reference materials

Takeda has implemented a product-specific in-house reference material (PC) which is used to continuously monitor method performance for measuring the potency, and as reference material for confirmation of the identity of Takeda's TDV finished product.

The applicant indicates that the potency of the material is not susceptible to change on long-term storage. Stable performance of the material used for release and stability testing of commercial finished product and associated active substance lots is continuously monitored during routine testing via the respective assay validity criteria. Information about the historical lots of PCs and qualification of future lots of PCs have been included and is deemed acceptable.

Container Closure System

The finished product primary container closure system includes a 2 mL Type 1 glass vial, elastomeric stopper, aluminium seal with plastic flip-off cap. The vial and the elastomeric stopper comply with pharmacopeial standards. Following fill/finish manufacture, the bulk unlabelled finished product vials are stored at long-term storage conditions until packaging. The in-process secondary packaging components do not come in contact with the product.

The labelled finished product will be co-packaged with the diluent (37 mM saline solution) in several combinations. Needles may be included in the secondary pack for PFS diluent images depending on individual country requirements. Two needles (23 gauge and 25 gauge) may be included. The needles are CE marked. Sufficient information is provided about the container closure system.

2.4.3.4. Stability of the product

Storage and distribution of the lyophilised TDV finished product are deemed acceptable. Dual long-term and stress stability studies have been conducted to examine the effects of temperature on the stability of the finished product over time. Long term and accelerated data have been presented with a suitable number of batches representative of the commercial process to support the shelf life of the finished product. A commitment post-approval stability protocol in compliance with the ICH Guidance on Stability Testing of New Drug Substances and Products Q1A (R2) and Quality of Biotechnological Products (Q5C), has been included. A stability study at temperatures above the long-term storage condition (5°C±3°C) has been provided to evaluate the impact to potency from accidental exposures beyond Climatic Zone II. The applicant has also implemented a temperature excursion model based on the decay profile.

The packaged product is photostable. An in-use compatibility study has been performed. The product is to be used within 2 hours of reconstitution for the final commercial product (see section 6.3 of the SmPC). This is endorsed. The applicant has provided transportation validation studies for the bulk unlabelled finished product. To evaluate the impact of movement stress (shock/vibration and low air pressure) during the transportation on the product, container closure integrity tests were executed. After the tests the container closure integrity of the product was shown not to be affected.

The approved finished product shelf-life is 18 months at the refrigerated (5±3°C) storage condition.

2.4.4. Finished Product (Diluent)

2.4.4.1. Description of the diluent and Pharmaceutical Development

Takeda's Dengue Tetravalent Vaccine (TDV) is reconstituted with diluent (supplied as 37 mM sodium chloride solution) so that 0.5 mL dose of vaccine can be withdrawn and administered. The diluent is provided in either a PFS or a glass vial.

The diluent is presented in either vials or pre-filled syringes but the composition is the same, i.e. 37 mM sodium chloride (NaCl) solution.

Different manufacturers are responsible for the diluent presentations. GMP certificates covering the importation and quality control testing of the diluent presentations is provided for all sites.

A Notified Body Opinion (Article 117 of the Medical Device Regulation (EU) 2017/745) for the PFS is provided.

A diluent manufacturing comparison and evaluation showed that while there are some differences between diluent manufacturing sites with respect to manufacturing processes, scales, equipment, facilities and in-process controls, these differences do not have an appreciable impact on the resulting diluent. The TDV 37 mM saline diluent has been shown to be comparable across all manufacturers. The density used in calculations for filling across all three diluent sites is within the range of 0.999 to 1.000 g/mL.

The two primary containers (vials and syringes) have been shown to be suitable for the diluent. All components were tested for leachables, extractables and elemental impurities. Finally, container closure integrity is tested as in-process control and at some stability time points, which is adequate.

2.4.4.2. Manufacture of the diluent and process controls

A batch numbering system is in place at each site. Critical steps and intermediates have been defined for each manufacturing site, together with acceptance limits. A high-level summary of these control strategy at each site has been included. In general, the control strategy is considered adequate.

Alternate validation approaches were taken at the different sites based on site procedures and differences in the processes and development history. Multiple batches were manufactured at each site in accordance with local procedures.

In conclusion, the process is considered validated at the manufacturing sites.

2.4.4.3. Diluent specification, analytical procedures, batch analysis

The components of the diluent are considered excipients. Both sodium chloride and WFI are of compendial quality.

Release and stability specifications were provided. The specifications for the diluent are harmonised across all attributes except for image-specific differences. Only reference to USP methods are presented for tests such as identity (sodium and chloride), content, iron or container closure based on a comparison of USP and Ph. Eur. methods. Compendial methods were shown to be fit for purpose at each manufacturing site. The proposed specifications are considered well justified. All batches manufactured complied with the specifications.

2.4.4.4. Stability of the diluent

A harmonised shelf-life is proposed across both long-term storage conditions at all manufacturing locations.

Stability studies are on-going at all manufacturing sites and data are available from multiple batches that support of the proposed shelf-life. The shelf-life assigned to diluent co-packaged with the vaccine will not exceed the remaining shelf-life of the first component to expire.

2.4.4.5. Post approval change management protocol(s)

N/A

2.4.4.6. Adventitious agents

CONTROL OF VIRAL ADVENTITIOUS AGENTS

The TDV is a live attenuated viral vaccine, therefore, viral inactivation/clearance steps are not applicable to this product. Thus, the risk mitigation strategy for control of viral adventitious agents for the TDV relied on:

• Qualification of raw materials.

• Qualification of biological starting materials.

• Comprehensive testing (viral) for verifying the absence of contaminants in the raw and biological materials, unprocessed bulk, end of production (EOP) cells, bulk drug substance.

CONTROL OF NON-VIRAL ADVENTITIOUS AGENTS

The control of non-viral adventitious agents was achieved with qualification of the starting materials (master and working cell banks, master and working viral seeds) and materials of biological origin. Animal origin materials were sourced from appropriate locations and documentation was provided. In addition, all lots were gamma-irradiated to further reduce the risk of viral contamination.

Comprehensive testing was performed to verify the absence of potential contaminants in the raw and biological test materials, unprocessed bulk, end of production (EOP) cells, bulk drug substance and the finished product similar to the viral adventitious agent control.

Bioburden testing is performed on the bulk finished product. Endotoxin and sterility release testing are performed on the finished product.

In the case of human serum albumin, which is used as a component of the formulation for the tetravalent bulk finished product. Human serum albumin was obtained from Octapharma GmbH (Dessau-Rosslau, Germany) as Albumin (Human). This product is authorised in the European Union (License No. PEI.H.04333.02.1 for Albunorm 20% (DE/H/0480/002/DC), and PEI.H.04333.06.1 for Albunorm 25% (DE/H/0480/004/DC)). Certificates of Compliance and CoA were provided showing the risk of adventitious agent contamination to be low.

2.4.4.7. GMO

The applicant conclude that the overall risk for human health and the environment is negligible. This conclusion is endorsed.

Refer to the section on Ecotoxicity/environmental risk assessment.

2.4.5. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Major Objections raised during the review of the application related to the GMP status of the manufacturing sites have been resolved.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product, which pertain to characterisation, validation of analytical methods, specifications, comparability and stability. These points have been agreed as recommendations for future quality development.

2.4.6. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.4.7. Recommendation(s) for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommended some points for investigation.

2.5. Non-clinical aspects

2.5.1. Introduction

The nonclinical pivotal safety studies and biodistribution were conducted in compliance with Good Laboratory Practice (GLP). There are some minor deviations from the GLP principles in the safety studies but they do not affect the overall integrity of the studies and their results.

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

Non-Good Laboratory Practice (GLP) primary pharmacodynamic nonclinical studies performed have assessed:

- TDV-1, -2, -3 and -4 replication in mosquito cell culture and infection, dissemination and transmission in Aedes mosquito vectors.
- Neurovirulence of TDV-1, -2, -3 and -4 in newborn ICR mice.
- Immune responses elicited by monovalent TDV-1, TDV-2, TDV-3, and TDV-4.
- Immune responses elicited by TDV.
- Efficacy against DENV challenge in AG129 mouse and Non-Human Primate models.

Primary pharmacodynamics was assessed in C3/36 mosquito cells, mosquitoes, AG129 mice and Non-Human Primates (NHPs).

The applicant has conducted a study in mosquito cells to address the replication competence of the vaccine candidates. The data provided indicate that in all the cases, the replication potential was reduced for the vaccine candidates, but at a different extent. Important differences were detected for the GMP-produced TDV-3, which are also more prominent considering that the C6/36 cells were infected at a lower starting multiplicity of infection (MOI) compared with the counterparts, TDV-1, TDV-2 and TDV-4. In this case, the viral titres compared to the wild-type virus (DEN-3 16562) are only 10- to100-fold lower, which is unexpected considering the lower starting MOI which should results in even a larger difference when compared with the other TDV candidates. It was clarified in this regard that DENV-3 16562 was used at a lower MOI compared to the other wild-type viruses. Upon revision of the data, MOI used for DENV-3 was only 0.77x that of DENV-1, a really small difference. This lower MOI, in theory, would compensate for the lower starting MOI used for TDV-3. In addition, it appears that DENV-3 wild-type replicates itself at a lower rate compared to the other wild-type viruses. All this could justify the lower attenuation on replication compared to the other constructs. Still, the differences observed are of great magnitude, but this issue will not be followed as *in vivo* assays in mosquitoes have been conducted by the applicant, showing attenuation in replication for the TDV-3 constructs.

The studies provided by the applicant demonstrate reduced vector competence for the vaccine candidates when addressing infection, dissemination and transmission in mosquitoes, both primary and secondary vectors (*Aedes aegypti* and *Aedes albopictus*, respectively) following infection by intrathoracic and/or oral infection. This is an important safety feature of the vaccine candidate, in the sense that attenuated vectors have low chances to be transmitted by mosquitoes from vaccinees to the unvaccinated population, through the natural infection route.

Neurovirulence studies were performed in newborn ICR mice. Advice was sought from CHMP in this regard, which confirmed that based on the genetics of the vaccine candidates (considered as homogenous dengue compared to the previously authorised dengue vaccine, based upon Yellow Fever backbone) and the well-known profile (in terms of no or limited neurovirulence) of both the wild type dengue viruses and the parental strains (DEN-2 PDK-52) used for the generation of the TDV vaccine candidates, there was no need to test for neurovirulence in NHP. Neurovirulence is then discussed based on the literature references and the results from the study in newborn ICR mice. In this regard, data with ChiDEN candidates were already generated in the same animal model (newborn ICR mice), indicating no neurovirulence. The only difference between the current and previous studies resides on the origin of the mice employed: while the ICR mice used in the testing of TDV were purchased from Taconic, the mice employed in the ChiDEN testing corresponded to an ICR mouse colony maintained at the Centers for Disease Control and Prevention (CDC).

Taconic mice were much more susceptible to infection with the candidates than were the ICR mice of the CDC colony, resulting in 68% of mortality for TDV-2 at a lower dose of 10E3 pfu. Similarly, the average mortality for ChiDEN-2 was 64%, at the same lower dose. For TDV-4, the mortality at 10E3 pfu resulted in 18.5%. When higher dose levels were used (10E4 pfu), no mortality was seen with TDV-1 and TDV-3, but TDV-2 and TDV-4 turned out in 100% mortality. Although completely unexpected, the results at the lower dose are of lower magnitude compared to the neurovirulence of the wildtype DEN-2 16681, which resulted in 100% mortality at 10E3 pfu, suggesting that indeed the vaccine candidates are attenuated for neurovirulence. However, the results are unforeseen compared to either the published literature and the previous results with ChiDEN viruses that showed no neurovirulence.

In addition, at the time advice was given, the biodistribution study was not yet completed. The results from the single dose biodistribution study showed that TDV2 was found in the brain of one animal at Day 42, the last time point addressed. In toxicity studies conducted in the same animal model (AG129 mice) TDV was safe and well tolerated, with no other central nervous system (CNS) TDV-related findings. Neurovirulence of TDV, and in more detail TDV-2, was further analysed and discussed, contextualising the findings from the biodistribution study and the additional histopathological assessment requested. The conclusions are summarised in the toxicology section.

In vivo immunogenicity and challenge studies

Several non-clinical *in vivo* studies were carried out to evaluate the immunogenicity and efficacy of subcutaneous (SC) administration of monovalent TDV-1, TDV-2, TDV-3 and TDV-4 and tetravalent TDV formulations. Most of the studies conducted employed the AG129 mouse model (deficient in interferon alpha/beta and gamma receptor signalling). In addition, a pilot study in a limited number of animals to assess immunogenicity and efficacy of different levels of all TDV components in cynomolgus macaques was also carried out. While both models have drawbacks, immunodeficiency in the former and the lack of overt DENV pathogenesis in the latter, they are considered relevant for the PD assessment of the vaccine candidate.

Protection against DENV-3 challenge has not been assessed in AG129 mice. Although some protection data is available in NHPs, the number of animals assessed (N=2) in a pilot study is considered insufficient for a proper assessment. Of note, successful DENV-3 challenge studies have been described in the literature with the same animal model by the time of MAA submission. The applicant claims that by the time this model was available, clinical immunogenicity data was already available, and therefore no additional challenge studies were carried out. Nonetheless, the challenge model in NHPs was available but no justification was provided for not carrying out the pivotal challenge study in this species. Since substantial clinical data is already available, carrying out additional challenge studies is not considered necessary.

Immunogenicity of DENVax master virus seeds was assessed for monovalent TDV-1, TDV-2, TDV-3 and TDV-4 MVS in the AG129 mouse model. Animals received a SC dose of 5.0 log10 PFU/per dose or control (PBS) on Day 0 and Day 42. Serum samples were analysed at Days 40 and 56 by a plaque reduction neutralisation test (PRNT50). All vaccines elicited strong response against its wild type homolog, and to a lesser extent to other serotypes, although cross-reactive response was higher for DENV-3. Neutralising antibodies (NAbs) increased approximately 2-fold with a second immunisation only for TDV-1 and TDV-4 for its wild type homolog. TDV-2 and TDV-3 showed little or no variation after boost dose administration. Of note control animals also displayed low level response for DENV-3. The applicant's attributes this issue to the neutralisation assay controls unexpected response only in DENV-3 as a result to a specific matrix interference which only occurs with DENV-3 and not with other serotypes. Since no pre-vaccination sera is available, this hypothesis cannot be confirmed or ruled out.

DENV-1 or DENV-2 challenge assessment in AG129 mice after single dose monovalent immunisation with all four serotypes (SC) resulted in protection against lethal challenge with DENV-1, while only TDV-1 and TDV-2 protected against lethal DENV-2 challenge. TDV-3 conferred only partially protection while TDV-4 only delayed onset of death. DENV-3 or DENV-4 serotypes were not included in this study. The applicant could not provide this information since, according to the applicant's claims the information is not available since the study was carried out by another company prior to the acquisition of the product by Takeda,

Different ratios of the monovalent vaccine components (TDV-2, TDV-2, TDV-3, TDV-4) impact on AG129 mice immunogenicity were assessed in a separate study. TDV 5:5:5:5 and 3:3:5:5 formulations administration appears to result in the highest neutralisation antibodies, although a high variability was observed in the data submitted. After two immunisations, increasing TDV-3 and TDV-4 dose from 3.0

to 5.0 log_{10} PFU while maintaining unchanged the TDV-1 and TDV-2 dose in study DEN-009 resulted in a more balanced neutralising antibody response, nonetheless increasing the dose for all TDVs did not result in any marked increases with the exception of TDV-4. The applicant claims that "*it is likely that it is the degree of local replication (even in the absence of detectable viraemia), rather than the absolute amount of input virus, that significantly drives the resulting neutralizing antibody responses to each vaccine component*", nonetheless this statement was not adequately justified or supported with data. In the same line the claim that the dose administered is insensitive to changes once the minimal replication dose is achieved, is unsupported by data.

When assessing single immunisation in mice (Study SR-15-010) with quadrivalent TDV at approximately the same doses as in the previous study (Study DEN-009), data provided shows that the levels of neutralising antibodies elicited for DENV-4 measured at Day 56 were more than 2 times higher than those reported after double immunisation (single dose 279 GMT vs double dose 113). In addition, single dose elicited antibodies were also significantly higher than those reported after two immunisations for DENV-2 and DENV-3 but measured at different time points (Day 30 and Day 56 respectively). Since Study SR-15-010 was a challenge study and because of immunisation all mice were protected against DENV-4, the applicant concluded that there was no interference. Nonetheless, this claim is not supported as the only issue that can be concluded is that all animals were protected in both groups in the conditions tested regardless the existence of any degree of interference or not.

The question of uneven neutralising antibodies and disproportional response to various tetravalent vaccine formulations remains unresolved from a non-clinical perspective and the applicant's response is deemed insufficient to clarify this concern. Since this issue can be better addressed in the clinical scenario, the concern will be not further pursued from a non-clinical perspective (see Clinical AR).

Viraemia assessment shows increased levels for TDV 5:5:5:5 in all instances while no viraemia was reported using 3:3:5:5 formulation for TDV-1 and TDV-4. The number of viraemic animals and levels reported correlates with overall NAbs data. TDV-4 viraemia was detected in only one animal from the TDV 5:5:5:5 group. Clinical data obtained in exploratory immunogenicity analyses show that DENV-4 type specific neutralising antibodies were detected in the majority of samples from baseline seronegative trial participants. Although no relevant supporting data were provided for the lack of viraemia observed, clinical data (Trial DEN-301) shows that participants developed neutralising antibodies to all 4 serotypes tested.

Mice immunised with all vaccine formulation levels were also challenged with either DENV-1 strain Mochizuki at 6.0 log10 PFU or DENV-2 strain New Guinea C at 4.0 log10 PFU. All control mice but one (DENV-2 strain) succumbed to infection while all vaccinated animals survived. Immunisation with TDV, TDV-2 and TDV-4 shows protection to lethal challenge to DENV-1 and DENV-2 strain in the conditions tested.

Single dose immunisation and protection to DENV-4 challenge was assayed also in the same mice species dosed SC with 0.2 mL of TDV (4.3 log10, 4.7 log10, 5.0 log10 and 5.5 log10 PFU/mL for TDV 1-4, respectively), monovalent TDV-4 (5.5 log10 PFU/mL), monovalent TDV-2 (4.7 log10 PFU/mL) or FTA. TDV-2 elicited neutralising antibodies to both DENV-3 and DENV-4. DENV-1 and 2 were not tested for monovalent assessment. TDV vaccine administration elicited neutralising antibodies to all DENV strains. As seen in other presented data, NAbs against DENV-4 have shown the lowest levels regardless the type of vaccine administered.

All DENV-4 challenged animals immunised with TDV-4 survived infection with no relevant clinical signs. One animal vaccinated with TDV-2 died on Day 27 post-challenge. Another vaccinated animal in the TDV group displayed reversible weight loss (>10%) and clinical signs. Viral ribonucleic acid (vRNA) (qRT-PCR) was only reported in some vaccinated animals by Day 3 post challenge, and all were RNA negative by Day 5. All control animals remained positive to vRNA in all time points and all succumbed

to infection. Of note, TDV-4 monovalent administration resulted in a much higher level of DENV-4 neutralising antibodies as compared to the same dose level of the TDV vaccine which adds to the concern of the potential of interference among vaccine components.

However, the detailed interference study data submitted by the applicant (SR-15-010) show that the mean of DENV-4 neutralisation antibody titres was slightly higher in the TDV vaccinated mice than in the mice vaccinated with the monovalent TDV-4. The presence of DENV-4 viral RNA could not be detected in the serum of any vaccinated mice after challenge, indicating that the immune response generated by the TDV vaccine is sufficient to clear DENV-4 virus. It is suggested that the cause of the weight loss that was observed in an animal in the TDV-vaccinated group after DENV-4 challenge could be an injury from handling or natural causes and it is not considered related to the viral infection. In addition, the death of an animal in the TDV-2 group is also not attributed to the DENV-4 challenge as it happened 19 days after the last death in the control group and no DENV-4 vRNA was detected in TDV-2 vaccinated mice after Day 2 post challenge.

An investigational study in order to select the most adequate species for DART evaluation, concluded that NZW rabbit was the optimal species of those tested (mice (CD1, Balb/C and C57Bl/6) or Sprague Dawley rats) since little to no antibody response was observed after immunisation with TDV (Days 0, 23 and 57) in rodents. All NZW rabbits elicited NAbs response also after the second TDV immunisation.

A pilot study in adult cynomolgus macaques vaccinated on Day 0 and 60 with a total of two SC doses of TDV formulations, composed of various ratios of TDV-1, -2, -3 and -4 viruses or FTA control, was carried out to assess immunogenicity and protection to all DENV serotypes. Animals were challenged on Day 90. This was the only study where protection against a DENV-3 challenge was evaluated.

Immunogenicity data shows that neutralising antibodies to all four dengue virus serotypes are detected following single and boost dose SC administration. Immunogenicity response was variable. Before the second boost dose administration, the formulation with higher percentage of seroconversion was the 3:3:3:3 group for DENV-1 and DENV-2, but the 5:5:5:5 and 3:3:5:5 groups seroconversion percentages were higher for the latter. the 3:3:3:3 group PRNT50 values were also the highest for DENV-2 only throughout the study. The 3:3:5:5 group was the group that displayed an overall immunogenicity profile for all serotypes. The lowest NAbs titre and percentage of seroconversion was observed for DENV-4 while complete seroconversion in all animals was reported for the other three serotypes. FTA control animals on the DENV-1 immunogenicity assessment group displayed a significant NAbs titre. The low number of animals included precludes reaching any conclusion.

The administration of TDV vaccine in this species shows also protective effects in challenged animals as compared to controls nonetheless the disease clinical signs induced are absent or too mild in order to take conclusions regarding protection to the clinical manifestations of the disease and protection assessment relied on plaque titration and vRNA detection which is considered acceptable. Viraemia duration post challenge was reduced in all vaccinated groups as compared to controls and did not last more than 3 days in vaccinated animals when plaque titration was used for the assessment. vRNA revealed longer viraemia duration in all groups, due to the higher sensitivity of the assay. The mean peak serum virus titre in vaccinated groups was lower than controls with levels ranging from 0-1.8 log10 PFU/mL. Due to the low number of animals (2 animals per challenge per formulation), no clear conclusions can be drawn from this study. The 5:5:5:5 group animals did not present infectious virus particles following challenge in contrast with positive detection in two animals presenting infectious virus in blood for 3:3:5:5 for DENV-1 and DENV-2 and another positive detection for 3:3:3:3 for DENV-2. Viral RNA could be detected in all positive animals with evidence of infectious virus in the blood. The lack of detection of live virus in vaccinated animals suggests that the RNA detection was potentially associated to non-infectious virus.

No nonclinical assessment of different circulating dengue strains was included in the immunogenicity assessment. However, sera obtained in vaccinated participants is able to neutralise vaccine-matched and genetically diverse DENV strains. The applicant proposes to use in patients the following serotypes and concentrations:

TDV-1: ≥ 3.3 log10 PFU/dose; TDV-2: ≥ 2.7 log10 PFU/dose; TDV-3: ≥ 4.0 log10 PFU/dose; TDV-4: ≥ 4.5 log10 PFU/dose. This formulation with the precise concentrations of each TDV component for use in human subjects has not been assessed in nonclinical PD studies. In study DEN-009 the concentration of TDV-1 was lower than the clinical formulation, whereas the concentration of TDV-2 was higher than the clinical formulation, and this study also supported the development of clinical formulations containing higher concentrations of TDV-3 and TDV-4. The formulation used in other pharmacology studies included higher concentrations of all TDV serotypes than the clinical formulation. Although the precise formulation was not evaluated in non-clinical studies, this is not considered a major concern since the limitations of animal testing in reflecting clinical efficacy in humans are acknowledged. Infection of vaccinated subjects with circulating wildtype virus may pose a risk of antibody-dependent enhancement based on observations of severe dengue disease in individuals following CYD-TDV dengue vaccination. This potential risk may be due to a lack of adequate immune response to all four serotypes, or due to waning antibody titres against one or several serotypes. The applicant has not provided any studies to address the potential risk of antibody dependent enhancement (ADE). Considering models to assess ADE have been described in the literature, the lack of such investigations is a limitation of the dossier. Although these data would have been welcomed, it is accepted that current ADE models are not necessarily predictive of the human situation and may be dispensable if the risk of ADE has been sufficiently considered and evaluated in the clinical development. The applicant provided a justification why small animal models are not appropriate (not permissive to a productive and symptomatic infection). Moreover, additional information regarding possible non-clinical antibody-dependent disease enhancement (ADE) has been incorporated in the RMP (SII). Overall, this is acceptable from a non-clinical point of view.

2.5.2.2. Secondary pharmacodynamic studies

The absence of secondary pharmacodynamics studies is deemed acceptable.

2.5.2.3. Safety pharmacology programme

While no dedicated safety pharmacology studies were performed, CNS effects were evaluated as part of neurovirulence studies (Report DEN-014). CNS-associated organs (brain and spinal cord) were assessed histopathologically as part of the biodistribution (Report 5002340 and Module 2.6.4) and toxicology (Reports DEN-004, 5001168, 5001446) studies. There were no adverse findings reported. The lack of these studies was considered acceptable for the proposed vaccine and in line with the WHO guideline on the nonclinical evaluation of vaccines (Annex 1, WHO TRS 927) as well as the Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (Annex 2, WHO TRS 979).

2.5.2.4. Pharmacodynamic drug interactions

Dedicated pharmacodynamic drug interaction studies, such as the investigation of co-administration with other vaccines were not performed. This is deemed acceptable.

2.5.3. Pharmacokinetics

BIODISTRIBUTION

No standard pharmacokinetic studies have been conducted by the applicant, which is in line with the WHO guideline on nonclinical valuation of vaccines. Nonetheless, due to the nature of the product, the biodistribution assessment of the product is considered relevant. Dedicated absorption, metabolism and excretion studies were not performed as is appropriate for a product such as TDV.

TDV was investigated for Biodistribution and viral shedding of TDV in a GLP-compliant single dose study in AG129 mice. This study included toxicity assessment and used a validated quantitative assay (TDV serotype-specific, tetraplex RT-qPCR).

According to validation report, the following parameters were validated: range of response, intra- and inter-assay precision and accuracy, specificity and selectivity, limit of detection, recovery, multiplex interference, and short-term, long-term and freeze-thaw stabilities of the isolated viral RNA. The sensitivity and specificity of the assay were satisfactory. The RT-qPCR method for quantification of viral RNA for TDV-1, TDV-2, TDV-3 and TDV-4 is deemed acceptable.

In this study, a multiple of the highest clinical trial dose of TDV was administered to AG129 mice through clinically intended route (SC). TDV administration did not result in deaths or severe adverse findings. The dose information provided in 2.6 section corresponds to: TDV 1: 5.1 log10 PFU/dose; TDV 2: 4.5 log10 PFU/dose; TDV 3: 5.4 log10 PFU/dose; TDV 4: 5.9 log10 PFU/dose. Five mice/sex/group were euthanised on Days 2 (24 hours post-dose), 6, 14, and 42 for the genomic viral RNA of each serotype in selected samples including the bone marrow, brain, heart, the injection site skin and muscle, kidney, liver, lung, lymph node (inguinal, axillary, mandibular), spleen, thymus, ovary, testis, saliva, serum, faeces, and urine. No other tissues (e.g. Peyer's patches, gastrointestinal) were analysed and thus distribution to such lymphoid and non-lymphoid tissues cannot be excluded.

Following SC product administration, all TDV serotypes were detected in the injection site on Day 2 and to a lesser extent in several lymph node samples. Maximal viraemia was observed by Day 6, being TDV-2 the serotype with higher levels of detection and to a lesser extent in TDV-3, TDV-1 and TDV-4. At Day 6, the maximal levels of TDV viral genomic RNA in the serum with widespread distribution to all tissues and organs analysed were evidenced. The main target tissues were the injection site (skin, muscle), spleen, the lymph nodes and liver. The viral RNA levels in other samples were apparently lower. As expected, TDV levels and distribution rapidly decreased over time. At Day 14, all samples of serum, spleen and liver which represent main target tissues became negative for TDV, and other TDV-positive samples (injection site, lymph nodes) had significantly lower levels, which further decreased at Day 42, compared to values obtained at Day 6. These results demonstrated viral clearance occurring between Days 14 and 42.

Shedding of viral vRNA was not reported in faeces, although low levels have been reported on Day 6 in urine in a single animal and a low level of vRNA was detected in saliva of 3 animals (TDV-2 group (1/10); TDV-3 (2/10).

By Day 42 a substantial clearance has been observed. Positive samples were reported in the thymus (2/10) and also brain for TDV-2 (2/10). The levels vRNA levels were reduced in the thymus as compared to Day 14 but levels and number of positive animals remained unchanged for brain samples only for TDV-2 (Day 42). Four samples were also positive for TDV-2 (1/10) and TDV-3 (3/10) at Day 42 in samples from the mandibular LN and only the one related to TDV-3 was > lower limit of quantification (LLOQ). In addition, only one injection site sample was positive for vRNA for TDV-2 but also >LLOQ at in the last day of sampling.

The biodistribution profile observed for TDV could be considered expected for a live-attenuated vaccine in the immunocompromised AG129 mice. The potential mechanism for the TDV distribution to brain of AG129 mice is not fully understood and may partially be ascribed to the sensitivity of the species to distribution or persistence of vRNA in brain, due to lack of interferon responses and increased vascular permeability. Based on the integrated nonclinical studies (toxicology, neurovirulence) and clinical trial safety data, the applicant states that the potential neurological risk associated with this finding was minimal. However, considering the unexpected neurovirulence and toxicological findings, this issue was further explored and the conclusions are summarised in the toxicology section. The observation of the injection site, lymph nodes, spleen and liver as potential target tissues for high TDV exposure was also supportive of toxicological findings. Interestingly, TAK-003-related increase in spleen (males) weight was noted at Day 2 (24 hours post dose), while no vRNA were detectable in this tissue at this time point, and development of adaptive immune response within 24 hours post dose is also unlikely. The applicant assumed that the increases in spleen weight on Day 2 are likely to be related to increased cellularity, described as extramedullary haematopoiesis that this is not an unexpected outcome in the spleen. This reasoning could be acceptable, since TDV is designed to stimulate an immune response and the time course, as represented by the incidence and severity of the findings in the spleen, is consistent with a typical response to an immunomodulatory agent. In the absence of virus mediated inflammation, the observed change at 24 hours in the spleen weight might result from haematopoiesis-related factors secondary to immune stimulation.

2.5.4. Toxicology

AG129 mice and NZW rabbits were selected as animal models for the general toxicity studies and reproductive toxicity studies, respectively. Although both species have limitations it is acknowledged that they are the most suitable species considering the almost negligible antibody response observed in other mouse strains and rats after TDV administration (see study SR-15-011 in section 2.1). Rabbits developed antibody response after 2 TDV doses but they are not permissive for viral replication. Therefore, the relevance of the results of the reproductive toxicity studies are limited (see additional discussion below).

Different TDV batches were used in four safety studies. Therefore, the applicant clarified the composition and nature of each batch used in the pivotal toxicity studies and justified their representativeness with respect to the clinical batches. Upon clarification, it can be confirmed that batches DToxB0010115 and DToxB0020115 (studies 5001168 and 5001446 respectively) were representative of Phase 2 clinical trials while batch PPQ0010617 (study 20129939) was representative of Phase 3 clinical trials.

2.5.4.1. Single and repeat dose toxicity

A series of nonclinical general toxicity studies were conducted with TDV in AG129 mice. One pilot single-dose toxicity study (non-GLP) was conducted in AG129 mice to evaluate acute toxic effects of the vaccine. After subcutaneous injection of 5.6x10^5 PFU Dengue Tetravalent Vaccine, animals were observed for 11 days. In most of the vaccine-treated animals, transient minimal to mild inflammation in skin, haematopoiesis in spleen, transient increase of white blood cells and platelets were noted. In general, the findings were less severe than in wild-type DENV-2 treated animals. Three vaccinated mice developed testicular teratoma and one animal showed a testicular infarct with necrosis. In contrast, only one DENV-2 treated mouse developed a teratoma and none were observed in the control group. AG129 mice are known to be susceptible for testicular teratoma. Nonetheless, vaccine-treated mice showed a tendency of increased testicular teratoma, compared to the control animals. Although the applicant could not provide any in-house historical background data regarding testicular teratoma

in AG129 mice, a published scientific article (Stevens and Little 1954) mentions a 1% mean incidence of testicular teratomas in this mice strain. Recent data about teratomas in AG129 mice were not provided. In contrast to this publication from 1954, vaccine administered AG129 mice showed a 0.68% incidence of testicular teratomas compared to a 0% incidence in control AG129 mice. It is considered that the higher incidence of testicular teratomas in the vaccine-treated mice compared to control mice is not toxicological relevant because this incidence is still below the historical background incidence of 1%. However, a vaccine-related effect cannot be fully excluded. Viraemia was also addressed in this study. Upon a single dose, AG129 mice treated with TDV or the wild type strain DENV-2 showed detectable viraemia through Day 7 in DENV-2 infected animals and Day 5 in TDV-vaccinated animals. Viraemia was highest on Day 3 post treatment in both groups with peak titres lower in the TDV group than in the DENV-2 group. Virus was isolated from clarified brain homogenates of the DENV-2 group primarily at Day 5 and no virus was detected in brain homogenates of any TDV-vaccinated animals.

The applicant conducted three different GLP repeat toxicity studies in AG129 mice with TDV. In the first study (DEN-004), wild type parental DENV-2 strain was included as a comparator to establish the suitability of the AG129 mice as a relevant species for toxicity testing of TDV. Marked differences in the severity of treatment-related effects were observed between the TDV and DENV-2 groups, with organ weight changes and histopathology observations more extensive in the wild type DENV-2 strain-treated animals, including histological signs of encephalitis in the brain of two DENV-2 treated mice. In addition, clinical signs (rough fur coat) were observed in the DENV-2 group only. Therefore, it is acknowledged that the AG129 mouse is an appropriate model for detection of dengue virus-mediated pathological effects.

In each GLP toxicity study a different TDV dose was administered ($3.1x10^5$, $5.93x10^6$ and $1.07x10^7$ pfu/dose, corresponding to $5.5log_{10}$, $6.8log_{10}$ and $7.0log_{10}$ pfu/dose), these viral concentrations were equal to or above the human clinical dose. In all studies TDV was immunogenic and well tolerated. Replication of all four vaccine components was detected after the first administered dose, and treatment-related effects observed after the first administration were reversible or partially reversible and in most cases were considered expected observations following vaccination: increased in spleen weight that correlated with increased haematopoiesis, bone marrow myeloid cellularity, mild alterations in haematology and clinical chemistry, and inflammation at the injection site/skin.

However, in all three studies there were histological signs of lung inflammation, apparently with greater severity after the last dose, although in study 5001446 the incidence and severity of histopathological findings in the lung were greater in the control group than in the treated group when a concurrent control group was available for comparison (Days 8 and 76). Unfortunately, there was no control group for the assessment of the findings on Day 49. In addition, per protocol histopathological assessment was not conducted in the two mice found dead in this study with dark discoloration of the lung accompanied by dark fluid accumulation in the thoracic cavity in one of them and a conclusion cannot be drawn.

Taken together, a relationship between the vaccine and the lung findings in mice cannot be entirely ruled out. However, in the clinical assessment of Dengue Tetravalent Vaccine no safety signals regarding lung toxicity were observed.

Another unexpected finding in the histopathology report was the presence of findings in the brain of a female animal (No. 2508) in study 5001446 (neutrophilic infiltration and mild multifocal, white matter/grey matter, with micro-abscesses/neuron degeneration/malacia). The applicant has provided an additional histopathology report concerning animal 2508 (euthanised at D49). The conclusions of the new report are in accordance with the previous pathology analysis and stated that findings within the brain consisted of inflammation, necrosis, perivascular cuffing, meningeal inflammatory cell

infiltration, and endothelial cell hypertrophy and were of uncertain relationship to administration of TDV. In addition, several points in support of this conclusion are also raised in the pathology report: the absence of detectable vRNA in blood beyond D8 post dose, no comparability between the observed histopathological lesions and characteristic viral or immune-mediated encephalitis (although similarities were detected when the comparison was performed against Zika infection in AG129 mice), and the absence of acute liver failure (as a leading factor in dengue-associated CNS pathology). However, microscopic findings in the brain from this single test article-administered animal could not be ruled out as potentially test article-related, even in the absence of any other findings in this animal or in other vaccinated animals.

In this regard, it should be noted that the biodistribution study (5002340), with single SC administration of TDV in AG129 mice showed presence of TDV-2 in brain in animals at Day 6, 14 and 42. TDV-1 and TDV-3 were also detected in brain at Day 6, but not beyond that time point. This result indicated that TDV could reach the brain. Regarding the neurovirulence study, discordant results were found when different mouse colonies were used, which are presumably linked to the mice background. Although less virulent than the respective wildtype virus, 10^3 PFU of DENVax-2 still resulted in approximately 68% neurovirulence in ICR newborn mice, being this the lowest result obtained in three experimental rounds (Report DEN-014).

The applicant concludes after examination of all the data that the risk for clinical neurovirulence for TDV is negligible and substantiates this conclusion on the inherent permissive features of the non-clinical models used. The rationale for the animal model selection with the purpose of non-clinical assessment has been justified by the applicant in several sections of the dossier. However, the relevance of both models, suckling mice and AG129 mice can be considered as limited with the purpose of safety assessment of candidate dengue vaccines following WHO guidelines (Annex 2 Guidelines on the quality, safety and efficacy of dengue tetravalent vaccines (live, attenuated) Replacement of Annex 1 of WHO Technical Report Series, No. 932).

The pathologist report concludes on an uncertain relationship to the administration of the vaccine and indicates that the findings cannot be ruled out as potentially test-article related. This conclusion is supported. However, no neurological symptoms were found in the clinical trials following administration of TDV.

2.5.4.2. Genotoxicity

No genotoxicity studies were conducted with TDV, which is acceptable.

2.5.4.3. Carcinogenicity

No carcinogenicity studies were conducted with TDV, which is acceptable.

2.5.4.4. Reproductive and developmental toxicity

The reproductive toxicity assessment, pilot study 20153701 was conducted according to the CHMP recommendation (Scientific Advice EMA/CHMP/SAWP/629331/2014) to consider the vaccination of a group of animals during gestation period only, without pre-mating dose, to assess the potential risk associated with TDV vector infection and replication during gestation period and TDV vector transmission into the foetus. In this study no significant or adverse inflammation was observed in rabbits after vaccine administration and therefore in the pivotal reproductive toxicity study an additional group vaccinated shortly after mating was not included.

A combined embryo-foetal and pre/post-natal reproductive toxicity study was conducted in the New Zealand White rabbits. The test article was clinical lot TAK-003, which was produced using the final manufacturing process. A multiple (i.e. 2 times) of highest clinical dose of TAK-003 was administered subcutaneously (SC) to female rabbits on Study Days (SDs) 1, 21 and 42 (day of mating), and then on Gestation Days (GDs) 7 and 28 for a total of 5 occasions. The dose level and schedule were selected according to the pilot studies in this species and strain and is acceptable. The Caesarean sectioning group was terminated on GD29 and the natural delivery group was terminated at Lactation Day (LD) 29. TAK-003-specific antibodies were detected in all F0 female rabbits and in 100% of foetuses at the end of gestation (GD29) and the natural delivery kits were 95%, 100%, 83% and 93% seropositive to DENV-1, -2, -3 and -4, respectively, at the end of lactation (LD29).

Administration of TAK-003 caused transient grade 1 or 2 erythema and grade 1 oedema, but did not increase the incidence of clinical signs, maternal gross lesions or have an adverse effect on body weights, food consumption, and mating, fertility, or reproductive performance of the F0 generation does, as compared with the group administered the saline control. There were 5 unscheduled deaths of F0 generation does: 2 in saline control group euthanised on GD29 or LD26, and 3 in TAK-003 group all found dead on LD2, LD21 or LD25. Regarding the cause of death, the applicant reasoned that they may have been related to the reduced food consumption and bodyweight loss. The observed rate of unscheduled deaths of F0 generation falls into the historical range of variability in the testing facility. Histopathology analysis has not been conducted on these unscheduled deaths of animals and reasons for these deaths or abortions are not typically documented.

Intrauterine examination or litter parameters evaluated at GD 29 were not adversely affected by maternal administration of TAK-003. No adverse effects were observed on foetal weight and no foetal gross external, soft tissue, or skeletal abnormalities related to administration of TAK-003.

Maternal administration with TAK-003 did not result in adverse effects on clinical observations, body weights, organ weights, gross pathology observations or pre-weaning developmental observations in the F1 generation kits. At natural delivery, there were a total of 19 stillborn kits from 7 (29.2%) out of 24 does in the TAK-003 group, which was higher than a total of 4 stillborn kits from 3 (15.8%) out of 19 does in saline control group. However, the observed rate of does with stillborn in both study groups falls into historical range of variability in the testing facility for the tested species. Therefore, the observed imbalance is considered incidental and not TAK-003 related. It is also agreed that stillbirth is common and its rate is high in NZW rabbits.

In addition, a total of 43 kits in saline control and 44 kits in TAK-003 group were found dead or euthanised early and necropsied. Only a fraction of them were found with no milk in the stomach as potential cause of death (22 in saline and 15 in TAK-003). According to the applicant, the cause for death of F1 kits is not limited to no milk in stomach, but may also include insufficient feeding of the kits or abandonment of the kits, etc. Death of F1 kits due to malformation was not mentioned but an imbalance in this aspect appears unlikely, given the foetal examination data from the Caesarean section of the study. In compliance with the study protocol, histopathology analysis was not conducted in these kits found dead or euthanised.

TDV excretion in milk was not assessed in rabbits.

As rabbits are not permissive for dengue virus replication, the relevance of the fertility and reproductive toxicity assessment in this species is limited.

2.5.4.5. Local Tolerance

Local tolerance was assessed in repeat-dose studies in AG129 mice and in the reproductive and developmental toxicity study in rabbits. In these studies, non-adverse transient findings related to administration of TDV were observed both macroscopically (abnormal appearance) and microscopically (mixed cellular inflammation, necrosis, oedema, haemorrhage, vascular degeneration/necrosis, thrombosis, fibrosis, epidermal hyperplasia, hyperkeratosis, and cellular crusts) at the site of administration. These findings are consistent with vaccine administration and, generally, TDV was well tolerated.

A separate local tolerance study is not warranted.

2.5.5. Ecotoxicity/environmental risk assessment

An environmental risk assessment was submitted by the applicant. This is not a mandatory requirement for a scientific opinion on a medicinal product under Article 58 of Regulation (EC) No 726/2004.

The characterisation of the risk scenarios in relation to both seriousness and probability of harm as well as benefit of the TDV product, in the context of release of the GMO in endemic and non-endemic areas justify the use of TDV. The assessment did not identify any risks that require further control. The reasons for this include:

• Safe history of evaluation of TDV in conventional studies of single dose, local tolerance, repeated dose toxicity, and toxicity to reproduction and development in AG129 mice and New Zealand White rabbits.

• TDV viruses are dengue viruses, and as such are not known to be transmitted by shedding, but rather by bite from an infected mosquito vector. In a GLP-compliant single dose biodistribution and shedding study in AG129 mice, no shedding of TDV vRNA was detected in faeces and urine, and TDV-3 was detected in saliva from one animal at day 6 only, confirming a low risk for vaccine shedding to the environment or transmission from vaccinees by shedding.

• No serious allergic reactions or toxicity in people and other organisms have been observed to date.

• TDV was generally well tolerated in clinical trials. To date, an estimated total of more than 19,000 subjects have received more than 38,000 doses of different formulations of TDV. This included adults and children in both endemic and non-endemic areas and in these trials the TDV vaccine has been generally well tolerated, with an overall adverse event profile that is considered acceptable.

• Limited ability of TDV to replicate in humans, nonhuman primates and AG129 mice.

• Limited ability of TDV to replicate in the mosquito vectors Ae. aegypti and Ae. Albopictus and in C6/36 cells (Ae. albopictus derived) compared to wild type dengue viruses.

• Highly unlikely contribution of maternal-foetal or mother to infant transmission of TDV to dissemination of the attenuated vaccine viruses in the environment.

• Based on the MID50 for both *Ae. aegypti* and *Ae. albopictus* and the vaccine viral RNA levels present in TDV vaccinees, there is very limited potential for human-to-human TDV transfer by mosquito vector.

• Limited ability and opportunity for TDV to undergo recombination with other viruses based on low levels of viral RNA present in vaccinees, and thereby transferring the introduced prM and E genes or transferring genes from the attenuated TDV-2 backbone.

• TDV viruses have been shown to be genetically stable in vitro through the cell culture passages required for vaccine manufacture and *in vivo* in humans and animals. Limited reversion events have been observed for TDV, most often the 5 prime non-coding region locus and rarely at the NS1 locus. In all cases, two of the three attenuation loci were maintained which are sufficient to retain the TDV attenuation phenotype

The applicant should liaise with the relevant local authorities where the medicinal product is intended to be authorised as to ensure that the documentation complies with the relevant national legislation with regard to ecotoxicity and environmental risk assessment.

2.5.6. Discussion on non-clinical aspects

Several experimental models were used for the assessment of the pharmacology of TDV vaccine candidates. Mosquitoes were employed for addressing the attenuation of TDV: the studies provided by the applicant demonstrate reduced vector competence for the vaccine candidates when addressing infection, dissemination and transmission in mosquitoes.

Neurovirulence was tested in newborn ICR mice. Although almost absent neurovirulence was expected, that was not the case for either ChiDEN-2 and TDV-2, but the percentage of mortality was reduced compared with wild-type DENV-2. Several non-clinical *in vivo* studies were carried out to evaluate the immunogenicity and efficacy of subcutaneous (SC) administration of monovalent TDV-1, TDV-2, TDV-3 and TDV-4 and tetravalent TDV formulations. Most of the studies conducted employed the AG129 mice model (deficient in interferon alpha/beta and gamma receptor signalling). In addition, a pilot study in a limited number of animals to assess immunogenicity and efficacy of different levels of all TDV components in cynomolgus macaques was also carried out.

Although the overall assessment of the immunogenicity elicited by the TDV vaccine and monovalent components and protection to wild-type DENVs infection in mice and cynomolgus macaques is promising, some other concerns have been identified. Those issues were further clarified and addressed below.

Protection against DENV-3 challenge has not been assessed in AG129 mice. Although some protection data is available in NHPs, the number of animals assessed (N=2) is considered insufficient for a proper assessment. No adequate discussion has been provided. Successful DENV-3 challenge studies have been described in the literature with the same animal model (DENV-3). However, since substantial clinical data is already available, carrying out additional challenge studies is not considered necessary. The applicant's attributes this issue to the neutralisation assay controls unexpected response only in DENV-3 as a result to a specific matrix interference which only occurs with DENV-3 and not with other serotypes. Since no pre-vaccination sera is available, this hypothesis cannot be confirmed or ruled out**.** The assessors could not locate the full report of the study titled "Immunogenicity and Efficacy of Chimeric Dengue Vaccine (DENVax) Formulations in Interferon-Deficient AG129 Mice". This report was not made available since, according to the applicant's claims, the information is not available as the study was carried out by another company prior to the acquisition of the product by Takeda. The Nabs response to various tetravalent vaccine formulations across pharmacology studies, is uneven and not proportional to the doses administered but no relevant justification has been submitted by the applicant. This issue remains unresolved from a non-clinical perspective and the applicant's response is deemed insufficient to clarify this concern. Since this issue can be better addressed in the clinical scenario, the concern will be not further pursued from a non-clinical perspective (see Clinical AR).

Although TDV-4 viraemia was detected in only one animal from the TDV 5:5:5:5 group (Report DEN-009), clinical data (Trial DEN-301) shows that participants developed neutralising antibodies to all 4 serotypes tested. TDV-4 monovalent administration resulted in a much higher level of DENV-4 NAbs as

compared to the same dose level of the TDV vaccine which adds to the concern of the potential of interference among vaccine components that should be discussed by the applicant. In addition, one TDV vaccinated animal displayed weight loss, which contrasts with the lack of clinical effects in those animals vaccinated with monovalent TDV-4.

No assessment of different circulating dengue strains was included in the immunogenicity assessment; however, sera obtained in vaccinated participants is able to neutralise vaccine-matched and genetically diverse DENV strains. The applicant proposes to use in patients the following serotypes and concentrations: TDV-1: ≥ 3.3 log10 PFU/dose; TDV-2: ≥ 2.7 log10 PFU/dose; TDV-3: ≥ 4.0 log10 PFU/dose; TDV-4: ≥ 4.5 log10 PFU/dose. Although the precise formulation proposed for clinical use was not evaluated in non-clinical studies, this is not considered a major concern. Infection of vaccinated subjects with circulating wildtype virus may pose a risk of antibody-dependent enhancement based on observations of severe dengue disease in individuals following CYD-TDV dengue vaccination. This potential risk may be due to a lack of adequate immune response to all four serotypes, or due to waning antibody titres against one or several serotypes. The applicant has not provided any studies to address the potential risk of antibody dependent enhancement (ADE). Considering that models to assess ADE have been described in the literature, the lack of such investigations are a limitation of the dossier. Although these data would have been welcomed, it is accepted that current ADE models are not necessarily predictive of the human situation and may be dispensable if the risk of ADE has been sufficiently considered and evaluated in the clinical development. The applicant provided a justification why small animal models are not appropriate (not permissive to a productive and symptomatic infection). Moreover, additional information regarding possible non-clinical antibody-dependent disease enhancement (ADE) has been incorporated in the RMP. No standard pharmacokinetic studies have been conducted by the applicant, which is in line with the WHO guideline on nonclinical evaluation of vaccines. A GLP-compliant study to assess the biodistribution and shedding of the four serotypes of the vaccine candidate in AG129 mice was performed in support of the biodistribution of the product. In this study, a multiple of highest clinical trial dose of TDV was administered to AG129 mice through clinically intended route (SC). TDV administration did not result in deaths or relevant clinical adverse findings. Upon administration, TDV-1, TDV-2, TDV-3 and TDV-4 were detected in the injection site at Day 2 and in several lymph nodes. Maximal viraemia was achieved at Day 6 being TDV-2 and TDV-4 the serotypes with the highest and lowest levels, respectively. During the course of the study, several microscopic and microscopic findings of minor or moderate relevance were found in some tissues, including bone marrow, spleen, mandibular lymph nodes and injection site; however, at Day 42, the last study day, those findings were reported as recovered or partially recovered, indicating a transient nature. By Day 42 most tissues were negative for vRNA, with several exceptions. These results demonstrated viral clearance occurring between Days 14 and 42. It should be noted that TDV-2 was detected in brain in one animal at Day 42 but this finding was not associated with clinical observations of histological hallmarks. Interestingly, Dengue Tetravalent Vaccine-related increase in spleen (males) weight was noted at Day 2 (24 hours post dose), while no vRNA were detectable in this tissue at this time point, and development of adaptive immune response within 24 hours post dose is also very unlikely. The applicant assumed that the increases in spleen weight on Day 2 are likely to be related to increased cellularity, described as extramedullary haematopoiesis that this is not an unexpected outcome in the spleen. This reasoning is accepted since TDV is designed to stimulate an immune response and the time course, as represented by the incidence and severity of the findings in the spleen, is consistent with a typical response to an immunomodulatory agent. In the absence of virus mediated inflammation, the observed change at 24 hours in the spleen weight might result from haematopoiesis-related factors secondary to immune stimulation.

Regarding the shedding, vRNA was not detected in faeces, albeit lower levels were seen in urine and saliva for some animals.

AG129 mice and NZW rabbits were selected as animal models for the general toxicity studies and reproductive toxicity studies, respectively. Although both species has limitations it is accepted that they are suitable species considering the almost negligible antibody response observed in other mouse strains and rats after TDV administration.

A series of non-clinical general toxicity studies were conducted with TDV in AG129 mice. Wild type DENV-2 was used as a comparator in the single dose study and one of the repeat-dose studies in order to establish the suitability of the AG129 mice as a relevant species for toxicity testing of TDV. Marked differences in the severity of treatment-related effects were observed between the TDV and DENV-2 groups, including histological signs of encephalitis in the brain of some DENV-2 treated mice.

In all general toxicity studies TDV was immunogenic and well tolerated. Replication of all four vaccine components was detected after the first administered dose, and treatment-related effects were observed after the first administration that were reversible or partially reversible and in most cases were considered expected observations following vaccination: increased in spleen weight that correlated with increased haematopoiesis, bone marrow myeloid cellularity, mild alterations in haematology and clinical chemistry and inflammation at the injection site/skin.

The parental AG129 sub-strain is known to be susceptible to spontaneous occurrence of testicular teratoma, and in this respect, great caution needs to be exercised to interpret the finding of testicular teratoma in the TDV-treated AG129 mice in the single dose toxicity study. Although it is considered that the higher incidence of testicular teratomas in the vaccine-treated mice compared to control mice is not toxicological relevant because this incidence is still below the historical background incidence of 1%, a vaccine-related effect cannot be fully excluded.

In all three studies there were histological signs of lung inflammation, apparently with greater severity after the last dose. Although some control animals showed similar findings, a relationship with the treatment cannot be excluded. In addition, two vaccine-treated mice found dead in study 5001446 showed dark discoloration of the lung accompanied by dark fluid accumulation in the thoracic cavity in one of them. Although in this study the incidence and severity of histopathological findings in the lung were greater in the control group than in the treated group, no control group at Day 49 was available. Moreover, per protocol histopathological assessment was not conducted in the two mice found dead in this study and a conclusion cannot be drawn. However, no safety signal related to lung toxicity was detected in the clinical trials.

Also, as unexpected finding in the histopathology report was the presence of findings in the brain of a female animal (No. 2508) in study 5001446 (neutrophilic infiltration and mild multifocal, white matter/grey matter, with micro-abscesses/neuron degeneration/malacia). There was no vRNA detected in serum on Day 49 (vRNA was not detectable in serum past Day 8, even with repeated dosing), indicating a low likelihood of infectivity. In addition, there were no correlative clinical observations or haematology changes indicative of infection, neuro-manifestations, or any adverse effects in Animal 2508. However, taking into account that encephalitis is an atypical manifestation of dengue and the unexpected results obtained in the neurovirulence study (68.8% neurovirulence of 10^3 PFU DENVax-2), further discussion of this issue was requested, contextualising the findings from the biodistribution study and the additional histopathological assessment requested for Animal 2508. The additional pathology report provided in the responses concludes on an uncertain relationship to the administration of the vaccine and indicates that the findings cannot be ruled out as potentially test-article related, even in the absence of any other findings in this animal or in other vaccinated animals. It should be noted as well that the biodistribution study (5002340) showed presence of TDV-2 in brain and despite being less virulent than the respective wildtype virus, 10^3 PFU of DENVax-2 still resulted in approximately 68% neurovirulence in ICR newborn mice. The applicant concludes on a negligible risk for neurovirulence, which is supported by the absence of neurological signs in the clinical trial and

ascribes the observed results to the inherent permissive features of the non-clinical models used although some uncertainties remain regarding the potential relationship of the described findings with vaccine administration.

The developmental and reproductive toxicity of TDV vaccine was assessed in NZW rabbits, according to the feedback of the CHMP/EMA. There were a few positive findings including unscheduled deaths of F0 generation of animals and imbalanced number of stillborn kits at natural delivery. Regarding the unscheduled deaths of F0 generation, this could be due to reduced food consumption and bodyweight loss with the observed rate of deaths falling into the historical range of variability in the testing facility. The observed rate of does with stillborn also falls into the historical range of variability.

TDV elicited an immune response in 100% of the rabbits prior to mating, and antibodies were detected in >83% of kits delivered by Caesarean section or natural delivery. However, as rabbits are not permissive for dengue virus replication the relevance of the fertility and reproductive toxicity assessment in this species is limited.

The omission of genotoxicity and carcinogenicity studies with TDV vaccine is acceptable, due to the nature of the product. Local tolerance of TDV vaccine was assessed in GLP-compliant repeat-dose toxicity studies, which is acceptable.

All potential hazards for both unintended recipients and the environment have been identified. Given the nature of the GMOs (four live attenuated virus strains), the manufacturing controls, the stability of the attenuating genotype, the lack of shedding, the need for a mosquito vector to transmit, the limited ability to replicate in humans, animals and mosquito vectors, etc, it is concluded that any risks of harm from the proposed use of TDV and release into the environment are considered negligible to either people and animals or the environment.

2.5.7. Conclusion on the non-clinical aspects

From a non-clinical point of view, there are no outstanding issues precluding a Scientific Opinion.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

It follows a list of all completed and ongoing Clinical Trials performed with this vaccine.

Table 2: Tabular Listing of Clinical Studies

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Controlled Clinical Studies							
Immunogenicity and Safety	INV-DEN-101 United States	Dose escalation - To investigate the safety and immunogenicity of the liquid TDV formulation. The trial evaluated low dose TDV and high dose TDV when given subcutaneously (SC) or intradermally as 2 doses 3 months apart.	Randomized Double-blind, Placebo-controlled Dose escalation	TDV TDV doses: Low dose TDV-1: 8 x 10^3 PFU TDV-2: 5 x 10^3 PFU TDV-3: 1 x 10^4 PFU TDV-4: 2 x 10^5 PFU High dose TDV-1: 2 x 10^4 PFU TDV-2: 5 x 10^4 PFU TDV-3: 1 x 10^5 PFU TDV-4: 3 x 10^5 PFU Subcutaneous or intradermal injection	72	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	INV-DEN-102 Colombia	Evaluating High Dose (HD) TDV and Low Dose (LD) TDV - To assess the safety and tolerability of TDV in healthy adults.	Randomized Double-blind, Placebo-controlled Dose escalation	TDV TDV doses: Low dose TDV-1: 8 x 10^3 PFU TDV-2: 5 x 10^3 PFU TDV-3: 1 x 10^4 PFU TDV-4: 2 x 10^5 PFU High dose TDV-1: 2 x 10^4 PFU TDV-2: 5 x 10^4 PFU TDV-3: 1 x 10^5 PFU TDV-4: 3 x 10^5 PFU Subcutaneous or intradermal injection	96	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	INV-DEN-103 United States	LD TDV evaluating different methods of delivery - To compare the safety and tolerability of varied dose schedules and methods of administration of a recombinant tetravalent dengue vaccine, TDV, when administered intradermally in healthy adults. - To compare the immunogenicity of varied dose schedules and different methods of administration of the vaccine against all 4 dengue serotypes in healthy adults.	Randomized Partial-blind, Parallel group	TDV TDV dose: Low Dose TDV-1: 8 x 10^3 PFU TDV-2: 5 x 10^3 PFU TDV-3: 1 x 10^4 PFU TDV-4: 2 x 10^5 PFU Intradermal Injection	67	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	INV-DEN-104 United States	- To compare the safety and tolerability of different dose schedules and level of SC administered recombinant TDV in healthy adults. - To compare the immunogenicity of different dose schedules and level of TDV against all four dengue serotypes in healthy adults.	Randomized Double-blind for Parts 1 and 2, Open-label for Part 3	TDV TDV doses: High dose TDV-1: 2 x 10^4 PFU TDV-2: 5 x 10^4 PFU TDV-3: 1 x 10^5 PFU TDV-4: 3 x 10^5 PFU High dose 2 TDV-1: 2 x 10^4 PFU TDV-2: 5 x 10^4 PFU TDV-3: 1 x 10^5 PFU TDV-4: 1 x 10^6 PFU High dose/10 TDV-1: 2 x 10^3 PFU TDV-2: 5 x 10^3 PFU TDV-3: 1 x 10^4 PFU TDV-4: 3 x 10^4 PFU Subcutaneous injection	140	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	DEN-106 United States	Evaluating lyophilized formulation of TDV - To evaluate the equivalence of the lyophilized formulation (Group D) compared to the Shantha liquid formulation (Groups A+B combined) based on the Geometric Mean Titers (GMTs) of neutralizing antibodies of each serotype measured at Month 1 among dengue seronegative subjects.	Randomized Double-blind	TDV TDV dose: High dose TDV-1: 2 x 10^4 PFU TDV-2: 5 x 10^4 PFU TDV-3: 1 x 10^5 PFU TDV-4: 3 x 10^5 PFU Subcutaneous injection	996	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	INV-DEN-203 Colombia, Puerto Rico, Singapore, Thailand	Age de-escalation - To evaluate the safety and tolerability of a subcutaneously administered recombinant tetravalent dengue vaccine in healthy adults and children. - To assess the immunogenicity of the vaccine against all four dengue serotypes in healthy adults and children.	Randomized Double blind, Placebo-controlled	TDV TDV dose: High dose TDV-1: 2 x 10^4 PFU TDV-2: 5 x 10^4 PFU TDV-3: 1 x 10^5 PFU TDV-4: 3 x 10^5 PFU Subcutaneous injection	360	Vaccination: Month 0, Month 3	Complete; Full
Immunogenicity and Safety	DEN-204 Dominican Republic, Panama, The Philippines	Immunogenicity and safety - To assess the humoral immune responses to subcutaneously administered TDV in a subset of healthy subjects aged between 2 and <18 years and living in dengue endemic countries.	Randomized Double-blind, Placebo-controlled	TDV TDV Dose: TDV-1: 4.4 $\log_{10}$ PFU TDV-2: 3.8 $\log_{10}$ PFU TDV-3: 4.5 $\log_{10}$ PFU TDV-4: 5.6 $\log_{10}$ PFU Subcutaneous injection	1794	Vaccination: Group 1 Month 0, Month 3 Group 2 Month 0 Group 3 Month 0, Month 12	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Efficacy and Safety	DEN-301 Thailand, The Philippines, Sri Lanka, Brazil, Panama, Dominican Republic, Colombia, Nicaragua	Efficacy, immunogenicity, and safety. - To evaluate the efficacy of 2 doses of TDV in preventing symptomatic dengue fever of any severity and due to any of the 4 dengue virus serotypes in 4-16 year old subjects	Randomized Double-blind, Placebo-controlled	TDV TDV dose: TDV-1: 3.6 $\log_{10}$ PFU TDV-2: 4.0 $\log_{10}$ PFU TDV-3: 4.6 $\log_{10}$ PFU TDV-4: 5.1 $\log_{10}$ PFU Subcutaneous injection	20,071	Vaccination: Month 0, Month 3 Booster dose: Planned between 4 years to approximately 4.5 years post second dose.	Ongoing; Interim (Completed Parts 1, 2, initial 6 months of Part 3, i.e. up to 24 months after second dose). Additional Tables/Listings/ Graphs (TLG) report for data up to 36 months after second dose.

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	DEN-304 United States	Lot to lot consistency - To demonstrate lot-to-lot consistency of 3 consecutive TDV lots in terms of equivalence of immune responses at 1 month post second dose.	Randomized Double-blind, Placebo-controlled	TDV TDV doses: TDV Lot 1 TDV-1: 5.1 $\log_{10}$ PFU TDV-2: 4.5 $\log_{10}$ PFU TDV-3: 5.4 $\log_{10}$ PFU TDV-4: 5.9 $\log_{10}$ PFU TDV Lot 2 TDV-1: 5.2 $\log_{10}$ PFU TDV-2: 4.5 $\log_{10}$ PFU TDV-3: 5.4 $\log_{10}$ PFU TDV-4: 6.2 $\log_{10}$ PFU TDV Lot 3 TDV-1: 5.2 $\log_{10}$ PFU TDV-2: 4.5 $\log_{10}$ PFU TDV-3: 5.2 $\log_{10}$ PFU TDV-4: 5.8 $\log_{10}$ PFU Subcutaneous injection	919	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	DEN-315 Mexico City	Immunogenicity and safety - To describe the neutralising antibody response against each dengue serotype at 1 month post second dose of TDV or placebo in dengue-naive adolescent subjects.	Randomized Double-blind, Placebo-controlled	TDV TDV dose TDV-1: 5.1 log_{10} PFU TDV-2: 4.5 log_{10} PFU TDV-3: 5.4 log_{10} PFU TDV-4: 5.9 log_{10} PFU Subcutaneous injection	400	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety Studies	DEN-305 United States	Co-administration: YF vaccine - To demonstrate non-inferiority (NI) of the YF seroprotection rate response to 1 dose of YF-17D vaccine, 1 month following concomitant administration with 1 dose of TDV compared to placebo.	Randomized Observer-blind, Placebo-controlled	TDV Yellow Fever YF-17D Vaccine TDV dose: TDV-1: 5.1 log10 PFU TDV-2: 4.5 log10 PFU TDV-3: 5.4 log10 PFU TDV-4: 5.9 log10 PFU YF-17D vaccine (Stamaril, Sanofi Pasteur) contained not less than 4.74 log_{10} PFU per dose of the 17D-204 strain of the YF virus. Subcutaneous injection	900	Vaccination: Group 1 Month 0: YF-17D + placebo Month 3: TDV Month 6: TDV Group 2 Month 0: TDV + placebo Month 3: TDV Month 6: YF-17D Group 3 Month 0: TDV + YF-17D Month 3: TDV Month 6: Placebo	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	DEN-314 United Kingdom	Co-administration: HAV vaccine - To demonstrate non-inferiority of the immune response to 1 dose of HAV vaccine in HAV/DENV-naive subjects 1 month following co-administration with 1 dose of TDV (Group 3) compared to 1 dose of HAV vaccine co administered with placebo (Group 1).	Randomized Observer-blind, Placebo controlled	TDV Hepatitis A virus (HAV) vaccine TDV dose: TDV-1: 5.1 log_{10} PFU TDV-2: 4.5 log_{10} PFU TDV-3: 5.4 log_{10} PFU TDV-4: 5.9 log_{10} PFU HAV vaccine dose: Each vaccine dose contained 1440 ELISA units adsorbed on aluminum hydroxide (0.5 mg/1.0 mL). Subcutaneous or intramuscular injection	897	Vaccination: Group 1 Month 0: HAV + placebo Month 3: Placebo Group 2 Month 0: TDV + placebo Month 3: TDV Group 3 Month 0: TDV + HAV Month 3: TDV	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Uncontrolled Clinical Studies							
Immunogenicity and Safety	INV-DEN-105 United States	Evaluating LD TDV administered SC or IM, using different delivery methods. - To evaluate the safety and immunogenicity of TDV when administered by 2 different routes (subcutaneous [SC] and intramuscular [IM]) in healthy adults. - To evaluate the safety and immunogenicity of TDV when administered SC and IM using the Pharmajet Stratis™ Food and Drug Administration (FDA)-cleared delivery device. - To evaluate the safety and immunogenicity of TDV on a compressed vaccination schedule of 2 injections on Day 0 (1 in each arm).	Randomized Non-blinded	TDV TDV dose: Low dose TDV-1: 8 x 10^3 PFU TDV-2: 5 x 10^3 PFU TDV-3: 1 x 10^4 PFU TDV-4: 2 x 10^5 PFU Subcutaneous or intramuscular injection	80	Vaccination: Month 0, Month 3	Complete; Full

Type of Study	Study Identifier; Country	Objectives of Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of subjects	Vaccination schedule	Study Status; Type of Report
Immunogenicity and Safety	DEN-205 Singapore	Compare final TDV and HD TDV formulation - To assess the post-vaccination neutralizing antibody response against each dengue serotype by vaccine group.	randomized Double-blind	TDV TDV doses: HD-TDV TDV-1: 4.3 x 10^4 PFU TDV-2: 4.8 x 10^4 PFU TDV-3: 5.1 x 10^5 PFU TDV-4: 5.4 x 10^5 PFU TDV TDV-1: 4.4 x 10^4 PFU TDV-2: 3.8 x 10^5 PFU TDV-3: 4.5 x 10^5 PFU TDV-4: 5.6 x 10^5 PFU Subcutaneous injection	351	Vaccination: Month 0	Complete; Full
Immunogenicity and Safety	DEN-313 Panama, The Philippines	Immunogenicity and long-term safety - To assess the cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years at 1 month post second vaccination.	Open label	TDV TDV dose TDV-1: 3.6 $\log_{10}$ PFU TDV-2: 4.0 $\log_{10}$ PFU TDV-3: 4.6 $\log_{10}$ PFU TDV-4: 5.1 $\log_{10}$ PFU Subcutaneous injection	200	Vaccination: Month 0, Month 3	Ongoing; Interim

Table 3: Ongoing Clinical Studies Included in Safety Snap Shot (CSRs not included in submission)

Ongoing Phase 2 and 3 Studies included in the safety snap shot presented in Module 2.7.4 and 5.3.5.3 (CSRs not included in the submission):

DEN-307, DEN-303 and DEN-210.

All studies are conducted in healthy subjects.

Table 2 Ongoing Clinical Studies Included in Safety Snap Shot (CSRs not included in submission)

Type of Study	Study Identifier; Country	Objective(s) of the Study	Study Design and Type of Control	Test Product(s); Dose; Route of Administration	Number of Subjects	Vaccination schedule	Study Status
Immunogenicity and Safety	DEN- 307 United States	Trial evaluating a naturally aged TDV lot (naturally aged: stored >12 months at 2°C to 8°C)	Open label	TDV TDV dose: TDV-1: ≥3.3 $\log_{10}$ PFU TDV-2: ≥2.7 $\log_{10}$ PFU TDV-3: ≥4.0 $\log_{10}$ PFU TDV-4: ≥4.5 $\log_{10}$ PFU Subcutaneous injection	200	Vaccination: Month 0, Month 3	Ongoing Enrollment completed March 2019
Immunogenicity and Safety	DEN-303 United States and Mexico	Trial evaluating long-term safety of previously vaccinated subjects, and the immunogenicity and safety of a single booster injection at Month 15 (subjects previously vaccinated with TDV in Trials DEN 304 and DEN 315)	Open label	TDV TDV dose: TDV-1: 5.2 $\log_{10}$ PFU TDV-2: 4.5 $\log_{10}$ PFU TDV-3: 5.2 $\log_{10}$ PFU TDV-4: 5.8 $\log_{10}$ PFU Subcutaneous injection	600	Vaccination: Month 15 (= Month 36 after the second dose of trial vaccine in the parent trial)	Ongoing Enrollment completed March 2020
Immunogenicity and Safety	DEN-210 United States	Trial evaluating TDV in seropositive and seronegative adult subjects. - To assess the neutralizing antibody response (Geometric Mean Titers [GMT]) against each dengue serotype post-vaccination.	Open label	TDV TDV dose: TDV-1: 5.1 $\log_{10}$ PFU TDV-2: 4.5 $\log_{10}$ PFU TDV-3: 5.4 $\log_{10}$ PFU TDV-4: 5.9 $\log_{10}$ PFU Subcutaneous injection	30	Vaccination: Month 0, Month 3	Ongoing Enrollment completed February 2020

A total of 18 trials (7 phase 3 trials, 6 phase 2 trials, and 5 phase 1 trials) with more than 27,000 subjects (19,980 subjects received any TDV) from dengue-endemic and non-endemic regions, and covering an age range from 1.5 to 60 years comprise the overall clinical development programme of TDV.

Data from these 18 clinical trials are included in this submission: phase 1 Trials DEN-101, DEN-102, DEN-103, DEN-104, and DEN-105; phase 2 Trials DEN-106, DEN-203, DEN-313, DEN-204, DEN-205, and DEN-210 (preliminary safety data only); phase 3 Trials DEN-301, DEN-304, DEN-315, DEN-305, DEN-314, DEN-303 (preliminary safety data only), and DEN-307 (preliminary safety data only).

The seven trials described in the Pharmacology section that contributed to the selection of TDV formulation, the selection of the lyophilised formulation and the selection of the subcutaneous route of administration (DEN-101, DEN-102, DEN-103, DEN-104, DEN-106, DEN-203, DEN-205). Other trials described in this section contributed to selection of the dosing schedule and the characterisation of the immune response induced by TDV (DEN-204, DEN-205, and DEN-313). The pivotal Phase 3 (efficacy and safety) was DEN-301, and additional phase 3 Trials (DEN-304 and DEN-315) allowed characterising the immunogenicity induced by the vaccine in subjects from non-endemic areas. An assessment of immunogenicity when TDV is co-administered with a vaccine against the yellow fever (YF) virus (DEN-305) or hepatitis A virus (DEN-305 and DEN-314) are also included in the submission.

For 3 ongoing trials (DEN-307, DEN-303 and DEN-210) only preliminary safety data are available. Overall, cumulative safety data up to a cut-off date of 01 October 2020 (approximately 6 months prior to first filing) are presented in this clinical overview.

Additionally, booster dose evaluations are planned in 2 studies (within dengue-endemic and non-endemic areas) with data becoming available at later date (ie, not included in this submission). This includes a protocol amendment for Trial DEN-301 and a planned booster dose as part of the ongoing phase 3 Trial DEN-303. Trial DEN-301 will investigate a booster injection in the subset of approximately 10,500 subjects aged 4 to 11 years at the time of enrolment into Trial DEN-301, to be given 4 to 4.5 years after the second dose of the primary vaccination in endemic regions, and safety follow-up will continue up to approximately 2 years after the booster dose.

Trial DEN-303 will investigate a booster injection given 36 months after the first dose of the primary vaccination in non-endemic regions, and follow-up for safety and immunogenicity will continue until 6 months after the booster dose.

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics and Pharmacodynamics

Clinical pharmacology trials, including pharmacokinetic trials, were not appropriate for this vaccine and were therefore not performed. Pharmacodynamic studies for this vaccine, as it is usual for many other vaccines, consists of the analyses of the immune response induced, and these data are described below.

Methods

- **Analytical methods**

All assays used in trials in this submission are listed in Table 4. Individual method and validation reports have been submitted.

A summary of the dengue-specific validated assays follows which explains the individual use of each assay and the scientific rationale for its selection.

Table 4: Assays Used in Each Trial

Assay	Validation	Trials
MNT Assays		
Dengue MNT	Research	DEN-101, DEN-102, DEN-103, DEN-104, DEN-203
Dengue MNT	Qualified	DEN-106, DEN-204, DEN-205
Dengue MNT	Validated	DEN-313, DEN-301, DEN-304, DEN-315, DEN-305, DEN-314
Molecular Assays		
Febrile illness dengue RT-PCR	Research	DEN-203
Qualitative dengue detection RT-PCR	Validated	DEN-204
Quantitative dengue detection RT-PCR	Validated	DEN-313, DEN-301
Viremia RT-PCR [(b)]	Research	DEN-101
Viremia RT-PCR [(b)]	Research	DEN-102, DEN-103, DEN-104, DEN-203
Vaccine screening RT-PCR [(b)]	Validated	DEN-204, DEN-205, DEN-313, DEN-301
Vaccine confirmation sequencing [(c)]	Validated	DEN-204, DEN-205, DEN-313, DEN-301
TDV sequencing	Validated	DEN-313, DEN-301
E-gene sequencing	Research	DEN-204
Virus propagation/ IFA	Research	DEN-101, DEN-102, DEN-103, DEN-104, DEN-203
Virus propagation	Fit-for-purpose [(d)]	DEN-313, DEN-301
Spot sequencing [(c)]	Research	DEN-101, DEN-102, DEN-103, DEN-104, DEN-203
Serology ELISAs		
Dengue IgG ELISA	Validated	DEN-205, DEN-301
Dengue IgM ELISA	Validated	DEN-205, DEN-301
NS1 antigen ELISA	Qualified	DEN-204, DEN-205
NS1 antigen ELISA	Validated	DEN-313, DEN-301
Cellular Immunity Assays		
CMI ICS	Research [(e)]	DEN-101, DEN-102
CMI ICS	Fit-for-purpose [(d)]	DEN-204
CMI ICS	Fit-for-purpose [(d)]	DEN-313
CMI ELISPOT	Fit-for-purpose [(d)]	DEN-205
CMI ELISPOT	Qualified	DEN-313
Other Exploratory Assays		
Transcriptional profile	Research [(d)]	DEN-102
Endothelial hyperpermeability (TEER)	Research [(e)]	DEN-203
Quad-color fluorospot	Research [(e)]	DEN-205
Breadth of neutralisation by MNT	Fit-for-purpose [(d)]	DEN-205
Dengue 2 depletion and RVP/dengue RVP neutralisation	Fit-for-purpose [(d)]	DEN-203, DEN-204, DEN-301, DEN-304
Neutralising antibody specificity (chimeric virus and blockade of binding)	Research [(e)]	DEN-203
Dengue total binding IgG ELISA	Fit-for-purpose [(d)]	DEN-203, DEN-204, DEN-301, DEN-304
Dengue IgG avidity	Fit-for-purpose [(d)]	DEN-203, DEN-204, DEN-301, DEN-304
Anti-dengue NS1 IgG	Fit-for-purpose [(d)]	DEN-203, DEN-204, DEN-301, DEN-304
Anti-dengue complement antibody	Fit-for-purpose [(d)]	DEN-203, DEN-204
Coadministration Assays		
Yellow fever PRNT	Validated	DEN-305
Hepatitis A ELISA	Validated	DEN-314

Abbreviations: CMI = cell-mediated immunity; CSR = clinical study report; ELISA = enzyme-linked immunosorbent assay; ELISPOT = enzyme-linked immunosorbent spot; ICS = intracellular cytokine staining; IFA = immunofocus assay; Ig(G/M)

= Immunoglobulin G/M; MNT = microneutralisation test; NS1/3 = nonstructural protein 1/3; PRNT = plaque reduction neutralisation test; RT-PCR = real-time polymerase chain reaction; RVP = reported virus particle.
Trial DEN-105 is not included in this table because only safety data from this trial are reported.
(a) Legacy study CSRs may indicate the use of a PRNT assay. Notably this neutralisation assay is following the same assay design concept as the qualified/validated MNT assay used in phase 2 and phase 3 studies.
(b) Assays follow the same design in respect to applied primer and probe sequences.
(c) Assays were used to determine the sequence of the 3 major TDV attenuation marker in the 5' NCR, NS1, and NS3 genes, respectively.
(d) Key assay parameters optimised; assay not qualified or validated.
(e) Research performed at academic institution.

Validated Assays

The bioanalytical methods used for characterisation of TDV can be grouped into 2 major categories depending on the assessed clinical objective; immunogenicity evaluation by using the functional dengue microneutralisation (MNT) assay and various enzyme-linked immunosorbent assays (ELISA) versus efficacy and viraemia evaluation by means of molecular real-time polymerase chain reaction (RT-PCR) and sequencing assays.

Dengue MNT

The plaque reduction neutralisation test titre resulting in a reduction of at least 50% (PRNT50) as described in the WHO Guidelines is a standardised method to test immunogenicity of dengue vaccine candidates. While other serological tests (eg, complement fixation and hemagglutinin inhibition) are available, only neutralising antibody (NAb) tests, including PRNT, measure functional antibody responses. The PRNT is currently considered the gold-standard assay for the measurement of functional, dengue neutralising antibodies in human serum.

The dengue MNT assay represents the primary assay for assessing the immune response following TDV administration. All trials included in this submission used this MNT assay format for assessing the immunogenicity endpoints. In the context of evaluating vaccine efficacy, the MNT assay is also used to determine subject serostatus at baseline. Furthermore, MNT results are used to support immunobridging across age groups, with the exploratory Anti-dengue NS1 IgG assay providing supportive data.

The MNT assay is applied to quantify virus-neutralising antibodies (i.e., antibody titres) present in human sera following vaccination with TDV. In order to best measure the neutralising response against the vaccine, the four wild-type virus strains representing TDV parental components are used in the assay.

The MNT assay is based on the inactivation of the dengue virus in the presence of NAbs in the serum of subjects dosed with a dengue vaccine or subjects infected with the DENV. The inactivation of the virus by these antibodies prevents the virus from replicating. This causes a reduction in the number of immunofoci in a dengue virus-susceptible cell culture, which is quantitatively related to the amount of NAbs in the serum.

The MNT assay was qualified during the end of phase 1 and beginning of phase 2 development. The MNT assay was validated prior to the start of the phase 3 trials.

RT-PCRs

RT-PCR was used to detect dengue viraemia either as an outcome of dosing (within 30 days of dosing) or as a means of detecting DENV during the period designated for evaluation of various efficacy endpoints. While TDV viraemia was evaluated using the validated vaccine screening RT-PCR, the validated, quantitative dengue detection RT-PCR served as primary tool for laboratory diagnosis of dengue infection and hence for evaluation of TDV efficacy in phase 3.

Dengue Detection RT-PCR

This tetraplex RT-PCR assay is designed to detect all 4 dengue serotypes testing virus RNA after purification from human serum. Both the qualitative and quantitative dengue detection RT-PCR methods are used to reverse transcribe and amplify a portion of the type-specific dengue virus genome.

To discern between wild type and vaccine virus Dengue RNA every positive RT-PCR result for febrile illness samples collected within 30 days of vaccination triggered the downstream sequencing of the affected samples by TDVSeq.

Vaccine Screening RT-PCR

This tetraplex RT-PCR assay is designed to detect and quantify all 4 serotypes testing virus RNA after purification from human serum. RT-PCR technology is used to reverse transcribe and amplify a portion of the serotype-specific dengue vaccine virus genome.

The technique is the same in terms of the assay steps as previously described for Dengue Detection RT-PCR.

This method was validated.

Sequencing Assays

As revealed experimentally, the dengue detection RT-PCR as well as the vaccine screening RT-PCR showed some level of cross-reactivity in detecting dengue wild-type and TDV strains. Thus, samples that tested positive within 30 days after vaccination for either of the 2 RT-PCR assays were subject to sequencing. To differentiate between wild-type and vaccine dengue virus as well as to reveal genetic TDV stability, 2 assays that sequence TDV unique regions were employed: *Vaccine Confirmation Sequencing (t*he validated vaccine confirmation sequencing assay was designed to sequence the dengue genome at 3 known TDV attenuation sites in the 5'-noncoding region) and the T*DV Sequencing (this* validated assay was designed to sequence and identify certain TDV RNA regions.

Serology ELISAs

The validated IgG, IgM, and NS1 ELISA assays were used to assess the serological profile of suspected dengue cases to support evaluation of exploratory clinical endpoints. All 3 ELISA assays were performed using commercially available kits that were validated for their specific application in measuring clinical samples. All three methods were validated and the corresponding validation reports have been submitted.

IgG and IgM ELISA

The principle of the IgG and IgM ELISA assays is based upon exposing sera to dengue antigens that are attached to the surface of the ELISA plate. Dengue-specific IgG or IgM antibodies bound to the dengue antigens are detected by the addition of an anti-IgG or anti-IgM NAb complexed to HRP, which following addition of a substrate effects a colorimetric change that is detected by the ELISA reader.

NS1 Antigen

The dengue virus NS1 antigen ELISA is designed to detect dengue NS1 protein present in human serum.

Assays for Coadministration Trials

Yellow Fever

The PRNT for yellow fever (YF) virus was used to determine the NAb levels following the administration of an YF vaccine (YFV) or in conjunction with trials for vaccines to be administered in YF virus endemic regions, such as dengue vaccines.

This assay was validated in 2018 and validation reports have been submitted.

Hepatitis A

Since there are currently no commercially available anti-hepatitis A ELISA kits that are validated to meet the international clinical standard for hepatitis A virus (HAV) seroprotection, an ELISA kit was developed.

This assay was validated.

Cellular Immunity

To further characterise the immune response induced by TDV, CMI was assessed using an enzyme-linked immunospot (ELISPOT) assay. The ELISPOT assay measured the production of antiviral cytokine interferon-gamma (IFN-γ) by dengue-specific T-cells.

This method was qualified.

An intracellular cytokine staining (ICS) assay was used to further characterise the CMI induced by TDV. During early clinical development the applied ICS assays were designed to measure antigen-specific T-cell responses by stimulating cryopreserved, peripheral blood mononuclear cells in vitro using dengue virus-specific peptide pools matching relevant virus-specific antigens. After stimulation, cells were fixed and permeabilised before staining for selected intracellular cytokines, which finally allowed measuring the frequency of activated, antigen-specific CD4 and CD8 cells.

This assay was optimised in order to make it fit-for-purpose, but it has not been qualified nor validated.

Other Exploratory Assays

Exploratory immunology testing of samples from Good Clinical Practice-compliant clinical phase 2 and 3 trials was performed in non-Good Laboratory Practice (GxP) laboratories to broadly characterise immune responses elicited by TDV in seronegative children and adults, and in seropositive children. Many of these assays have not been qualified nor validated. They are briefly described below.

Dengue 2 Depletion and Reporter Virus Particle Assay

To assess the type-specific neutralising antibody response to the other serotypes, antibody depletion was used to remove antibodies against DENV-2 from human serum samples with neutralising antibody titres of >20 for all 4 dengue serotypes. The DENV-2 antibody depleted samples were then assessed for type specific neutralising antibody response to DENV-1, DENV-3 and DENV-4 using an RVP assay.

Dengue IgG Avidity Assay

A Bio-Layer Interferometry (BLI) based avidity assay was developed to characterise avidity of polyclonal sera, as an indicator of affinity maturation of the polyclonal antibody response following vaccination.

Anti-Dengue NS1 IgG Assay

An indirect ELISA was developed to quantitate the IgG response to NS1 of DENV-1, DENV-2, DENV-3, and DENV-4.

Dengue Microneutralising Assay for Breadth of Neutralisation

This dengue virus neutralisation assay was used to measure the level of neutralising antibodies (MNT_{50}) to antigenically diverse DENV strains in order to determine the breadth of the neutralising antibody response elicited by TDV. Three strains representing 3 distinct genotypes within each serotype (DENV-1, DENV-2, DENV-3 and DENV-4) were used in this assay: the parental TDV wild-type strains and 2 other antigenically distinct strains.

Anti-Dengue Complement Antibody Assay

This assay was designed to simultaneously determine the magnitude of anti-dengue virus complement-fixing antibody responses directed against all 4 vaccine components.

Trials that contributed to the selection of TDV formulation, route of administration and vaccination schedule

This section describes:

- The formulation development and dose schedule selection of TDV.
- The formulations investigated in all trials included in the submission.
- Results from the exploratory immunogenicity characterisation analyses.

Figure 1 graphically illustrates the trials (summarised in this document) that contributed to the development of the formulation and the selection of the dose schedule.

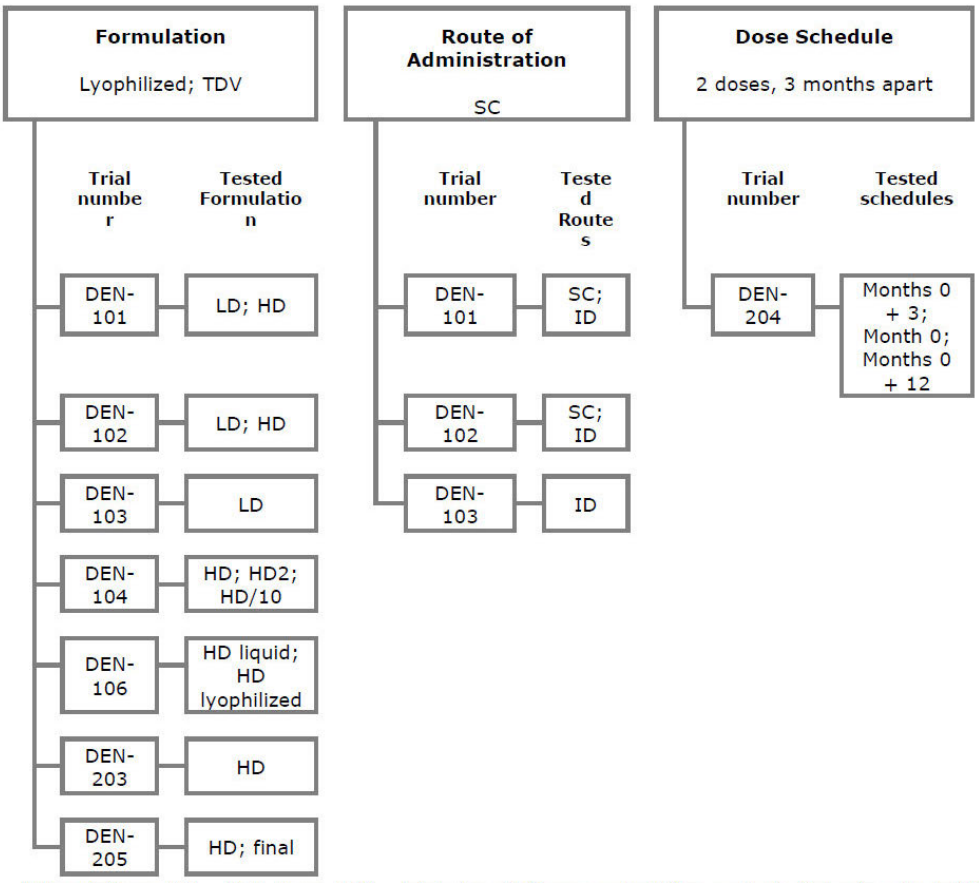
Trials DEN-101 to DEN-104 and DEN-203 and DEN-205 contributed to the selection of the TDV formulation and Trial DEN-106 also contributed to the selection of the lyophilised formulation. Trials DEN-101 to DEN-103 contributed to the selection of subcutaneous (SC) as route of administration, and Trial DEN-204 was the primary study contributing to the selection of the dosing schedule.

Table 5: List of Trials with Immunogenicity Data Included in the Submission

Phase	Trial Number	Formulation(s); Route of Administration	Number of Subjects [a]	Age Range (Years)
1	DEN-101	LD TDV (SC; ID), HD TDV (SC; ID)	48	18-45
1	DEN-102	LD TDV (SC; ID), HD TDV (SC; ID)	79	18-45
1	DEN-103	LD TDV (ID)	67	18-45
1	DEN-104	HD TDV (SC), HD2 TDV (SC), HD/10 TDV (SC)	140	18-45
1	DEN-105 [b]	LD TDV (SC; IM)	80	18-45
2	DEN-106	HD TDV (SC)	996	18-49
2	DEN-203	HD TDV (SC)	249	1.5-45
2	DEN-205	HD TDV (SC), TDV (SC)	351	21-45
2	DEN-204	TDV (SC)	1794	2-17
2	DEN-313	TDV (SC)	200	4-15
3	DEN-301	TDV (SC)	13,384	4-16
3	DEN-304	TDV (SC)	788	18-60
3	DEN-315	TDV (SC)	300	12-17
3	DEN-305	TDV (SC)	871	18-60
3	DEN-314	TDV (SC)	598	18-60

Abbreviations: HD = high dose; HD2 = high dose 2 (increased relative content of vaccine strain TDV-4); HD/10 = 1/10 dilution of high dose; ID = intradermal; IM = intramuscular; LD = low dose; SC = subcutaneous.
(a) Number of subjects who received LD TDV, HD TDV, or TDV.
(b) Only safety results from this trial are reported.

The trial designs are summarised below.



Abbreviations: HD = high dose; HD2 = high dose 2 (increased relative content of vaccine strain TDV-4); HD/10 = 1/10 dilution of high dose; ID = intradermal(ly); LD = low dose; SC = subcutaneous(ly).

Figure 2: Trials Contributing to the Formulation and Dose Schedule Selection

Formulation Development

The formulations used in the phase 1 and phase 2 studies that follow are represented in

Table 6.

Table 6: Viral Content as Specified in Clinical Study Protocols

	Low Dose (LD TDV)	High Dose (HD TDV)	High Dose 2 (HD2 TDV)	1/10 Dilution of HD TDV (HD/10 TDV)	TDV	ESL Specification Limits
TDV-1						
PFU/dose	3.9 $\log_{10}$	4.3 $\log_{10}$	4.3 $\log_{10}$	3.3 $\log_{10}$	4.3 $\log_{10}$	≥3.3 $\log_{10}$
	8×10^3	2×10^4	2×10^4	2×10^3	2×10^4	≥2×10^3
TDV-2						
PFU/dose	3.7 $\log_{10}$	4.7 $\log_{10}$	4.7 $\log_{10}$	3.7 $\log_{10}$	3.7 $\log_{10}$	≥2.7 $\log_{10}$
	5×10^3	5×10^4	5×10^4	5×10^3	5×10^3	≥5×10^2
TDV-3						
PFU/dose	4.0 $\log_{10}$	5.0 $\log_{10}$	5.0 $\log_{10}$	4.0 $\log_{10}$	5.0 $\log_{10}$	≥4.0 $\log_{10}$
	1×10^4	1×10^5	1×10^5	1×10^4	1×10^5	≥1×10^4
TDV-4						
PFU/dose	5.3 $\log_{10}$	5.5 $\log_{10}$	6.0 $\log_{10}$	4.5 $\log_{10}$	5.5 $\log_{10}$	≥4.5 $\log_{10}$
	2×10^5	3×10^5	1×10^6	3×10^4	3×10^5	≥3×10^4
Trials	DEN-101 DEN-102 DEN-103 DEN-105	DEN-101 DEN-102 DEN-104 DEN-106 DEN-203 DEN-205	DEN-104	DEN-104	DEN-205 DEN-204 (a) DEN-301 (b) DEN-313	DEN-304 DEN-315 DEN-305 DEN-314

Abbreviations: CSP = clinical study protocol; ESL = end-of-shelf-life; HD = high dose; HD2 = high dose 2 (increased relative content of vaccine strain TDV-4); HD/10 = 1/10 dilution of high dose; LD = low dose; PFU = plaque forming units.

(a) The TDV used in Trial DEN-204 had 10-fold reduced TDV-2, however, it met the ESL specification limits.

(b) The TDV used in Trial DEN-301 (pivotal trial) had 10-fold reduced viral contents, however they met the ESL specification limits.

SUMMARY OF IMMUNOGENICITY RESULTS OF INDIVIDUAL TRIALS

For the sake of brevity figures are only shown for the first trial (DEN-101) and for the rest of the trials the results are explained in text.

Trial DEN-101

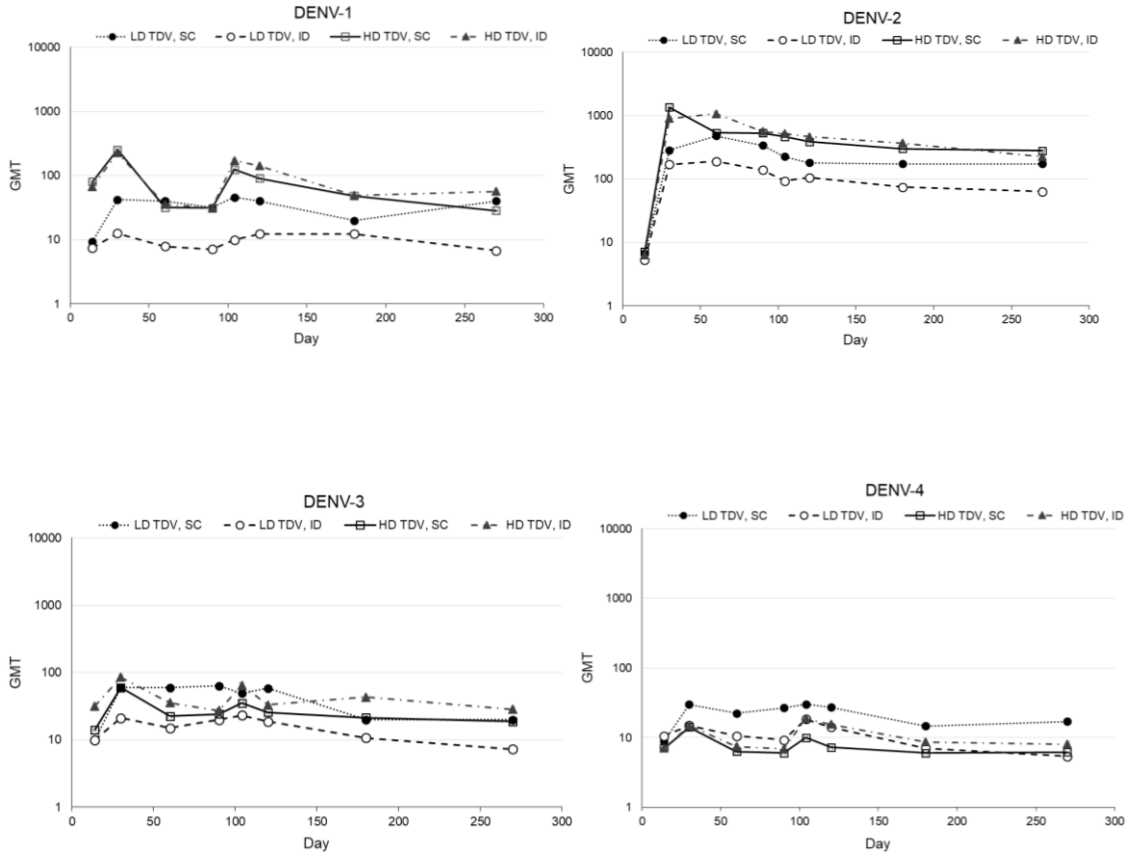
This was a double-blind, randomised, placebo-controlled, phase 1 dose-escalation trial conducted at a single site in the United States (a non-endemic region) to investigate the safety and immunogenicity of TDV in healthy adult subjects. The trial evaluated LD TDV and HD TDV when given subcutaneous (SC) or intradermal (ID).

A total of 72 healthy baseline seronegative male and female subjects (48 receiving TDV and 24 receiving placebo), 18 to 45 years of age were randomised in 1:1 ratio in four groups.

The primary objective was to evaluate the safety and tolerability of a 2-dose schedule of TDV given either SC or ID. The secondary objectives were to assess the immunogenicity of TDV to all 4 serotypes and to assess the viraemia derived from the attenuated vaccine viruses measured on Days 0 (baseline), 2, 4, 5/6, 7, 9, 11, and 14 after each dose.

Results

HD TDV and LD TDV elicited neutralising antibodies against all 4 serotypes; MNT GMTs were overall highest against DENV-2 and lowest against DENV-4 (see Figure 3). The MNT GMTs against DENV-1 and DENV-2 were notably higher in the HD TDV groups than in the LD TDV groups. There was no meaningful difference in the MNT GMTs based on the route of administration (SC or ID). MNT GMTs at Months 6 and 9 were lowest for DENV-4.



Source: DEN-101 CSR, Table 14.2.1.1.

Abbreviations: GMT = geometric mean titre; HD = high dose; ID = intradermal; LD = low dose; MNT = microneutralisation test; SC = subcutaneous.

MNT GMTs on Day 0 were not reported, but all subjects were seronegative at baseline.

Figure 3: Geometric Mean Titres of Dengue Neutralising Antibodies Over Time (All Subjects); Trial DEN-101

The seropositivity rates varied between the HD TDV and LD TDV groups at the different visits, but overall, the seropositivity rates were higher in the HD TDV groups than in the LD TDV groups for DENV-1 and DENV-2.

More subjects in the HD TDV groups (40.0% when given ID and 50.0% when given SC) had antibodies to at least 3 serotypes at Month 9 (ie, 6 months after the second dose) than subjects in the LD TDV groups (22.2% when given ID and SC).

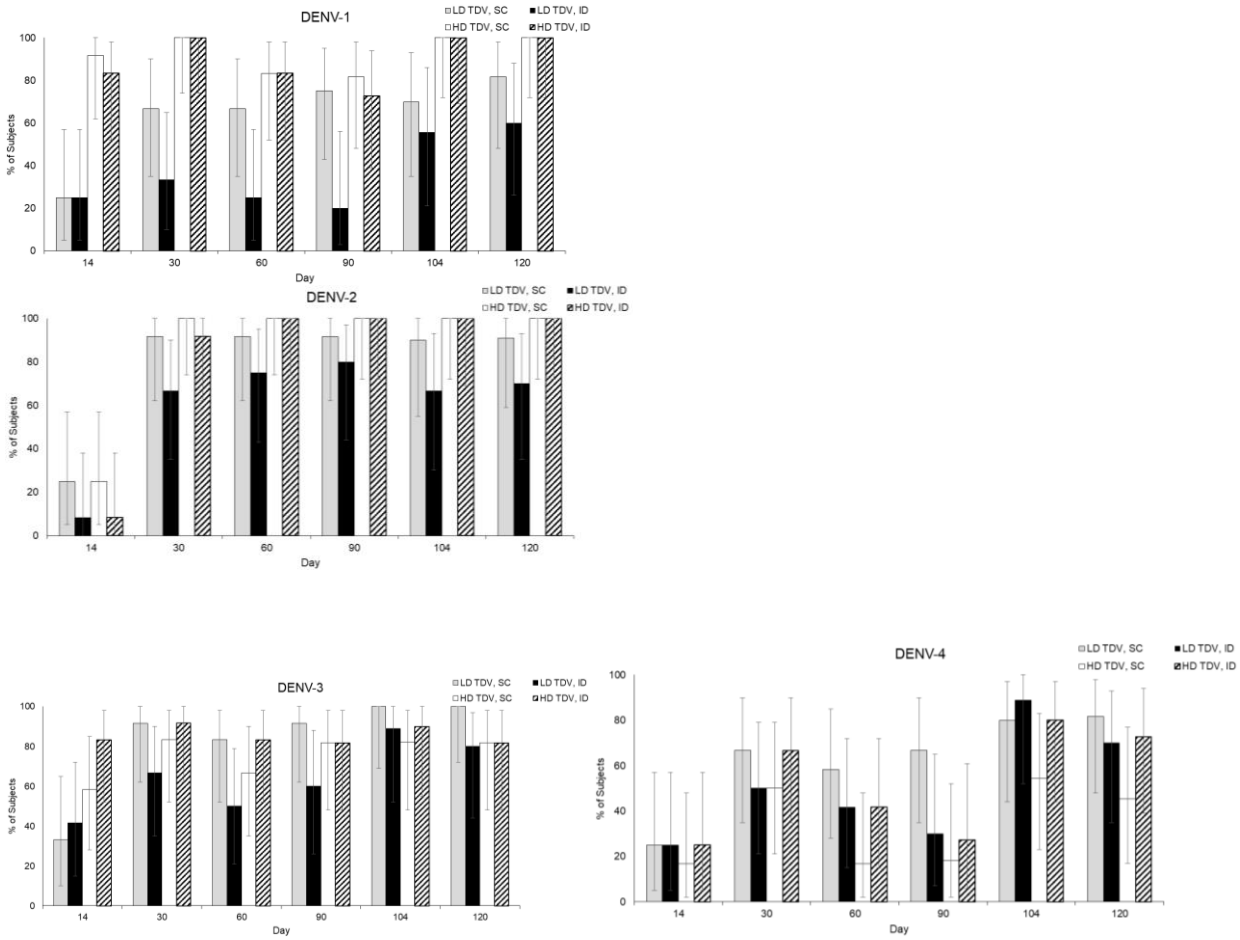


Figure 4: Seropositivity Rates for Each Dengue Serotype by Visit (All Subjects); Trial DEN-101

In conclusion, immunogenicity to all 4 serotypes was observed when administered as LD TDV or HD TDV and given SC or ID. The MNT GMTs were generally higher in the HD TDV groups than in the LD TDV groups, especially against DENV-1 and DENV-2. The SC route of administration was better tolerated than ID.

Trial DEN-102

This was a double-blind, randomised, placebo-controlled, phase 1, dose-escalation trial conducted at a single site in Colombia (a non-endemic region of the country) to investigate the safety and immunogenicity of LD TDV and HD TDV when given SC or ID in healthy adult subjects.

A total of 96 healthy baseline seronegative male and female subjects (79 receiving LD TDV or HD TDV and 17 receiving placebo), 18 to 45 years of age were randomised in a 23:5 ratio in four groups.

The primary objective of this trial was to assess the safety and tolerability of LD TDV and HD TDV administered SC or ID. The secondary objectives were to assess the immunogenicity of TDV. The

assessment of viraemia derived from the attenuated vaccine viruses was performed on Days 0 (baseline), 2, 4, 5/6, 7, 9, 11, and 14 after each dose.

Results

In general, the HD TDV groups (administered SC or ID) had higher MNT GMTs and seropositivity rates to at least 2 serotypes compared with the LD TDV groups. MNT GMTs in the HD TDV, SC group were higher against DENV-1 and DENV-2 and were lower against DENV-3 and DENV-4 compared with MNT GMTs in the HD TDV, ID group. However, results were similar between the ID and SC administration routes within the LD TDV or HD TDV groups, respectively.

At 1 month after the second dose, ie, Month 4 (Day 120), the HD TDV SC group had higher MNT GMTs compared with the LD TDV SC group against DENV-1 (181.8 vs 128.6) and DENV-2 (1536.1 vs 206.6). The responses against DENV-3 and DENV-4 were generally lower and varied between the doses and routes of administration.

Seropositivity rates were generally higher in the HD TDV groups than in the LD TDV groups). The durability of response was low against DENV-4. Seropositivity rates at Month 9 were highest in the HD TDV SC and ID groups against DENV-1 and DENV-2 (89.5%-100.0%), highest in the HD TDV ID group against DENV-3 (88.2%), and highest in the LD TDV ID group against DENV-4 (57.1%).

At Month 4, both LD TDV and HD TDV given ID returned higher seropositivity rates for all 4 serotypes compared with SC dosing (71.4% and 70.6%, respectively [ID] compared with 57.9% in the LD TDV, SC group and 47.4% in the HD TDV, SC group).

In conclusion, at Month 4, higher seropositivity rates for DENV-1, DENV-2, and DENV-3 were seen in the HD TDV groups, when given ID or SC, compared with the LD TDV groups. Higher seropositivity rates for DENV-4 were seen when HD TDV was given ID compared with SC. Higher seropositivity rates for all 4 serotypes were reported in subjects receiving ID compared with SC administration at Month 4. Results from this trial supported the use of HD TDV over LD TDV, but with a need to identify a viral content that elicits a stronger response against DENV-4.

Trial DEN-103

This was a partially blinded (ie, the subjects, investigators, and other staff were blinded to the trial vaccine [LD TDV or placebo], but not to the delivery device), randomised, parallel-group, phase 1b trial conducted at multiple sites in the United States (a non-endemic region) to investigate the safety and immunogenicity of LD TDV in healthy adult subjects. The trial evaluated various dose schedules of LD TDV: 2 injections given 3 months apart, 2 simultaneous injections in different arms, and 3 injections administered as 2 simultaneous injections in different arms and a single injection 3 months later; and when administered with 2 different devices: a PharmaJet needle-free injector and a needle-syringe.

However, enrolment for Trial DEN-103 was closed early when significant local reactogenicity was seen after ID administration compared to the SC administration in Trials DEN-101 and DEN-102. It was then decided not to pursue the ID route of administration for further development.

Prior to closing enrolment, a total of 67 healthy adult male and female subjects, 18 to 45 years of age were randomised in a 1:1:1:1 ratio in four groups.

The primary objective was to compare the safety and tolerability of various dose schedules and different devices for ID administration and to compare the immunogenicity to each of the 4 serotypes

between these dose schedules and devices. The secondary objectives were to assess the MNT GMTs in a population without previous exposure to dengue or other known flaviviruses after each immunisation and to assess the incidence of viraemia on Days 0 (baseline), 7, 10, 14, 21, 90, 97, and 104.

Results

The highest MNT GMTs after the first dose were observed for DENV-2 in all trial groups. MNT GMTs for DENV-2 were higher after 2 simultaneous injections of LD TDV than after a single LD TDV injection, regardless of the device. MNT GMTs for DENV-2 were also higher following administration with the PharmaJet needle-free injector than with the needle-syringe after a single LD TDV injection. MNT GMTs for DENV-1, DENV-3, and DENV-4 remained low in all trial groups.

A noticeable increase in MNT GMTs was observed for all 4 serotypes following second LD TDV injection at Month 3. Overall, MNT GMTs for each of the 4 serotypes decreased over time but GMTs for DENV-2 remained substantially above baseline at the end of the follow-up period in all groups.

Two simultaneous injections of LD TDV at Month 0, followed by a third LD TDV injection at Month 3 with the PharmaJet needle-free injector resulted in higher maintained seropositivity rates for DENV-1 and DENV-2 at Month 4 and Month 9 compared with the group which followed the same dose schedule, but with placebo instead of LD TDV at Month 3.

A single LD TDV injection at Month 0 with the PharmaJet needle-free injector resulted in higher seropositivity rates for DENV-1, DENV-2, and DENV-3 at Month 1 and Month 3 than injection by needle-syringe. Conversely, the seropositivity rates for DENV-4 were higher with the needle-syringe than with the PharmaJet needle-free injector at all time points.

A third injection of LD TDV at Month 3 induced higher seropositivity rates for DENV-2 at Month 3, Month 4, and Month 9 than a second LD TDV injection, regardless of the device.

In conclusion, two simultaneous injections of LD TDV showed the potential to improve seropositivity rates for DENV-1 and DENV-2 and to increase the MNT GMTs for DENV-2. A noticeable increase in MNT GMTs was observed for all 4 serotypes following second LD TDV injection at Month 3. Due to the higher local reactogenicity following ID administration observed in other trials without consistent improvement of immune responses, the ID administration route was not pursued for further development.

Trial DEN-104

This was a randomised, 3-part, multicentre, phase 1b trial conducted in the United States (a non-endemic region) to investigate the safety and immunogenicity of HD TDV in healthy adult subjects. The trial evaluated 2 potential strategies to improve immune responses against DENV-4, by increasing the content of TDV-4 in the product (named, HD2 TDV), and by administering 2 doses (one in each arm on the same day), to possibly activate immune cells and antigen presenting cells that traffic to 2 different sets of lymph nodes. The trial also explored the impact of a 10-fold reduction in potency of TDV by administering a 1/10 dilution of the HD TDV product (named, HD/10 TDV). All doses were given SC.

A total of 140 healthy male and female subjects, 18 to 45 years of age were randomised to the several trial groups.

The primary objective was to compare the safety and tolerability of different dose schedules and to compare the immunogenicity to all 4 serotypes between these dose schedules. The secondary

objectives were to assess the viraemia derived from the attenuated vaccine viruses measured on Days 0 (baseline), 7, 9, 11, 14, 17, 21, 90, 97, and 104, to assess the MNT GMTs to all 4 serotypes, and to assess the immune response induced in a population with no previous exposure to dengue.

Results

Substantial increases in MNT GMTs against DENV-1 and DENV-2 were observed following the first dose, regardless of the number of injections (single or 2 simultaneous). To address the low response against DENV-4 seen in previous trials, the DENV-4 dose was increased. However, this increase did not result in higher MNT GMTs for this serotype and no dose effect could be observed.

An additional dose at Month 3 with either HD TDV or HD2 TDV did not result in an increase of MNT GMTs for any of the serotypes, regardless of whether 1 or 2 injections were given at Month 0. Results from HD/10 TDV were similar to HD TDV

The seroconversion rates at Month 4 (the primary endpoint visit) for DENV 1 (84.2%-100%), DENV 2 (95.8%-100.0%), and DENV 3 (83.3%-100.0%) were high in all trial groups, while seroconversion for DENV 4 was lower (33.3%-76.5%). The seroconversion rates for DENV 1, DENV 2, and DENV 3 were also high at Month 1 for all trial groups. Even though MNT GMTs against DENV 1 and DENV 2 decreased following the first dose, a high percentage of subjects maintained seropositivity prior to the second dose at Month 3.

At Month 4, the seroconversion rates for DENV 4 were higher in the HD2 TDV groups (>70.0% compared with ≤60.0% in other groups) but was still not as high as for the other serotypes and no dose effect was observed.

A 3-injection dose schedule with HD TDV did not have an effect on seroconversion rates compared with a 2-injection dose schedule.

In conclusion, HD2 TDV did not result in a meaningful difference in the response against DENV-4 compared with HD TDV. Results from this trial supported the use of HD TDV administered 3 months apart.

A 10-fold reduction in overall viral content (ie, HD/10 TDV) resulted in improved immune responses against DENV-4, compared with HD TDV. The resulting hypothesis from these data was that the TDV-2 in HD TDV was interfering with the replication of the other vaccine viruses and thus inhibiting the immune response to the remaining serotypes.

Trial DEN-106

This was a double-blind, randomised, 4-parallel groups, phase 2 trial conducted at multiple sites in the United States (a non-endemic region) to investigate the safety and immunogenicity of HD TDV in healthy adult subjects. The trial evaluated the equivalence of 3 formulations: liquid and lyophilised formulations, which was developed to meet World Health Organization (WHO) prequalification storage conditions, to the liquid formulation manufactured by Shantha Biotechnics. The Shantha liquid formulation was used in all phase 1 trials and in 1 other phase 2 trial, Trial DEN-203. All doses in Trial DEN-106 were given SC.

A total of 996 healthy baseline seronegative male and female subjects, 18 to 49 years of age were randomised in a 2:1:1:6 ratio in four groups.

The primary objective was to test the equivalence of the IDT lyophilised formulation to the Shantha liquid formulation at Month 1. The secondary objectives were to assess the immunogenicity of the IDT

lyophilised formulation compared with 2 doses of the Shantha liquid formulation to all 4 serotypes at Month 4 and to compare the safety of these formulations.

Results

Equivalence in MNT GMTs was demonstrated between the IDT lyophilised formulation and the Shantha liquid formulation (combined groups) after the first dose for DENV-2 (CI, 0.90-1.26), but not for DENV-1 (CI, 0.23-0.36), DENV-3 (CI, 1.13-1.89), or DENV-4 (CI, 0.57-0.95). The MNT GMTs for DENV-1 were approximately 71% lower for the IDT lyophilised formulation than for the Shantha liquid formulation (combined groups) at Month 1, MNT GMTs for DENV-2 were similar (only 6% higher), MNT GMTs for DENV-3 were 46% higher, and MNT GMTs for DENV-4 were 26% lower.

For the secondary endpoint, MNT GMTs were equivalent between the IDT lyophilised formulation and the 2-dose Shantha liquid dose schedule for DENV-2 (CI, 0.70-1.15) after the first dose and for DENV-2 (CI, 0.75-1.09) and DENV-4 (CI, 0.70-1.18) after the second dose. The differences in the MNT GMTs between the formulations were less pronounced at Month 4 than at Month 1.

Given that differences in MNT GMTs between formulations were not consistent for each of the serotypes, it is possible that the failure to demonstrate equivalence was not related to the lyophilisation process.

The seropositivity rates for DENV-1 was notably lower for the IDT lyophilised formulation compared with the Shantha liquid formulation (combined groups) after the first dose ($p = 0.024$. Seropositivity rates for DENV-2, DENV-3, and DENV-4 were similar between the formulations. Seropositivity rates were similar between the IDT lyophilised formulation and the 2-dose Shantha liquid schedule after both the first and the second dose.

In conclusion, the immune response against DENV-2 was equivalent between the IDT lyophilised and the Shantha liquid formulations.

Trial DEN-203

This was a double-blind, randomised, placebo-controlled, 2-part, multicentre, age-descending and expansion, phase 2 trial conducted in Puerto Rico, Colombia, Singapore, and Thailand (all endemic regions) to investigate safety and immunogenicity of HD TDV in adult and paediatric male and female subjects, 1.5 to 45 years of age. Both baseline seronegative and baseline seropositive subjects were enrolled. The trial evaluated 2 HD TDV doses, given SC and 3 months apart, in different age groups and was conducted in 2 parts.

Adult and paediatric subjects in Part 1 were enrolled into 1 of 4 age cohorts and randomised in a 2:1 ratio to receive HD TDV (90 subjects) or placebo (58 subjects) as follows:

- 21 to 45 years cohort: HD TDV (N = 24) at Month 0 (Day 0) and at Month 3 (Day 90).
- 12 to 20 years cohort: HD TDV (N = 22) at Month 0 (Day 0) and at Month 3 (Day 90).
- 6 to 11 years cohort: HD TDV (N = 21) at Month 0 (Day 0) and at Month 3 (Day 90).
- 1.5 to 5 years cohort: HD TDV (N = 23) at Month 0 (Day 0) and at Month 3 (Day 90).

Part 2 comprised only 1 age cohort and subjects were randomised in a 3:1 ratio to receive HD TDV (159 subjects) or placebo (53 subjects):

- 1.5 to 11 years cohort: HD TDV at Month 0 (Day 0) and at Month 3 (Day 90).

The primary objectives were to assess the safety and tolerability of a 2-dose schedule of HD TDV administered SC, and to assess the immunogenicity to all 4 serotypes. The secondary objectives were to assess the viraemia due to the attenuated vaccine viruses measured on Days 0 (baseline), 7, 14, 90, 97, and 104 in Part 1, to assess the safety and immunogenicity of HD TDV during long-term follow-up (up to 36 months post-first dose), and to assess the immune response induced in a population with previous exposure to dengue.

Results

The number of subjects seropositive to at least 1 serotype at baseline increased with age (see Table 7).

Table 7: Trial DEN-203: Number (%) of Subjects Seronegative or Seropositive at Baseline (Full Analysis Set)

	Number (%) of Subjects in the HD TDV Group				
	Age (Years)				
	Part 1				Part 2
Serostatus	21-45 N = 24	12-20 N = 22	6-11 N = 21	1.5-5 N = 23	1.5-11 N = 159
Seronegative to all serotypes	5 (20.8)	7 (31.8)	12 (57.1)	16 (69.6)	93 (58.0)
Seropositive to at least 1 serotype	19 (79.2)	15 (68.2)	9 (42.9)	7 (30.4)	66 (42.0)
DENV-1	19 (79.2)	14 (63.6)	8 (38.1)	5 (21.7)	51 (32.1)
DENV-2	19 (79.2)	13 (59.1)	5 (23.8)	3 (13.0)	43 (27.0)
DENV-3	19 (79.2)	15 (68.2)	7 (33.3)	6 (26.1)	58 (36.5)
DENV-4	19 (79.2)	12 (54.5)	5 (23.8)	3 (13.0)	43 (27.0)

Source: DEN-203 CSR, Table 15.1.4.1, Table 15.1.4.2, Table 15.2.1.1.1, and Table 15.2.2.1.1.

Abbreviations: HD = high dose.

MNT GMTs at each time point have been presented for all subjects (both baseline seropositive and baseline seronegative) Overall, the MNT GMTs for DENV-2 were the highest in the 21 to 45 years cohort. The lowest MNT GMTs were observed for DENV-4. The MNT GMTs for each of the 4 serotypes decreased over time.

Seropositivity rates for DENV-1, DENV-2, and DENV-3 ranged from 95.5% to 100.0% at Month 4. Notably, these high seropositivity rates persisted up to 36 months after the first dose and was observed regardless of the presence of dengue antibodies at baseline.

In conclusion, this trial showed that HD TDV induced a response in MNT GMTs and high seropositivity to all 4 serotypes, regardless of the presence of dengue antibodies at baseline. In addition, the trial demonstrated persistence of vaccine-induced titres throughout the 36-month trial duration.

Trial DEN-205

This was a double-blind, randomised, single dose, phase 2 trial conducted at multiple sites in Singapore (an endemic country) to investigate the safety and compare the immunogenicity of lyophilised TDV (named, TDV) with a 10-fold lower viral content of TDV-2 with that of HD TDV liquid formulation. Both baseline seronegative and baseline seropositive (to at least 1 dengue serotype) subjects were enrolled.

A total of 351 male and female subjects 21 to 45 years of age were randomised in a 1:1 ratio in two groups.

The primary objective was to assess the immunogenicity of the two vaccine formulations to all 4 serotypes. The secondary objectives were to assess the safety of HD TDV and TDV by baseline serostatus and to assess the viraemia derived from the attenuated vaccine viruses on Days 1, 5, 7, 9, 11, 15, 17, 21, and 30.

Results

Subject recruitment was stopped after 351 subjects were randomised, before the target of 400 subjects had been reached. The decision to stop the recruitment prematurely was due to difficulty in the recruitment of subjects who were seropositive at baseline (the required number of subjects seronegative at baseline had already been enrolled) which resulted in a risk of not meeting the target enrolment of 400 subjects before the expiry date of the vaccine. The decision to stop enrolment was considered to have limited impact on the evaluation of the trial objectives and scientific integrity of the trial because >85% of the planned subjects had been randomised. The percentage of subjects seropositive to at least 1 serotype at baseline was similar between the trial groups (52.6% in the HD TDV group and 54.6% in the TDV group).

Table 8: Trial DEN-205: Number (%) of Subjects Seropositive at Baseline (Per-Protocol Set)

	Number (%) of Subjects	
Serotype	**HD TDV N = 173**	**TDV N = 174**
At least 1 serotype	91 (52.6)	95 (54.6)
DENV-1	84 (48.6)	85 (48.9)
DENV-2	82 (47.4)	88 (50.6)
DENV-3	76 (43.9)	81 (46.6)
DENV-4	72 (41.6)	71 (40.8)

Source: DEN-205 CSR, Table 15.1.8.2.
Abbreviations: HD = high dose.

Peak MNT GMTs were higher for DENV-1 and DENV-3 in the HD TDV group than in the TDV group). The decrease between Month 1 and Month 3 was smaller for the TDV than HD TDV. MNT GMTs in the HD TDV group were higher for DENV-2 and marginally lower for DENV-4 than in the TDV group at most visits.

By baseline serotype, differences between HD TDV and the TDV were more pronounced in baseline seronegative subjects. These results suggest that a different balance of the immune response was achieved with the TDV compared with HD TDV. In subjects who were seronegative at baseline, the response against DENV-2 was less prominent with a lower incidence of DENV-2 viraemia in the TDV group than the HD TDV group, while DENV-4 seropositivity rates and MNT GMTs were higher in the TDV group.

Seropositivity rates were similar between HD TDV and TDV for DENV-1 and DENV-3, higher in the HD TDV group for DENV-2 than the TDV group, and lower in the HD TDV group for DENV-4 than the TDV group.

The seropositivity rates were higher in the HD TDV group than in the TDV group for DENV-2 and lower for DENV-4 in subjects seronegative at baseline.

In conclusion, vaccine viraemia following HD TDV administration has most commonly been associated with TDV-2, suggesting a greater potential for TDV-2 to replicate, theoretically inhibiting replication of the other serotypes and interfering with the overall immune response. The results of the trial supported the selection of the TDV (viral content in the lyophilised TDV product with a 10-fold lower viral content of TDV-2) for future clinical development since they suggest that a different balance of the immune response was achieved with the TDV compared to HD-TDV. The results showed a less

dominant response to DENV-2 (including lower incidence of TDV-2 viraemia) in the TDV group compared to the HD TDV group. In addition, DENV-4 seropositivity rates and GMTs were improved in the TDV group.

Trial DEN-204

This was a double-blind, randomised, placebo-controlled, phase 2 trial conducted in The Dominican Republic, Panama, and The Philippines (all endemic regions), to investigate the safety and immunogenicity of different dose schedules of TDV, given SC. The trial compared three dose schedules: 2 doses given 3 months apart, a single dose followed by a booster at Month 12, and a single dose only. The trial aimed to support the use of the lyophilised TDV product and the final dose schedule, including long-term persistence of immunogenicity. All analyses presented were descriptive with no statistical comparisons. Both baseline seronegative and baseline seropositive (to at least 1 dengue serotype) subjects were enrolled.

Only the data contributing to the dose schedule selection (ie, through Month 18) are described in this summary document. The complete immunogenicity results from this trial (ie, through Month 48) are described (after discussion on pivotal trial DEN-310).

A total of 1794 male and female subjects (1596 TDV and 198 placebo), 2 to 17 years of age were randomised in a 1:2:5:1 ratio into 4 groups.

The primary objective was to assess the humoral immune responses to TDV administered SC on different dosing schedules. The secondary objectives were to assess the immunogenicity of TDV to all 4 serotypes and to assess the safety of this formulation.

Results

The percentage of subjects seropositive to at least 1 serotype at baseline varied from 49.4% to 57.3% between the trial groups. Results from the 18-month interim analysis showed that TDV induced a response to all 4 serotypes regardless of serostatus at baseline and dosing schedule.

In the overall population, no major differences were noted in MNT GMTs or seropositivity rates between subjects who received 1 or 2 doses of TDV. A second dose at Month 3 improved the immune responses in subjects without a serotype-specific response to the first dose (ie, increased the seropositivity rate). A dose at Month 12 led to higher GMTs to all serotypes than when the second dose was given at Month 3. Seropositivity rates were similar whether the second dose of TDV was administered at Month 3 or Month 12, and this was observed regardless of serostatus at baseline.

Therefore, the dose schedule which results in the highest percent of tetravalent seropositivity regardless of serostatus at baseline, was selected for further development.

In conclusion, the decision on dose schedule to be used in the pivotal Trial DEN-301 was made based on 6-month data, however, the 18-month data ultimately validated this decision. Even though the second dose given at 12 months after the first dose resulted in higher GMTs compared to the second dose given at 3 months, the seropositivity rates were comparable and validated the decision on optimal dosing schedule, as tetravalent seropositivity is achieved in a higher proportion of subjects more rapidly after the 0, 3 months schedule.

Exploratory Immunogenicity Characterisation

The applicant has characterised the immune responses elicited by TDV in a broad way in order to analyse the range of immune responses encompassing multiple arms of the immune system that may contribute to prevention and clearance of viral infections, in addition to serum neutralising antibodies. For that aim, exploratory immunology testing of samples from Good Clinical Practice-compliant clinical

trials was performed in non- Good Practice (GxP) laboratories to broadly characterise immune responses elicited by TDV in seronegative children and adults, and in seropositive children.

Most of the serum samples tested in the post-hoc exploratory immunology assays were collected from subjects who were included in the immune subsets (randomly selected at enrolment) for Trials DEN-203, DEN-204, DEN-205, and DEN-301. For Trial DEN-304, the samples tested were collected from the group including subjects who received TDV Lot 3). Cellular immunology analyses were also conducted on samples from Trials DEN-204, DEN-205 and DEN-313 as part of the primary or secondary trial objectives as specified in the respective trial protocols and clinical study reports (CSRs).

A table illustrating the exploratory analysis carried out in each study follows:

Table 9: Overview of Exploratory Immunology Data in this Report

Trial	Population	Trial Group, Formulation (Dosing Schedule)	Assay[a]	Number of Subjects		Sampling Days
				Seronegative[b]	Seropositive[c]	
DEN-102	Adult	Group 1, LD TDV (Day 0 and Day 90)	Transcriptional profile[d]	20	0	0, 2, 4, 7, 90, 92
DEN-203	Pediatric, adolescent, adult	Groups 1-5, HD TDV (Day 0 and Day 90)	Anti-dengue NS1 IgG ELISA[e] (DENV-1, -2, -3, -4)	33	20	0, 120
			Anti-dengue NS1 IgG ELISA[e] (DENV-2 only)	33	22	0, 28, 90, 120, 180, 360
			DENV total binding IgG ELISA[e]	23	21	0, 120, 180
		Groups 2-5, HD TDV (Day 0 and Day 90)	DENV-2 depletion and RVP neutralization assay (type-specific neutralizing antibodies)[e]	10	4	120 (seronegative subjects) 0,120 (seropositive subjects)
		Groups 1-4, HD TDV (Day 0 and Day 90)	Anti-dengue IgG avidity assay[e]	37	19	0, 28, 90, 120, 180, 360
			Anti-dengue complement antibody assay[e]	28	11	0, 28, 90, 120, 180, 360
DEN-204	Pediatric, adolescent	Group 1, TDV (Day 1 and Day 91)	Anti-dengue NS1 IgG ELISA[e]	27	18	1, 180
			DENV total binding IgG ELISA[e]	21	15	1, 180
			Anti-dengue IgG avidity assay[e]	21	15	1, 180
			Anti-dengue complement antibody assay[e]	11	10	1, 180, 360
			DENV-2 depletion and RVP neutralization assay (type-specific neutralizing antibodies) [e]	17	13	180 (seronegative subjects) 1,180 (seropositive subjects)
		Groups 1-4, Immunogenicity subset, Panama only, TDV (Day 1 and Day 91/Day 1 only/Day 1 and Day 365) or Placebo (Days 1, 91, and 365)	ICS[f]	6[g]	4[g]	1, 91, 180, 365, 540
				10[h]	14[h]	
				13[i]	8[i]	
				12[j]	0	

Trial	Population	Trial Group, Formulation (Dosing Schedule)	Assay[a]	Number of Subjects		Sampling Days
				Seronegative[b]	Seropositive[c]	
DEN-205	Adult	TDV TDV (Day 0)	Quad-color fluorospot[k]	8	7	0, 30, 180
		HD TDV, HD TDV (Day 0)	Dengue MNT – breadth of neutralization[e]	0	9	30 (seronegative subjects)
				9	0	0, 30 (seropositive subjects)
		TDV, TDV (Day 0)	Dengue MNT – breadth of neutralization[e]	0	1	30 (seronegative subjects)
				1	0	0, 30 (seropositive subjects)
		TDV, TDV (Day 0)	IFNγ ELISpot[f]	18	15	0, 30, 180, 365
DEN-301	Pediatric, adolescent	TDV, immunogenicity subset, TDV (Day 1 and Day 90)	Anti-dengue NS1 IgG ELISA[e]	24	0	1, 120, 270, 450
			DENV total binding IgG ELISA[e]	24		1, 120, 270, 450
			Anti-dengue IgG avidity assay[e]	24		1, 120, 270, 450
			DENV-2 depletion and RVP neutralization assay (type-specific neutralizing antibodies)[e]	24[l]		120, 270
DEN-304	Adult	Group 3, TDV Lot 3 (Day 1 and Day 90)	Anti-dengue NS1 IgG ELISA[e]	48	0	1, 120, 270
			DENV total binding IgG ELISA[e]	48		1, 120, 270
			Anti-dengue IgG avidity assay[e]	48		1, 120, 270
			DENV-2 depletion and RVP neutralization assay (type-specific neutralizing antibodies)[e]	25		120, 270
DEN-313	Pediatric, adolescent	Immunogenicity subset, TDV (Day 1 and Day 90)	IFNγ ELISpot[f]	36	NA	1, 30, 120, 270

See Module 3.2.P for formulation potency and TDV properties.

Abbreviations: DENV, wild-type dengue virus; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunosorbent spot; HD TDV, high dose TDV; ICS, intracellular cytokine staining; IFN, interferon; LD TDV, low dose TDV; MNT, microneutralization test; NA, not applicable; NS1, nonstructural protein 1; RVP, reporter virus particle; TDV, TDV, selected formulation.

(a) Assay methods are described in Module 2.7.2, Section 3.2.3.

(b) Baseline seronegative - baseline reciprocal neutralizing titer of <10 for all 4 dengue serotypes as determined by MNT 50% assay.

(c) Baseline seropositive - baseline reciprocal neutralizing titer of ≥10 for at least 1 dengue serotype as determined by MNT 50% assay.

(d) As reported in Kim et al (manuscript submitted, 2020) [9].

(e) Post-hoc analysis, not specified in the clinical trial protocol.

(f) As specified in the respective trial protocols and clinical study reports (Module 5.3.5.1 and Module 5.3.5.2).

(g) Number of samples tested from Group 1 (TDV on Day 1 and Day 91).

(h) Number of samples tested from Group 2 (TDV on Day 1 only).

(i) Number of samples tested from Group 3 (TDV on Day 1 and Day 365).

(j) Number of samples tested from Group 4 (placebo on Days 1, 91, and 365).

(k) As reported in Michlmyer et al (2020) [10].

(l) Only 20 of 24 subjects had sufficient Day 270 serum volume for type-specific neutralizing antibody testing.

All the trials in which exploratory immunogenicity was assessed have been described above except study DEN-313, which is described below:

Trial DEN-313

Trial DEN-313 was an open-label, single-arm, phase 2 trial, designed to evaluate the cell mediated immunity after vaccination with TDV in healthy children and adolescents aged 4 to 16 years from Panama and The Philippines (endemic regions). All subjects were vaccinated with TDV administered at Month 0 and Month 3, and were to be followed up for immunogenicity and safety assessments for 3 years after the second TDV dose. Both baseline seropositive and seronegative subjects were enrolled, and subjects were screened prior to enrolment to ensure that each site enrolled at least 40% of subjects with each serostatus.

The aim of this study was to assess the cellular immune responses to 2 doses of TDV at 1 month post second vaccine dose. Additionally, further characterisation of the immune response was carried out including to assess the cellular immune responses up to 3 years post second vaccination, overall and by region and baseline serostatus, to characterise the phenotype of cellular immune responses to TDV by intracellular cytokine staining and to assess the post-vaccination neutralising antibody response against each dengue serotype and against multiple dengue serotypes. These objectives were assessed by measuring the frequency and the magnitude of IFN-γ ELISPOT responses to TDV, by phenotype

characterisation of cellular immune responses to TDV by intracellular cytokine staining and by evaluating GMTs of neutralising antibodies (by MNT_{50}) and seropositivity rates.

Cellular immunogenicity data from the DEN-313 study confirm that TDV elicits a potent cross-reactive T cell response against all 4 dengue serotypes that is maintained through Month 9, irrespective of the subject's baseline dengue serostatus (as investigated using the IFN γ ELISpot assay). A multifunctional CD8+ and CD4+ T cell response against the NS targets of DENV-2 was observed at Month 4 (as investigated using the ICS assay). GMTs of neutralising antibodies against dengue and dengue seropositivity rates (tetravalent responses after the second dose of 99.5% at Month 4 and 93.0% at Month 9) showed good maintenance of dengue neutralising antibody titres for all serotypes through Month 9, irrespective of the baseline dengue serostatus.

It follows a brief description of the exploratory immunogenicity characterisation and their results:

Early immune response

To study the molecular events that trigger NAb responses following TDV vaccination, a comprehensive analysis was conducted to examine differential expression of coding and noncoding genes and isoform expression changes. Samples were selected from Trial DEN-102 subjects who developed low and high titre NAbs and the results were then compared.

The results showed that the early events that occur after vaccination with TDV, that lead to potent Nabs, include type I IFN signalling and expansion of immune cells; these events are similar to those triggered by YFV 17D vaccination and DENV infection. The early events leading to effective CD8+ TCRs elicited by TDV vaccination have also been investigated.

Humoral Immune Responses to DENV Structural Proteins

Several humoral immune response parameters elicited by TDV that might contribute to preventing DENV infection were characterised, including: NAbs, (type-specific [TS] and cross-reactive [CR]), neutralisation of diverse DENV genotypes, binding antibodies, complement-fixing antibodies and polyclonal antibody avidity.

NAbs are generally regarded as the most relevant marker for protective immunity against DENV. DENV NAbs are most commonly measured using the plaque reduction neutralisation test. DENV NAbs can be type-specific (TS) ie, neutralise a single serotype only, or cross-reactive (CR) ie, neutralise more than one DENV serotype. Amino acid homology of the envelope (E) protein, the major surface protein, ranges from 66% to 78% among DENV-1, DENV-2, DENV-3, and DENV-4. Determination and quantitation of TS NAbs is complicated by the serological relationship among the 4 DENV serotypes.

- TS and CR Nabs

Samples from 3 clinical trials (DEN-203, DEN-204, and DEN-301) were selected for characterisation of NAb specificity following vaccination with TDV. The results showed that in exploratory, non-GxP analyses of baseline seronegative vaccinated subjects, TDV elicits tetravalent NAbs that are a combination of TS and CR for DENV-1 and DENV-4, and primarily CR for DENV-3. In baseline seropositive vaccinated subjects, results were variable and likely influenced by prior DENV exposure. Further studies are required to characterise DENV-2 TS NAbs in more detail, but the available results suggest that vaccination can elicit DENV-2 TS NAbs in the majority of subjects. Specificity of DENV NAbs is complex and available methods have significant technical limitations. The relationship between TS or CR NAbs and the outcome of infection has not been established.

- TS and CR MBCs

The results demonstrate TS MBC responses against all 4 DENV serotypes after tetravalent TDV vaccination in both baseline seronegative and baseline seropositive individuals. Therefore, the pattern

of MBC specificity after tetravalent TDV vaccination is different from the pattern after primary or secondary DENV infection. DENV infection only activates TS MBCs to 1 serotype at a time (eg, during each sequential infection). These results demonstrate that all 4 components of TDV contribute to the DENV-specific MBC response. The repertoire of tetravalent DENV TS and CR MBCs generated by vaccination represent a pool of MBCs primed to produce anamnestic antibody responses upon subsequent DENV exposure.

- Neutralisation of Diverse Genotypes

DENV strains are categorised into genotypes within each serotype. Despite the complex serological relationships among DENV strains, genotype can be employed as an indicator of the antigenic differences among strains within a serotype.

For the neutralisation of diverse genotypes studies, sera from Trial DEN-205 were selected. A limitation of the data is the small number of samples that were tested against a small panel of DENV strains.

Overall, exploratory, non-GxP analyses demonstrate that TDV can elicit NAbs capable of neutralising diverse DENV-1, DENV-2, DENV-3, and DENV-4 strains across multiple genotypes.

- Binding Antibodies

Exploratory, non-GxP analyses demonstrate that TDV elicits DENV-1, DENV-2, DENV-3, and DENV-4 virion-binding antibodies that persist for at least 450 days post-vaccination. The relationship between titres in the DENV Total Binding IgG ELISA and the iELISA or the relationship between specific levels of virion-binding antibodies and the outcome of infection have not been established.

- Complement-Fixing Antibodies

Non-GxP analyses demonstrate that TDV elicits complement-fixing antibodies to DENV-1, DENV-2, DENV-3, and DENV-4 that persist for at least 360 days post-vaccination in baseline seronegative and seropositive subjects. The correlation observed between DENV total binding IgG and complement-fixing antibodies suggests that the DENV total binding IgG produced in response to TDV vaccination includes antibodies with functional complement-fixing activity.

- Antibody Affinity Maturation and High Avidity Antibodies

The results demonstrate that TDV can elicit DENV-specific antibody affinity maturation, with high affinity antibodies generated to all 4 DENV serotypes that persist through 450 days post-vaccination.

Immune Responses to Nonstructural Proteins

- Anti-Nonstructural Protein 1 IgG Antibodies

In vitro and *in vivo* studies have demonstrated that antibodies to NS1 can block the pathogenic mechanism of DENV NS1. Although the relationship between NS1-specific IgG elicited by vaccination and the outcome of DENV infection is not known, results of exploratory non-GxP studies demonstrate that TDV can elicit an IgG response in baseline seronegative subjects and boosts responses in baseline seropositive subjects to DENV-1, DENV-2, DENV-3, and DENV-4 NS1 that persist through Day 450 post-vaccination.

Cell-Mediated Immunity

Trials that used earlier TDV formulations and only included flavivirus-naïve adults have shown that like natural DENV infection, TDV elicits broad IFN-ɣ ELISpot responses in adults against TDV components, including the structural proteins of DENV-1, -2, -3, and -4, the DENV-2, in addition to CR responses against the non-structural proteins of DENV-1, -3, and -4, as measured by IFN-γ ELISpot using DENV

peptide pools. To study CMI with the final TDV formulation, samples have been collected from participants in studies DEN-205, DEN-204 and DEN-313.

These CMI analyses represent an extensive dataset clearly showing that TDV elicits cellular immune responses in adults, adolescents and children. IFN-ɣ ELISpot assays with peptide pools spanning the DENV proteome demonstrate that cellular immune responses were elicited to all of the TDV components, this included responses to the structural proteins of DENV-1, -2, -3, and -4, responses to the non-structural proteins of DENV-2, and CR responses against the non-structural proteins of DENV-1, -3, and -4. NS1, NS3, NS5 and prM/E were the most frequently recognised proteins. The magnitude of responses to DENV-2 was higher than to the other 3 serotypes. ICS identified a higher magnitude of CD8+ T cells compared to CD4+ T cells in addition to multi-functional CD4+ and CD8+ responses, with IFN-ɣ+/TNF-α+ or IFN-ɣ+ CD8+ cells being the most frequent phenotype. Similar responses were seen in all CMI assessments in baseline seropositive and seronegative subjects.

Other immunogenicity data

Results from humoral immunogenicity from pivotal trial DEN-301 are detailed at the end of the following section. Similarly, comparison of humoral immunogenicity results from studies that used the final formulation, the analysis of humoral immune response in endemic and non-endemic areas as well as in dengue seronegative subjects is described following discussion of pivotal trial DEN-301.

Mechanism of action

The primary mechanism of action of TDV is to replicate locally and elicit neutralising antibodies to confer protection against dengue disease caused by any of the 4 dengue virus serotypes. TDV activates multiple arms of the immune system, including binding antibodies, complement fixing antibodies, functional antibodies to dengue non-structural protein 1 (NS1), and cell mediated immune responses (CD4+, CD8+, and natural killer cells).

2.6.3. Discussion on clinical pharmacology

Analytical tests

A comprehensive collection of assays has been used for the analysis of the endpoints throughout the clinical development plan of TDV. Overall, the assays performed by the applicant are suitable for their purpose and the scientific rationale for their selection is endorsed.

The assays involved in the analysis of the primary and secondary endpoints of the phase 2 and 3 clinical studies have all been qualified or validated. Validated assays include the dengue MNT, the dengue detection RT-PCR, the vaccine screening RT-PCR, the vaccine confirmation sequencing, the TDV sequencing, the IgG, IgM and NS1 ELISAs, the Yellow Fever PRNT and the anti-hepatitis A ELISA. It is noted that the Vaccine Screening RT-PCR is designed to detect and quantify the RNA of TDV strains, a characteristic that is utilised for both TDV efficacy evaluations within 30 days after vaccination and determination of TDV viraemia following vaccination. The only qualified assay used for the analysis of primary or secondary endpoints was the ELISPOT assay used to characterise cellular-mediated immunity. For the validated or qualified assays, the corresponding validation/qualification reports have been submitted.

The two most important tests for the primary endpoints of the phase 3 pivotal study DEN-301 are the MNT test and the dengue detection RT-PCR, used for case confirmation by detecting the presence of wild type dengue RNA from serum samples of subjects suffering from febrile illness. Both these tests are well described and validated. Nonetheless, in relation to these two assays, there was no information on the handling and storage of the samples to ensure its integrity until final testing. Details

on these procedures were requested and have been provided, showing that there were precise instructions to ensure the integrity of the samples until final testing.

The applicant has developed the MNT assay with reference to the WHO guidance specific to PRNT for dengue. Compared to the PRNT50, the MNT allows a higher throughput and better performance than the classical PRNT50. The Takeda MNT assay used during the entire clinical development of TDV is in line with the WHO Dengue PRNT testing guidance, although it was not validated against the PRNT, as was indicated in the WHO guidance. The applicant states that this is because the WHO Dengue PRNT guideline allows for many different assay variations, and assay methodologies and critical reagents have not been standardised between laboratories. Additionally, a well characterised reference sample panel resulting from either natural dengue infection or generated serum samples is not available. Since many variables such as reagents, virus and cell line that have been shown to have an impact on the assay and have not been standardised and harmonised between laboratories, the applicant has not considered meaningful an experimental comparison between a large format PRNT and the Takeda MNT assay. This approach for using the MNT was already accepted in the final Scientific advice letter sent by CHMP and therefore the assay validation provided is considered sufficient.

Several assay-specific questions were raised for the PRNT50 but have been resolved after the applicant's answers. In addition to validated and qualified assays, exploratory immunity testing was performed with assays in the research status. These assays were used for the analysis of the exploratory endpoints of phase 2 and 3 studies in the clinical development of TDV. These assays are ICS, dengue 2 depletion and reported virus particle assay, dengue total binding IgG assay, dengue IgG avidity assay, anti-dengue NS1 IgG assay, dengue MNT assay for breadth of neutralisation, and anti-dengue complement antibody assay. It is considered that the exploratory nature of these trials did not require using validated assays.

Selection of TDV formulation and dosing schedule

Overall, the total number of subjects that were enrolled in the phase 1 trials was 1371 participants for studies DEN-101, DEN-102, DEN-103, DEN-104 and DEN-106. Phase 2 studies enrolled 2485 subjects in trials DEN-203, DEN-204 and DEN-205. All the phase 1 studies were carried out in non-endemic settings and the phase 2 studies were done in endemic countries and therefore included both seropositive and seronegative participants. All these trials were carried out to determine the final formulation, number of doses, schedule and route of administration. All phase 1 studies enrolled adult subjects, and children (aged 1.5 years and above) were included in the phase 2 studies. All trials, except DEN-203, DEN-204 and DEN-205 were carried out in non-endemic areas.

The first trials DEN-101 and DEN-102 explored two formulations [LD (low dose) and HD (high dose)] using with two different administration routes (SC and ID) in a 2-dose schedule with 3 months separation. Regarding the route of administration, the results from these initial trials showed that the intradermal administration presented a higher reactogenicity in studies DEN-101 and DEN-102, and thus the subcutaneous route was selected for future trials. This selection is sensible. The results from these two trials showed that TDV was immunogenic, although neutralising antibody GMTs induced were not equally high for all serotypes. Based on these immunogenicity results, the applicant continued the clinical programme with this vaccine aimed to determine in additional trials the formulation and schedule that yielded a balanced strong response against the four dengue virus serotypes.

In order to achieve the highest and more balanced immune response, several options were assayed in additional phase 1 trials: vaccines containing high and low doses of the 4 viral components (Trial DEN-103), formulations with different ratios of the four-dengue virus (trial DEN-104), lyophilised and the liquid formulation (DEN-106) and also the administration of two simultaneous doses in separate arms (Trial DEN-103, DEN-104). It was also hypothesised that administering 2 doses at the same visit would increase compliance by reducing the number of visits required. However, this did not result in an

improved response. These trials fixed the lyophilised formulation for future development. The selection of the optimal formulation was complex, since it appeared that TDV-2 had a higher replication rate than the others and specially interfered with TDV-4 replication. These data were used in the design of trials DEN-203, DEN-204 and DEN-205, which were carried out in endemic areas and therefore included both dengue seropositive and seronegative subjects.

Trial DEN-203 was the first study to include children 1.5 years of age and older and seropositive adult subjects, and was carried out in endemic regions (Puerto Rico, Colombia, Singapore, and Thailand). The trial evaluated 2 HD TDV doses, given SC and 3 months apart. This trial provided support for a 2-dose schedule with 3 months interval between doses. This schedule was further supported from data from trials DEN-204 and DEN-205.

The final formulation for the TDV was decided in trial DEN-205, in which the immunogenicity of HD TDV formulation and a TDV with 10-fold lower content of the TDV-2 component (named TDV) was compared. This study was carried out in Singapore (endemic region). The results showed that participants in the TDV group (10-fold lower content of the TDV-2) presented higher MNT GMTs and seroconversion rates against DENV-4 than those in the HD TDV group. Therefore, the applicant selected the TDV (viral content with a 10-fold reduction of TDV-2) for future clinical development and this decision is supported.

The final schedule for further phase 3 development was based on data from trial DEN-204 in which three schedules were compared: 2 doses with 3 months interval; 2 doses given as primary vaccination and a booster at 12 months; and a single dose. The study was conducted in endemic regions (The Dominican Republic, Panama, and The Philippines). The criterion used to decide the final schedule was seroconversion rates rather than MTN GMTs, since seropositivity is likely to be a prerequisite for protection. In the absence of a correlate of protection, this approach is supported. Results from an interim analysis performed at month 6 showed that a second dose at month 3 enhanced immunogenicity against DENV-3 and to a lesser extent against DENV4 in subjects who were seronegative at baseline. Because a 0–3-month schedule ensured a more rapid vaccine response and higher tetravalent seropositivity response in baseline seronegative subjects compared to the 0-12 month schedule, the applicant chose the two dose vaccination 3 months apart for the Phase 3 studies. The applicant's decision is endorsed.

The results of trial DEN-205 supported the selection of the TDV (viral content in the lyophilised TDV product with a 10-fold lower viral content of TDV-2) for future clinical development since this formulation results in a more balanced immune response with the TDV compared to HD-TDV. The results showed a less dominant response to DENV-2 (including lower incidence of TDV-2 viraemia) in the TDV group compared to the HD TDV group.

In conclusion, the results of the phase 1 and 2 studies led to the selection for the Phase 3 trials of a lyophilised TDV formulation in which the Dengue virus 2 component is reduced 10-fold as compared to the initial HD-TDV formulation. The vaccine should be administered subcutaneously, with a 2-dose schedule given three months apart. The final formulation of TDV at release is ≥ 3.3; ≥ 2.7; ≥ 4.0; ≥ 4.5 log10 PFU/dose for TDV-1, TDV-2, TDV-3, and TDV-4, respectively.

Overall, the rationale that supports the formulation, number of doses, schedule and route of administration to be used for phase 3 development, is supported. No safety concerns were raised.

Exploratory Immunogenicity Characterisation

The immune response to dengue infection is complex and involves both the humoral and cellular arms of the immune system; however, the exact mechanisms that underlie protective immunity are not yet fully understood. TDV was specifically designed to activate multiple arms of the immune response; therefore the applicant has carried out a very comprehensive collection of immunologic assays to

characterise the immune response elicited by TDV. This exploratory immunogenicity characterisation was done by testing of samples from Good Clinical Practice-compliant clinical trials but performed in non-Good Practice (GxP) laboratories. This approach is considered acceptable considering the exploratory nature of the assays. The samples have been obtained from subjects who were included in the immune subsets for Trials DEN-203, DEN-204, DEN-205, DEN-301, DEN-304 and DEN-313.

The exploratory immunogenicity characterisation includes the following analysis: Type-specific (TS) and cross-reactive (CR) neutralising antibodies, TS and CR MBCs, neutralisation of diverse genotypes, binding antibodies, complement-fixing antibodies, antibody affinity maturation and high avidity antibodies, anti-NS1 IgG antibodies and cell-mediated immunity.

There were some issues that needed to be solved in relation to the functional role of the complement binding antibodies in disease mitigation (trial DEN-204); the ADE-inducing potential of cross-reactive and binding antibodies; and the role of CD8+ and CD4+ T cell responses in disease mitigation. These issues have been appropriately addressed by the applicant.

Taken together, the results obtained showed that TDV induces a diverse antibody response that includes both type-specific and cross-reactive between DENV serotypes, binding antibodies, and complement fixing antibodies to all 4 DENV serotypes in addition to DENV-2 NS1-specific antibodies that are cross-reactive with NS1 from DENV-1, -3, and -4. Cellular immunity was elicited to all the structural and non-structural proteins of the four DENV serotypes. The magnitude of the response was higher for CD8+ compared to CD4+ T cells, being IFN-γ+/TNF-α+ or IFN-γ+ CD8+ cells the most frequent phenotype. These results agree with evidence from the literature that suggests that each of the studied components of the immune response can contribute to protection against dengue infection.

Nonetheless, there were some issues, specifically regarding cellular immunogenicity (study DEN-313) that were requested to be addressed by the applicant. The applicant has now provided valuable additional information and explanations for the specific points that were raised regarding the evaluation of the vaccine induced cellular immune response.

2.6.4. Conclusions on clinical pharmacology

In total, eight Phase I and II trials tested different formulations, administration routes and schedules of the Takeda dengue vaccine. From these trials, a lyophilised TDV formulation (in which the Dengue virus 2 component is reduced 10-fold as compared to the initial formulation) to be administered subcutaneously, with a 2-dose schedule given three months apart was selected for the pivotal efficacy trial DEN-301.

An extensive analysis on the humoral and cellular immune response induced by the vaccine has been made. It has been shown that TDV induces: i) a diverse antibody response that is both type-specific and cross-reactive between DENV serotypes, binding antibodies, and complement fixing antibodies to all 4 DENV serotypes; ii) a cellular immunity to all the structural and non-structural proteins of the four DENV serotypes. The magnitude of the response was higher for CD8+ compared to CD4+ T cells, being IFN-γ+/TNF-α+ or IFN-γ+ CD8+ cells the most frequent phenotype.

2.6.5. Clinical efficacy

2.6.5.1. Dose response studies

See clinical Pharmacology section.

2.6.5.2. Main studies

Study DEN-301: **Phase III, Double-Blind, Randomised, Placebo-Controlled Trial to Investigate the Efficacy, Safety and Immunogenicity of a Tetravalent Dengue Vaccine (TDV) Administered Subcutaneously in Healthy Children Aged 4–16 Years Old.**

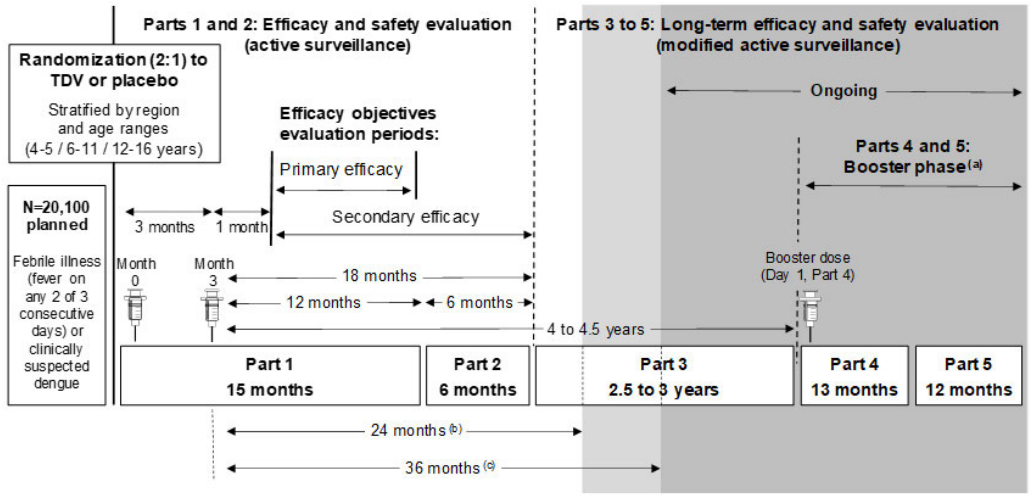
Trial Design

Pivotal Trial DEN-301 is a phase 3, double-blind, randomised, placebo-controlled trial with 5 trial parts (Parts 1-5) for surveillance of febrile illness with potential dengue aetiology in subjects aged 4 to 16 years living in endemic regions.

The age range of 4 to 16 years of age at trial entry was chosen based on the epidemiology of dengue in the endemic Asia Pacific and Latin American countries included in the trial and to have sufficient subjects who were seronegative at baseline to allow an analysis of vaccine efficacy (VE) by serostatus. Symptomatic dengue is most common during a second infection, less common during a primary infection, and unusual during a third or fourth infection. An efficacy assessment based on the detection of symptomatic dengue is therefore best performed in age groups for which a secondary infection is likely. An age range of 4 to 16 years is an age range for which febrile surveillance and long-term follow-up is more successful.

The trial is conducted in 3 countries of the Asia Pacific region (The Philippines, Sri Lanka, and Thailand) and in 5 countries of Latin America (Brazil, Colombia, The Dominican Republic, Nicaragua, and Panama). Eligible subjects aged 4 to 16 years were randomised in a 2:1 ratio to receive either TDV or placebo (normal saline for injection) by a subcutaneous (SC) injection into the upper arm (planned number of subjects: 13,400 TDV; 6700 placebo).

The trial comprises 5 consecutive trial parts (see next Figure): Part 1 was the primary analysis period, a 15-month period lasting until 12 months post second dose, and included the primary efficacy analysis. This part started on the day of vaccination and ended once both of the following 2 criteria had been fulfilled: i) 120 cases of confirmed dengue fever; ii) minimum duration of subject follow-up of 12 months post-second vaccination. The end of Part 1 was defined for each subject so that the duration of follow-up after the second vaccination was approximately the same for all subjects. Virologically confirmed cases in Part 1 counted towards the primary efficacy objective if they occurred at least 30 days post-second vaccination. Part 2 was a 6-month period lasting until 18 months post second dose, at the end of which the secondary efficacy endpoints were analysed. In fact, virologically confirmed cases in Parts 1 and 2 contributed towards the secondary efficacy objectives; Part 3 is a 2.5- to 3-year period for the assessment of long-term efficacy and safety; and Parts 4 and 5 comprise the booster phase of at least 25 months for the assessment of the efficacy, immunogenicity, and safety following an additional placebo or TDV booster dose administered 48 to 54 months (4-4.5 years) after the second vaccine dose.



Source: Module 5.3.5.1, DEN-301 Protocol Amendment 4.

Abbreviations: N, total number of subjects.

(a) Only subjects from the per-protocol set who were 4 to 11 years of age at the time of randomization in the trial (Month 0) can participate in the booster phase. Eligible subjects will receive a single dose of placebo or TDV, depending on the assignment at randomization at Month 0. The target sample size is approximately 10,500 subjects (placebo: approximately 3500 subjects; TDV: approximately 7000 subjects).

(b) Data up to 24 months post second vaccine dose are described in this document.

(c) Summaries of data from 36 months post second vaccine dose are available in Module 5.3.5.1, DEN-301,

Figure 5: Trial DEN-301: Trial Schematic

Subjects could have been enrolled into a dry run to commence and test the febrile surveillance methodology. This dry-run involved pre-vaccination surveillance for dengue and was conducted for up to 10 months prior to vaccination on Day 1. The need for and duration of the dry run at an individual site depended on the experience of the site in conducting similar trials.

At baseline, blood samples were taken from all subjects to determine the dengue serostatus. During Parts 1 and 2 (as well as the dry run), active surveillance was applied (see Table 10). Any subject with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) was asked to return to the site for dengue fever evaluation by the investigator. Subjects/guardians were contacted at least weekly to ensure robust identification of febrile illness and to remind them of their obligation to return to the site in the event of febrile illness.

During Part 3 and the booster phase (Parts 4 and 5), modified active surveillance is applied to detect dengue cases of any severity in a tiered approach based on the need for hospitalisation (see Table 10). The active surveillance was modified for Parts 3 to 5 to enable detection of symptomatic dengue while minimising the burden on subjects and their guardians to maintain compliance and subject retention during this long period of follow-up. Any subject with febrile illness is asked to return to the site for evaluation by the investigator. Subjects presenting with febrile illness not requiring hospitalisation are to be screened for dengue disease (by RT-PCR) unless there is an alternative laboratory confirmed aetiology (note: only occasionally an acute sample for RT-PCR was not taken because of an alternative laboratory confirmed diagnosis). Subjects with febrile illness requiring hospitalisation are evaluated as during active surveillance. During modified active surveillance, subjects/guardians are contacted at least weekly. Thus, consistency with the active surveillance in Parts 1 and 2 is maintained in terms of frequency of contact and the definition of febrile illness. The principal difference between the surveillance methods is that clinical chemistry (alanine aminotransferase and aspartate aminotransferase), hematology (hematocrit and platelets), and dengue serology (IgG, IgM, and NS1) in acute or convalescent samples are not mandated by the protocol during Parts 3 to 5 unless the

subject requires hospitalisation. Any relevant clinical data, however, were to be collected where testing was performed as part of clinical care regardless of hospitalisation.

Table 10: Trial DEN-301: Differences Between Active Surveillance (Dry-Run, Parts 1 and 2) and Modified Active Surveillance (Parts 3-5)

	Active Surveillance (Dry-Run, Parts 1 and 2)	Modified Active Surveillance (Parts 3-5)	
Contact frequency	At least weekly	At least weekly	
Threshold for evaluation	All febrile illness (irrespective of the need for hospitalization)	Febrile illness requiring hospitalization	Febrile illness not requiring hospitalization (unless with alternate laboratory confirmed etiology) [a]
Laboratory evaluations	• Within 5 days of onset of fever: RT-PCR, ELISA (NS1, IgM, IgG); platelet count, hematocrit, liver function (ALT, AST) • 7-14 days after obtaining acute sample: ELISA (IgM, IgG); platelet count, hematocrit, liver function (ALT, AST) • Other laboratory evaluations as per standard of care		• Within 5 days of onset of fever: RT-PCR • Other laboratory evaluations as per standard of care

Source: Module 5.3.5.1, DEN-301 CSR, Table 9.a.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELISA, enzyme-linked immunosorbent assay; IgG/IgM, immunoglobulin G/M; NS1, nonstructural protein 1; RT-PCR, reverse transcriptase polymerase chain reaction.

RT-PCR and ELISA assays performed at a central laboratory; platelet counts, hematocrit, liver function, and "other" laboratory evaluations performed at local sites.

(a) No laboratory evaluation for a febrile illness that does not require hospitalization and has an alternative laboratory confirmed etiology during Parts 3 to 5.

The trial protocol, which initially included Parts 1 to 3 only, has been amended to introduce a booster phase comprising Parts 4 and 5, to assess the efficacy, immunogenicity, and safety following an additional placebo or TDV booster dose administered 48 to 54 months (4-4.5 years) after the second vaccine dose. Only subjects who were 4 to 11 years of age at the time of randomisation in the trial (Month 0) are eligible to participate in the booster phase.

Parts 1, 2and 3 have been completed; the remainder of the trial (parts 4 and 5) is still ongoing. In this MAA, the unblinded data from Part 1, Part 2, and until 12 months after the end of Part 1 were initially submitted. In addition, during the assessment procedure, data from first dose to 57 months post second vaccine dose (parts 1, 2 and 3) have been provided.

Methods

- **Study participants**

Inclusion Criteria

Subject eligibility was determined according to the following criteria:

1. The subject was aged 4 to 16 years, inclusive, at the time of randomisation.

2. Individuals who were in good health at the time of entry into the trial as determined by medical history, physical examination (including vital signs), and clinical judgment of the Investigator.

3. The subject and/or the subject`s parent(s)/guardian(s) signed and dated an assent/written ICF where applicable, and any required privacy authorisation prior to the initiation of any trial procedures, after the nature of the trial had been explained according to local regulatory requirements.

4. Individuals who could comply with trial procedures and were available for the duration of follow-up.

Booster Phase (Parts 4 and 5)

Subject eligibility for enrollment in the booster phase will be determined according to the following key criteria:

1. The subject is included in the per-protocol set (PPS).

2. The subject is aged 4 to 11 years at the time of randomisation (Day 1 [M0]).

Exclusion Criteria

Trial Entry

Any subject who met any of the following criteria did not qualify for entry into the trial:

1. Febrile illness (temperature ≥38° C) or moderate or severe acute illness or infection at the time of randomisation.

2. History or any illness that, in the opinion of the Investigator, could have interfered with the results of the trial or pose an additional risk to the subject due to participation in the trial, including but not limited to (only key criteria follow): Known hypersensitivity or allergy to any of the vaccine components, Female subjects (post-menarche) who were pregnant or breastfeeding, individuals with any serious chronic or progressive disease according to judgment of the Investigator (eg, neoplasm, insulin-dependent diabetes, cardiac, renal, or hepatic disease, neurologic or seizure disorder or Guillain-Barré syndrome), known or suspected impairment/alteration of immune function, and females of childbearing potential who were sexually active, and who had not used any of the acceptable contraceptive methods for at least 2 months prior to Day 1 (M0).

- **Treatments**

In order to demonstrate clinical effectiveness of the product at or near the predicted end of shelf-life, the clinical trial material was intentionally formulated at lower potencies than those intended for commercial lots, and the potency values for this clinical trial form the basis for the stability specifications, referred to as the end of shelf life specifications.

Identity, manufacturers, and batch numbers of TDV, diluent, and placebo were provided.

Investigational TDV was provided in single use 2 mL glass vials with to be reconstituted by adding approximately 0.7 mL diluent to facilitate withdrawal of 1 dose (0.5 mL) for SC injection.

Placebo was provided by the sponsor as single use 2 mL vials.

All products were labelled and packaged according to applicable regulatory requirements.

All subjects were to receive a 2-dose regimen (Day 1 [M0]) and Day 90 [M3]) of either TDV or placebo according to their random assignment; subjects enrolled in the booster phase (Parts 4 and 5) will additionally receive a single dose of TDV or placebo according to the randomisation from Day 1 ([M0]).

- **Objectives**

Primary Objective

To evaluate the efficacy of 2 doses of TDV in preventing symptomatic dengue fever of any severity and due to any of the 4 dengue virus serotypes in 4 to 16 year old subjects.

Secondary Objectives

Assessed post-second vaccination:

Efficacy:

• To assess the efficacy of TDV in preventing symptomatic dengue fever of any severity induced by individual dengue serotypes.

• To assess the efficacy of TDV in preventing symptomatic dengue fever of any severity by dengue exposure status at baseline.

• To assess the efficacy of TDV in preventing hospitalisation due to VCD fever.

• To assess the efficacy of TDV in preventing severe dengue induced by any dengue serotype.

Immunogenicity:

• To assess the immunogenicity of TDV in a subset1 of subjects.

Exploratory Objectives

Parts 1, 2, and 3

• To describe the efficacy of TDV in preventing VCD fever between first and second vaccinations.

• To describe the efficacy of TDV in preventing VCD fever from first vaccination until end of Part 2.

• To describe virologically confirmed and hospitalised dengue fever identified during Part 3.

• To describe VCD fever identified during Part 3.

For the correlate of protection, a threshold antibody titre value may be evaluated to predict VE using descriptive methodology.

• To describe the profiles of immunoglobulin G (IgG), IgM, and Nonstructural Protein 1 (NS1) antigen in episodes of febrile illness.

Booster Phase (Parts 4 and 5)

Efficacy:

• To assess the efficacy of a TDV booster dose in preventing symptomatic dengue fever of any severity induced by any dengue serotype.

• To assess the efficacy of a TDV booster dose in preventing symptomatic dengue fever of any severity by dengue exposure status at baseline.

• To assess the efficacy of a TDV booster dose in preventing hospitalisation due to VCD fever induced by any dengue serotype.

• To assess the efficacy of a TDV booster dose in preventing severe dengue induced by any dengue serotype.

Booster immunogenicity:

• To assess the immunogenicity of a TDV booster dose in a subset of subjects.

- **Outcomes/endpoints**

The endpoints listed in this section reflect those in the statistical analysis plan (SAP) (Version 4.0), which was approved prior to the database lock for the first interim analysis and included certain exploratory endpoints additional to those listed in the protocol. There were no differences between the protocol and SAP for the primary or secondary endpoints.

Primary Endpoint

Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by any dengue serotype occurring from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 1, with VE defined as 1 - ($\lambda V/\lambda C$) (where λV and λC denote the hazard rates for the TDV and placebo arms, respectively).

The primary analysis of VE occurred at the end of Part 1, i.e. after both of the following 2 criteria were fulfilled: (1) 120 cases of VCD had accrued, and (2) a minimum duration of subject follow-up of 12 months post-second vaccination.

Secondary Endpoints

Key secondary efficacy endpoint

• Vaccine efficacy of 2 doses of TDV in preventing hospitalisation due to VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2.

Additional secondary efficacy endpoints

• Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by each dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2.

• Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2 in subjects dengue seronegative at baseline.

• Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2 in subjects dengue seropositive at baseline.

• Vaccine efficacy of 2 doses of TDV in preventing severe VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2.

Immunogenicity

Subset (post-first and post-second vaccinations):

• Seropositivity rate (% of seropositive subjects) for each of the 4 dengue serotypes at prevaccination on Day 1 (M0), post-first vaccination on Day 30 (M1), pre-vaccination on Day 90 (M3); post-second vaccination at Day 120 (M4), Day 270 (M9), Day 450 (M15), and then annually.

• Seropositivity rate (% of seropositive subjects) for any 1 (monovalent), 2 (bivalent), 3 (trivalent), and 4 (tetravalent) dengue serotypes, as well as at least bivalent (seropositive for ≥2 dengue serotypes) and at least trivalent (seropositive for ≥3 dengue serotypes) at prevaccination on Day 1 (M0), post-first vaccination on Day 30 (M1), pre-vaccination on Day 90 (M3), post-second vaccination on Day 120 (M4), Day 270 (M9), Day 450 (M15), and then annually. (Data after 12 months after end of Part 1 will be reported in future CSRs for this trial.). (Note: Seropositivity is defined as a reciprocal neutralising titre ≥10.)

• Geometric mean titres of neutralising antibodies (microneutralisation test [MNT]) for each dengue serotype at pre-vaccination on Day 1 (M0), post-first vaccination on Day 30 (M1), pre-vaccination on Day 90 (M3); post-second vaccination at Day 120 (M4), Day 270 (M9), Day 450 (M15), and then annually.

Exploratory Endpoints

Parts 1, 2, and 3

• Vaccine efficacy of two doses of TDV in preventing VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [Month 4]) until the end of Part 2.

• Vaccine efficacy of TDV in preventing VCD fever identified between the first and second vaccination (ie, Day 1 until Day 90).

• Vaccine efficacy of TDV in preventing VCD fever identified from 30 days post-first vaccination until second vaccination (ie, Day 31 until Day 90).

• Vaccine efficacy of TDV in preventing VCD fever identified from first vaccination (Day 1) until end of Part 1.

• Vaccine efficacy of TDV in preventing VCD fever identified from first vaccination (Day 1) until end of Part 2.

• Vaccine efficacy of TDV in preventing VCD fever identified from first vaccination (Day 1) until end of Part 3.

• Analyses to describe virologically confirmed and hospitalised dengue fever identified during the first half (18 months) of Part 3.

•Analyses to describe virologically confirmed and hospitalised dengue fever identified during the second half (18 months) of Part 3.

• Analyses to describe VCD fever induced by any dengue serotype identified during the first half (18 months) of Part 3.

• Analyses to describe VCD fever induced by any dengue serotype identified during the second half (18 months) of Part 3.

• Analyses to assess VE of TDV in preventing VCD fever identified from 30 days post-second vaccination (Day 120) until end of Part 3.

• Analyses to assess VE of TDV in preventing VCD fever identified during Part 3.

• Analyses to assess VE of TDV in preventing VCD fever identified in 12 months intervals after completion of Part 1 (ie, Part 2 and the first 6 months of Part 3, Months 7-18 within Part 3, and Months 19-30 within Part 3). (Data for Months 7-18 within Part 3, and Months 19-30 within Part 3 will be reported in future CSRs for this trial.)

• Analyses to describe severe VCD fever induced by any dengue serotype identified during the first half (18 months) of Part 3.

• Analyses to describe severe VCD fever induced by any dengue serotype identified during the second half (18 months) of Part 3.

Post-first and post-second vaccinations:

• To examine the relationship between dengue neutralising antibodies (MNT) and protection from dengue infection (correlate of protection).

• To describe the profiles of IgG, IgM, and NS1 during episodes of febrile illness.

Efficacy Measurements: Handling of Febrile Illness Cases (Suspected Dengue Cases)

Subjects presenting with febrile illness (defined as temperature ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue during the dry-run, Parts 1 and 2 or requiring hospitalisation during Parts 3, 4, or 5 had/will have 2 blood samples taken to confirm dengue infection. These were/will be in addition to those taken as part of the clinical care of the subject.

The main trial assessment/measurement of febrile illness was defined as fever ≥38° C on any 2 of 3 consecutive days was chosen rather than "2 consecutive days" to increase the likelihood of capturing more dengue cases. This approach was considered to be the most appropriate to maximise the likelihood of capturing all dengue cases. All dengue cases were confirmed by dengue detection RT-PCR and severity was assessed by the Dengue Case Adjudication Committee (DCAC). All other measurements were made according to standard methods. Each local laboratory provided their reference ranges to the sponsor and centralised laboratory analyses were performed in validated laboratories designated by the sponsor.

The first or acute blood sample was taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever). Testing included dengue IgM and IgG enzyme-linked immunosorbent assay (ELISA), dengue NS1 antigen ELISA, dengue detection RT-PCR, haematocrit, platelet count, and liver function tests (LFTs [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]).

The second or convalescent blood sample was taken during the convalescent phase of the disease (ie, between 7 and 14 days after the acute sample) and tested for dengue IgM/IgG ELISA, haematocrit, platelet count, and LFTs.

Local standards of care may have required additional tests, based on clinical presentation and at medical discretion. Additional dengue neutralising antibody and other laboratory tests could have been performed. In addition to blood tests, clinical evaluation was performed for signs of haemorrhage or plasma leakage as well as any other abnormal signs or symptoms.

In addition, during Parts 3, 4, and 5, subjects presenting with febrile illness (defined as temperature ≥ 38ºC on any 2 of 3 consecutive days) or clinically suspected dengue and not requiring hospitalisation had/will have a blood sample taken for dengue infection confirmation by dengue detection RT-PCR unless there was an alternate laboratory-confirmed aetiology. The blood sample was/will be taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever). Febrile illness cases within 30 days after vaccination were investigated for the presence of wild-type or vaccine-derived dengue virus. Clinical evaluation was/will be performed for signs of haemorrhage or plasma leakage as well as any other abnormal signs or symptoms. Local standards of care could have required additional tests, based on clinical presentation and at medical discretion. Approximate blood volumes and analyses for febrile surveillance are presented in Table 11.

A febrile illness as described above required/will require an interval of at least 14 days from a previous febrile illness to avoid overlap of acute and convalescent visits from 1 episode with those from a second episode.

Table 11: Blood Volumes and Analyses for Febrile Surveillance

Timing	Blood Volume	Assessment(s)
During the dry-run, Parts 1 and 2, and if hospitalization was required during Part 3, 4, and 5:		
Acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever)	4 mL (dry-run, Parts 1, 2, & 3) 5 mL (Parts 4 & 5)	Dengue detection RT-PCR, NS1 antigen ELISA, IgM and IgG ELISA
	3-5 mL [(a)] (dry-run, Parts 1, 2, & 3) 4-6 mL [(a)] (Parts 4 & 5)	Hematocrit, platelet count and LFTs (AST and ALT)
Convalescent phase of the disease (ie, between 7 and 14 days after the acute sample)	3 mL (dry-run, Parts 1, 2, & 3) 4 mL (Parts 4 & 5)	IgM and IgG ELISA
	3-5 mL [(a)] (dry-run, Parts 1, 2, & 3) 4-6 mL (Parts 4 & 5)	Hematocrit, platelet count and LFTs (AST and ALT)
During Parts 3, 4, and 5 without alternate laboratory-confirmed etiology and not requiring hospitalization:		
Acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever)	3 mL (dry-run, Parts 1, 2, & 3) 4 mL (Parts 4 & 5)	Dengue detection RT-PCR

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELISA, enzyme-linked immunosorbent assay; IgG/IgM, Immunoglobulin G/M; LFT, liver function test; NS1, nonstructural protein 1; RT-PCR, reverse transcriptase polymerase chain reaction.

Only samples for dengue detection RT-PCR, NS1 antigen ELISA, IgM ELISA and IgG ELISA were sent to the central laboratory. Data from the central laboratory were not available for real-time case management. Diagnostic tests were to be performed locally as per standard of care for case management.

(a) Blood volume could have varied per local laboratory requirements.

Severity of VCD Fever Assessed by Independent DCAC

The criteria used for the assessment of VCD fever severity by the independent DCAC, which assessed all hospitalised VCD cases based on criteria pre-defined in a charter are summarised below:

i. Bleeding abnormality

• For a case to be considered severe there must have been a significant intervention needed in response to the bleeding episode, such as: - Blood transfusion, - Nasal packing, or - Hormonal therapy.

or,

• Bleeding occurred into critical organs such as the brain.

ii. Plasma leakage

• For a case to be considered severe there must have been evidence of both plasma leakage AND functional impairment.

- Plasma leakage includes clinical evidence, radiological evidence, or haematocrit elevated >20% above normal levels or baseline.

- Functional impairment is defined as: · Shock; · Respiratory distress.;

Liver

• For a case to be considered severe, there must have been evidence of both hepatitis and functional impairment.

- Hepatitis was defined as AST or ALT >10 × the upper limit of normal.

- Functional impairment was defined as prothrombin time >1.5 × the upper limit of normal or hypalbuminaemia.

iv. Renal

• Serum creatinine >2.5 × the upper limit of normal.

or,

• Requiring dialysis.

v. Cardiac

• Abnormalities intrinsic to the heart (ie, not resulting from intravascular volume depletion) and with evidence of functional impairment.

- Examples of intrinsic abnormality: myocarditis, pericarditis, myoepicarditis.

- Example of functional impairment: new conduction abnormality resulting in irregular heart rhythm (ie, not transient first-degree heart block).

vi. Central nervous system

• Any abnormality with the exception of a simple febrile convulsion or brief delirium.

vii. Shock

• All shock cases were considered severe.

All non-hospitalised VCD cases were considered "non-severe" for the corresponding endpoint (ie, VE of 2 doses of TDV in preventing severe VCD dengue).

Severity of VCD fever assessed according to the WHO criteria (1997) for dengue haemorrhagic fever (DHF)

According to the WHO criteria (1997) for DHF, VCD fever severity was analysed programmatically without applying medical judgment.

According to the WHO, typical cases of DHF are characterised by four major clinical manifestations: high fever, haemorrhagic phenomena, and often, hepatomegaly and circulatory failure.

Immunogenicity Measurements

Blood samples for serological immunogenicity testing were collected from all subjects in Part 1 at pre-vaccination on Day 1 (M0) and on Day 120 (post-second vaccination [M4]) and will be collected from all subjects in the booster phase at pre -booster vaccination on Day 1b (M0b) and post-booster vaccination on Day 30b (M1b).

Additional samples were collected from the subset in Part 1, 2, and 3 post-first vaccination on Day 30 (M1), pre-vaccination on Day 90 (M3), post-second vaccination on Day 270 (M9) and Day 450 (M15), and then every 12 months until the end of Part 3 and will be collected from the booster immunogenicity subset post-booster vaccination on Day 180b (M6b), Day 395b (M13b), and Day 760b (M25b).

Blood samples taken at pre-vaccination on Day 1 (M0) of all subjects were analysed for dengue serostatus at baseline. Samples taken at Day 120 (M4) for subjects not in the subset were stored for future analysis.

For immunogenicity endpoints including seropositivity (SP) rate and geometric mean titres (GMTs) for dengue neutralising antibodies (and geometric mean ratios [GMRs] for the booster phase), descriptive statistics and 95% CIs are provided.

- **Sample size**

This was a partially case-driven trial.

Assuming true VE of 60% and a randomisation ratio of 2:1 (TDV: placebo) a total of 120 cases of VCD fever induced by any dengue serotype occurring from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 1 would provide at least 90% power to rule out a vaccine effect of ≤25% (with a 2-sided significance level of 0.05).

Assuming a background incidence rate of 1.0% by the end of Part 1 (minimum 12 months after the second vaccination for each subject), randomisation of 20,100 subjects in a 2:1 ratio with follow-up for a minimum of 12 months would allow the accrual of at least 120 dengue fever cases. Exclusion of subjects from the PPS was compensated for by a potentially longer duration of Part 1.

For the booster phase, no formal sample size calculations were performed.

- **Randomisation**

The Investigator or designee accessed the IWRS at subject enrolment to obtain the subject number, which was used throughout the trial, and to randomise the subject into the trial on the first day of dosing (Day 1 [M0]). Subjects assigned to participate in the booster phase of the trial will receive a single dose of TDV or placebo, depending on the assignment at the randomisation performed on Day 1 (M0) by IWRS/IVRS.

The Investigator or designee accessed the IWRS at each dispensing visit to obtain the Vaccination Identification number for the vaccine dose. The vaccines were prepared and administered by the unblinded pharmacist or unblinded administrator according to the instructions in the Pharmacy Manual or per manufacturer´s instructions.

The Investigator or designee was responsible for overseeing the administration of vaccine to subjects randomised in the trial according to the procedures stipulated in this trial protocol. All vaccines were administered only by unblinded personnel who were qualified to perform that function under applicable laws and regulations for that specific trial. The unblinded personnel who administered the vaccine did not assess AEs.

Randomisation by using an interactive system (Interactive Web Response System [IWRS] or Interactive Voice Response System [IVRS]) was stratified by region (Asia Pacific and Latin America) and age range (children aged 4-5 years, 6-11 years, and 12-16 years) to ensure that each age range had the appropriate ratio of TDV to placebo in each region. In addition, recruitment followed an enrolment plan to ensure representative enrolment across the age ranges and regions. This was considered necessary to mitigate the relative difficulty of recruiting subjects at the extremes of the age ranges in this trial.

- **Blinding (masking)**

As a double-blind trial, the subjects, data collectors (eg, investigators), designated pharmacists/vaccine administrators and data evaluators (eg, trial statisticians) were blinded to TDV or placebo administration until the time of randomisation code break.

- **Statistical methods**

Analysis Sets

Dry Run Set**:** The Dry Run Set consists of all subjects (regardless of whether they were randomised or not) who participated in the dry-run.

Parts 1, 2, and 3

Randomised Set: The Randomised Set consists of all randomised subjects, regardless of whether any dose of TDV or placebo was received. Subjects are summarised according to the IP to which they were assigned.

Safety Set: The SS consists of all randomised subjects who received at least 1 dose of TDV or placebo. For analyses of solicited AEs (reactogenicity) and unsolicited AEs, only subjects in the subset were included. For all subjects in the SS, SAEs were assessed during Parts 1, 2 and 3. Subjects are summarised according to the IP received.

Full Analysis Set: The FAS consists of all randomised subjects who received at least 1 dose of the TDV or placebo. Subjects are summarised according to the IP to which they were assigned.

Full Analysis Set for Immunogenicity (FASI): The FASI is based on the FAS and consisted of all randomised subjects in the subset for whom a valid pre-dosing and at least 1 valid post-dosing blood sample was received for immunogenicity. Subjects are summarised according to the IP to which they were assigned.

Per-Protocol Set: The PPS consists of all subjects in the FAS who had no major protocol violations as presented in Table 12.

Per-Protocol Set for Immunogenicity (PPSI): The PPSI consists of all subjects in the FASI who had no major protocol violations as presented in Table 12.

Table 12: Criteria for Exclusion from the PPS and PPSI

Criteria for Exclusion	Probable Method of Identification
Not receiving both doses (TDV or placebo)	Identified programmatically using dosing data
Not receiving both doses in the correct interval (refer to windows defined in Table 9.h)	Identified programmatically using dosing data
Not receiving the assigned product (TDV or placebo)/dosing schedule (refers to correct administration of active or placebo on Day 1 or Day 90)	Identified after unblinding (eg, a subject who was randomized to TDV but received placebo, or randomized to placebo but received TDV)
Product preparation error	Identified through source documents and provided in blinded fashion to the statistician
Subject met any of Exclusion Criteria 2d, 3, 4 or 5 (subject to blinded medical review)	Subjects identified programmatically using CRF-recorded data. Subjects were identified before unblinding, and a blinded review list was sent for clinical review to determine evaluability status for each identified subject. Note that Exclusion Criteria 2d and 3 identify subjects' use of prohibited medications prior to enrollment
Use of prohibited medications/vaccines (subject to blinded medical review)	Potential prohibited medications were identified by sending a blinded review list of CRF-recorded medication data for clinical review to determine the evaluability status for each identified subject

Efficacy analysis

Primary Efficacy Endpoint

The primary efficacy endpoint in this trial was VE of 2 doses of TDV in preventing VCD fever induced by any dengue serotype occurring from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 1, with VE defined as 1 - (λV/λC) (where λV and λC denote the hazard rates for the TDV and placebo arms, respectively).

The primary analysis of VE was done after the 2 criteria for the end of Part 1 had been fulfilled, ie: (1) at least 120 cases of VCD fever had accrued, and (2) the minimum duration of subject follow-up was at least 12 months post-second vaccination.

The primary efficacy objective was considered to have been met if the lower bound of the 2-sided 95% CI for the VE was above 25%. The following hypotheses were tested in a confirmatory manner 2-sided at a significance level of 5%:

H0: $1 - \lambda V/\lambda C \leq 0.25$

H1: $1 - \lambda V/\lambda C > 0.25$

For the primary efficacy evaluation, a case of VCD fever was defined as febrile illness with a positive serotype-specific dengue detection RT-PCR (ie, positive dengue detection RT-PCR) and occurring at any time starting from 30 days post-second vaccination (Day 120 [M4]) through the end of Part 1.

The primary analysis was performed on the PPS (ie, for the primary analysis at the end of Part 1).

Vaccine efficacy and the 2-sided 95% CI were estimated using the Cox proportional-hazard model with trial vaccine group as a factor and age at baseline as a continuous covariate, with stratification by region. A p-value associated with the primary objective was also calculated.

Subjects who withdrew consent or were lost to follow-up were censored at the time of last contact. Cases of VCD fever reported in the dry-run period were not taken into account for censoring.

For the analyses based on the PPS and FAS, discontinued subjects were censored at the day of discontinuation.

Sensitivity analyses of the primary endpoint included:

1. Analysis using exact 95% CIs calculated as described by Breslow & Day.

2. Analysis based on the FAS.

3. Analysis in which cases of VCD fever were observed at any time post-second vaccination.

Subgroup analyses were performed based on the PPS in analogy to the primary analysis of the primary efficacy endpoint with a null hypothesis of $1 - \lambda V/\lambda C \leq 0$ and included analyses by (only the most relevant are listed here):Age group (4-5 years/6-11 years/12-16 years).; Region (Asia Pacific/Latin America); Baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes); Combination of age group and region; Combination of age group and baseline seropositivity status; Combination of baseline seropositivity status and region and Prior vaccination against Yellow Fever (YF) or Japanese encephalitis (JE).

Key Secondary Efficacy Endpoint

The key secondary efficacy endpoint in this trial is VE of two doses of TDV in preventing hospitalisation due to VCD fever induced by any dengue serotype occurring from 30 days post second vaccination (Day 120 [M4]) until the end of Part 2 (see Section 9.5.3.2).

A hierarchical testing strategy was used, and the key secondary efficacy endpoint was only to be tested in a confirmatory manner if statistical significance for the primary efficacy endpoint was achieved. Thus, the family wise Type I error rate was strongly controlled for the primary endpoint and the key secondary endpoint.

After demonstration of statistical significance for the primary endpoint, the following hypotheses were tested in a confirmatory manner 2-sided at a significance level of 5%:

H0: $1 - \lambda V/\lambda C \leq 0$

H1: 1 - λV/λC >0

The analysis of the key secondary efficacy endpoint was based on the PPS and assessed using data from Parts 1 and 2. The same analysis method as for the primary efficacy endpoint was used but the objective was considered to have been met if the lower bound of the 95% CI was >0. Subgroup analyses were performed for the key secondary efficacy endpoint in a similar fashion to the subgroup analyses for the primary endpoint.

Additional Secondary Efficacy Endpoints

Other secondary efficacy endpoints were analysed (including subgroup analyses) based on the PPS similar to the key secondary efficacy endpoint. The null hypotheses for these endpoints was 1 - λV/λC ≤0. Consistent with other multivalent vaccines, efficacy against individual serotypes was analysed without formal adjustment for multiplicity.

Severity of VCD fever was determined in two different ways, according to the independent DCAC and according to the WHO criteria (1997) for DHF, as described below.

Immunogenicity Analyses (Parts 1, 2, and 3)

Immunogenicity endpoints are summarised using descriptive statistics and 95% CIs by trial group and for each visit (post-first dose and post-second dose). (Note that for GMTs summary statistics were calculated based on log-transformed data.)

The primary immunogenicity analyses were performed using the PPSI. Supportive analyses are provided using the FASI.

Results

- **Participant flow**

Study Participant flow

The number of subjects is presented by region and country for the randomised set in Table 13 (all subjects, aged 4-5 years, aged 6-11 years, aged 12-16 years). Overall, the 2:1 ratio for TDV: placebo was achieved (13,401:6698) with this also being achieved in each age group and region as planned.

Table 13: Number of Subjects by Region, and Country (Randomised Set) (All Subjects, Age 4-5 Years, Age 6-11 Years, Age 12-16 Years)

			Number (%) of Subjects		
Age Group	Region	Country	Placebo (N = 6,698)	TDV (N = 13,401)	Total (N = 20,099)
All subjects	Asia Pacific		3000 (44.8)	6000 (44.8)	9000 (44.8)
		Philippines	1328 (19.8)	2599 (19.4)	3927 (19.5)
		Sri Lanka	703 (10.5)	1397 (10.4)	2100 (10.4)
		Thailand	969 (14.5)	2004 (15.0)	2973 (14.8)
	Latin America		3698 (55.2)	7401 (55.2)	11099 (55.2)
		Brazil	561 (8.4)	1213 (9.1)	1774 (8.8)
		Colombia	1322 (19.7)	2578 (19.2)	3900 (19.4)
		Dominican Republic	557 (8.3)	1043 (7.8)	1600 (8.0)
		Nicaragua	270 (4.0)	555 (4.1)	825 (4.1)
		Panama	988 (14.8)	2012 (15.0)	3000 (14.9)

			Number (%) of Subjects		
Age Group	Region	Country	Placebo (N = 6,698)	TDV (N = 13,401)	Total (N = 20,099)
4-5 years	Asia Pacific		474 (55.8)	948 (55.6)	1422 (55.7)
		Philippines	299 (35.2)	595 (34.9)	894 (35.0)
		Sri Lanka	74 (8.7)	136 (8.0)	210 (8.2)
		Thailand	101 (11.9)	217 (12.7)	318 (12.5)
	Latin America		375 (44.2)	757 (44.4)	1132 (44.3)
		Brazil	74 (8.7)	125 (7.3)	199 (7.8)
		Colombia	123 (14.5)	264 (15.5)	387 (15.2)
		Dominican Republic	48 (5.7)	113 (6.6)	161 (6.3)
		Nicaragua	32 (3.8)	53 (3.1)	85 (3.3)
		Panama	98 (11.5)	202 (11.8)	300 (11.7)
6-11 years	Asia Pacific		1496 (40.4)	2991 (40.4)	4487 (40.4)
		Philippines	487 (13.1)	973 (13.1)	1460 (13.1)
		Sri Lanka	435 (11.7)	834 (11.3)	1269 (11.4)
		Thailand	574 (15.5)	1184 (16.0)	1758 (15.8)
	Latin America		2208 (59.6)	4412 (59.6)	6620 (59.6)
		Brazil	325 (8.8)	714 (9.6)	1039 (9.4)
		Colombia	803 (21.7)	1534 (20.7)	2337 (21.0)
		Dominican Republic	341 (9.2)	617 (8.3)	958 (8.6)
		Nicaragua	154 (4.2)	334 (4.5)	488 (4.4)
		Panama	585 (15.8)	1213 (16.4)	1798 (16.2)
12-16 years	Asia Pacific		1030 (48.0)	2061 (48.0)	3091 (48.0)
		Philippines	542 (25.3)	1031 (24.0)	1573 (24.4)
		Sri Lanka	194 (9.0)	427 (9.9)	621 (9.6)
		Thailand	294 (13.7)	603 (14.0)	897 (13.9)
	Latin America		1115 (52.0)	2232 (52.0)	3347 (52.0)
		Brazil	162 (7.6)	374 (8.7)	536 (8.3)
		Colombia	396 (18.5)	780 (18.2)	1176 (18.3)
		Dominican Republic	168 (7.8)	313 (7.3)	481 (7.5)
		Nicaragua	84 (3.9)	168 (3.9)	252 (3.9)
		Panama	305 (14.2)	597 (13.9)	902 (14.0)

Subject disposition to the end of Part 2 is presented graphically for placebo and TDV for the efficacy and immunogenicity analyses in Figure 6.

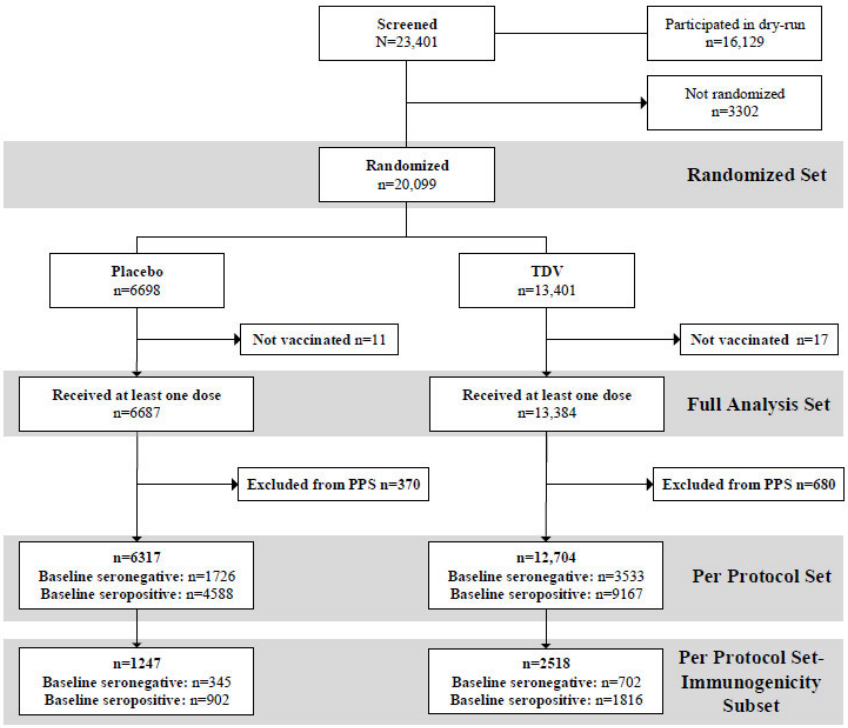


Figure 6: Disposition of Subjects (end of Part 2) for the Efficacy and Immunogenicity Analyses (All Subjects)

Of the 20,099 randomised subjects, 20,071 subjects received at least one vaccination. Of these subjects, 98.4% received 2 vaccinations in Part 1. Overall, 97.4%, 96.9%, and 96.3% of subjects completed Part 1, Part 2, and until 12 months after end of Part 1, respectively, with no important difference in premature discontinuations between placebo and TDV.

The reasons for discontinuation of subjects before receiving the second vaccination were mainly withdrawal of consent by either the subject or a parent(s)/guardian(s) (83.0% and 78.9% for placebo and TDV). Similarly, in Part 1 overall the most frequent reason for discontinuation was withdrawal of consent by either the subject or a parent(s)/guardian(s) (72.6% and 70.6% for placebo and TDV).

For Part 2, the most frequent reason for discontinuation was also withdrawal of consent by either the subject or a parent(s)/guardian(s) (37.5% and 45.8% for placebo and TDV) but there was an increased proportion of withdrawals due to pregnancy (34.4% and 23.6% for placebo and TDV).

A total of 6573 (placebo) and 13,164 (TDV) subjects received two vaccinations, 6,519 (placebo), 13,033 (TDV) subjects completed Part 1, and 6,487 (placebo) and 12,961 (TDV) subjects completed Part 2.

Overall, significant protocol deviations were reported for 7.4% of subjects, with most occurring in Part 1 (overall 6.8% of subjects at the end of Part 1 vs 7.4% at the end of Part 2 and 2.6% at 12 months after end of Part 1) and there was no difference between placebo and TDV.

The most common significant protocol deviations that occurred at an incidence of >1% were study procedures and assessments, which occurred in 5.1% of subjects overall to the end of Part 1, 5.7% of subjects overall to the end of Part 2, and 1.1% of subjects overall to 12 months after end of Part 1. There was no difference between placebo and TDV, however these occurred more frequently in Latin America. The only other significant protocol deviation that occurred at an incidence of >1% in Part 1 and Part 2 was "other GCP deviation" (1.1% of placebo subjects in Asia Pacific in Part 1 and Part 2). These were most commonly temperature excursions (excursions in IP storage temperatures), data

protection deviations (inadvertently recording personal data during SAE reporting), and deviations in safety reporting (not reporting SAEs within the required timelines).

- **Recruitment**

Trial initiation date (date first subject signed informed consent form): 26 April 2016.

Date of last subject's last visit/contact (Part 2)*: 07 January 2019

Date of last subject's last visit/contact (12 months after end of Part 1)*: 11 July 2019

Date of last subject's last visit/contact (18 months after end of Part 2)*: 8 July 2020

Date of last subject's last visit/contact (end of Part 3)*: 11 January 2022

Data lock point: 15 November 2018 (Part 1), 1 April 2019 (Part 2), 25 October 2019

(12 months after end of Part 1), and 5 October 2020 (18 months after end of Part 2),

21 March 2022 (end of Part 3)

*Date refers to the latest cut-off date used for the interim analysis

- **Conduct of the study**

The original protocol had 4 amendments, which are summarised below.

There have been in total four protocol amendment 1. The first one was implemented before trial initiation and regarding the other three, the protocol Amendment 4 (dated 18 May 2020) was the only with a substantial amendment: Modification of the trial design to include a booster vaccination in a subgroup of subjects aged 4-11 years at trial entry. As detailed in the Clinical trial protocol the rationale is the following. This protocol amendment is due to the fact that, although a generally consistent overall efficacy and continued protection against hospitalised dengue is observed after a 2-dose vaccination regimen with TDV (2 single doses 3 months apart), data also indicate a trend towards a reduction in overall vaccine efficacy in the second year after vaccination. This observation suggests a waning in vaccine efficacy and therefore the present ongoing phase III trial will evaluate the effect of a booster dose. Due to the absence of a correlate of protection in dengue, the booster effect can only be demonstrated by efficacy data. Adding a booster phase to the present ongoing DEN-301 trial gives the opportunity to administer a booster dose of TDV to assess vaccine efficacy against virologically confirmed dengue.

- **Baseline data**

Overall demographic and baseline characteristics for the PPS are presented in Table 14.

Table 14: Summary of Demographic and Baseline Characteristics (Per-Protocol Set)

	Placebo (N = 6317)	TDV (N = 12,704)	Total (N = 19,021)
Age (years), mean (SD)	9.6 (3.34)	9.6 (3.35)	9.6 (3.35)
Age categories (n[%])			
4-5 years	801 (12.7)	1620 (12.8)	2421 (12.7)
6-11 years	3492 (55.3)	7010 (55.2)	10,502 (55.2)
12-16 years	2024 (32.0)	4074 (32.1)	6098 (32.1)
Gender (n[%])			
Male	3219 (51.0)	6390 (50.3)	9609 (50.5)
Female	3098 (49.0)	6314 (49.7)	9412 (49.5)
Race (n[%])			
American Indian or Alaska Native [a]	2378 (37.6)	4819 (37.9)	7197 (37.8)
Asian	2934 (46.4)	5888 (46.3)	8822 (46.4)
Black or African American	706 (11.2)	1351 (10.6)	2057 (10.8)
Native Hawaiian/Other Pacific Islander	1 (<0.1)	2 (<0.1)	3 (<0.1)
White	131 (2.1)	284 (2.2)	415 (2.2)
Multiracial	165 (2.6)	360 (2.8)	525 (2.8)
Missing	2 (<0.1)	0	2 (<0.1)
Country (n[%])			
Brazil	504 (8.0)	1091 (8.6)	1595 (8.4)
Colombia	1155 (18.3)	2268 (17.9)	3423 (18.0)
Dominican Republic	533 (8.4)	1007 (7.9)	1540 (8.1)
Nicaragua	239 (3.8)	512 (4.0)	751 (3.9)
Panama	944 (14.9)	1930 (15.2)	2874 (15.1)
Philippines	1306 (20.7)	2554 (20.1)	3860 (20.3)
Sri Lanka	683 (10.8)	1368 (10.8)	2051 (10.8)
Thailand	953 (15.1)	1974 (15.5)	2927 (15.4)
Region (n[%])			
Asia Pacific	2942 (46.6)	5896 (46.4)	8838 (46.5)
Latin America	3375 (53.4)	6808 (53.6)	10,183 (53.5)
BMI (kg/m^2)			
n	6317	12,696	19,013
Mean (SD)	17.67 (3.644)	17.76 (3.831)	17.73 (3.770)
Median	16.70	16.80	16.80
Min, max	8.8, 42.1	8.5, 64.8	8.5, 64.8

Source: Section 15, Table 15.1.1.8.1.3.

(a) Due to limited check-box options for "Race" on the eCRF, investigators mostly chose "American Indian/ Alaskan Native" to describe the race of subjects in Latin America.

Baseline Dengue Serostatus: Baseline seropositivity was defined as seropositive (reciprocal neutralising titre ≥10) for at least 1 dengue serotype or seronegative (reciprocal neutralising titre <10 for all dengue serotypes). Baseline dengue serostatus data are summarised for the PPS in Table 15 (all subjects), Table 16 (by region and country), and Table 17 (by age group).

Table 15: Summary of Baseline Seropositivity Status (Per Protocol Set) (All Subjects)

	Placebo (N = 6317)	TDV [a] (N = 12,704)	Total (N = 19,021)
Baseline seropositivity status			
Number evaluated	6314	12,700	19,014
Seropositive	4588 (72.7)	9167 (72.2)	13,755 (72.3)
Seronegative	1726 (27.3)	3533 (27.8)	5259 (27.7)
Baseline seropositivity rate to each serotype			
DENV-1: Number evaluated	6314	12,700	19,014
n(%)	4103 (65.0)	8248 (64.9)	12,351 (65.0)
DENV-2: Number evaluated	6314	12,698	19,012
n(%)	4452 (70.5)	8919 (70.2)	13,371 (70.3)
DENV-3: Number evaluated	6314	12,698	19,012
n(%)	3953 (62.6)	7984 (62.9)	11,937 (62.8)
DENV-4: Number evaluated	6314	12,700	19,014
n(%)	4002 (63.4)	8086 (63.7)	12,088 (63.6)
Monovalent			
Number evaluated	6314	12,700	19,014
n(%)	414 (6.6)	783 (6.2)	1197 (6.3)
Bivalent			
Number evaluated	6314	12,700	19,014
n(%)	210 (3.3)	372 (2.9)	582 (3.1)
Trivalent			
Number evaluated	6314	12,698	19,012
n(%)	180 (2.9)	338 (2.7)	518 (2.7)
Tetravalent			
Number evaluated	6314	12,698	19,012
n(%)	3784 (59.9)	7674 (60.4)	11,458 (60.3)
At least bivalent			
Number evaluated	6314	12,700	19,014
n(%)	4174 (66.1)	8384 (66.0)	12,558 (66.0)
At least trivalent			
Number evaluated	6314	12,698	19,012
n(%)	3964 (62.8)	8012 (63.1)	11,976 (63.0)

Source: Section 15, Table 15.1.1.9.2.
(a) For some samples no result was reported for DENV-2 and DENV-3 but reported for DENV-1 and DENV-4.

Overall, 27.7% of subjects were seronegative (ie, seronegative against all 4 dengue serotypes) and 72.3% seropositive at baseline, with similar proportions per trial group.

Table 16: Summary of Baseline Seropositivity Status (Per-protocol Set) (by Region and Country)

Region / Country		Placebo (N = 6317)	TDV (N = 12,704)	Total (N = 19,021)
Asia Pacific	Number of subjects	2942	5896	8838
	Number evaluated	2940	5895	8835
	Seropositive	2167 (73.7)	4392 (74.5)	6559 (74.2)
	Seronegative	773 (26.3)	1503 (25.5)	2276 (25.8)
Philippines	Number evaluated	1305	2553	3858
	Seropositive	1145 (87.7)	2233 (87.5)	3378 (87.6)
	Seronegative	160 (12.3)	320 (12.5)	480 (12.4)
Sri Lanka	Number evaluated	682	1368	2050
	Seropositive	420 (61.6)	841 (61.5)	1261 (61.5)
	Seronegative	262 (38.4)	527 (38.5)	789 (38.5)
Thailand	Number evaluated	953	1974	2927
	Seropositive	602 (63.2)	1318 (66.8)	1920 (65.6)
	Seronegative	351 (36.8)	656 (33.2)	1007 (34.4)
Latin America	Number of subjects	3375	6808	10,183
	Number evaluated	3374	6805	10,179
	Seropositive	2421 (71.8)	4775 (70.2)	7196 (70.7)
	Seronegative	953 (28.2)	2030 (29.8)	2983 (29.3)
Brazil	Number evaluated	504	1091	1595
	Seropositive	367 (72.8)	769 (70.5)	1136 (71.2)
	Seronegative	137 (27.2)	322 (29.5)	459 (28.8)
Colombia	Number evaluated	1155	2268	3423
	Seropositive	979 (84.8)	1917 (84.5)	2896 (84.6)
	Seronegative	176 (15.2)	351 (15.5)	527 (15.4)
Dominican Republic	Number evaluated	533	1006	1539
	Seropositive	515 (96.6)	981 (97.5)	1496 (97.2)
	Seronegative	18 (3.4)	25 (2.5)	43 (2.8)
Nicaragua	Number evaluated	239	511	750
	Seropositive	180 (75.3)	403 (78.9)	583 (77.7)
	Seronegative	59 (24.7)	108 (21.1)	167 (22.3)
Panama	Number evaluated	943	1929	2872
	Seropositive	380 (40.3)	705 (36.5)	1085 (37.8)
	Seronegative	563 (59.7)	122 (63.5)	1787 (62.2)

As expected, the proportion of baseline seropositive subjects was lower in subjects aged 4 to 5 years (58.7%) compared with those aged 6 to 11 years (68.9%) or 12 to 16 years (83.7%). The overall baseline serostatus was similar for subjects in Asia Pacific (74.2% seropositive, 25.8% seronegative) and Latin America (70.7% seropositive, 29.3% seronegative).

Table 17: Summary of Baseline Seropositivity Status (Per-Protocol Set) (By Age Group)

Age Group		Placebo (N = 6317)	TDV (N = 12,704)	Total (N = 19,021)
4-5 years	Number evaluated	801	1620	2421
	Seropositive	464 (57.9)	957 (59.1)	1421 (58.7)
	Seronegative	337 (42.1)	663 (40.9)	1000 (41.3)
6-11 years	Number evaluated	3489	7007	10,496
	Seropositive	2424 (69.5)	4806 (68.6)	7230 (68.9)
	Seronegative	1065 (30.5)	2201 (31.4)	3266 (31.1)
12-16 years	Number evaluated	2024	4073	6097
	Seropositive	1700 (84.0)	3404 (83.6)	5104 (83.7)
	Seronegative	324 (16.0)	669 (16.4)	993 (16.3)

The history of prior vaccinations against JE or YF also varied by region. Prior vaccination against JE only occurred in the Asia Pacific region (53.2% of subjects), primarily in Sri Lanka (95.6%) and Thailand (91.9%) where subjects are routinely vaccinated against JE; only 1.4% of subjects in The Philippines were vaccinated against JE. Prior vaccination against YF only occurred in Latin America (38.2% of subjects overall; in Colombia [81.7%], Brazil [49.1%], and Panama [6.5%]), with the exception of 1 subject in Sri Lanka.

- **Numbers analysed**

The number of subjects included in the Dry-Run Set, Randomised Set, SS, FAS, FASI, PPS, and PPSI are summarised in Table 18.

Table 18: Analysis Sets Overall and by Region

Region		Number (%) of Subjects		
		Placebo	TDV	Total
Overall	All screened			23,401
	Dry-run set			16,129
	Randomized set	6698	13401	20,099
	Immunogenicity subset	1333 (19.9)	2667 (19.9)	4000 (19.9)
	Safety set	6687 (99.8)	13380 (99.8)	20,071 (99.9)
	Safety set-immunogenicity subset	1329 (99.7)	2663 (99.9)	3993 (99.8)
	Full analysis set	6687 (99.8)	13384 (99.9)	20,071 (99.9)
	Full analysis set for immunogenicity	1319 (98.9)	2635 (98.8)	3954 (98.9)
	Per-protocol set	6317 (94.3)	12704 (94.8)	19,021 (94.6)
	Per-protocol set for immunogenicity	1247 (93.5)	2518 (94.4)	3765 (94.1)
Asia Pacific	All screened			10,436
	Dry-run set			7153
	Randomized set	3000	6000	9000
	Immunogenicity subset	595 (19.8)	1191 (19.9)	1786 (19.8)
	Safety set	2993 (99.8)	5996 (>99.9)	8991 (>99.9)
	Safety set-immunogenicity subset	592 (99.5)	1191 (100.0)	1783 (99.8)
	Full analysis set	2994 (99.8)	5997 (>99.9)	8991 (>99.9)
	Full analysis set for immunogenicity	589 (99.0)	1188 (99.7)	1777 (99.5)
	Per-protocol set	2942 (98.1)	5896 (98.3)	8838 (98.2)
	Per-protocol set for immunogenicity	580 (97.5)	1173 (98.5)	1753 (98.2)
Latin America	All screened			12,965
	Dry-run set			8976
	Randomized set	3698	7401	11,099
	Immunogenicity subset	738 (20.0)	1476 (19.9)	2214 (19.9)
	Safety set	3694 (99.9)	7384 (99.8)	11,080 (99.8)
	Safety set-immunogenicity subset	737 (99.9)	1472 (99.7)	2210 (99.8)
	Full analysis set	3693 (99.9)	7387 (99.8)	11,080 (99.8)
	Full analysis set for immunogenicity	730 (98.9)	1447 (98.0)	2177 (98.3)
	Per-protocol set	3375 (91.3)	6808 (92.0)	10,183 (91.7)
	Per-protocol set for immunogenicity	667 (90.4)	1345 (91.1)	2012 (90.9)

Source: Section 15, Table 15.1.1.7.
Four subjects received both placebo and TDV vaccinations in error. For the SS, the sum of counts in placebo and TDV columns may not add up to the counts in the Total column since these subjects are included only in the Total column.

Of 23,401 screened subjects, 16,129 subjects were included in the dry-run and 20,099 subjects were randomised, of whom 20,071 subjects (99.9%) were included in the FAS and SS and 19,021 subjects (94.6%) were included in the PPS. Overall, 4000 subjects (19.9%) were included in the immunogenicity subset as planned, and of these, 3993 subjects (99.8%), 3954 subjects (98.9%), and 3765 subjects (94.1%) were included in the subset, FASI, PPSI, respectively.

The trends for Asia Pacific and Latin America were similar to the overall group.

Surveillance performance metrics

It follows a description of the surveillance performance metrics, according to region and country, for the dry run, Part 1 and part 2. Data from the first vaccination to the end of Part 2 are presented by region and country in Table 19.

Table 19: Surveillance Performance Metrics by Region, and Country From First Vaccination to End of Part 2 (safety Set)

Region	Country	Country	Placebo (N = 6687) n (%)	TDV (N = 13,380) n (%)	Total (N = 20,071) n (%)
Overall	Overall	Febrile illness cases	4884	8991	13,881
		Acute samples taken	4810 (98.5)	8841 (98.3)	13,657 (98.4)
		Acute samples taken within 5 days	4580 (93.8)	8407 (93.5)	12,993 (93.6)
Asia Pacific	Overall	Febrile illness cases	2616	4782	7402
		Acute samples taken	2606 (99.6)	4756 (99.5)	7366 (99.5)
		Acute samples taken within 5 days	2581 (98.7)	4707 (98.4)	7292 (98.5)
	Philippines	Febrile illness cases	1497	2713	4210
		Acute samples taken	1496 (>99.9)	2710 (99.9)	4206 (>99.9)
		Acute samples taken within 5 days	1489 (99.5)	2694 (99.3)	4183 (99.4)
	Sri Lanka	Febrile illness cases	441	704	1149
		Acute samples taken	439 (99.5)	700 (99.4)	1143 (99.5)
		Acute samples taken within 5 days	427 (96.8)	678 (96.3)	1109 (96.5)
	Thailand	Febrile illness cases	678	1365	2043
		Acute samples taken	671 (99.0)	1346 (98.6)	2017 (98.7)
		Acute samples taken within 5 days	665 (98.1)	1335 (97.8)	2000 (97.9)
Latin America		Febrile illness cases	2268	4209	6479
		Acute samples taken	2204 (97.2)	4085 (97.1)	6291 (97.1)
		Acute samples taken within 5 days	1999 (88.1)	3700 (87.9)	5701 (88.0)
	Brazil	Febrile illness cases	310	584	895
		Acute samples taken	308 (99.4)	577 (98.8)	886 (99.0)
		Acute samples taken within 5 days	282 (91.0)	520 (89.0)	803 (89.7)
	Colombia	Febrile illness cases	1003	1856	2860
		Acute samples taken	977 (97.4)	1816 (97.8)	2794 (97.7)
		Acute samples taken within 5 days	883 (88.0)	1662 (89.5)	2546 (89.0)
	Dominican Republic	Febrile illness cases	500	954	1454
		Acute samples taken	479 (95.8)	910 (95.4)	1389 (95.5)
		Acute samples taken within 5 days	424 (84.8)	788 (82.6)	1212 (83.4)
	Nicaragua	Febrile illness cases	80	153	233
		Acute samples taken	79 (98.8)	149 (97.4)	228 (97.9)
		Acute samples taken within 5 days	77 (96.3)	143 (93.5)	220 (94.4)
	Panama	Febrile illness cases	375	662	1037
		Acute samples taken	361 (96.3)	633 (95.6)	994 (95.9)
		Acute samples taken within 5 days	333 (88.8)	587 (88.7)	920 (88.7)

Similar results were obtained for the PP set when the period analysed was 12 Months After End of Part 1 (data not shown).

- **Outcomes and estimation**

Efficacy

Virologically Confirmed Dengue Case and Serotype Distribution

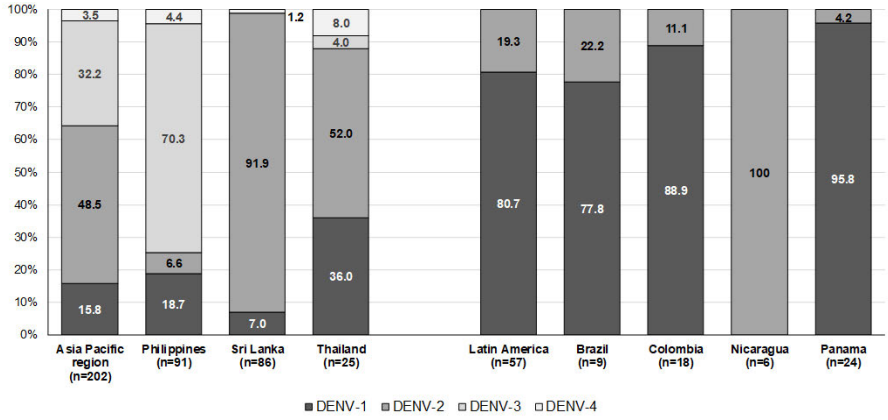
Incidence rates and the dengue serotype distribution for VCD fever from first dose to 18 months post second vaccine dose (Parts 1 and 2 combined) varied by region and country, as shown in Table 20 for the safety set.

Table 20: Trial DEN-301: Serotype Distribution by Country for Virologically Confirmed Dengue From First Vaccine Dose to 18 Months Post Second Vaccine Dose (Until End of Part 2) (Safety Set)

	Number (%) of VCD cases							
		Asia Pacific Region				Latin America [a]		
	Overall	Philippines	Sri Lanka	Thailand	Brazil	Colombia	Nicaragua	Panama
Total VCD	390	177	96	34	12	29	9	33
Placebo								
VCD	259	91	86	25	9	18	6	24
DENV-1	78 (30.1)	17 (18.7)	6 (7.0)	9 (36.0)	7 (77.8)	16 (88.9)	0	23 (95.8)
DENV-2	109 (42.1)	6 (6.6)	79 (91.9)	13 (52.0)	2 (22.2)	2 (11.1)	6 (100.0)	1 (4.2)
DENV-3	65 (25.1)	64 (70.3)	0	1 (4.0)	0	0	0	0
DENV-4	7 (2.7)	4 (4.4)	1 (1.2)	2 (8.0)	0	0	0	0
TDV								
VCD	131	86	10	9	3	11	3	9
DENV-1	41 (31.3)	10 (11.6)	3 (30.0)	6 (66.7)	3 (100.0)	10 (90.9)	0	9 (100.0)
DENV-2	14 (10.7)	4 (4.7)	6 (60.0)	1 (11.1)	0	0	3 (100.0)	0
DENV-3	69 (52.7)	67 (77.9)	1 (10.0)	1 (11.1)	0	0	0	0
DENV-4	7 (5.3)	5 (5.8)	0	1 (11.1)	0	1 (9.1)	0	0

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.g, Pt2 Table 15.2.2.32.1, and Pt2 Table 15.2.2.32.2.
Abbreviations: VCD, virologically confirmed dengue.
(a) There were no VCD fever cases in The Dominican Republic.

The serotype distribution for VCD fever cases in the placebo group, ie, unaffected by active vaccine administration, from the first placebo dose to 18 months post second placebo dose is depicted in Figure 7, showing the varying distribution of serotypes in the participating countries.



Source: Module 5.3.5.1, DEN-301 CSR, Table 11.f and Table 11.g.
Abbreviations: n, total number of VCD cases in the region or country; VCD, virologically confirmed dengue.
Numbers in bars show the percentage among all cases in that region or country. There were no VCD fever cases in The Dominican Republic.

Figure 7: Trial DEN-301: Serotype Distribution of Virologically Confirmed Dengue Cases in the Placebo Group From First Placebo Dose to 18 Months Post Second Placebo Dose (Until End of Part 2), by Region and Country (Safety Set)

Primary Efficacy Analysis

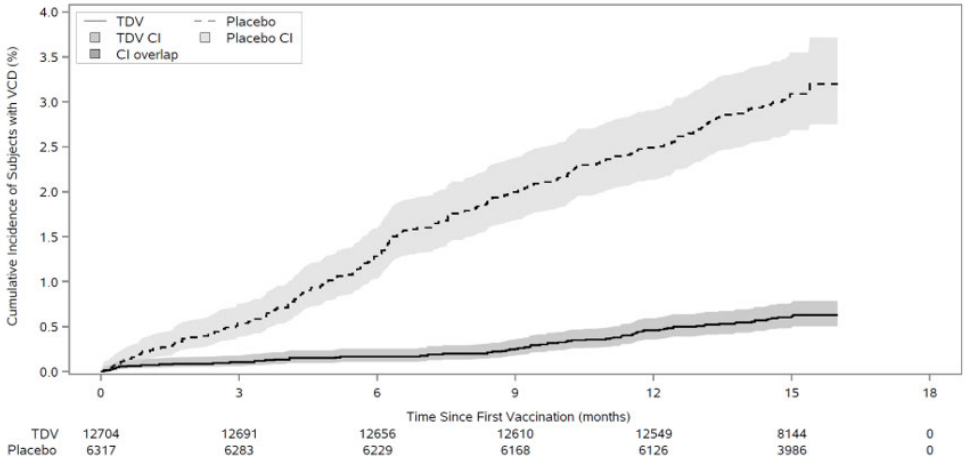
The primary objective to show efficacy in the prevention of VCD fever caused by any serotype (ie, all 4 serotypes combined) from 30 days to 12 months post second vaccine dose (until the end of Part 1; primary endpoint) was met. Rates of VCD fever were 2.4% in the placebo group and 0.5% in the TDV group, with an associated VE of 80.2% (95% CI: 73.3%, 85.3%; p<0.001) (see Table 21). The lower bound of the 2-sided 95% CI for the VE was above 25%, and thus the criterion to meet the primary objective was fulfilled. As shown in the placebo group, all 4 dengue serotypes contributed to the primary efficacy result.

Table 21: Trial DEN-301: Vaccine Efficacy in Preventing Virologically Confirmed Dengue Fever From 30 Days to 12 Months Post Second Vaccine Dose (Primary Endpoint) (Per-Protocol Set)

Statistic	Placebo N = 6317	TDV N = 12,704
Number of subjects evaluated [a]	6316	12,700
Number of subjects with febrile illness (%) [b]	1712 (27.1)	3195 (25.2)
Number of febrile illness cases (episodes) [c]	2591	4692
VCD fever, n (%)	149 (2.4)	61 (0.5)
DENV-1	30 (0.5)	16 (0.1)
DENV-2	64 (1.0)	3 (<0.1)
DENV-3	51 (0.8)	39 (0.3)
DENV-4	4 (<0.1)	3 (<0.1)
Person-years at risk	5671.1	11,586.0
Incidence density [d]	2.6	0.5
Relative risk (95% CI)	0.20 (0.15, 0.27)	
Vaccine efficacy (95% CI) (%)	80.2 (73.3, 85.3)	
p-value	<0.001	

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.j and Table 11.u.
Abbreviations: CI, confidence interval; n, number of subjects with fever; N, total number of subjects; VCD, virologically confirmed dengue.
(a) Five subjects (1 in the placebo group, 4 in the TDV group) were not evaluable for analysis because they discontinued between the second vaccine dose and 30 days post second dose.
(b) Percentages were hand-calculated.
(c) Some subjects had more than 1 case (episode) of febrile illness.
(d) Number of VCD fever cases per 100 person-years.

The cumulative incidence of VCD fever and associated 95% CIs are presented over time during Part 1 (up to 12 months post second vaccine dose) in Figure 8, showing a steady increase in the cumulative incidence in both groups, with a steeper slope in the placebo group and the separation between curves starting during the first month.



Source: Module 5.3.5.1, Supplemental Analysis Table and Figures, Figure 2.c.
Abbreviations: CI, (95%) confidence interval; VCD, virologically confirmed dengue.
The number of subjects at risk per trial group and time point are shown below the graph.

Figure 8: Trial DEN-301: Cumulative Incidence of Virologically Confirmed Dengue Fever Over Time Until 12 Months Post Second Vaccine Dose (End of Part 1; Month 15) (Per-Protocol Set)

Three sensitivity analyses of the primary endpoint were performed (analysis using exact 95% CIs calculated as described by Breslow & Day on the PPS; analysis based on the FAS, and analysis in which cases of VCD fever were observed at any time post-second vaccination). The results are shown in the next three tables.

Table 15.2.1.1.2
Vaccine Efficacy of TDV in Preventing VCD Fever from 30 Days Post-Second Vaccination until end of Part 1
– Sensitivity Analysis 1: Exact 95% CI
Per-Protocol Set

	Placebo (N=6,317)	TDV (N=12,704)
Number of Subjects Evaluated	6316	12700
Number of Subjects with Febrile Illness	1712	3195
Number of Febrile Illness Cases	2591	4692
Virologically-Confirmed Dengue Fever (n[%])	149 (2.4)	61 (0.5)
Person-Years at Risk	5671.1	11586.0
Incidence Density	2.6	0.5
Relative Risk	0.20	
95% CI	(0.15, 0.28)	
Vaccine Efficacy (%)	79.6	
Exact 95% CI	(72.4, 85.1)	

Table 15.2.1.1.3
Vaccine Efficacy of TDV in Preventing VCD Fever from 30 Days Post-Second Vaccination until end of Part 1
– Sensitivity Analysis 2
Full Analysis Set

	Placebo (N=6,687)	TDV (N=13,384)
Number of Subjects Evaluated	6573	13163
Number of Subjects with Febrile Illness	1768	3287
Number of Febrile Illness Cases	2667	4811
Virologically-Confirmed Dengue Fever (n[%])	152 (2.3)	61 (0.5)
Person-Years at Risk	5901.6	12006.4
Incidence Density	2.6	0.5
Relative Risk	0.20	
95% CI	(0.15, 0.27)	
Vaccine Efficacy (%)	80.6	
95% CI	(73.9, 85.6)	

Table 15.2.1.1.4
Vaccine Efficacy of TDV in Preventing VCD Fever from Second Vaccination until end of Part 1
– Sensitivity Analysis 3
Per-Protocol Set

	Placebo (N=6,317)	TDV (N=12,704)
Number of Subjects Evaluated	6317	12704
Number of Subjects with Febrile Illness	1845	3440
Number of Febrile Illness Cases	2847	5211
Virologically-Confirmed Dengue Fever (n[%])	160 (2.5)	65 (0.5)
Person-Years at Risk	6186.7	12628.1
Incidence Density	2.6	0.5
Relative Risk	0.20	
95% CI	(0.15, 0.27)	
Vaccine Efficacy (%)	80.3	
95% CI	(73.7, 85.2)	

Secondary Efficacy Analyses

Hospitalisation due to Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose (Key Secondary Endpoint)

After successfully meeting the primary objective of the trial, the VE of TDV in the prevention of hospitalisation due to VCD fever from 30 days to 18 months post second vaccine dose (until the end of Part 2), the key secondary endpoint of the trial, was tested. The rates of hospitalisation due to VCD fever were 1.0% in the placebo group and 0.1% in the TDV group, with an associated VE against hospitalisation due to VCD fever of 90.4% (95% CI: 82.6%, 94.7%; p<0.001). The lower bound of the 2-sided 95% CI for the VE was above 0%, and thus the criterion to meet this key secondary objective was fulfilled.

Table 22: Trial DEN-301: Vaccine Efficacy in Preventing Hospitalisation Due to Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose (Key Secondary Endpoint) (Per-Protocol Set)

Statistic	Placebo N = 6317	TDV N = 12,704
Number of subjects evaluated [a]	6316	12,700
Number of subjects hospitalized due to febrile illness (%) [b]	140 (2.2)	146 (1.1)
Number of febrile illness cases (episodes) leading to hospitalization [c]	148	159
Hospitalization due to VCD fever, n (%)	66 (1.0)	13 (0.1)
Person-years at risk	8708.3	17,721.6
Incidence density [d]	0.8	<0.1
Relative risk (95% CI)	0.10 (0.05, 0.18)	
Vaccine efficacy (95% CI) (%)	90.4 (82.6, 94.7)	
p-value	<0.001	

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.k.

Abbreviations: CI, confidence interval; n, number of subjects with fever; N, total number of subjects; VCD, virologically confirmed dengue.

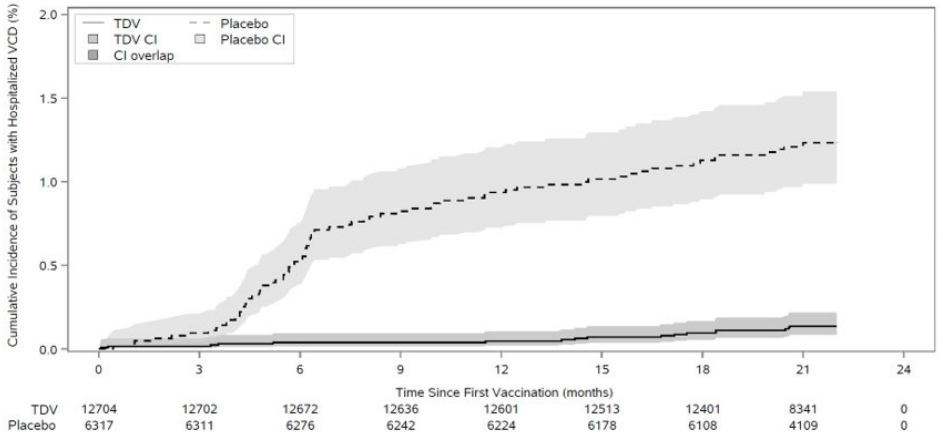
(a) Five subjects (1 in the placebo group, 4 in the TDV group) were not evaluable for analysis because they discontinued between the second vaccine dose and 30 days post second dose.

(b) Percentages were hand-calculated.

(c) Some subjects had more than 1 case (episode) of febrile illness.

(d) Number of VCD fever cases per 100 person-years.

The cumulative incidence of hospitalised VCD fever and associated 95% CIs are presented over time in Figure 9 showing a separation of curves after Month 1, with a steeper increase in the placebo group, in particular from Month 3 to Month 6, which was due to a rapid acquisition of cases during an outbreak of DENV-2 in Sri Lanka in 2017.



Source: Module 5.3.5.1, Supplemental Analysis Table and Figures, Figure 2.d.

Abbreviations: CI, (95%) confidence interval; VCD, virologically confirmed dengue.

The number of subjects at risk per trial group and time point are shown below the graph.

Figure 9: Trial DEN-301: Cumulative Incidence of Virologically Confirmed Dengue Fever Leading to Hospitalisation Over Time Until 18 Months Post Second Vaccine Dose (End of Part 2; Month 21) (Per-Protocol Set)

To help interpreting the VE data against hospitalised VCD, the following tables have been prepared to describe useful information. They describe the number of hospitalised cases observed in the different countries (and also according to each dengue serotype) for the placebo group for the time period 30 days post second dose until end of Part 2. The tables also include the "Percent of hospitalised VCD Cases Among VCD Cases".

Table 23: Hospitalised VCD (from 30 Days post-second vaccination to end Part 2)- Subgroup analysis per country.

Taken from TABLE 15.2.2.32.4				
Serotype Distribution in VCD, Hospitalized VCD, from 30 Days Post-Second Vaccination to end of Part 2 - Subgroup Analysis by Country. Per-Protocol Set				
	PLACEBO (% of cases vs total)	TDV	TOTAL (Placebo +TDV)	Percent of Hospitalized VCD Cases Among VCD cases
(VCD) (Number of Cases)				
Philippines	77	75	152	
Sri Lanka	61 (30%)	5	66	
Thailand	23	9	32	
Brazil	8	3	11	
Colombia	17	10	27	
Dominican Republic	0	0	0	
Nicaragua	5	3	8	
Panama	16	9	25	
TOTAL	207	114	321	
Hospitalized VCD Number of Cases				
Philippines	6 (9)	8	14	7.8
Sri Lanka	44 (66.6)	1	45	72.1
Thailand	11 (16.6)	3	14	47.8
Brazil	1 (1.5)	0	1	12.5
Colombia	4 (6)	1	5	23.5
Dominican Republic	0	0	0	--
Nicaragua	0	0	0	0
Panama	0	0	0	0
TOTAL	66	13	79	

Table 24: Hospitalised VCD (from 30 Days post-2nd vaccination to end Part 2)- Subgroup analysis per serotype and by country

Taken from TABLE 15.2.2.32.4						
Serotype Distribution in Hospitalized VCD, from 30 Days Post-Second Vaccination to end of Part 2 – Placebo-Subgroup Analysis by serotype and Country. Per-Protocol Set						
Serotypes	Philippines	Sri Lank(% total Hosp cases)	Thailand	Brazil	Colombia	Total (Percent of Hospitalized per serotype)
DENV-1	1	3	5	1	3	13 (19)
DENV-2	0	41 (62%)	4	0	1	46 (70)
DENV-3	5	0	1	0	0	6 (9)
DENV-4	0	0	1	0	0	1 (1.5)

The country with more hospitalised VCD cases was Sri Lanka (44 out 66 cases in the placebo group) followed by Thailand with 11 cases. When examining the "Percent of hospitalised VCD Cases Among VCD Cases", the country with the highest percentage was Sri Lanka (72.1%) again followed by Thailand (47.8%). It is noted that 41 out of the 44 cases in Sri Lanka were due to DENV-2, whereas in

Thailand (5, 4, 1 and 1 cases were due to DENV-1, DENV-2, DENV-3 and DENV4, respectively). Thus, the main contributors to the estimate of VE against hospitalised VCD were cases in Sri Lanka due to an outbreak of DENV-2.

The following table describes vaccine efficacy against hospitalisation due to VCD from 30 days post second dose to the end of part 2, per dengue serotype and according to the baseline serostatus.

Table 25: VE of TDV against hospitalisation due to VCD (from 30 days post 2nd dose to the end of part 2) per dengue serotype and by baseline serostatus

Taken from 15.2.2.11.1 and 15.2.2.11.2				
Vaccine efficay of TVD in preventing hospitalizacion due to VCD fever from 13 days post second vaccination until end of part 2. (analysis by Dengue Serotype and Serostatus)				
		TDV	**PLACEBO**	**V.E.**
DEN-1	**TOTAL**	6	13	77,3 (40;91)
	SERO +	4	9	78,2 (29;93)
	SER0 -	2	4	75,1 (-35;95)
DEN-2	**TOTAL**	0	46	100 (-:-)
	SERO +	0	30	100 (-:-)
	SER0 -	0	16	100 (-:-)
DEN-3	**TOTAL**	7	6	42,9 (-69;80)
	SERO +	4	5	61 (-43;89)
	SER0 -	3	1	-51(-1356;84)
DEN-4	**TOTAL**	0	1	100 (-:-)
	SERO +	0	1	100 (-:-)
	SER0 -	0	0	0 (-:-)

Secondary vaccine efficacy Endpoints

It follows the analysis of a number of secondary endpoints aimed at measuring vaccine efficacy of 2 doses of TDV from 30 days post-second vaccination (Day 120) until the end of Part 2 (18 months post second dose).

Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose, Overall and by Serostatus

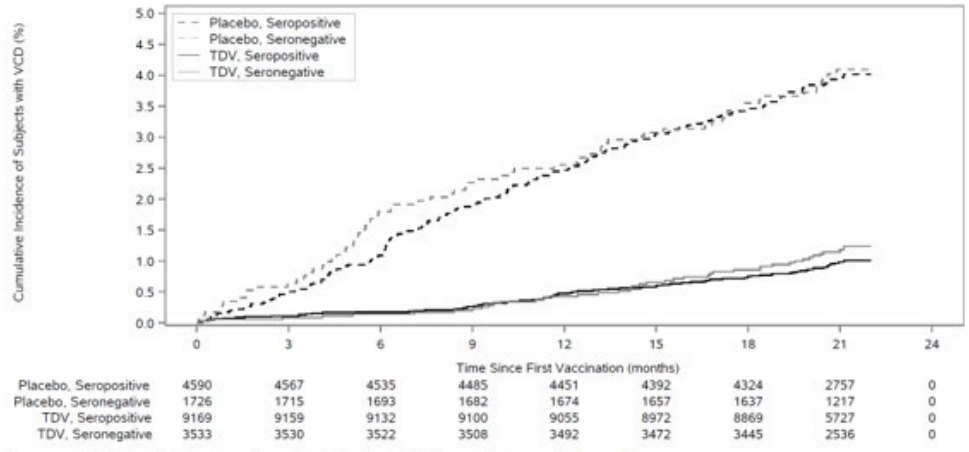
TDV was also shown to be efficacious in preventing VCD fever caused by any serotype (ie, all 4 serotypes combined) when the period of observation was extended by 6 additional months compared with the period for the primary efficacy endpoint, ie, until 18 months post second vaccine dose (end of Part 2).

Table 26: Trial DEN-301: Vaccine Efficacy in Preventing Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose – Overall and by Baseline Serostatus (Per-Protocol Set)

Baseline Serostatus	Statistic	Placebo N = 6317	TDV N = 12,704
All subjects	Number of subjects evaluated [a]	6316	12,700
	Number of subjects with febrile illness (%) [b]	2106 (33.3)	4024 (31.7)
	Number of febrile illness cases (episodes) [c]	3756	6909
	VCD fever, n (%)	206 (3.3)	114 (0.9)
	Person-years at risk	8611.0	17,664.1
	Incidence density [d]	2.4	0.6
	Relative risk (95% CI)	0.28 (0.22, 0.35)	
	Vaccine efficacy (95% CI) (%)	73.3 (66.5, 78.8)	
Seropositive	Number of subjects evaluated	4589	9167
	Number of subjects with febrile illness (%) [b]	1575 (34.3)	2989 (32.6)
	Number of febrile illness cases (episodes) [c]	2785	5203
	VCD fever, n (%)	150 (3.3)	75 (0.8)
	Person-years at risk	6252.3	12,732.7
	Incidence density [d]	2.4	0.6
	Relative risk (95% CI)	0.25 (0.19, 0.33)	
	Vaccine efficacy (95% CI) (%)	76.1 (68.5, 81.9)	
Seronegative	Number of subjects evaluated	1726	3531
	Number of subjects with febrile illness (%) [b]	531 (30.8)	1034 (29.3)
	Number of febrile illness cases (episodes) [c]	971	1704
	VCD fever, n (%)	56 (3.2)	39 (1.1)
	Person-years at risk	2357.3	4928.6
	Incidence density [d]	2.4	0.8
	Relative risk (95% CI)	0.34 (0.23, 0.51)	
	Vaccine efficacy (95% CI) (%)	66.2 (49.1, 77.5)	

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.m.
Abbreviations: CI, confidence interval; n, number of subjects with fever; N, total number of subjects; VCD, virologically confirmed dengue.
(a) Five subjects (1 in the placebo group, 4 in the TDV group) were not evaluable for analysis because they discontinued between the second vaccine dose and 30 days post second dose.
(b) Percentages were hand-calculated.
(c) Some subjects had more than 1 case (episode) of febrile illness.
(d) Number of VCD fever cases per 100 person-years.

The cumulative incidence of VCD fever over time by baseline serostatus is presented in Figure 10, showing a separation between trial groups from first dose onwards and a steady increase throughout the entire period from first dose to Month 21 (end of Part 2), with a steeper slope in the placebo group than in the TDV group. The patterns were similar between baseline seropositive and baseline seronegative subjects within each trial group.



Source: Module 5.3.5.1, Supplemental Analysis Table and Figures, Figure 2.e.
Abbreviations: VCD, virologically confirmed dengue.
The number of subjects at risk per time point are shown below the graph.

Figure 10: Trial DEN-301: Cumulative Incidence of Virologically Confirmed Dengue Fever over Time Until 18 Months Post Second Vaccine Dose (End of Part 2; Month 21) by Baseline Serostatus (Per-Protocol Set)

Virologically Confirmed Dengue Fever Caused by Each Individual Serotype From 30 Days to 18 Months Post Second Vaccine Dose

Analyses of VCD fever from 30 days to 18 months post second vaccine dose (until end of Part 2) showed varying efficacy of TDV against each individual dengue serotype (see Table 27).

Table 27: Trial DEN-301: Vaccine Efficacy in Preventing Virologically Confirmed Dengue Fever by Each Individual Serotype From 30 Days to 18 Months Post Second Vaccine Dose (Per-Protocol Set)

Serotype	Summary Statistics	Placebo N = 6317	TDV N = 12,704
	Number of subjects evaluated [a]	6316	12,700
DENV-1	Number of subjects with febrile illness (%) [b]	2106 (33.3)	4024 (31.7)
	Number of febrile illness cases (episodes) [c]	3756	6909
	VCD fever, n (%)	62 (1.0)	38 (0.3)
	Person-years at risk	8738.6	17,707.3
	Incidence density [d]	0.7	0.2
	Relative risk (95% CI)	0.30 (0.20, 0.46)	
	Vaccine efficacy (95% CI) (%)	69.8 (54.8, 79.9)	
DENV-2	Number of subjects with febrile illness (%) [b]	2106 (33.3)	4024 (31.7)
	Number of febrile illness cases (episodes) [c]	3756	6909
	VCD fever, n (%)	80 (1.3)	8 (<0.1)
	Person-years at risk	8693.9	17,722.5
	Incidence density [d]	0.9	<0.1
	Relative risk (95% CI)	0.05 (0.02, 0.10)	
	Vaccine efficacy (95% CI) (%)	95.1 (89.9, 97.6)	
DENV-3	Number of subjects with febrile illness (%) [b]	2106 (33.3)	4024 (31.7)
	Number of febrile illness cases (episodes) [c]	3756	6909
	VCD fever, n (%)	60 (0.9)	63 (0.5)
	Person-years at risk	8725.4	17,689.6
	Incidence density [d]	0.7	0.4
	Relative risk (95% CI)	0.52 (0.37, 0.74)	
	Vaccine efficacy (95% CI) (%)	48.9 (27.2, 64.1)	
DENV-4	Number of subjects with febrile illness (%) [b]	2106 (33.3)	4024 (31.7)
	Number of febrile illness cases (episodes) [c]	3756	6909
	VCD fever, n (%)	5 (<0.1)	5 (<0.1)
	Person-years at risk	8768.3	17,724.1
	Incidence density [d]	<0.1	<0.1
	Relative risk (95% CI)	0.50 (0.14, 1.72)	
	Vaccine efficacy (95% CI) (%)	51.0 (-69.4, 85.8)	

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.1.

Abbreviations: CI, confidence interval; n, number of subjects with fever; N, total number of subjects; VCD, virologically confirmed dengue.

(a) Five subjects (1 in the placebo group, 4 in the TDV group) were not evaluable for analysis because they discontinued between the second vaccine dose and 30 days post second dose.

(b) Percentages were hand-calculated.

(c) Some subjects had more than 1 case (episode) of febrile illness.

(d) Number of VCD fever cases per 100 person-years.

Severe Forms of Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose

From 30 days to 18 months post second vaccine dose (until end of Part 2), 1 subject in the placebo group (N evaluated: 6316), who was seropositive at baseline, and 2 subjects in the TDV group (N evaluated: 12,700), who were seronegative at baseline, had severe VCD fever as determined by the Dengue Case Adjudication Committee (DCAC) (see Table 31 below for individual subject details). Due to these low rates, VE could not be demonstrated for DCAC-defined severe VCD fever (VE: 2.3%; 95% CI: -977.5%, 91.1%). All of these 3 cases, which occurred in The Philippines, were caused by DENV-3 and had functional impairment among the clinical presentations (reduced pulse pressure, hypotensive shock, or respiratory distress).

Seven subjects in the placebo group compared with 2 subjects in the TDV group had DHF based on WHO criteria (1997) from 30 days to 18 months post second vaccine dose (see Table 28). The efficacy

of TDV against DHF was shown with an associated VE of 85.9% (95% CI: 31.9%, 97.1%). In the placebo group, 1 of these cases occurred in a baseline seronegative subject and was caused by DENV-3, while the remaining 6 cases occurred in baseline seropositive subjects and were caused by either DENV-1 (2 cases) or DENV-2 (4 cases) (see Table 31 for individual subject details). The 2 cases in the TDV group were both caused by DENV-3; one of these cases occurred in a baseline seronegative subject and the other in a baseline seropositive subject. Of note, 1 of the 2 cases in the TDV group was classified as both DHF and DCAC-defined severe VCD and was therefore included in both respective analyses. Thus, taking both definitions together, there were 8 cases in the placebo group versus 3 cases in the TDV group who had severe forms of dengue.

Table 28: Trial DEN-301: Vaccine Efficacy in Preventing Dengue Haemorrhagic Fever From 30 Days to 18 Months Post Second Vaccine Dose (Per-Protocol Set)

	Placebo N = 6317	TDV N = 12,704
Number of subjects evaluated [a]	6316	12,700
DHF based on WHO criteria (1997), n (%)	7 (0.1)	2 (<0.1)
Person-years at risk	8767.9	17725.6
Incidence density [b]	<0.1	<0.1
Relative risk (95% CI)	0.14 (0.03, 0.68)	
Vaccine efficacy (95% CI) (%)	85.9 (31.9, 97.1)	

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.o.

Abbreviations: CI, confidence interval; DHF, dengue hemorrhagic fever; n, number of subjects with DHF; N, total number of subjects; WHO, World Health Organization.

(a) Five subjects (1 in the placebo group, 4 in the TDV group) were not evaluable for analysis because they discontinued between the second vaccine dose and 30 days post second dose.

(b) Number of DHF cases per 100 person-years.

Exploratory Efficacy Analyses

In the following subsections, results from additional efficacy analyses are summarised focusing on the data collected up to 24 months post second vaccine dose. These exploratory analyses have not been powered to draw any confirmatory conclusions, and results are descriptive only. It should also be noted that in several sub-analyses (eg, by time period, dengue serotype, or baseline serostatus) and the combination thereof, sample sizes and incidence rates became small, often precluding any robust conclusions due to the limited data.

Virologically Confirmed Dengue Fever From 30 Days to 24 Months Post Second Vaccine Dose

Exploratory analyses of the incidence of VCD fever from 30 days to 24 months post second vaccine dose support the results seen for the primary and secondary endpoints as described before, substantiating the efficacy of TDV.

Table 29: Trial DEN-301: Virologically Confirmed Dengue From 30 Days to 24 Months Post Second Vaccine Dose (Per-Protocol Set)

	Number (%) of VCD Cases		
	Placebo N = 6317	TDV N = 12,704	Vaccine Efficacy (95% CI) (%)
Number of evaluable subjects [a]	6316	12,700	
Overall VCD fever	255 (4.0)	158 (1.2)	70.2 (63.7, 75.6)
Leading to hospitalization	75 (1.2)	16 (0.1)	89.6 (82.1, 93.9)
Caused by DENV-1	79 (1.3)	57 (0.4)	64.5 (50.1, 74.7)
Caused by DENV-2	92 (1.5)	17 (0.1)	91.0 (84.8, 94.6)
Caused by DENV-3	77 (1.2)	76 (0.6)	52.0 (34.1, 65.0)
Caused by DENV-4	8 (0.1)	8 (<0.1)	51.1 (-30.3, 81.6)
Severe forms of dengue fever			
DCAC-defined severe VCD	2 (<0.1)	2 (<0.1) [b]	50.8 (-249.0, 93.1)
DHF (WHO criteria 1997)	8 (0.1)	3 (<0.1) [b]	81.4 (30.0, 95.1)
Baseline seropositive			
Number of evaluable subjects	4589	9167	
Overall VCD fever	187 (4.1)	105 (1.1)	73.2 (66.0, 78.9)
Leading to hospitalization	51 (1.1)	9 (<0.1)	91.4 (82.6, 95.8)
Baseline seronegative			
Number of evaluable subjects	1726	3531	
Overall VCD fever	68 (3.9)	53 (1.5)	62.2 (45.9, 73.6)
Leading to hospitalization	24 (1.4)	7 (0.2)	85.5 (66.4, 93.8)

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.aa, Table 11.cc, and Table 11.jj.
Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue hemorrhagic fever; N, total number of subjects; VCD, virologically confirmed dengue; WHO, World Health Organization.
(a) Five subjects (1 in the placebo group, 4 in the TDV group) were not evaluable for analysis due to early discontinuation prior to 30 days post second vaccine dose.
(b) Includes 1 case who was classified as both DHF and DCAC-defined severe VCD.

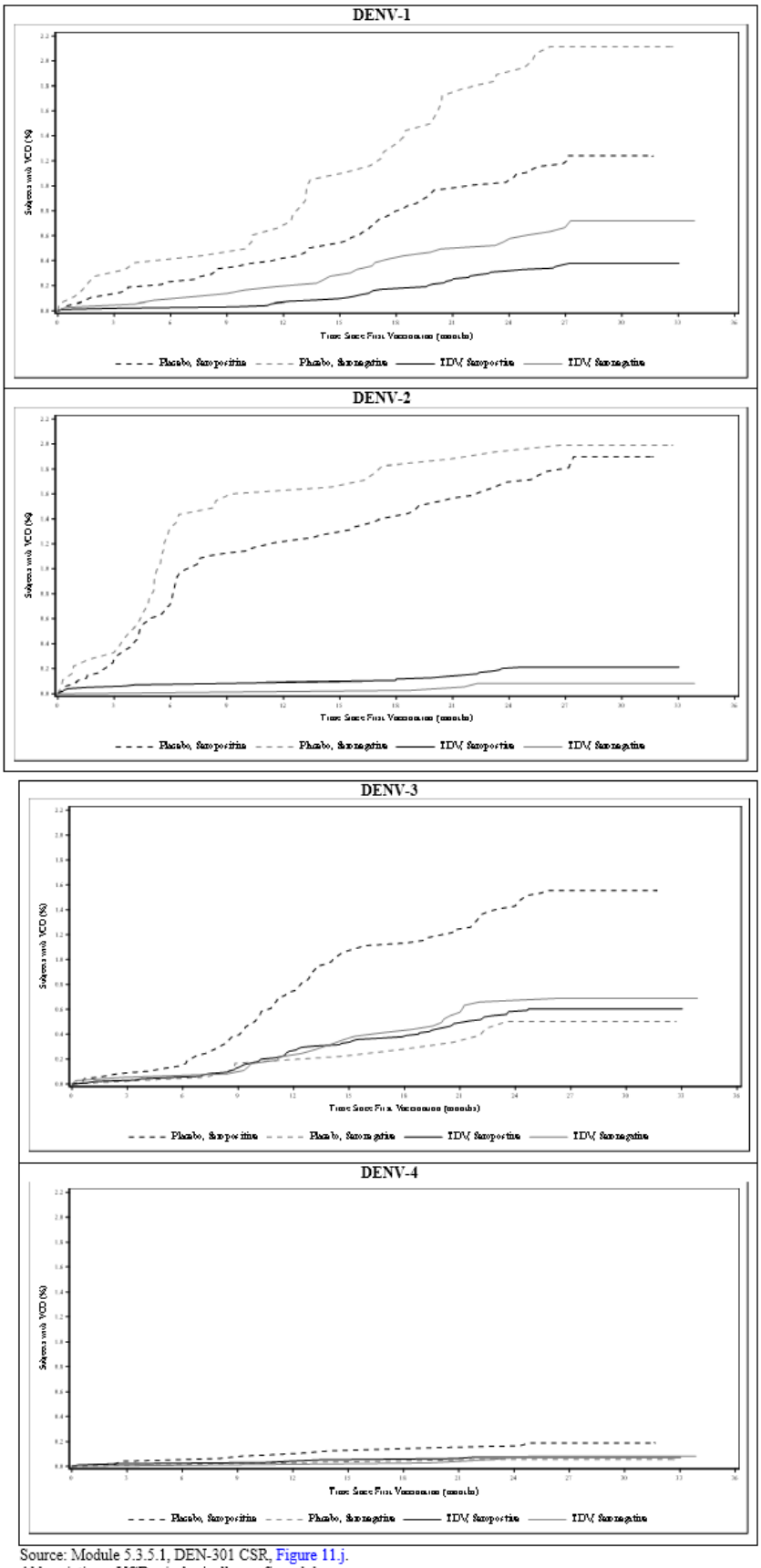
In further exploratory subgroup analyses of VE is provided against each dengue serotype by baseline serostatus, (see Table 30). This Table also includes data on VE against VCD leading to hospitalisation.

Table 30: Trial DEN-301: Vaccine Efficacy by Serotype and Baseline Serostatus From 30 Days to 24 Months Post Second Vaccine Dose (Per-Protocol Set)

		Placebo		TDV		
Serotype	Baseline Serostatus	n/N (%)	Incidence Density [a]	n/N (%)	Incidence Density [a]	Vaccine Efficacy (95% CI) (%)
Overall VCD fever						
DENV-1	Seropositive	48/4589 (1.0)	0.6	33/9167 (0.4)	0.2	66.0 (47.1, 78.2)
	Seronegative	31/1726 (1.8)	1.0	24/3531 (0.7)	0.4	63.3 (37.4, 78.4)
DENV-2	Seropositive	64/4589 (1.4)	0.7	14/9167 (0.2)	<0.1	89.3 (80.9, 94.0)
	Seronegative	28/1726 (1.6)	0.9	3/3531 (<0.1)	<0.1	94.6 (82.4, 98.4)
DENV-3	Seropositive	68/4589 (1.5)	0.8	53/9167 (0.6)	0.3	62.7 (46.6, 74.0)
	Seronegative	9/1726 (0.5)	0.3	23/3531 (0.7)	0.3	-29.0 (-178.8, 40.3)
DENV-4	Seropositive	7/4589 (0.2)	<0.1	5/9167 (<0.1)	<0.1	65.4 (-8.9, 89.0)
	Seronegative	1/1726 (<0.1)	<0.1	3/3531 (<0.1)	<0.1	-47.7 (-1320.5, 84.6)
VCD fever leading to hospitalization						
DENV-1	Seropositive	10/4589 (0.2)	0.1	5/9167 (<0.1)	<0.1	75.4 (28.1, 91.6)
	Seronegative	6/1726 (0.3)	0.2	3/3531 (<0.1)	<0.1	75.4 (1.5, 93.8)
DENV-2	Seropositive	33/4589 (0.7)	0.4	0/9167 (0)	0.0	100.0 (NE, NE)
	Seronegative	17/1726 (1.0)	0.5	0/3531 (0)	0.0	100.0 (NE, NE)
DENV-3	Seropositive	7/4589 (0.2)	<0.1	4/9167 (<0.1)	<0.1	72.5 (5.9, 91.9)
	Seronegative	1/1726 (<0.1)	<0.1	4/3531 (0.1)	<0.1	-101.9 (-1706.1, 77.4)
DENV-4	Seropositive	1/4589 (<0.1)	<0.1	0/9167 (0)	0.0	100.0 (NE, NE)
	Seronegative	0/1726 (0)	0.0	0/3531 (0)	0.0	NE (NE, NE)

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.cc.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue.
(a) Number of VCD fever cases per 100 person-years.

The plot of the cumulative incidence of VCD fever for each dengue serotype from first doses until 24 months post second dose, by baseline serostatus, are shown in the next figure.



Source: Module 5.3.5.1, DEN-301 CSR, Figure 11.j.
Abbreviations: VCD, virologically confirmed dengue.

Figure 11: Trial DEN-301: Cumulative Incidence of Virologically Confirmed Dengue Fever for Each Dengue Serotype Over Time From First Dose Until 24 Months Post Second VaccineDose, by Baseline Serostatus (Safety Set)

Details for all DCAC-defined severe cases up to 24 months post second dose are provided in Table 31, including 1 additional case in a placebo subject who was excluded from the PPS because the subject received only 1 placebo dose resulting in a protocol deviation.

Table 31: Trial DEN-301: Cases of Severe Dengue (per Adjudication Committee) or Dengue Haemorrhagic Fever (per WHO 1997 Criteria) From First Dose to 24 Months Post Second Vaccine Dose (Safety Set)

Subject Number, Age at Randomization, Country	Baseline Serostatus	Severe Dengue (DCAC-Defined)/ DHF (WHO Criteria)	Clinical Diagnosis	Time Since Last Dose (Days)	Hospital-ization	Serotype
Placebo group						
First dose to 18 months post second vaccine dose (Parts 1 and 2)						
[redacted]	Seropositive	Severe dengue	DHF	208	Yes	DENV-3
	Seronegative	DHF	DHF	347	Yes	DENV-3
	Seropositive	DHF	DHF	297	Yes	DENV-2
	Seropositive	DHF	DHF	105	Yes	DENV-2
	Seropositive	DHF	DHF	78	Yes	DENV-2
	Seropositive	DHF	DHF	452	Yes	DENV-1
	Seropositive	DHF	DHF	419	Yes	DENV-1
	Seropositive	DHF	Dengue fever	494	No	DENV-2
End of Part 2 to 24 months post second vaccine dose						
[redacted]	Seropositive	Severe dengue	DHF	692	Yes	DENV-3
	Seropositive	Severe dengue	Dengue fever	686	Yes	DENV-2
	Seropositive	DHF	DHF	640	Yes	DENV-1
TDV group						
First dose to 18 months post second vaccine dose (Parts 1 and 2)						
[redacted]	Seronegative	Severe dengue	Dengue fever	349	Yes	DENV-3
	Seronegative	Severe dengue/DHF	DHF	524	Yes	DENV-3
	Seropositive	DHF	DHF	255	Yes	DENV-3
End of Part 2 to 24 months post second vaccine dose						
[redacted]	Seronegative	DHF	DHF	573	Yes	DENV-3

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.lck, Table 11.ll, Appendix 16.2.6.2.7.1 and 16.2.6.3.7.1.
Abbreviations: DCAC, Dengue Case Adjudication Committee (blinded); DHF, dengue hemorrhagic fever; WHO, World Health Organization.

:

Virologically Confirmed Dengue Fever From 30 Days to 12 Months (Year 1) and From 13 to 24 Months (Year 2) Post Second Vaccine Dose

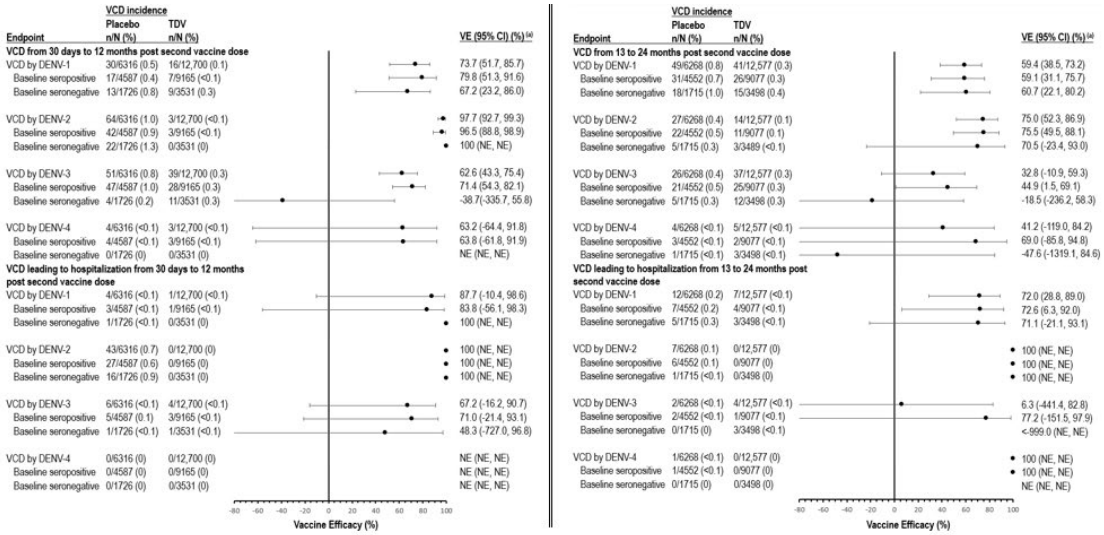
Of the total of 413 VCD fever cases (placebo: 255 [4.0%], TDV: 158 [1.2%]) that occurred from 30 days to 24 months post second vaccine dose, 210 cases occurred from 30 days to 12 months post second vaccine dose (referred to as Year 1), and 203 from 13 months (or end of Part 1) to 24 months post second vaccine dose (referred to as Year 2) (see Table 32). The next Table shows the VE results from year-by-year analyses of the 24-month efficacy data, ie, for Year 1 (from 30 days to 12 months [end of Part 1] post second dose) and for Year 2 (from 12 months [end of Part 1] to 24 months post second dose). These analyses are purely exploratory, and results are descriptive only.

Table 32:Trial DEN-301: Virologically Confirmed Dengue From 30 Days to 12 Months (Year 1) and From 13 to 24 Months (Year 2) Post Second Vaccine Dose (Per-Protocol Set)

	Year 1 Post Second Vaccine Dose [a]			Year 2 Post Second Vaccine Dose [b]		
	Number (%) of VCD Cases			Number (%) of VCD Cases		
	Placebo N = 6317	TDV N = 12,704	Vaccine Efficacy (95% CI) (%)	Placebo N = 6317	TDV N = 12,704	Vaccine Efficacy (95% CI) (%)
No. of evaluable subjects	6316	12,700		6268	12,577	
Overall VCD fever	149 (2.4)	61 (0.5)	80.2 (73.3, 85.3)	106 (1.7)	97 (0.8)	56.2 (42.3, 66.8)
Leading to hospitalization	53 (0.8)	5 (<0.1)	95.4 (88.4, 98.2)	22 (0.4)	11 (<0.1)	76.1 (50.8, 88.4)
VCD by serotype						
DENV-1	30 (0.5)	16 (0.1)	73.7 (51.7, 85.7)	49 (0.8)	41 (0.3)	59.4 (38.5, 73.2)
DENV-2	64 (1.0)	3 (<0.1)	97.7 (92.7, 99.3)	27 (0.4)	14 (0.1)	75.0 (52.3, 86.9)
DENV-3	51 (0.8)	39 (0.3)	62.6 (43.3, 75.4)	26 (0.4)	37 (0.3)	32.8 (-10.9, 59.3)
DENV-4	4 (<0.1)	3 (<0.1)	63.2 (-64.6, 91.8)	4 (<0.1)	5 (<0.1)	41.2 (-119.0, 84.2)
Severe forms of dengue						
DCAC-defined severe VCD	1 (<0.1)	1 (<0.1)	50.8 (-686.1, 96.9)	1 (<0.1)	1 (<0.1)	51.9 (-669.7, 97.0)
DHF (WHO criteria 1997)	4 (<0.1)	1 (<0.1)	87.3 (-13.5, 98.6)	4 (<0.1)	2 (<0.1)	75.9 (-31.5, 95.6)
VCD by baseline serostatus						
Baseline seropositive						
No. of evaluable subjects	4587	9165		4552	9077	
Overall VCD fever	110 (2.4)	41 (0.4)	82.2 (74.5, 87.6)	77 (1.7)	64 (0.7)	60.3 (44.7, 71.5)
Leading to hospitalization	35 (0.8)	4 (<0.1)	94.4 (84.4, 98.0)	16 (0.4)	5 (<0.1)	85.2 (59.6, 94.6)
Baseline seronegative						
No. of evaluable subjects	1726	3531		1715	3498	
Overall VCD fever	39 (2.3)	20 (0.6)	74.9 (57.0, 85.4)	29 (1.7)	33 (0.9)	45.3 (9.9, 66.8)
Leading to hospitalization	18 (1.0)	1 (<0.1)	97.2 (79.1, 99.6)	6 (0.3)	6 (0.2)	51.4 (-50.7, 84.3)

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.aa, Table 11.cc, Pt1 Table 15.2.1.13.1 and Table 15.2.1.17.1, and Pt1+12m Table 15.2.3.13.1.1 and Table 15.2.3.17.1.1.
Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue hemorrhagic fever; N, total number of evaluable subjects; VCD, virologically confirmed dengue; WHO, World Health Organization.
(a) Defined as 30 days to 12 months post second vaccine dose (end of Part 1). The overall incidence corresponds to the predefined primary efficacy endpoint of the trial (see Section 2.1.4.2.2).
(b) Defined as 13 months (or from end of Part 1) to 24 months post second vaccine dose.

The exploratory year-by-year analyses of efficacy by serotype and serostatus is shown in Figure 12.



Source: Module 5.3.5.1, DEN-301 CSR, Table 11.bb and Table 11.cc.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.
(a) VE was defined as 1 - (λV/λC), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 12: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for VCD (Overall and Leading to Hospitalisation) During 30 Days to 24 Months post Second Vaccine Dose by Yearly Intervals by Serotype and Baseline Serostatus (Per-Protocol Set)

Virologically Confirmed Dengue Fever From First Vaccine Dose Onwards

Results from efficacy analyses based on time periods starting at first vaccine dose are summarised in the following Table 33 based on the safety set to reflect the real-life clinical setting as close as

possible, ie, including subjects regardless of the number of doses received or any other protocol deviations.

Table 33: Trial DEN-301: Virologically Confirmed Dengue From First Vaccine Dose to 24 Months Post Second Vaccine Dose (Safety Set)

	Number (%) of VCD Cases		
	Placebo N = 6687	TDV N = 13,380	Vaccine Efficacy (95% CI) (%)
Number of evaluable subjects	6687	13,380	
Overall VCD fever	310 (4.6)	175 (1.3)	72.7 (67.1, 77.3)
Leading to hospitalization	91 (1.4)	20 (0.1)	89.2 (82.4, 93.3)
Caused by DENV-1	96 (1.4)	60 (0.4)	69.0 (57.1, 77.5)
Caused by DENV-2	123 (1.8)	23 (0.2)	90.8 (85.6, 94.1)
Caused by DENV-3	83 (1.2)	82 (0.6)	51.4 (34.0, 64.2)
Caused by DENV-4	10 (0.1)	10 (<0.1)	50.4 (-19.3, 79.3)
Severe forms of dengue fever			
DCAC-defined severe VCD	3 (<0.1)	2 (<0.1)	66.9 (-97.8, 94.5)
DHF (WHO criteria 1997)	8 (0.1)	3 (<0.1)	81.2 (29.3, 95.0)
Baseline seropositive			
Number of evaluable subjects	4854	9663	
VCD fever	227 (4.7)	119 (1.2)	74.8 (68.6, 79.8)
Leading to hospitalization	64 (1.3)	13 (0.1)	90.0 (81.9, 94.5)
Baseline seronegative			
Number of evaluable subjects	1832	3714	
VCD fever	83 (4.5)	56 (1.5)	67.0 (53.6, 76.5)
Leading to hospitalization	27 (1.5)	7 (0.2)	87.0 (70.1, 94.3)

Source: Module 5.3.5.1, DEN-301 CSR, Pt1+12m Table 15.2.3.3.2, Table 15.2.3.3.4, Table 15.2.3.8.1, Table 15.2.3.10.1, Table 15.2.3.10.4, Table 15.2.3.14.1, and Table 15.2.3.18.1.

Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue hemorrhagic fever; N, total number of subjects; VCD, virologically confirmed dengue; WHO, World Health Organization.

From First Vaccine Dose Until Second Vaccine Dose

During the 3 months between the first and second vaccine dose, the incidence of VCD fever was 34/6317 cases (0.5%) in the placebo group and 13/12,704 (0.1%) in the TDV group, with an associated VE of 81.1% (95% CI: 64.1%, 90.0%), indicating a rapid onset of the effect of TDV from the first vaccine dose onwards. Among the VCD cases reported between the first and second vaccine doses there were 8 cases leading to hospitalisation (6 in the placebo group and 2 in the TDV group). In addition, it is important to note that all 4 dengue serotypes were reported to have induced VCD fever in this 3-month period.

Characteristics of Virologically Confirmed Dengue Fever Based on Clinical Signs, Symptoms, and Laboratory Data

Results from an analysis of clinical signs and laboratory data of subjects with VCD fever during the period from first vaccine dose to 24 months post second dose suggested some impact on the dengue pathophysiology. There were higher proportions of subjects in the placebo group than in the TDV group with evidence of bleeding (8.0% versus 4.6%), a positive tourniquet test (11.8% versus 9.2%), plasma leakage (5.4% versus 2.3%), hematocrit increase ≥20% (10.1% versus 4.3%), and platelet count ≤100 × 10^9/L (22.0% versus 5.8%) or ≤50 10^9/L (10.5% versus 2.6%) (Table not included). These differences were more pronounced in baseline seropositive subjects than in baseline seronegative subjects, or only seen in baseline seropositive subjects. However, the data in baseline seronegative subjects did not suggest any change in the severity in presentation of VCD fever. Similar findings were seen for the period from first vaccine dose until 18 months post second dose, ie, for Part 1 and 2 of the trial combined.

Subgroup Analyses of Efficacy

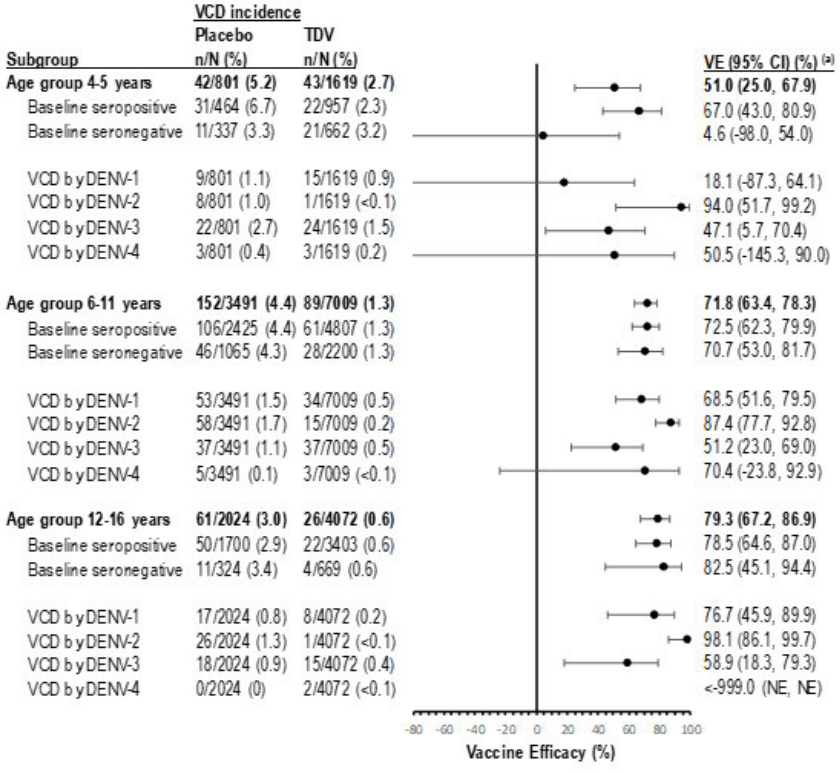
Comprehensive subgroup analyses were performed to better understand the VE data and to identify potential determinants of VE. In general, VE in the subgroups as described in the following sections could largely be explained by the serotype distribution in respective subgroups and the associated variation of efficacy across the 4 serotypes. In the following sections, results from subgroup analyses by age group (4-5, 6-11, and 12-16 years), region (Asia Pacific region, Latin America), country, and prior vaccination against YF or JE are summarised.

In addition, regression models assessed the potential impact of confounding variables. These models included factors for vaccine group, age group, baseline serostatus, prior YF or JE vaccination, region, and gender, as well as various interaction terms between these factors. Results were largely consistent with those from the preplanned subgroup analyses described in the following sections (Figures not shown in this assessment report).

It should be noted that these subgroup analyses are exploratory, and results are descriptive only. Furthermore, due to multiple levels of subgroup analyses (eg, by prespecified subgroup, dengue serotype, and baseline serostatus), sample sizes and incidence rates were sometimes small, which does not allow for any robust conclusions.

Vaccine Efficacy by Age Group

The VE of TDV in preventing VCD fever, from 30 days to 24 months post second dose, across predefined age groups (4 to 5 years, 6 to 11 years, and 12 to 16 years) is shown in Figure 13.



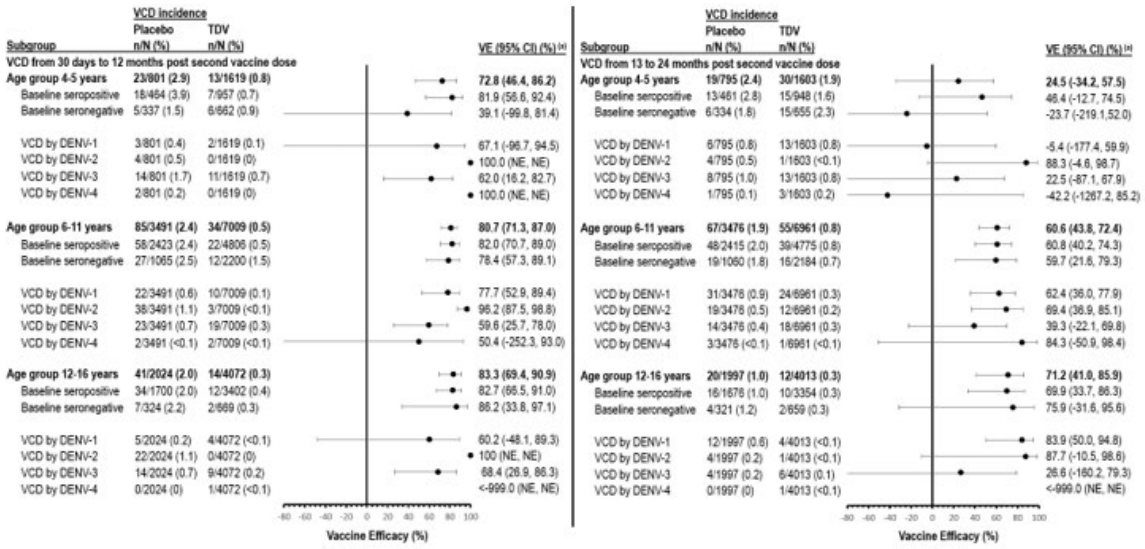
Source: Module 5.3.5.1, DEN-301 CSR, Table 11.dd.

Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) VE was defined as 1 - ($\lambda V/\lambda C$), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 13: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for VCD (Overall) During 30 Days to 24 Months Post Second Vaccine Dose by Age Group (Per-Protocol Set)

The exploratory year-by-year analysis of the VE by age group is shown in Figure 14.



Source: Module 5.3.5.1, DEN-301 CSR, Table 11.dd.

Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) VE was defined as 1 - ($\lambda V/\lambda C$), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 14: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for VCD (Overall) during 30 Days to 24 Months Post Second Vaccine Dose by Yearly Intervals by Age Group (Per-Protocol Set)

The VE analysis against VCD fever leading to hospitalisation is shown in Table 34. Incidences were small, precluding any conclusions to be drawn from these data.

Table 34: Trial DEN-301: Vaccine Efficacy Against Virologically Confirmed Dengue Leading to Hospitalisation From 30 Days to 24 Months Post Second Vaccine Dose by Age Group (Per-Protocol Set)

	Placebo		TDV		
	n/N (%)	Incidence Density [a]	n/N (%)	Incidence Density [a]	Vaccine Efficacy (95% CI) (%)
From 30 days to 24 months post second vaccine dose					
4-5 years	7/801 (0.9)	0.5	6/1619 (0.4)	0.2	58.0 (-25.0, 85.9)
Baseline seropositive	4/464 (0.9)	0.5	3/957 (0.3)	0.2	63.8 (-62.0, 91.9)
Baseline seronegative	3/337 (0.9)	0.5	3/662 (0.5)	0.2	47.7 (-159.5, 89.4)
6-11 years	48/3491 (1.4)	0.7	7/7009 (<0.1)	<0.1	92.9 (84.4, 96.8)
Baseline seropositive	30/2425 (1.2)	0.7	3/4807 (<0.1)	<0.1	95.2 (84.3, 98.5)
Baseline seronegative	18/1065 (1.7)	0.9	4/2200 (0.2)	<0.1	89.0 (67.4, 96.3)
12-16 years	20/2024 (1.0)	0.5	3/4072 (<0.1)	<0.1	92.6 (75.2, 97.8)
Baseline seropositive	17/1700 (1.0)	0.5	3/3403 (<0.1)	<0.1	91.2 (70.1, 97.4)
Baseline seronegative	3/324 (0.9)	0.5	0/669 (0)	0.0	100.0 (NE, NE)
From 30 days to 12 months post second vaccine dose					
4-5 years	3/801 (0.4)	0.4	1/1619 (<0.1)	<0.1	83.7 (-56.5, 98.3)
Baseline seropositive	1/464 (0.2)	0.2	1/957 (0.1)	0.1	52.3 (-662.3, 97.0)
Baseline seronegative	2/337 (0.6)	0.7	0/662 (0)	0.0	100.0 (NE, NE)
6-11 years	33/3491 (0.9)	1.0	2/7009 (<0.1)	<0.1	97.1 (87.8, 99.3)
Baseline seropositive	19/2423 (0.8)	0.9	1/4806 (<0.1)	<0.1	97.5 (81.5, 99.7)
Baseline seronegative	14/1065 (1.3)	1.5	1/2200 (<0.1)	<0.1	96.4 (72.5, 99.5)
12-16 years	17/2024 (0.8)	0.9	2/4072 (<0.1)	<0.1	94.2 (74.9, 98.7)
Baseline seropositive	15/1700 (0.9)	1.0	2/3402 (<0.1)	<0.1	93.4 (71.0, 98.5)
Baseline seronegative	2/324 (0.6)	0.7	0/669 (0)	0.0	100.0 (NE, NE)
From 13 to 24 months post second vaccine dose					
4-5 years	4/795 (0.5)	0.5	5/1603 (0.3)	0.3	40.1 (-123.0, 83.9)
Baseline seropositive	3/461 (0.7)	0.7	2/948 (0.2)	0.2	68.7 (-87.1, 94.8)
Baseline seronegative	1/334 (0.3)	0.3	3/655 (0.5)	0.5	-54.6 (-1387.6, 83.9)
6-11 years	15/3476 (0.4)	0.4	5/6961 (<0.1)	<0.1	84.1 (56.1, 94.2)
Baseline seropositive	11/2415 (0.5)	0.5	2/4775 (<0.1)	<0.1	91.4 (61.0, 98.1)
Baseline seronegative	4/1060 (0.4)	0.4	3/2184 (0.1)	0.1	64.4 (-59.0, 92.0)
12-16 years	3/1997 (0.2)	0.2	1/4013 (<0.1)	<0.1	84.2 (-52.1, 98.4)
Baseline seropositive	2/1676 (0.1)	0.1	1/3354 (<0.1)	<0.1	75.9 (-166.4, 97.8)
Baseline seronegative	1/321 (0.3)	0.3	0/659 (0)	0.0	100.0 (NE, NE)

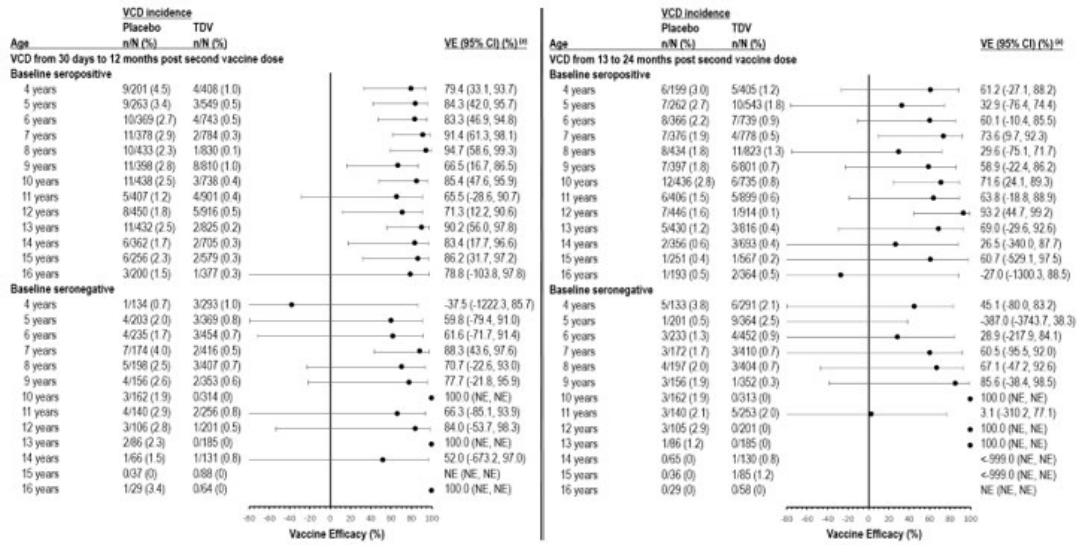
Source: Module 5.3.5.1, DEN-301 CSR, Table 11.dd.

Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue.

(a) Number of VCD fever cases per 100 person-years.

Exploratory Analyses by Individual Age

To further explore the above described results from the prespecified subgroup analyses by age category, the VE of TDV against VCD fever was analysed by individual age from 4 to 16 years. It should be noted that the number of cases were very small in this exploratory sub-analysis, precluding any robust conclusions from these data.



Source: Module 5.3.5.1, DEN-301 CSR, Table P-15.2.1.2.9 and Table P-15.2.3.2.9.1.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.
(a) VE was defined as 1 - (λV/λC), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 15: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Virologically Confirmed Dengue (Overall) During 30 Days to 12 Months and 13 to 24 Months Post Second Vaccine Dose by Individual Age and Baseline Serostatus (Per-Protocol Set)

Vaccine Efficacy by Region and Country

Results from subgroup analyses by region and country were reflective of the respective epidemiological setting, ie, the serotype distribution, in the respective country and region.

During Year 1, 181 VCD fever cases (placebo: 127 [4.3%]; TDV: 54 [0.9%]) occurred in the Asia Pacific region and 29 (placebo: 22 [0.7%]; TDV: 7 [0.1]) in Latin America. During Year 2, 126 cases (60 [2.1%] and 66 [1.1%], respectively) occurred in the Asia Pacific region and 77 (46 [1.4%] and 31 [0.5%], respectively) in Latin America.

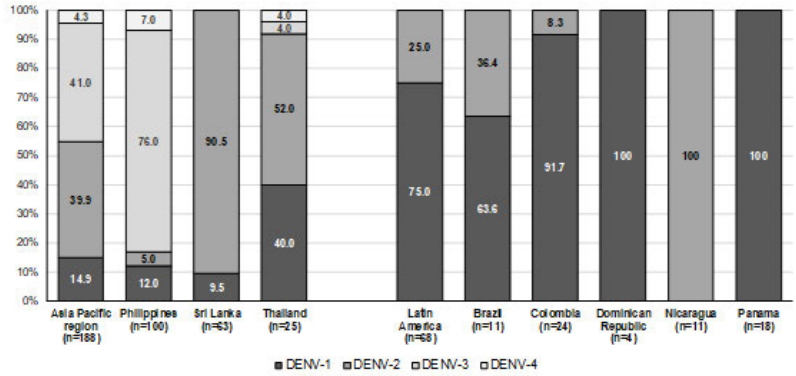
A descriptive comparison of the incidence density (incidence per 100 person-years) between Year 1 and Year 2 in the Asia Pacific region showed a decrease in the incidence density for any VCD in the placebo group (ie, unaffected by the administration of an active vaccine) from 4.9 in Year 1 to 2.2 in Year 2. Such decreases in the placebo group were seen in The Philippines (5.4-3.1) and especially in Sri Lanka (9.9-1.0), but not in Thailand where the incidence density increased from 0.9 to 1.8. The marked decrease in the VCD incidence in the placebo group in Sri Lanka is attributable to an outbreak during Year 1 that led to a rapid acquisition of VCD cases. In Latin America, the incidence density increased in all countries from Year 1 to Year 2, with the exception of Panama where it decreased. In Nicaragua, where no VCD cases were reported during Year 1 (incidence density of 0), VCD reports led to incidence densities of 4.8 (placebo) and 1.2 (TDV) during Year 2, which were the highest rates among the Latin American countries during Year 2.

Table 35: Trial DEN-301: Incidence per 100 Person-Years of Virologically Confirmed Dengue From 30 Days to 12 Months and From 13 to 24 Months Post Second Vaccine Dose, by Region and Country (Per-Protocol Set)

Region / Country	Incidence per 100 Person-Years			
	Year 1: 30 Days to 12 Months Post Second Dose		Year 2: 13 to 24 Months Post Second Dose	
	Placebo	TDV	Placebo	TDV
Asia Pacific region	4.9	1.0	2.2	1.2
The Philippines	5.4	2.0	3.1	2.1
Sri Lanka	9.9	0.3	1.0	0.3
Thailand	0.9	0.2	1.8	0.6
Latin America	0.7	0.1	1.4	0.5
Brazil	0.7	0.0	1.6	0.5
Colombia	0.4	<0.1	1.8	0.6
The Dominican Republic	0.0	0.0	0.8	0.2
Nicaragua	0.0	0.0	4.8	1.2
Panama	1.8	0.3	0.3	0.2

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.ff.

The serotype distribution for VCD fever cases from 30 days to 24 months post second vaccine dose in the placebo group, ie, unaffected by active vaccine administration, is depicted in Figure 16, showing the varying distribution of serotypes in the participating regions and countries.

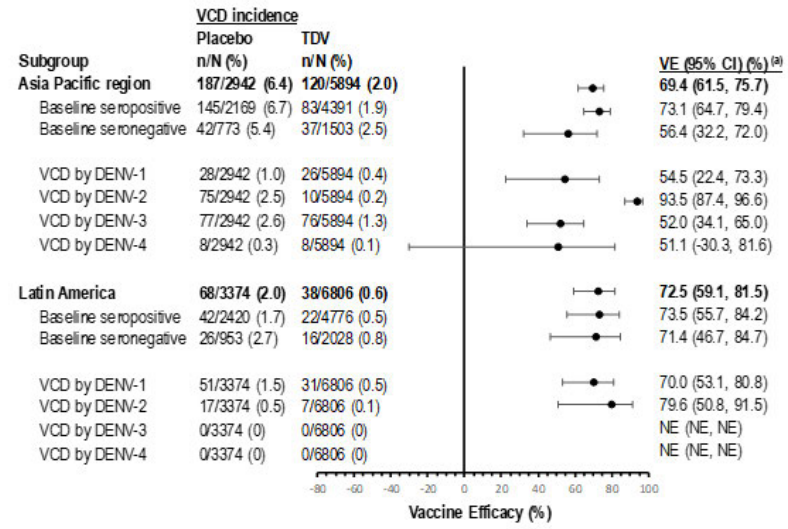


Source: Module 5.3.5.1, DEN-301 CSR, Pt1+12m Table 15.2.3.32.4.2.
Abbreviations: n, total number of VCD cases in the region or country; VCD, virologically confirmed dengue.
Numbers in bars show the percentage among all cases in that region or country; the presented data include repeat infections.

Figure 16: Trial DEN-301: Serotype Distribution in the Placebo Group From 30 Days to 24 Months Post Second Vaccine Dose, by Region and Country (Per-Protocol Set)

Vaccine Efficacy by Region (Asia Pacific Region vs Latin America)

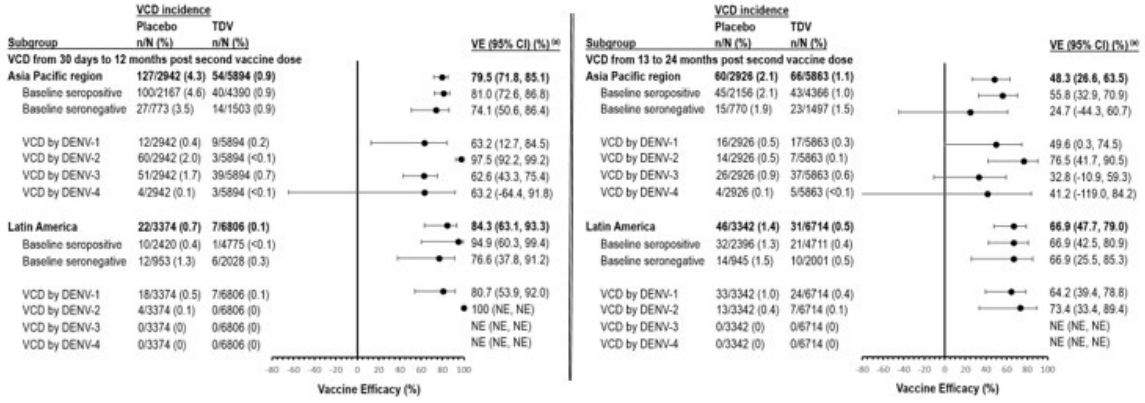
In the period from 30 days to 24 months post second vaccine dose, VE of TDV was seen in both the Asia Pacific region and Latin America, overall as well as in subgroups by baseline serostatus (see Figure 17).



Source: Module 5.3.5.1, DEN-301 CSR, Table 11.ee.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.
(a) VE was defined as 1 - (λV/λC), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 17: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for VCD (Overall) During 30 Days to 24 Months Post Second Vaccine Dose by Yearly Intervals by Region (Per-Protocol Set)

The exploratory year-by-year analysis of VE by region also showed the VE of TDV in each of the time periods, with slightly lower VE values in Year 2 compared with Year 1 (see Figure 18)



Source: Module 5.3.5.1, DEN-301 CSR, Table 11.ee.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.
(a) VE was defined as 1 - (λV/λC), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 18: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for VCD (Overall) During 30 Days to 24 Months Post Second Vaccine Dose by Yearly Intervals by Region (Per-Protocol Set)

The VE against VCD fever leading to hospitalisation was high in both the Asia Pacific region (88.9%) and Latin America (94.5%) as shown in Table 36.

Table 36: Trial DEN-301: Vaccine Efficacy Against Virologically Confirmed Dengue Leading to Hospitalisation From 30 Days to 24 Months Post Second Vaccine Dose by Region (Per-Protocol Set)

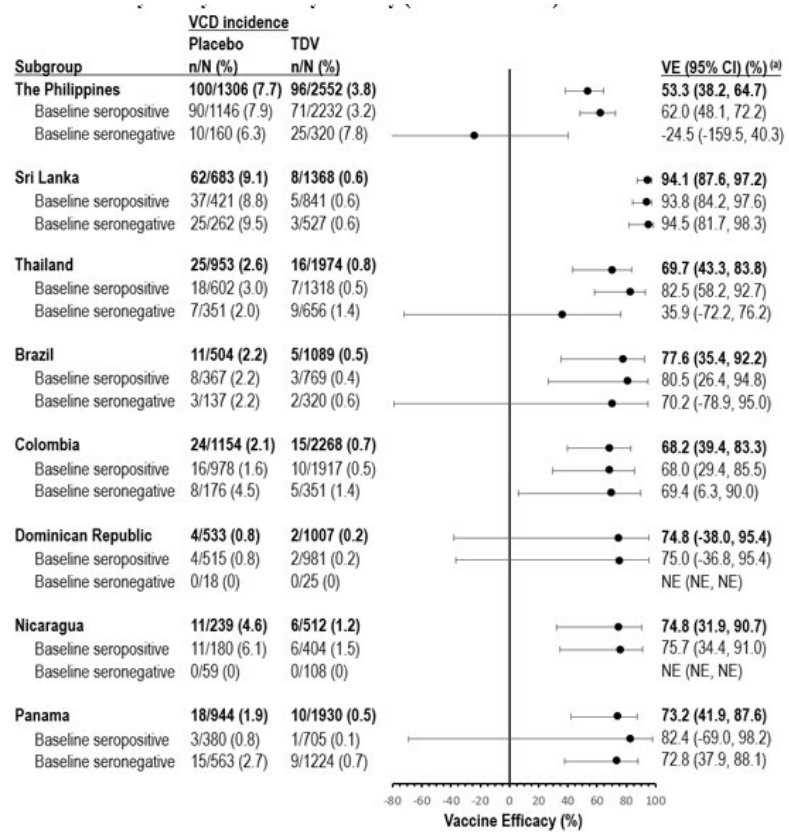
	Placebo		TDV		
	n/N (%)	Incidence Density [a]	n/N (%)	Incidence Density [a]	Vaccine Efficacy (95% CI) (%)
From 30 days to 24 months post second vaccine dose					
Asia Pacific region	66/2942 (2.2)	1.2	15/5894 (0.3)	0.1	88.9 (80.6, 93.7)
Baseline seropositive	45/2169 (2.1)	1.1	8/4391 (0.2)	<0.1	91.4 (81.8, 96.0)
Baseline seronegative	21/773 (2.7)	1.5	7/1503 (0.5)	0.2	83.3 (60.8, 92.9)
Latin America	9/3374 (0.3)	0.1	1/6806 (<0.1)	<0.1	94.5 (56.5, 99.3)
Baseline seropositive	6/2420 (0.2)	0.1	1/4776 (<0.1)	<0.1	91.5 (29.7, 99.0)
Baseline seronegative	3/953 (0.3)	0.2	0/2028 (0)	0.0	100.0 (NE, NE)
From 30 days to 12 months post second vaccine dose					
Asia Pacific region	52/2942 (1.8)	2.0	5/5894 (<0.1)	<0.1	95.3 (88.2, 98.1)
Baseline seropositive	34/2167 (1.6)	1.7	4/4390 (<0.1)	<0.1	94.3 (83.9, 98.0)
Baseline seronegative	18/773 (2.3)	2.6	1/1503 (<0.1)	<0.1	97.2 (79.1, 99.6)
Latin America	1/3374 (<0.1)	<0.1	0/6806 (0)	0.0	100.0 (NE, NE)
Baseline seropositive	1/2420 (<0.1)	<0.1	0/4775 (0)	0.0	100.0 (NE, NE)
Baseline seronegative	0/953 (0)	0.0	0/2028 (0)	0.0	NE (NE, NE)
From 13 to 24 months post second vaccine dose					
Asia Pacific region	14/2926 (0.5)	0.5	10/5863 (0.2)	0.2	66.3 (24.2, 85.0)
Baseline seropositive	11/2156 (0.5)	0.5	4/4366 (<0.1)	<0.1	83.2 (47.1, 94.6)
Baseline seronegative	3/770 (0.4)	0.4	6/1497 (0.4)	0.4	1.5 (-293.9, 75.4)
Latin America	8/3342 (0.2)	0.2	1/6714 (<0.1)	<0.1	93.8 (50.8, 99.2)
Baseline seropositive	5/2396 (0.2)	0.2	1/4711 (<0.1)	<0.1	89.9 (13.6, 98.8)
Baseline seronegative	3/945 (0.3)	0.3	0/2001 (0)	0.0	100.0 (NE, NE)

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.ee; Supplemental Analysis Table and Figures, Table P-15.2.1.9.8.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue.
(a) Number of VCD fever cases per 100 person-years.

Vaccine Efficacy by Country

Similar to the efficacy results by region, results from subgroup analyses by country were reflective of the varying epidemiological setting in each country, mainly the serotype distribution, and the observed efficacy of TDV by serotype and baseline serostatus as described previously. It should be noted that due to multiple levels of subgroup analyses (eg, by country, dengue serotype, and baseline serostatus), sample sizes and incidence rates were sometimes small, which does not allow for any robust conclusions.

The VE of TDV in preventing VCD was seen in each of the 8 participating countries (see Figure 19). Results also suggest the efficacy of TDV in subgroups by baseline serostatus within each country, with the exception of baseline seronegative subjects in The Philippines where the VE value was negative. This finding can be explained by the serotype distribution in The Philippines where the majority of VCD cases were caused by DENV-3 and the efficacy results by dengue serotype that suggested no efficacy of TDV against VCD caused by DENV-3 in baseline seronegative subjects.



Source: Module 5.3.5.1, DEN-301 CSR, Table 11.ff and Pt1+12m Table 15.2.3.2.8.2.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.
(a) VE was defined as 1 - (λV/λC), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 19: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for VCD (Overall) During 30 Days to 24 Months Post Second Vaccine Dose by yearly Intervals by Country (Per Protocol Set)

Where estimable, VE values for the prevention of VCD fever leading to hospitalisation were generally higher than for overall VCD. However, due to the very low number of cases in each country no robust conclusions can be drawn from these data (Table 37).

Table 37: Trial DEN-301: Vaccine Efficacy Against Virologically Confirmed Dengue Leading to Hospitalisation From 30 Days to 24 Months Post Second Vaccine Dose by Country (Per-Protocol Set)

	Placebo		TDV		
	n/N (%)	Incidence Density [a]	n/N (%)	Incidence Density [a]	Vaccine Efficacy (95% CI) (%)
From 30 days to 24 months post second vaccine dose					
Asia Pacific					
The Philippines	9/1306 (0.7)	0.4	9/2552 (0.4)	0.2	50.5 (-24.6, 80.4)
Sri Lanka	45/683 (6.6)	3.8	1/1368 (<0.1)	<0.1	99.0 (92.5, 99.9)
Thailand	12/953 (1.3)	0.7	5/1974 (0.3)	0.1	80.1 (43.5, 93.0)
Latin America					
Brazil	2/504 (0.4)	0.2	0/1089 (0)	0.0	100.0 (NE, NE)
Colombia	5/1154 (0.4)	0.2	1/2268 (<0.1)	<0.1	89.8 (12.4, 98.8)
The Dominican Republic	0/533 (0)	0.0	0/1007 (0)	0.0	NE (NE, NE)
Nicaragua	2/239 (0.8)	0.4	0/512 (0)	0.0	100.0 (NE, NE)
Panama	0/944 (0)	0.0	0/1930 (0)	0.0	NE (NE, NE)
From 30 days to 12 months post second vaccine dose					
Asia Pacific					
The Philippines	6/1306 (0.5)	0.5	4/2552 (0.2)	0.2	66.7 (-18.1, 90.6)
Sri Lanka	42/683 (6.1)	7.3	1/1368 (<0.1)	<0.1	98.9 (91.9, 99.8)
Thailand	4/953 (0.4)	0.5	0/1974 (0)	0.0	100.0 (NE, NE)
Latin America					
Brazil	0/504 (0)	0.0	0/1089 (0)	0.0	NE (NE, NE)
Colombia	1/1154 (<0.1)	<0.1	0/2268 (0)	0.0	100.0 (NE, NE)
The Dominican Republic	0/533 (0)	0.0	0/1007 (0)	0.0	NE (NE, NE)
Nicaragua	0/239 (0)	0.0	0/512 (0)	0.0	NE (NE, NE)
Panama	0/944 (0)	0.0	0/1930 (0)	0.0	NE (NE, NE)
From 13 to 24 months post second vaccine dose					
Asia Pacific					
The Philippines	3/1296 (0.2)	0.2	5/2535 (0.2)	0.2	21.2 (-229.5, 81.2)
Sri Lanka	3/683 (0.4)	0.5	0/1367 (0)	0.0	100.0 (NE, NE)
Thailand	8/947 (0.8)	0.9	5/1961 (0.3)	0.3	70.4 (9.6, 90.3)
Latin America					
Brazil	2/498 (0.4)	0.4	0/1079 (0)	0.0	100.0 (NE, NE)
Colombia	4/1142 (0.4)	0.4	1/2223 (<0.1)	<0.1	87.3 (-13.7, 98.6)
The Dominican Republic	0/527 (0)	0.0	0/997 (0)	0.0	NE (NE, NE)
Nicaragua	2/237 (0.8)	0.9	0/506 (0)	0.0	100.0 (NE, NE)
Panama	0/938 (0)	0.0	0/1909 (0)	0.0	NE (NE, NE)

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.ff.
Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue.

Note: The following table has been prepared from Table 37 and Figure 19 to help interpreting the VE data against hospitalised VCD. The table shows the number of hospitalised cases observed in the different countries (and also according to each dengue serotype) for the placebo group for the time period 30 days post second dose until end of Part 2. The tables also include the "Percent of hospitalised VCD Cases Among VCD Cases".

Data taken from Figure 19 and Table 37

Serotype Distribution in VCD, Hospitalized VCD, from 30 Days Post-Second Vaccination to 24 months post second vaccine dose Subgroup Analysis by Country, Per-Protocol Set		
	PLACEBO (% of cases)	Percent of Hospitalized VCD Cases Among VCD cases
(VCD) (Number of Cases)		
Philippines	100	
Sri Lanka	62 (24 %)	
Thailand	25	
Brazil	11	
Colombia	24	
Dominican Republic	4	
Nicaragua	11	
Panama	18	
TOTAL	255	
Hospitalized VCD (Number of Cases		
Philippines	9 (12)	9 %
Sri Lanka	45 (60)	72 %
Thailand	12 (16.6)	48 %
Brazil	2 (2.6)	18 %
Colombia	5 (6.6)	20 %
Dominican Republic	0	--
Nicaragua	2 (2.6)	18 %
Panama	0	0 %
TOTAL	75	

Vaccine Efficacy by Prior Vaccination Against Yellow Fever or Japanese Encephalitis

Subgroup analyses by prior vaccination against YF or JE showed VE values of 86.1% in those subjects previously vaccinated against JE, 68.8% in those previously vaccinated against YF, and 60.6% in those not previously vaccinated against either JE or YF (see Table 38), suggesting an impact of prior JE or YF vaccination on the VE of TDV. However, this finding can be explained by regional differences in JE and YF vaccination as well as by the serotype distribution.

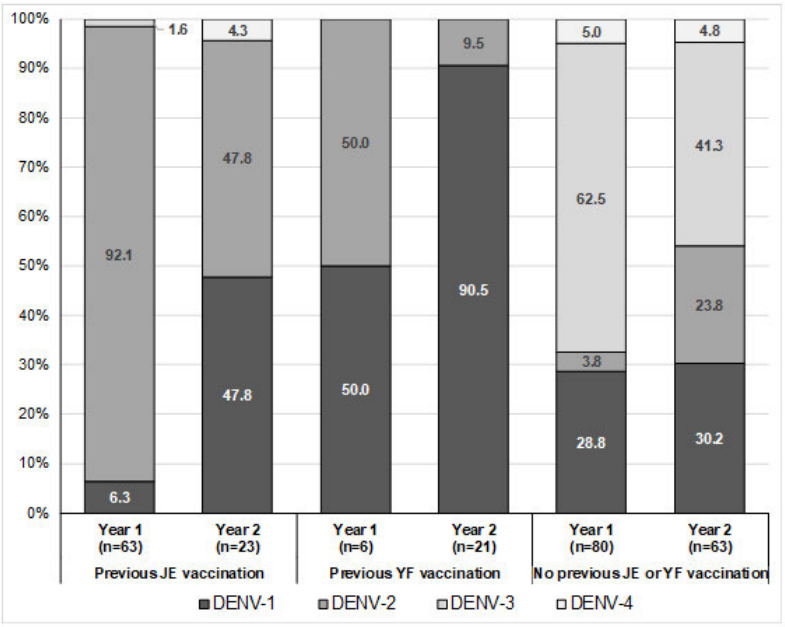
A vast majority of subjects vaccinated against JE were from Sri Lanka and Thailand, where subjects are routinely vaccinated against JE. Because VCD cases in Sri Lanka and Thailand were predominantly caused by DENV-2, also the cases in the subgroup of subjects with prior JE vaccination were predominantly caused by DENV-2, which resulted in the high VE in this subgroup. Similarly, as almost all subjects vaccinated against YF were from Latin America, predominantly from Colombia and Brazil, VCD cases in this subgroup were predominantly caused by DENV-1. As a result, in the subgroup of subjects who were not vaccinated against either JE or YF, the largest proportion of VCD fever cases were caused by DENV-3, which were predominantly reported in The Philippines.

The changes between Year 1 and Year 2 were reflective of the changes in the serotype distribution in these subgroups in Year 2 compared with Year 1, as shown for the placebo group in Figure 20.

Table 38: Trial DEN-301: Vaccine Efficacy Against Virologically Confirmed Dengue From 30 Days to 24 Months Post Second Vaccine Dose by Previous Japanese Encephalitis or Yellow Fever Vaccination (Per-Protocol Set)

	Placebo		TDV		
	n/N (%)	Incidence Density [a]	n/N (%)	Incidence Density [a]	Vaccine Efficacy (95% CI) (%)
From 30 days to 24 months post second vaccine dose					
Previous JE vaccination	85/1552 (5.5)	3.0	25/3157 (0.8)	0.4	86.1 (78.4, 91.1)
Previous YF vaccination	27/1355 (2.0)	1.1	17/2719 (0.6)	0.3	68.8 (42.8, 83.0)
No previous JE or YF vaccination	143/3409 (4.2)	2.3	116/6825 (1.7)	0.9	60.6 (49.7, 69.2)
From 30 days to 12 months post second vaccine dose					
Previous JE vaccination	63/1551 (4.1)	4.6	9/3157 (0.3)	0.3	93.2 (86.4, 96.6)
Previous YF vaccination	6/1355 (0.4)	0.5	2/2719 (<0.1)	<0.1	83.4 (17.6, 96.6)
No previous JE or YF vaccination	80/3410 (2.3)	2.6	50/6825 (0.7)	0.8	69.4 (56.4, 78.5)
From 13 to 24 months post second vaccine dose					
Previous JE vaccination	22/1547 (1.4)	1.5	16/3145 (0.5)	0.5	66.3 (35.8, 82.3)
Previous YF vaccination	21/1339 (1.6)	1.6	15/2679 (0.6)	0.6	64.7 (31.5, 81.8)
No previous JE or YF vaccination	63/3382 (1.9)	2.0	66/6754 (1.0)	1.0	49.6 (28.8, 64.3)

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.gg and Table P-15.2.1.2.6.
Abbreviations: CI, confidence interval; JE, Japanese encephalitis; n, number of subjects with VCD fever; N, total number of evaluable subjects; VCD, virologically confirmed dengue; YF, yellow fever.
(a) Number of VCD fever cases per 100 person-years.



Source: Module 5.3.5.1, DEN-301 CSR, Table P-15.2.1.32.9.3 and Table P-15.2.3.32.5.5.
Abbreviations: JE, Japanese encephalitis; n, total number of VCD cases in the region or country; VCD, virologically confirmed dengue; YF, yellow fever.
The presented data include repeat infections and therefore the number of cases per category might deviate from those given in Table 2.z. Numbers in bars show the percentage among all cases in the given subgroup.

Figure 20: Trial DEN-301: Serotype Distribution in the Placebo Group From 30 Days to 12 Months and From 13 to 24 Months Post Second Placebo Dose by Previous Japanese Encephalitis or Yellow Fewer Vaccination (Per-Protocol Set)

Virologically Confirmed Dengue Fever From First Dose to 36 Months Post Second Vaccine Dose.

The overall VE in preventing VCD fever from 30 days post second vaccination up to 36 months is 59.6% (95%CI: 53.6, 64.9), according to the PP set.

Table 15.2.4.1.1.2
Vaccine Efficacy of TDV in Preventing VCD Fever from 30 Days Post-Second Vaccination until 18 months after end of Part 2
Per-Protocol Set

	Placebo (N=6,317)	TDV (N=12,704)
Number of Subjects Evaluated	6316	12700
Number of Subjects with Febrile Illness	2911	5534
Number of Febrile Illness Cases	7002	13175
Virologically-Confirmed Dengue Fever (n[%])	434 (6.9)	366 (2.9)
Person-Years at Risk	17387.6	35949.4
Incidence Density	2.5	1.0
Relative Risk	0.42	
95% CI	(0.37, 0.48)	
Vaccine Efficacy (%)	59.6	
95% CI	(53.6, 64.9)	

Efficacy results from first dose until 36 months post second dose are briefly summarised using the safety set (to reflect the real-life clinical setting as close as possible):

- **VCD caused by any serotype:** The efficacy of TDV against overall VCD was seen in the total safety set (VE: 62.0%; 95% CI: 56.6%, 66.7%) as well as in baseline seronegative subjects (VE: 54.3%; 95% CI: 41.9%, 64.1%) and baseline seropositive subjects (VE: 65.0%; 95% CI: 58.9%, 70.1%).
- **VCD leading to hospitalisation:** For VCD leading to hospitalisation, the VE of TDV was 83.6% (95% CI: 76.8%, 88.4%) for the total safety set, 77.1% (95% CI: 58.6%, 87.3%) for baseline seronegative subjects, and 86.0% (95% CI: 78.4%, 91.0%) for baseline seropositive subjects.
- Efficacy against individual dengue serotypes varied by serotype, largely consistent with the results based on data up to 24 months post second dose:
  - **VCD caused by individual serotypes:** VE estimates were 51.8% for DENV-1 (95% CI: 41.1%, 60.5%), 86.0% for DENV-2 (95% CI: 80.9%, 89.8%), and 41.9% for DENV-3 (95% CI: 25.2%, 55.0%). Due to the lower incidence of VCD caused by DENV-4 compared with other dengue serotypes, the results for this serotype were inconclusive, but the VE estimate of 41.5% (95% CI: -11.7%, 69.4%) was similar to that for DENV-1 or DENV-3 and thus suggested efficacy. In baseline seropositive subjects, efficacy was seen against each individual dengue serotype. In baseline seronegative subjects, efficacy was seen for DENV-1 and DENV-2, while for DENV-3, results did not suggest efficacy (VE: -23.4; 95%CI: -125.3, 32.4); the small number of 10 cases of VCD caused by DENV-4 in baseline seronegative subjects as well as their occurrence in time over the 3-year period do not allow for a robust conclusion. It is however noted that in the safety set for dengue seronegative subjects at baseline, there were more cases of VCD due to DENV-4 in the TDV group (8 cases) than in the control group (2 cases) which resulted in a negative VE although with very wide CI. (VE -105%; 95%CI: -867; 56).
  - **VCD leading to hospitalisation caused by individual serotypes:** VE estimates were 72.0% for DENV-1 (95% CI: 50.1%, 84.3%), 96.7% for DENV-2 (95% CI: 91.8%, 98.7%), and 41.1% for DENV-3 (95% CI: -14.6%, 69.7). There were only 3 cases of DENV-4 induced VCD leading to hospitalisation (all in the placebo group). Results for VCD leading to hospitalisation by baseline serostatus were in line with those for overall VCD as described above, with generally higher VE estimates. There were no DENV-4 induced VCD cases leading to hospitalisation in baseline seronegative subjects.

Severe forms of dengue: A total of 8 cases of DCAC-defined severe VCD were reported in the safety set, 5 cases in the placebo group and 3 in the TDV group (VE: 70.2%; 95% CI: -24.7%, 92.9%). In

the same period, 13 cases of DHF occurred in the placebo group and 9 cases in the TDV group (VE: 65.4%; 95% CI: 19.0%, 85.2%), including 3 cases (1 in the placebo group, 2 in the TDV group) that were classified as both DCAC-defined severe and DHF. Thus, taken together, there were 17 cases of DCAC-defined severe VCD or DHF in the placebo group and 10 such cases in the TDV group (the randomisation ratio of 1:2 [placebo:TDV] should be considered. As can be seen in the next two Tables (further discussed in the safety section) the efficacy in terms of prevention of DHF and severe cases is only observed in Dengue seropositive subjects at baseline and not in those seronegative.

Table 15.2.4.18.3
Vaccine Efficacy of TDV in Preventing Severe VCD Determined by Adjudication Committee from First Vaccination until 18 months after end of Part 2 – Subgroup Analysis by Baseline Seropositivity Status
Safety Set

Baseline Seropositivity Status	Summary Statistics	Placebo (N=6,687)	TDV (N=13,380)
Seropositive	Number of Subjects Evaluated	4854	9663
	Severe VCD Fever (n[%])	5 (0.1)	1 (<0.1)
	Person-Years at Risk	15331.4	30497.5
	Incidence Density	<0.1	<0.1
	Relative Risk	0.10	
	95% CI	(0.01, 0.86)	
	Vaccine Efficacy (%)	90.1	
	95% CI	(15.3, 98.8)	

Table 15.2.4.18.3
Vaccine Efficacy of TDV in Preventing Severe VCD Determined by Adjudication Committee from First Vaccination until 18 months after end of Part 2 – Subgroup Analysis by Baseline Seropositivity Status
Safety Set

Baseline Seropositivity Status	Summary Statistics	Placebo (N=6,687)	TDV (N=13,380)
Seronegative	Number of Subjects Evaluated	1832	3714
	Severe VCD Fever (n[%])	0	2 (<0.1)
	Person-Years at Risk	5802.5	11732.6
	Incidence Density	0.0	<0.1
	Relative Risk	NE	
	95% CI	(NE, NE)	
	Vaccine Efficacy (%)	<-999.0	
	95% CI	(NE, NE)	

Table 15.2.4.14.3
Vaccine Efficacy of TDV in Preventing DHF Determined by WHO Criteria (1997) from First Vaccination until 18 months after end of Part 2 – Subgroup Analysis by Baseline Seropositivity Status
Safety Set

Baseline Seropositivity Status	Summary Statistics	Placebo (N=6,687)	TDV (N=13,380)
Seropositive	Number of Subjects Evaluated	4854	9663
	Dengue Hemorrhagic Fever (n[%])	12 (0.2)	5 (<0.1)
	Person-Years at Risk	15320.2	30493.3
	Incidence Density	<0.1	<0.1
	Relative Risk	0.21	
	95% CI	(0.07, 0.59)	
	Vaccine Efficacy (%)	79.3	
	95% CI	(41.1, 92.7)	

Table 15.2.4.14.3
Vaccine Efficacy of TDV in Preventing DHF Determined by WHO Criteria (1997) from First Vaccination until 18 months after end of Part 2 – Subgroup Analysis by Baseline Seropositivity Status
Safety Set

Baseline Seropositivity Status	Summary Statistics	Placebo (N=6,687)	TDV (N=13,380)
Seronegative	Number of Subjects Evaluated	1832	3714
	Dengue Hemorrhagic Fever (n[%])	1 (<0.1)	4 (0.1)
	Person-Years at Risk	5801.1	11732.4
	Incidence Density	<0.1	<0.1
	Relative Risk	1.97	
	95% CI	(0.22, 17.64)	
	Vaccine Efficacy (%)	-106.2	
	95% CI	(-1744.8, 77.0)	

- **Characteristics of VCD fever based on clinical signs, symptoms, and laboratory data:** Results from an exploratory analysis of clinical signs and laboratory data of subjects with VCD fever showed no major changes in the trend seen in the period up to 24 months post second dose. The favorable impact of TDV on the dengue pathophysiology (eg, signs of plasma leakage, bleeding, or thrombocytopenia) was mainly seen in baseline seropositive subjects). In baseline seronegative subjects, an imbalance was noted for the key parameter of plasma leakage, mainly explainable by the local clinical practices at a site in Sri Lanka (ie, high hospitalisation rate and close monitoring with serial ultrasonography) that prevent a robust treatment comparison. Four of the 7 VCD cases in the TDV group with plasma leakage were reported from this Sri Lankan site. In addition, the low frequencies (3 of 138 VCD cases in the placebo group vs 7 of 130 VCD cases in the TDV group) prevent a robust comparison between the 2 groups. It is also important to consider that the comparisons are based on the smaller denominator of the number of VCD cases and not based on all subjects in the analysis set, and thus do not consider the vaccine efficacy. Review of overall clinical symptomatology along with potential confounding factors did not suggest a worsening in severity of VCD cases in the TDV group.
- **Subgroup analyses by age group and country:** Exploratory analyses by age group (4-5, 6-11, and 12-16 years) of efficacy against overall VCD or VCD leading to hospitalisation did not show clear evidence of an age effect. Exploratory subgroup analyses of efficacy by country showed positive VE values across all countries, ranging from 39.6% to 84.4% for overall VCD and from 64.6% to 100% for VCD leading to hospitalisation. As seen in the data up to 24 months post second dose, differences between countries were mainly attributable to the serotype distribution in the respective country. Although frequencies of VCD leading to hospitalisation varied between countries, as evident in the placebo group, likely due to the local clinical practices, the VE estimates for VCD leading to hospitalisation were consistently higher than for overall VCD in each participating country.
- **Second episodes of VCD:** During the period from first dose to 36 months post second dose, a total of 11 subjects reported a second VCD episode after having had a previous episode: 9 of 6687 subjects in the placebo group, including 1 whose second episode was caused by the same dengue serotype as the first episode (DENV-3), and 2 of 13,380 subjects in the TDV group, resulting in a relative risk of 0.11 based on the total safety set (95% CI: 0.02, 0.51). None of these second episodes led to hospitalisation. Of note, second episodes were not considered in analyses of overall VCD, but in cumulative analyses by dengue serotype except for the 1 case of a homotypic second episode.

Virologically Confirmed Dengue Fever From 25 to 36 Months (Year 3) Post Second Vaccine Dose

In this section, efficacy results are briefly summarised based on data from 25 to 36 months (Year 3) post second dose (based on the PPS). Results from a year-by-year analysis of the data up to 24 months post second dose have been summarised above.

During Year 3 post second dose, efficacy results were as follows:

- **VCD caused by any serotype:** The protection against overall VCD continued, in the total PPS (VE: 44.7%; 95% CI: 32.5%, 54.7%) as well as in baseline seronegative subjects (VE: 35.5%; 95% CI: 7.3%, 55.1%) and seropositive subjects (VE: 48.3%; 95% CI: 34.2%, 59.3%). Compared with Year 2, some decline in the efficacy against overall VCD was observed during Year 3, which was mainly driven by VCD that did not lead to hospitalisation.
- **VCD leading to hospitalisation:** Little change from Year 2 to Year 3 was observed in the efficacy against VCD leading to hospitalisation with VE values during Year 3 of 70.8% (95% CI: 49.6%, 83.0%) for the total PPS, 45.0% (95% CI: -42.6%, 78.8%) for baseline seronegative subjects, and

78.4% (95% CI: 57.1%, 89.1%) for baseline seropositive subjects. Differences in the VE estimates against VCD leading to hospitalisation between baseline seronegative and seropositive subjects are mainly attributable to the efficacy seen against VCD caused by DENV-3 (see next bullet); it should also be noted that the overall number of cases was small in baseline seronegative subjects, resulting in wide CIs.

- **VCD caused by individual serotypes:** With regard to the efficacy against VCD (overall and leading to hospitalisation) caused by individual dengue serotypes and by baseline serostatus in Year 3 compared with Year 2, results were as follows:
  - DENV-1: For any VCD caused by DENV-1, in baseline seronegative subjects, the vaccine efficacy estimate was marginally positive (VE: 17.2%; 95% CI: -31.8%, 47.9%), while in baseline seropositive subjects, efficacy was observed with only a modest decline (VE: 45.4%; 95% CI: 24.5%, 60.6%). For VCD leading to hospitalisation, high VE estimates similar to those in Year 2 were seen without any decline in efficacy in both baseline seronegative and seropositive subjects.
  - DENV-2: For both DENV-2 induced overall VCD and VCD leading to hospitalisation, robust efficacy was seen in Year 3 compared with Year 2 without any decline in both baseline seronegative and seropositive subjects.
  - DENV-3: For any VCD caused by DENV-3, a marginally positive efficacy estimate was seen in both baseline seronegative subjects (VE: 9.5%; 95% CI: -144.7%, 66.5%) and seropositive subjects (VE: 15.2%; 95% CI: -46.1%, 50.8%). Of note, in baseline seronegative subjects, the VE value was positive in Year 3 whereas it was negative in the 2 preceding years. For VCD leading to hospitalisation during Year 3, in baseline seronegative subjects, an imbalance in the number of cases was seen in the TDV group (7 hospitalisations of 11 VCD cases) compared with the placebo group (1 hospitalisation out of 6 VCD cases) (the randomisation ratio of 2:1 [TDV:placebo] should be considered). This imbalance is likely impacted by the different thresholds for hospitalisation at the sites in The Philippines and Sri Lanka, the 2 countries where the 17 VCD cases occurred: 6 in Sri Lanka (all in the TDV group and at 1 site and all leading to hospitalisation) and 11 in The Philippines (1 of 6 in the placebo group and 1 of 5 in the TDV group leading to hospitalisation). In the cumulative data from first dose onwards (safety set), high rates of hospitalisation have been observed in the placebo group in Sri Lanka (68 of 100 VCD cases [68.0%]) compared with The Philippines (17 of 181 VCD cases [9.4%]. The high rate of hospitalisation in Sri Lanka is likely for enhanced clinical monitoring as per local clinical practice. In baseline seropositive subjects, high efficacy similar to Year 2 was seen for VCD leading to hospitalisation in Year 3.

    -DENV-4: In baseline seronegative subjects, there were too few cases of VCD caused by DENV-4 (5 cases in total) to allow for meaningful conclusions. In baseline seropositive subjects, there was little change in the VE estimate compared to Year 2, although frequencies were lower compared to the other dengue serotypes. In a cumulative analysis from the first dose onwards (safety set), a VE value of 60.7% (95% CI: 16.0%, 81.6%) was seen in baseline seropositive subjects. Only 1 case of DENV-4 induced VCD led to hospitalisation in Year 3 (in the placebo group).

The following two tables have been prepared to illustrate the number of hospitalised cases observed in the different countries (and also according to each dengue serotype) for the placebo group for the time period 30 days to month 36 post second dose. The tables also include the "Percent of hospitalised VCD Cases Among VCD Cases".

Table 39: Hospitalised VCD (from 30 Days to month 36 post-second vaccination)- Subgroup analysis per country

Taken from TABLE 15.2.4.32.4.2				
Serotype Distribution in VCD, Hospitalized VCD, from 30 Days to 36 months Post-Second Vaccination - Subgroup Analysis by Country. Per-Protocol Set				
	PLACEBO (% of cases vs total)	TDV	TOTAL (Placebo +TDV)	Percent of Hospitalized VCD Cases Among VCD cases
(VCD) (Number of Cases)				
Philippines	165	166	331	
Sri Lanka	75	29	104	
Thailand	51	33	84	
Brazil	19	9	28	
Colombia	62	66	128	
Dominican Republic	21	26	47	
Nicaragua	17	13	30	
Panama	32	26	58	
TOTAL	442	368	810	
Hospitalized VCD Number of Cases				
Philippines	16	12	28	10 %
Sri Lanka	54 (49%)	13	67	72 %
Thailand	20	8	28	39 %
Brazil	2	0	2	10 %
Colombia	9	2	11	14 %
Dominican Republic	4	1	5	19 %
Nicaragua	3	1	4	18 %
Panama	1	0	1	3 %
TOTAL	109	37	146	

Table 40: Hospitalised VCD (from 30 Days to 36 months post-2nd vaccination)- Subgroup analysis per serotype and by country

Taken from TABLE 15.2.4.32.4.2									
Serotype Distribution in Hospitalized VCD, from 30 Days P30 Days to 36 months Post-Second Vaccination– Placebo-Subgroup Analysis by serotype and Country. Per-Protocol Set									
Serotypes	Philippines	Sri Lank(% total Hosp cases)	Thailand	Brazil	Colombia	Dominican Republic	Nicaragua	Panama	Total (Percent of Hospitalized per serotype)
DENV-1	3	4	11	2	7	4	0	0	31 (28%)
DENV-2	2	46 (46%)	7	0	2	0	3	1	61 (56%)
DENV-3	10	4	1	0	0	0	0	0	15 (14%)
DENV-4	1	0	1	0	0	0	0	0	2 (0.02%)

Virologically confirmed dengue (VCD) fever from First Dose to 54 Months Post Second Dose in Baseline Seronegative Subjects.

In response to the outstanding MO raised at D180 that questioned the indication of the TDV vaccine for baseline seronegative subjects, the applicant provided the final data of pivotal Trial DEN-301 after completion of the pre-booster phase until 54 months post second dose (Parts 1, 2 and 3 of the trial), focusing on the benefit-risk profile in baseline seronegative subjects.

Of the total of 20,071 subjects in the safety set, 18,260 subjects (91.0%) completed Part 3 of the trial, ie, 4 to 4.5 years of follow-up after the second vaccine dose; this percentage was similar in the 2 trial groups.

The following analyses were made:

- Incidences of Virologically Confirmed Dengue Cases: Compared with the 36 months data, the additional 18-month follow-up period, including 7 cases leading to hospitalisation, 1 case of DHF, and no DCAC-defined severe cases (Table 41).

Table 41: Trial DEN-301: Virologically Confirmed Dengue Episodes (Overall, Hospitalised, and Severe Forms) from First Vaccine Dose to 36 and to 54 Months Post Second Vaccine Dose in Baseline Seronegative Subjects (Safety Set)

	Number of Episodes from First Dose to 36/54 Months Post Second Dose			
	VCD	VCD Leading to Hospitalization	DHF (WHO 1997 Criteria)	DCAC-Defined Severe VCD
Total number of episodes	268/308	51/58	5/6 [a]	2/2 [a]
DENV-1	143/169	16/20	0/1	0/0
DENV-2	64/72	22/23	0/0	0/0
DENV-3	51/52	13/14	5/5 [a]	2/2 [a]
DENV-4	10/15	0/1	0/0	0/0

Source: DEN-301 CSR Pt2+18m Table 15.2.4.32.2; DEN-301 36M CSR, Table 11.ss and 11.tt, DEN-301 Pt3 Table 15.2.5.32.2 and Appendix 16.2.6.5.7.1.

Abbreviations: DCAC, dengue case adjudication committee; DHF, dengue hemorrhagic fever; VCD, virologically confirmed dengue; WHO, World Health Organization.

(a) One case reported during Year 2 post second dose was classified as both DHF and DCAC-defined severe VCD.

Vaccine against VCD and hospitalised VCD from 30 days to 54 months post second vaccine dose. These data are shown in the following Table.

Table 42: Trial DEN-301: Virologically Confirmed Dengue From 30 Days to 54 Months Post Second Vaccine Dose (Per-Protocol Set)

	Number (%) of VCD Cases		
	Placebo N = 6317	TDV N = 12,704	Vaccine Efficacy (95% CI) (%)
Number of evaluable subjects [a]	6316	12,700	
Overall VCD fever	486 (7.7)	415 (3.3)	59.2 (53.5, 64.2)
Leading to hospitalization	125 (2.0)	40 (0.3)	84.4 (77.8, 89.1)
Caused by DENV-1	210 (3.3)	213 (1.7)	50.3 (39.9, 58.9)
Caused by DENV-2	159 (2.5)	58 (0.5)	82.3 (76.1, 86.9)
Caused by DENV-3	107 (1.7)	126 (1.0)	42.8 (26.0, 55.8)
Caused by DENV-4	21 (0.3)	22 (0.2)	48.8 (6.9, 71.8)
Severe forms of dengue fever			
DCAC-defined severe VCD [b]	4 (<0.1)	3 (<0.1)	63.1 (-64.9, 91.7)
DHF (WHO criteria 1997) [b]	14 (0.2)	8 (<0.1)	71.7 (32.6, 88.1)
Baseline seropositive			
Number of evaluable subjects	4589	9167	
Overall VCD fever	350 (7.6)	274 (3.0)	62.7 (56.3, 68.2)
Leading to hospitalization	87 (1.9)	23 (0.3)	87.2 (79.7, 91.9)
Baseline seronegative			
Number of evaluable subjects	1726	3531	
Overall VCD fever	136 (7.9)	141 (4.0)	50.2 (37.0, 60.7)
Leading to hospitalization	38 (2.2)	17 (0.5)	77.9 (60.8, 87.5)

Source: DEN-301 54M CSR, Table 11.ww, Table 11.yy, and Table 11.fff.

Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue hemorrhagic fever; N, total number of subjects; VCD, virologically confirmed dengue; WHO, World Health Organization.

(a) Five subjects (1 in the placebo group, 4 in the TDV group) were not evaluable for analysis due to early discontinuation prior to 30 days post second vaccine dose.

(b) Includes 3 cases, 1 in the placebo group and 2 in the TDV group, who were classified as both DHF and DCAC-defined severe VCD.

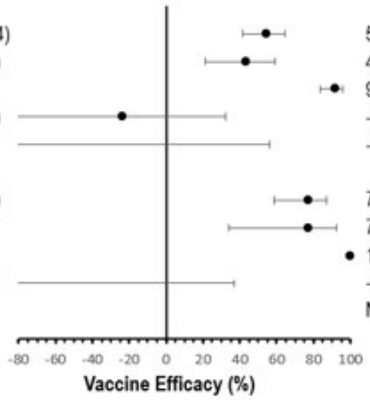
It follows further detailed analyses regarding the results obtained in baseline seronegative and seropositive subjects from 30 days to 54 months post second vaccine dose.

- **Vaccine Efficacy in Baseline Seronegative Subjects.**

The benefit of TDV against overall VCD in baseline seronegative subjects was confirmed in the analysis of VCD from first dose to 54 months post second vaccine dose with a VE estimate of 53.5% (95% CI: 41.6%, 62.9% [safety set]) (see Figure 21). The VE against VCD leading to hospitalisation was 79.3% (95% CI: 63.5%, 88.2%). Detailed analysis according to the infecting dengue virus serotype, and different periods of time are shown in Figure 21. Because of these different hospitalisation rate of VCD cases, analyses were repeated excluding the data from Sri Lanka (Figure 22).

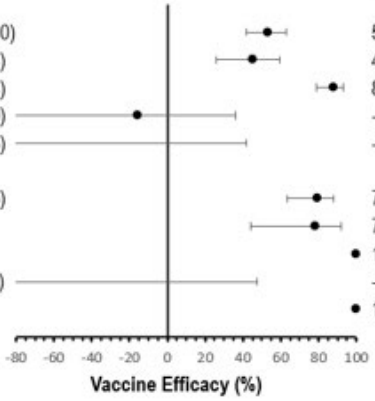
From first dose to 36 months post second dose

Variable	VCD incidence		
	Placebo	TDV	
Serotype	n/N (%)	n/N (%)	VE (95% CI) (%) [a]
Overall VCD			
Any serotype	136/1832 (7.4)	128/3714 (3.4)	54.3 (41.9, 64.1)
DENV-1	66/1832 (3.6)	77/3714 (2.1)	43.5 (21.5, 59.3)
DENV-2	55/1832 (3.0)	9/3714 (0.2)	91.9 (83.6, 96.0)
DENV-3	15/1832 (0.8)	36/3714 (1.0)	-23.4 (-125.3, 32.4)
DENV-4	2/1832 (0.1)	8/3714 (0.2)	-105.5 (-867.5, 56.4)
VCD leading to hospitalization			
Any serotype	35/1832 (1.9)	16/3714 (0.4)	77.1 (58.6, 87.3)
DENV-1	11/1832 (0.6)	5/3714 (0.1)	77.2 (34.3, 92.1)
DENV-2	22/1832 (1.2)	0/3714 (0)	100.0 (NE, NE)
DENV-3	2/1832 (0.1)	11/3714 (0.3)	-183.4 (-1178.3, 37.2)
DENV-4	0/1832 (0)	0/3714 (0)	NE (NE, NE)



From first dose to 54 months post second dose

Variable	VCD incidence		
	Placebo	TDV	
Serotype	n/N (%)	n/N (%)	VE (95% CI) (%) [a]
Overall VCD			
Any serotype	153/1832 (8.4)	147/3714 (4.0)	53.5 (41.6, 62.9)
DENV-1	79/1832 (4.3)	89/3714 (2.4)	45.4 (26.1, 59.7)
DENV-2	58/1832 (3.2)	14/3714 (0.4)	88.1 (78.6, 93.3)
DENV-3	16/1832 (0.9)	36/3714 (1.0)	-15.5 (-108.2, 35.9)
DENV-4	3/1832 (0.2)	12/3714 (0.3)	-105.6 (-628.7, 42.0)
VCD leading to hospitalization			
Any serotype	41/1832 (2.2)	17/3714 (0.5)	79.3 (63.5, 88.2)
DENV-1	14/1832 (0.8)	6/3714 (0.2)	78.4 (43.9, 91.7)
DENV-2	23/1832 (1.3)	0/3714 (0.0)	100.0 (NE, NE)
DENV-3	3/1832 (0.2)	11/3714 (0.3)	-87.9 (-573.4, 47.6)
DENV-4	1/1832 (<0.1)	0/3714 (0.0)	100.0 (NE, NE)

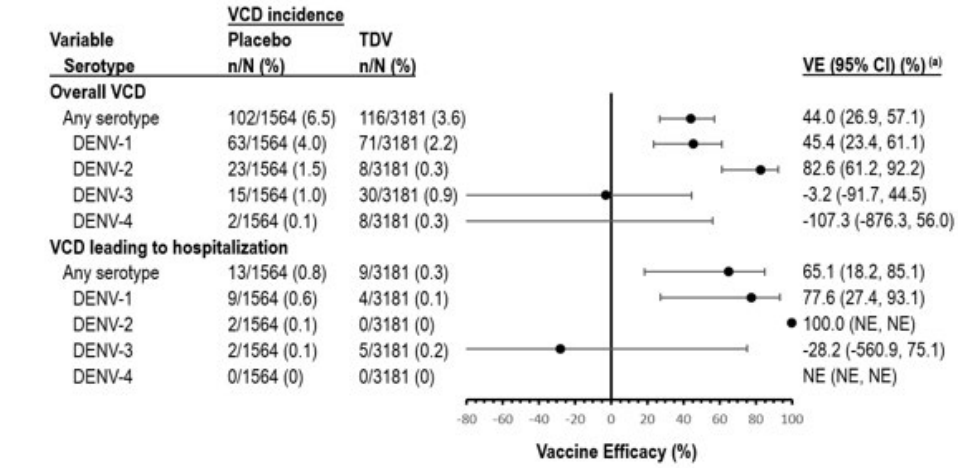


Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

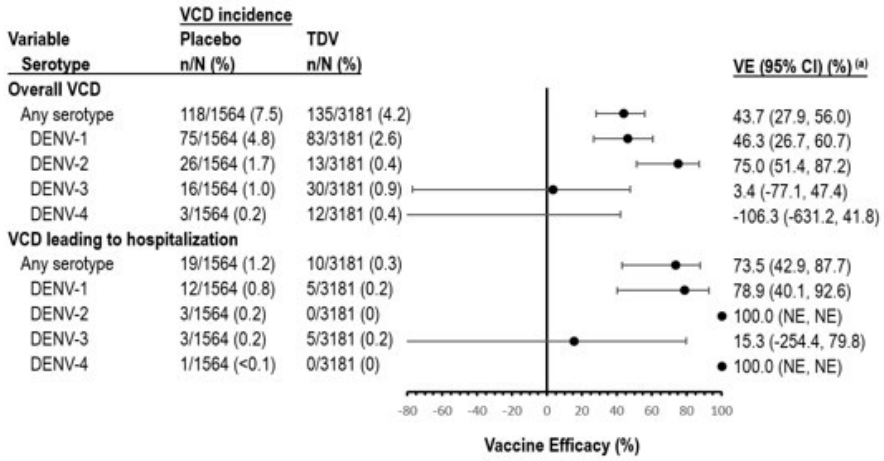
(a) VE was defined as 1 - ($\lambda V/\lambda C$), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 21: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD Leading to Hospitalisation from First Vaccine Dose to 36 and to 54 Months Post Second Dose in Baseline Seronegative Subjects (Safety Set; 1:2 Randomisation [Placebo: TDV] to Be Considered).

From first dose to 36 months post second dose



From first dose to 54 months post second dose



Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) VE was defined as 1 - ($\lambda V/\lambda C$), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 22: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD leading to Hospitalisation from First Vaccine Dose to 36 and to 54 Months Post Second Dose in Baseline Seronegative Subjects (Safety Set; 1:2 Randomisation [Placebo:TDV] to Be Considered) – Excluding Sri Lanka

During the last 18 months up to 54 months post second dose in TDV Trial DEN-301, there was 1 case of a severe form of dengue (DCAC-defined severe VCD or DHF) in baseline seronegative subjects (Table 41): a case of DHF caused by DENV-1 in the placebo group. Thus, during the entire period from first dose to 54 months post second dose, 2 cases of DHF occurred in the placebo group versus 4 cases in the TDV group (VE: 3.4%; 95% CI: -464.7%, 81.1%), the 1:2 randomisation (placebo: TDV) should be considered. For severe forms of dengue combined (DCAC-defined severe VCD and/or DHF), the frequencies were 2 versus 5 cases (VE: -29.2%; 95% CI: 566.1%, 74.9%). Of note, 1 case was classified as both DHF and DCAC-defined severe VCD (during Year 2 post second dose in the TDV group; Table 43).

Because 2 of the DHF cases in the TDV group were from Sri Lanka (both occurred during Year 3 post second dose; Table 43), incidences in corresponding sensitivity analyses excluding Sri Lanka changed to 2 versus 2 DHF cases (VE: 47.5%; 95% CI: -272.9%, 92.6%) or 2 versus 3 cases of severe forms of dengue (VE: 21.3%; 95% CI: -371.6%, 86.9%).

Table 43: Trial DEN-301: Virologically Confirmed Dengue (Overall and Leading to Hospitalisation) and Severe Forms of Dengue Caused by DENV-3 in Baseline Seronegative Subjects, in and Outside Sri Lanka (Safety Set; 1:2 Randomisation [Placebo:TDV] to Be Considered)

	Number (%) of Cases					
Period	**Total**		**Sri Lanka**		**Excluding Sri Lanka**	
Parameter	**Placebo**	**TDV**	**Placebo**	**TDV**	**Placebo**	**TDV**
From first dose to 54 months post second vaccine dose						
Number of subjects evaluated	1832	3714	268	533	1564	3181
Overall VCD	16 (0.9)	36 (1.0)	0	6 (1.1)	16 (1.0) (a)	30 (0.9) (a)
Leading to hospitalisation	3 (0.2)	11 (0.3)	0	6 (1.1)	3 (0.2)	5 (0.2)
Severe forms of dengue	1 (<0.1)	5 (0.1)	0	2 (0.4)	1 (<0.1)	3 (<0.1)
DCAC-defined severe VCD	0	2 (<0.1) (b)	0	0	0	2 (<0.1) (b)
DHF (WHO criteria 1997)	1 (<0.1)	4 (0.1) (b)	0	2 (0.4)	1 (<0.1)	2 (<0.1) (b)
From 30 days to 12 months post second dose (Year 1)						
Number of subjects evaluated	1804	3660	265	531	1539	3129
Overall VCD	4 (0.2)	11 (0.3)	0	0	4 (0.3)	11 (0.4)
Leading to hospitalisation	1 (<0.1)	1 (<0.1)	0	0	1 (<0.1)	1 (<0.1)
Severe forms of dengue	1 (<0.1)	1 (<0.1)	0	0	1 (<0.1)	1 (<0.1)
DCAC-defined severe VCD	0	1 (<0.1)	0	0	0	1 (<0.1)
DHF (WHO criteria 1997)	1 (<0.1)	0	0	0	1 (<0.1)	0
From 13 to 24 months post second vaccine dose (Year 2)						
Number of subjects evaluated	1795	3634	266	532	1529	3102
Overall VCD	5 (0.3) (c)	12 (0.3) (c)	0	0	5 (0.3) (c)	12 (0.4) (c)
Leading to hospitalisation	0	3 (<0.1) (c)	0	0	0	3 (<0.1) (c)
Severe forms of dengue	0	2 (<0.1)	0	0	0	2 (<0.1)
DCAC-defined severe VCD	0	1 (<0.1) (b)	0	0	0	1 (<0.1) (b)
DHF (WHO criteria 1997)	0	2 (<0.1) (b)	0	0	0	2 (<0.1) (b)

Period	Number (%) of Cases					
	Total		Sri Lanka		Excluding Sri Lanka	
Parameter	Placebo	TDV	Placebo	TDV	Placebo	TDV
From 25 to 36 months post second vaccine dose (Year 3)						
Number of subjects evaluated	1780	3603	266	530	1514	3073
Overall VCD	6 (0.3)	11 (0.3)	0	6 (1.1)	6 (0.4) [(c)]	5 (0.2) [(c)]
Leading to hospitalisation	1 (<0.1)	7 (0.2)	0	6 (1.1)	1 (<0.1) [(c)]	1 (<0.1) [(c)]
Severe forms of dengue	0	2 (<0.1)	0	2 (0.4)	0	0
DCAC-defined severe VCD	0	0	0	0	0	0
DHF (WHO criteria 1997)	0	2 (<0.1)	0	2 (0.4)	0	0
From 37 to 48 months post second vaccine dose (Year 4)						
Number of subjects evaluated	1761	3552	264	527	1497	3025
Overall VCD	1 (<0.1) [(d)]	0	0	0	1 (<0.1) [(d)]	0
Leading to hospitalisation	1 (<0.1) [(d)]	0	0	0	1 (<0.1) [(d)]	0
Severe forms of dengue	0	0	0	0	0	0
DCAC-defined severe VCD	0	0	0	0	0	0
DHF (WHO criteria 1997)	0	0	0	0	0	0

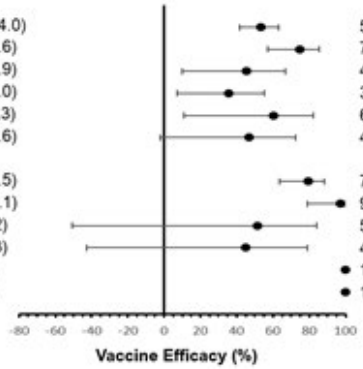
Source: DEN-301 Pt3 Table 15.2.5.300.
Abbreviations: DCAC, Dengue Case Adjudication Committee; DHF, dengue haemorrhagic fever; VCD, virologically confirmed dengue; WHO, World Health Organization.
(a) All cases from The Philippines except for 2 cases from Thailand (1 in each group) and 1 case from Colombia (in the placebo group).
(b) Includes 1 case classified as both DHF and DCAC-defined severe VCD.
(c) All cases from The Philippines.
(d) From Colombia.

- **Vaccine Efficacy by Trial Interval in Baseline Seronegative Subjects**

Results from the year-by-year analysis across dengue serotypes showed continued VE against VCD and VCD leading to hospitalisation during Year 4, ie, 37 to 48 months post second vaccine dose, in baseline seronegative subjects with VE estimates of 60.2% and 100%, respectively (Figure 23).

In the last 18 months of the cumulative period (including Year 4), the VE against overall VCD was 46.9% and against hospitalised VCD 100% (Figure 23). It should be noted that not all subjects completed the last 6 months following Year 4, as some were enrolled into the booster phase. Also, not all subjects will have passed through the peak dengue season during the months following Year 4, which may have introduced bias.

Variable Period [a]	VCD incidence Placebo n/N (%)	TDV n/N (%)	VE (95% CI) (%) [b]
Overall VCD			
Cumulative	153/1832 (8.4)	147/3714 (4.0)	53.5 (41.6, 62.9)
Year 1	39/1726 (2.3)	20/3531 (0.6)	74.9 (57.0, 85.4)
Year 2	29/1715 (1.7)	33/3498 (0.9)	45.3 (9.9, 66.8)
Year 3	51/1698 (3.0)	69/3465 (2.0)	35.5 (7.3, 55.1)
Year 4	13/1677 (0.8)	11/3403 (0.3)	60.2 (11.1, 82.1)
Last 18 months	17/1676 (1.0)	19/3402 (0.6)	46.9 (-2.1, 72.4)
VCD leading to hospitalization			
Cumulative	41/1832 (2.2)	17/3714 (0.5)	79.3 (63.5, 88.2)
Year 1	18/1726 (1.0)	1/3531 (<0.1)	97.2 (79.1, 99.6)
Year 2	6/1715 (0.3)	6/3498 (0.2)	51.4 (-50.7, 84.3)
Year 3	8/1698 (0.5)	9/3465 (0.3)	45.0 (-42.6, 78.8)
Year 4	5/1677 (0.3)	0/3403 (0)	100.0 (NE, NE)
Last 18 months	5/1676 (0.3)	0/3402 (0)	100.0 (NE, NE)



Source: DEN-301 36M CSR, Table 11.kk; DEN-301 Pt3 Table 15.2.5.2.5.1, 15.2.5.2.9.1.6, 15.2.5.3.3, 15.2.5.9.5.1, 15.2.5.9.8.1.5, 15.2.5.10.3.

Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) Cumulative (safety set): from first dose to 54 months post second dose; Time intervals (per-protocol set): Year 1: from 30 days to 12 months post second dose; Year 2: from 13 to 24 months post second dose; Year 3: from 25 to 36 months post second dose; Year 4: from 37 to 48 months post second dose; last 18 months: from 37 to 54 months post second dose.

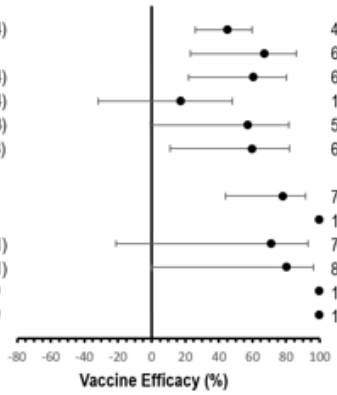
Figure 23: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD leading to Hospitalisation on Baseline Seronegative subjects, Cumulative (Safety Set) and by Yearly Intervals (Per-Protocol Set) (1:2 Randomisation [Placebo: TDV] to Be Considered)

- **Vaccine Efficacy by Dengue Serotype in Baseline Seronegative Subjects**

The data from Year 4 showed continued protection against overall VCD caused by the 2 most common dengue serotypes DENV-1 and DENV-2, with VE estimates of 57.1% and 100%, respectively (see Figure 24).

DENV-1:

Variable	VCD incidence	
	Placebo	TDV
Period [a]	n/N (%)	n/N (%)
Overall VCD		
Cumulative	79/1832 (4.3)	89/3714 (2.4)
Year 1	13/1726 (0.8)	9/3531 (0.3)
Year 2	18/1715 (1.0)	15/3498 (0.4)
Year 3	28/1698 (1.6)	49/3465 (1.4)
Year 4	11/1677 (0.7)	10/3403 (0.3)
Last 18 months	13/1676 (0.8)	11/3402 (0.3)
VCD leading to hospitalization		
Cumulative	14/1832 (0.8)	6/3714 (0.2)
Year 1	1/1726 (<0.1)	0/3531 (0)
Year 2	5/1715 (0.3)	3/3498 (<0.1)
Year 3	5/1698 (0.3)	2/3465 (<0.1)
Year 4	3/1677 (0.2)	0/3403 (0) [c]
Last 18 months	3/1676 (0.2)	0/3402 (0) [c]



DENV-2:

Variable	VCD incidence	
	Placebo	TDV
Period [a]	n/N (%)	n/N (%)
Overall VCD		
Cumulative	58/1832 (3.2)	14/3714 (0.4)
Year 1	22/1726 (1.3)	0/3531 (0)
Year 2	5/1715 (0.3)	3/3498 (<0.1)
Year 3	16/1698 (0.9)	5/3465 (0.1)
Year 4	1/1677 (<0.1)	0/3403 (0)
Last 18 months	3/1676 (0.2)	4/3402 (0.1)
VCD leading to hospitalization		
Cumulative	23/1832 (1.3)	0/3714 (0)
Year 1	16/1726 (0.9)	0/3531 (0)
Year 2	1/1715 (<0.1)	0/3498 (0)
Year 3	2/1698 (0.1)	0/3465 (0)
Year 4	1/1677 (<0.1)	0/3403 (0)
Last 18 months	1/1676 (<0.1)	0/3402 (0)

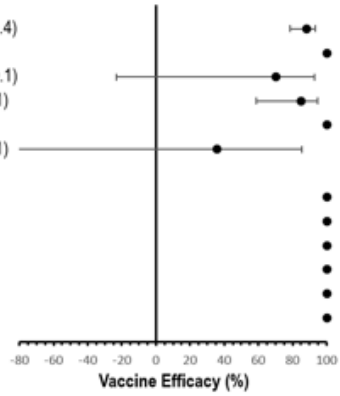
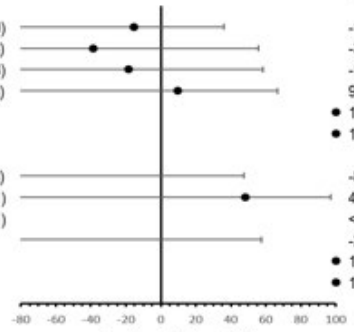


Figure 24: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD leading to Hospitalisation in Baseline Seronegative Subjects, by Dengue Serotype – Cumulative (Safety Set) and by Yearly Intervals (Per-Protocol Set) (1:2 Randomisation [Placebo: TDV] to Be Considered)

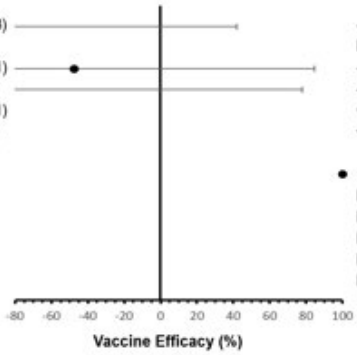
DENV-3:

Variable	VCD incidence Placebo	TDV	VE (95% CI) (%) [b]
Period [a]	n/N (%)	n/N (%)	
Overall VCD			
Cumulative	16/1832 (0.9)	36/3714 (1.0)	-15.5 (-108.2, 35.9)
Year 1	4/1726 (0.2)	11/3531 (0.3)	-38.7 (-335.7, 55.8)
Year 2	5/1715 (0.3)	12/3498 (0.3)	-18.5 (-236.2, 58.3)
Year 3	6/1698 (0.4)	11/3465 (0.3)	9.5 (-144.7, 66.5)
Year 4	1/1677 (<0.1)	0/3403 (0)	100.0 (NE, NE)
Last 18 months	1/1676 (<0.1)	0/3402 (0)	100.0 (NE, NE)
VCD leading to hospitalization			
Cumulative	3/1832 (0.2)	11/3714 (0.3)	-87.9 (-573.4, 47.6)
Year 1	1/1726 (<0.1)	1/3531 (<0.1)	48.3 (-727.0, 96.8)
Year 2	0/1715 (0)	3/3498 (<0.1)	<-999.0 (NE, NE)
Year 3	1/1698 (<0.1)	7/3465 (0.2)	-246.6 (-2716.1, 57.3)
Year 4	1/1677 (<0.1)	0/3403 (0)	100.0 (NE, NE)
Last 18 months	1/1676 (<0.1)	0/3402 (0)	100.0 (NE, NE)



DENV-4:

Variable	VCD incidence Placebo	TDV	VE (95% CI) (%) [b]
Period [a]	n/N (%)	n/N (%)	
Overall VCD			
Cumulative	3/1832 (0.2)	12/3714 (0.3)	-105.6 (-628.7, 42.0)
Year 1	0/1726 (0)	0/3531 (0)	NE (NE, NE)
Year 2	1/1715 (<0.1)	3/3498 (<0.1)	-47.6 (-1319.1, 84.6)
Year 3	1/1698 (<0.1)	4/3465 (0.1)	-99.0 (-1680.3, 77.8)
Year 4	0/1677 (0)	1/3403 (<0.1)	<-999.0 (NE, NE)
Last 18 months	0/1676 (0)	4/3402 (0.1)	<-999.0 (NE, NE)
VCD leading to hospitalization			
Cumulative	1/1832 (<0.1)	0/3714 (0)	100.0 (NE, NE)
Year 1	0/1726 (0)	0/3531 (0)	NE (NE, NE)
Year 2	0/1715 (0)	0/3498 (0)	NE (NE, NE)
Year 3	0/1698 (0)	0/3465 (0)	NE (NE, NE)
Year 4	0/1677 (0) [c]	0/3403 (0)	NE (NE, NE)
Last 18 months	0/1676 (0) [c]	0/3402 (0)	NE (NE, NE)



Source: DEN-301 36M CSR, Table 11.kk; DEN-301 Pt3 Table 15.2.5.7.2.1, 15.2.5.7.3.2, 15.2.5.8.2, 15.2.5.11.2.1, 15.2.5.11.3.2, 15.2.5.12.2.

Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) Cumulative (safety set): from first dose to 54 months post second dose; Time intervals (per-protocol set): Year 1: from 30 days to 12 months post second dose; Year 2: from 13 to 24 months post second dose; Year 3: from 25 to 36 months post second dose; Year 4: from 37 to 48 months post second dose; last 18 months: from 37 to 54 months post second dose.

(b) VE was defined as 1 - (λV/λC), where λV and λC denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

(c) One case of hospitalized VCD that occurred during Year 4/last 18 months was not counted in the interval analysis because of the prespecified censoring rules.

The reference point for the yearly intervals is the date of second vaccination, while the reference point for the interval "Last 18 months" is the End of Part 2 date, which was not fixed for all subjects (some subjects had a duration of follow-up in Part 2 of >6 months).

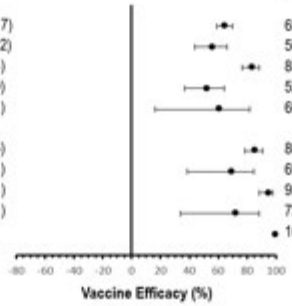
Figure 24 (continued): Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD Leading to Hospitalisation in Baseline Seronegative Subjects, by Dengue Serotype – Cumulative (Safety Set) and by Yearly Intervals (Per-Protocol Set) (1:2 Randomisation [Placebo:TDV] to Be Considered

- **Vaccine Efficacy in Baseline Seropositive Subjects**

Detailed VE analysis according to the infecting dengue virus serotype, and different periods of time are shown in the next two figures. Because of the different hospitalisation rate of VCD cases, analyses were repeated excluding the data from Sri Lanka, which was the county with the highest VCD hospitalisation rate.

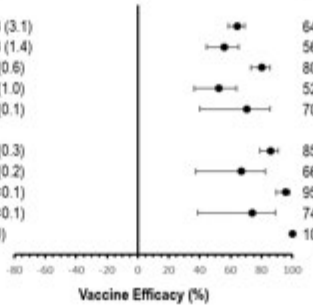
From first dose to 36 months post second dose

Variable	VCD incidence Placebo	TDV	VE (95% CI) (%) [a]
Serotype	n/N (%)	n/N (%)	
Overall VCD			
Any serotype	358/4854 (7.4)	262/9663 (2.7)	65.0 (58.9, 70.1)
DENV-1	130/4854 (2.7)	114/9663 (1.2)	56.2 (43.7, 66.0)
DENV-2	124/4854 (2.6)	42/9663 (0.4)	83.4 (76.4, 88.3)
DENV-3	95/4854 (2.0)	94/9663 (1.0)	52.3 (36.6, 64.2)
DENV-4	15/4854 (0.3)	12/9663 (0.1)	60.7 (16.0, 81.6)
VCD leading to hospitalization			
Any serotype	91/4854 (1.9)	26/9663 (0.3)	86.0 (78.4, 91.0)
DENV-1	21/4854 (0.4)	13/9663 (0.1)	69.2 (38.5, 84.6)
DENV-2	53/4854 (1.1)	5/9663 (<0.1)	95.3 (88.4, 98.1)
DENV-3	14/4854 (0.3)	8/9663 (<0.1)	72.1 (33.6, 88.3)
DENV-4	3/4854 (<0.1)	0/9663 (0)	100.0 (NE, NE)



From first dose to 54 months post second dose

Variable	VCD incidence Placebo	TDV	VE (95% CI) (%) [a]
Serotype	n/N (%)	n/N (%)	
Overall VCD			
Any serotype	394/4854 (8.1)	295/9663 (3.1)	64.2 (58.4, 69.2)
DENV-1	151/4854 (3.1)	133/9663 (1.4)	56.1 (44.6, 65.2)
DENV-2	135/4854 (2.8)	54/9663 (0.6)	80.4 (73.1, 85.7)
DENV-3	97/4854 (2.0)	96/9663 (1.0)	52.3 (36.7, 64.0)
DENV-4	20/4854 (0.4)	12/9663 (0.1)	70.6 (39.9, 85.6)
VCD leading to hospitalization			
Any serotype	101/4854 (2.1)	29/9663 (0.3)	85.9 (78.7, 90.7)
DENV-1	24/4854 (0.5)	16/9663 (0.2)	66.8 (37.4, 82.3)
DENV-2	59/4854 (1.2)	5/9663 (<0.1)	95.8 (89.6, 98.3)
DENV-3	15/4854 (0.3)	8/9663 (<0.1)	74.0 (38.6, 89.0)
DENV-4	3/4854 (<0.1)	0/9663 (0)	100.0 (NE, NE)



Source: DEN-301 CSR, Pt2+18m Table 15.2.4.3.3, 15.2.4.8.2, 15.2.4.10.3, and 15.2.4.12.2; DEN-301, Pt3 Table 15.2.5.3.3, 15.2.5.8.2, 15.2.5.10.3, and 15.2.5.12.2.

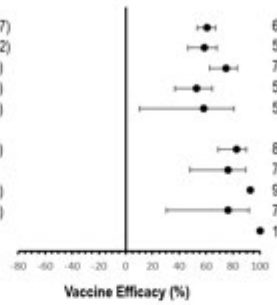
Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) VE was defined as 1 - (λ_V/λ_C), where λ_V and λ_C denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 25: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD leading to Hospitalisation From First Vaccine Dose to 36 and to 54 Months Post Second Dose in Baseline Seropositive Subjects (Safety Set; 1:2 Randomisation [Placebo:TDV] to Be Considered)

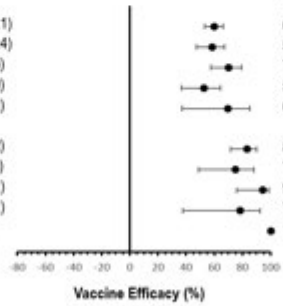
From first dose to 36 months post second dose

Variable	VCD incidence		
	Placebo	TDV	
Serotype	n/N (%)	n/N (%)	VE (95% CI) (%) [a]
Overall VCD			
Any serotype	294/4422 (6.6)	241/8802 (2.7)	60.7 (53.4, 66.9)
DENV-1	124/4422 (2.8)	103/8802 (1.2)	58.6 (46.3, 68.1)
DENV-2	71/4422 (1.6)	36/8802 (0.4)	74.9 (62.5, 83.2)
DENV-3	90/4422 (2.0)	90/8802 (1.0)	52.6 (36.5, 64.6)
DENV-4	14/4422 (0.3)	12/8802 (0.1)	58.4 (10.1, 80.8)
VCD leading to hospitalization			
Any serotype	45/4422 (1.0)	16/8802 (0.2)	82.5 (69.1, 90.1)
DENV-1	19/4422 (0.4)	9/8802 (0.1)	76.5 (48.1, 89.4)
DENV-2	14/4422 (0.3)	2/8802 (<0.1)	92.8 (68.5, 98.4)
DENV-3	10/4422 (0.2)	5/8802 (<0.1)	76.1 (30.2, 91.8)
DENV-4	2/4422 (<0.1)	0/8802 (0)	100.0 (NE, NE)



From first dose to 54 months post second dose

Variable	VCD incidence		
	Placebo	TDV	
Serotype	n/N (%)	n/N (%)	VE (95% CI) (%) [a]
Overall VCD			
Any serotype	328/4422 (7.4)	273/8802 (3.1)	60.1 (53.2, 66.1)
DENV-1	145/4422 (3.3)	121/8802 (1.4)	58.5 (47.2, 67.4)
DENV-2	80/4422 (1.8)	48/8802 (0.5)	70.3 (57.5, 79.2)
DENV-3	92/4422 (2.1)	92/8802 (1.0)	52.5 (36.6, 64.4)
DENV-4	19/4422 (0.4)	12/8802 (0.1)	69.6 (37.3, 85.2)
VCD leading to hospitalization			
Any serotype	53/4422 (1.2)	18/8802 (0.2)	83.3 (71.4, 90.2)
DENV-1	22/4422 (0.5)	11/8802 (0.1)	75.1 (48.7, 87.9)
DENV-2	18/4422 (0.4)	2/8802 (<0.1)	94.4 (76.0, 98.7)
DENV-3	11/4422 (0.2)	5/8802 (<0.1)	78.3 (37.4, 92.4)
DENV-4	2/4422 (<0.1)	0/8802 (0)	100.0 (NE, NE)



Source: DEN-301 Tables and Figures Supporting CHMP Response, Table P-15.2.4.10.13, R-15.2.4.3.12, R-15.2.4.8.4, and R-15.2.4.12.4; DEN-301, Pt3 Table 15.2.5.3.3.1, 15.2.5.8.2.1, 15.2.5.10.3.1, and 15.2.5.12.2.1.

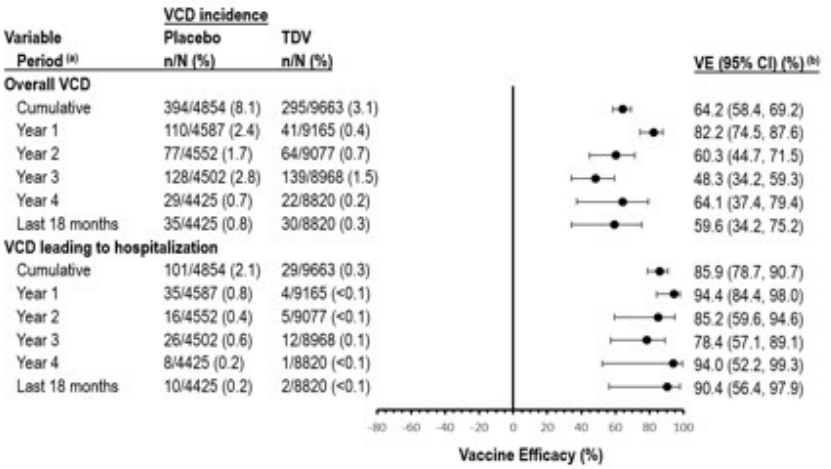
Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) VE was defined as 1 - (λ_V/λ_C), where λ_V and λ_C denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 26: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD leading to Hospitalisation From First Vaccine Dose to 36 and to 54 Months Post Second Dose in Baseline Seropositive Subjects (Safety Set; 1:2 Randomisation [Placebo:TDV] to Be Considered) – excluding Sri Lanka

- **Vaccine Efficacy by Trial Interval in Baseline Seropositive Subjects**

Results from the year-by-year analysis across dengue serotypes is shown in the next Figure.



Source: DEN-301 36M CSR, Table 11.kk; DEN-301 Pt3 Table 15.2.5.2.5.1, 15.2.5.2.9.1.6, 15.2.5.3.3, 15.2.5.9.5.1, 15.2.5.9.8.1.5, 15.2.5.10.3.

Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

Note: The reference point for the yearly intervals is the date of second vaccination, while the reference point for the interval "Last 18 months" is the End of Part 2 date, which was not fixed for all subjects (some subjects had a duration of follow-up in Part 2 of >6 months).

(a) Cumulative (safety set): from first dose to 54 months post second dose; Time intervals (per-protocol set): Year 1: from 30 days to 12 months post second dose; Year 2: from 13 to 24 months post second dose; Year 3: from 25 to 36 months post second dose; Year 4: from 37 to 48 months post second dose; last 18 months: from 37 to 54 months post second dose.

(b) VE was defined as 1 - (λ_V/λ_C), where λ_V and λ_C denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

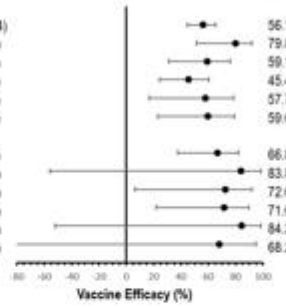
Figure 27: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD Leading to Hospitalisation in Baseline Seropositive Subjects, Cumulative (Safety Set) and by Yearly Intervals (Per-Protocol Set) (1:2 Randomisation [Placebo: TDV] to Be Considered)

- **Vaccine Efficacy by Dengue Serotype in Baseline Seropositive Subjects**

The results are shown in the next representations:

DENV-1:

Variable Period [a]	VCD incidence Placebo n/N (%)	TDV n/N (%)	VE (95% CI) (%) [b]
Overall VCD			
Cumulative	151/4854 (3.1)	133/9663 (1.4)	56.1 (44.6, 65.2)
Year 1	17/4587 (0.4)	7/9165 (<0.1)	79.8 (51.3, 91.6)
Year 2	31/4552 (0.7)	26/9077 (0.3)	59.1 (31.1, 75.7)
Year 3	69/4502 (1.5)	77/8968 (0.9)	45.4 (24.5, 60.6)
Year 4	18/4425 (0.4)	16/8820 (0.2)	57.7 (17.0, 78.4)
Last 18 months	20/4425 (0.5)	17/8820 (0.2)	59.6 (22.8, 78.8)
VCD leading to hospitalization			
Cumulative	24/4854 (0.5)	16/9663 (0.2)	66.8 (37.4, 82.3)
Year 1	3/4587 (<0.1)	1/9165 (<0.1)	83.8 (-56.1, 98.3)
Year 2	7/4552 (0.2)	4/9077 (<0.1)	72.6 (6.3, 92.0)
Year 3	10/4502 (0.2)	6/8968 (<0.1)	71.6 (21.7, 89.7)
Year 4	3/4425 (<0.1)	1/8820 (<0.1)	84.2 (-51.9, 98.4)
Last 18 months	3/4425 (<0.1)	2/8820 (<0.1)	68.2 (-90.2, 94.7)



DENV-2:

Variable Period [a]	VCD incidence Placebo n/N (%)	TDV n/N (%)	VE (95% CI) (%) [b]
Overall VCD			
Cumulative	135/4854 (2.8)	54/9663 (0.6)	80.4 (73.1, 85.7)
Year 1	42/4587 (0.9)	3/9165 (<0.1)	96.5 (88.8, 98.9)
Year 2	22/4552 (0.5)	11/9077 (0.1)	75.5 (49.5, 88.1)
Year 3	34/4502 (0.8)	20/8968 (0.2)	72.1 (51.6, 84.0)
Year 4	6/4425 (0.1)	4/8820 (<0.1)	68.3 (-12.5, 91.1)
Last 18 months	9/4425 (0.2)	11/8820 (0.1)	42.6 (-38.7, 76.2)
VCD leading to hospitalization			
Cumulative	59/4854 (1.2)	5/9663 (<0.1)	95.8 (89.6, 98.3)
Year 1	27/4587 (0.6)	0/9165 (0)	100.0 (NE, NE)
Year 2	6/4552 (0.1)	0/9077 (0)	100.0 (NE, NE)
Year 3	9/4502 (0.2)	2/8968 (<0.1)	89.4 (51.1, 97.7)
Year 4	4/4425 (<0.1)	0/8820 (0)	100.0 (NE, NE)
Last 18 months	6/4425 (0.1)	0/8820 (0)	100.0 (NE, NE)

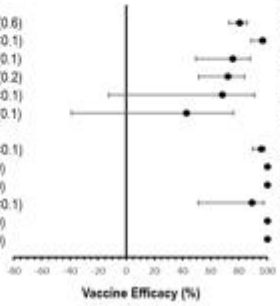
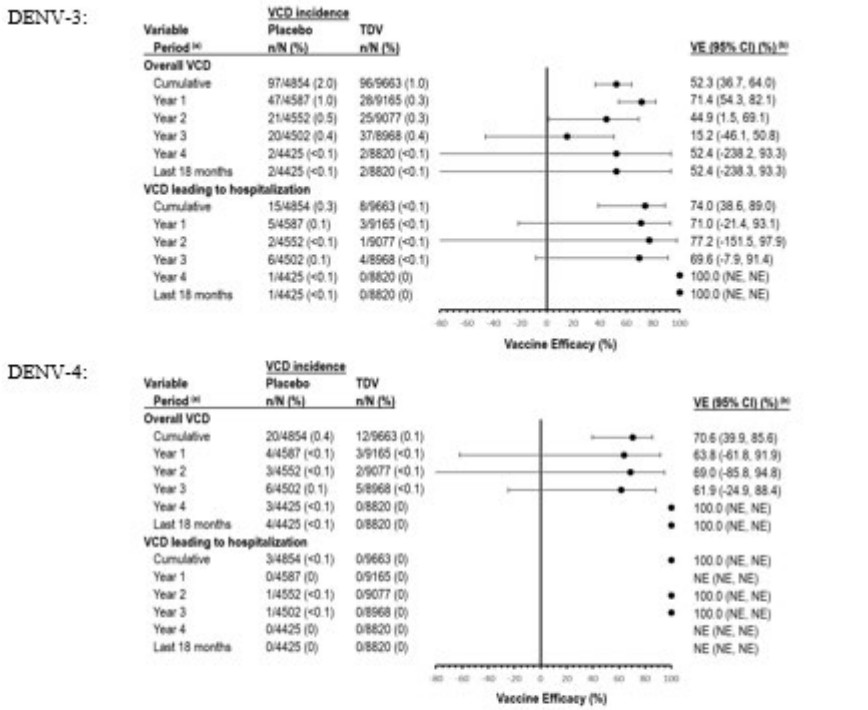


Figure 28: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD Leading to Hospitalisation in Baseline Seropositive Subjects, by Dengue Serotype – Cumulative (Safety Set) and by Yearly Intervals (Per-Protocol Set) (1:2 Randomisation [Placebo:TDV] to Be Considered)



Source: DEN-301 36M CSR, Table 11.kk; DEN-301 Pt3 Table 15.2.5.7.2.1, 15.2.5.7.3.2, 15.2.5.8.2, 15.2.5.11.2.1, 15.2.5.11.3.2, 15.2.5.12.2.

Abbreviations: CI, confidence interval; n, number of subjects with VCD; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

Note: The reference point for the yearly intervals is the date of second vaccination, while the reference point for the interval "Last 18 months" is the End of Part 2 date, which was not fixed for all subjects (some subjects had a duration of follow-up in Part 2 of >6 months).

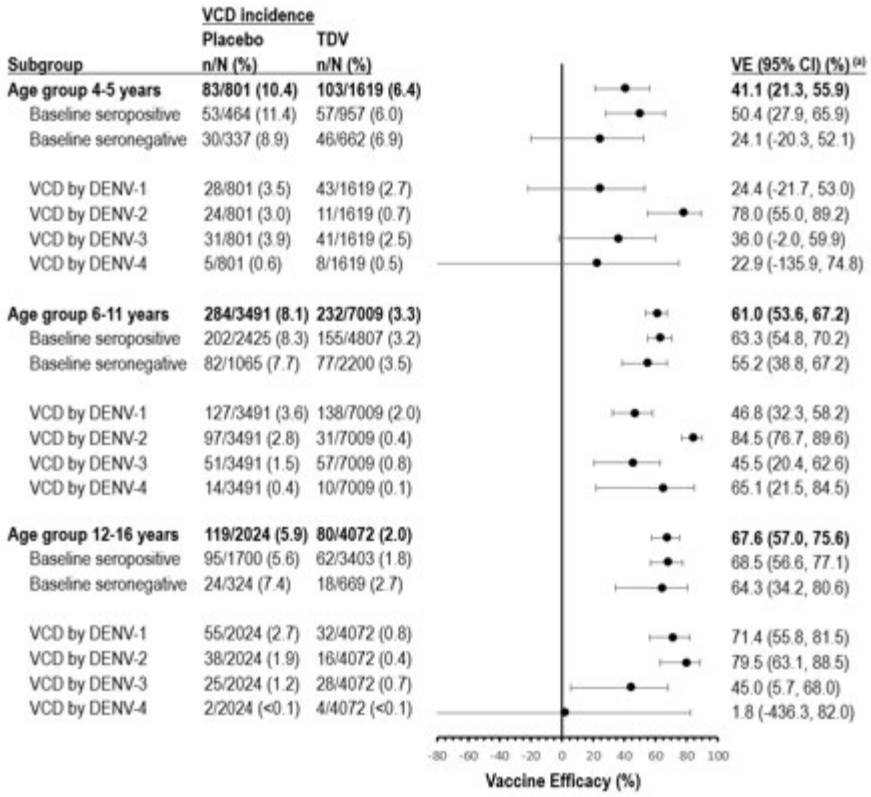
(a) Cumulative (safety set): from first dose to 54 months post second dose; Time intervals (per-protocol set): Year 1: from 30 days to 12 months post second dose; Year 2: from 13 to 24 months post second dose; Year 3: from 25 to 36 months post second dose; Year 4: from 37 to 48 months post second dose; last 18 months: from 37 to 54 months post second dose.

(b) VE was defined as 1 - (λ_V/λ_C), where λ_V and λ_C denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 28 (continued): Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for Overall VCD and VCD Leading to Hospitalisation in Baseline Seropositive Subjects, by Dengue Serotype – Cumulative (Safety Set) and by Yearly Intervals (Per-Protocol Set) (1:2 Randomisation [Placebo:TDV] to Be Considered)

- **VE data against VCD fever and VCD leading to hospitalisation up to 54 months post second dose by age group and baseline serostatus**

Upon request, the applicant provided vaccine efficacy data against VCD fever and VCD leading to hospitalisation up to 54 months post second vaccine dose by age group and baseline serostatus. These results are shown in the following representations.



Source: DEN-301 54M CSR, Table 11.zz

Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue; VE, vaccine efficacy.

(a) VE was defined as 1 - (λ_V/λ_C), where λ_V and λ_C denote the hazard rates for developing VCD fever for the TDV and placebo arms, respectively.

Figure 29: Trial DEN-301: Forest Plot of Vaccine Efficacy (95% Confidence Interval) for VCD (Overall) During 30 Days to 54 Months Post Second Vaccine Dose by Age Group (Per-Protocol Set)

Table 44: Trial DEN-301: Vaccine Efficacy Against Virologically Confirmed Dengue Leading to Hospitalisation From 30 Days to 54 Months Post Second Vaccine Dose by Age Group (Per-Protocol Set)

	Placebo		TDV		
	n/N (%)	Incidence Density [a]	n/N (%)	Incidence Density [a]	Vaccine Efficacy (95% CI) (%)
From 30 days to 54 months post second vaccine dose					
4-5 years	14/801 (1.7)	0.4	11/1619 (0.7)	0.2	61.5 (15.1, 82.5)
Baseline seropositive	7/464 (1.5)	0.4	7/957 (0.7)	0.2	51.7 (-37.7, 83.1)
Baseline seronegative	7/337 (2.1)	0.5	4/662 (0.6)	0.1	70.2 (-2.0, 91.3)
6-11 years	81/3491 (2.3)	0.5	22/7009 (0.3)	<0.1	86.8 (78.9, 91.8)
Baseline seropositive	55/2425 (2.3)	0.5	10/4807 (0.2)	<0.1	91.3 (82.9, 95.5)
Baseline seronegative	26/1065 (2.4)	0.6	12/2200 (0.5)	0.1	76.9 (54.2, 88.4)
12-16 years	30/2024 (1.5)	0.4	7/4072 (0.2)	<0.1	88.6 (74.1, 95.0)
Baseline seropositive	25/1700 (1.5)	0.4	6/3403 (0.2)	<0.1	88.2 (71.2, 95.2)
Baseline seronegative	5/324 (1.5)	0.4	1/669 (0.1)	<0.1	91.0 (23.0, 99.0)
From 30 days to 12 months post second vaccine dose (Year 1)					
4-5 years	3/801 (0.4)	0.4	1/1619 (<0.1)	<0.1	83.7 (-56.5, 98.3)
Baseline seropositive	1/464 (0.2)	0.2	1/957 (0.1)	0.1	52.3 (-662.3, 97.0)
Baseline seronegative	2/337 (0.6)	0.7	0/662 (0)	0.0	100.0 (NE, NE)
6-11 years	33/3491 (0.9)	1.0	2/7009 (<0.1)	<0.1	97.1 (87.8, 99.3)
Baseline seropositive	19/2423 (0.8)	0.9	1/4806 (<0.1)	<0.1	97.5 (81.5, 99.7)
Baseline seronegative	14/1065 (1.3)	1.5	1/2200 (<0.1)	<0.1	96.4 (72.5, 99.5)
12-16 years	17/2024 (0.8)	0.9	2/4072 (<0.1)	<0.1	94.2 (74.9, 98.7)
Baseline seropositive	15/1700 (0.9)	1.0	2/3402 (<0.1)	<0.1	93.4 (71.0, 98.5)
Baseline seronegative	2/324 (0.6)	0.7	0/669 (0)	0.0	100.0 (NE, NE)
From 13 to 24 months post second vaccine dose (Year 2)					
4-5 years	4/795 (0.5)	0.5	5/1603 (0.3)	0.3	40.1 (-123.0, 83.9)
Baseline seropositive	3/461 (0.7)	0.7	2/948 (0.2)	0.2	68.7 (-87.1, 94.8)
Baseline seronegative	1/334 (0.3)	0.3	3/655 (0.5)	0.5	-54.6 (-1387.6, 83.9)
6-11 years	15/3476 (0.4)	0.4	5/6961 (<0.1)	<0.1	84.1 (56.1, 94.2)
Baseline seropositive	11/2415 (0.5)	0.5	2/4775 (<0.1)	<0.1	91.4 (61.0, 98.1)
Baseline seronegative	4/1060 (0.4)	0.4	3/2184 (0.1)	0.1	64.4 (-59.0, 92.0)
12-16 years	3/1997 (0.2)	0.2	1/4013 (<0.1)	<0.1	84.2 (-52.1, 98.4)
Baseline seropositive	2/1676 (0.1)	0.1	1/3354 (<0.1)	<0.1	75.9 (-166.4, 97.8)
Baseline seronegative	1/321 (0.3)	0.3	0/659 (0)	0.0	100.0 (NE, NE)

Table 44 (continued): Trial DEN-301: Vaccine Efficacy Against Virologically Confirmed Dengue Leading to Hospitalisation From 30 Days to 54 Months Post Second Vaccine Dose by Age Group (Per-Protocol Set)

	Placebo		TDV		
	n/N (%)	Incidence Density [a]	n/N (%)	Incidence Density [a]	Vaccine Efficacy (95% CI) (%)
From 25 to 36 months post second vaccine dose (Year 3)					
4-5 years	3/788 (0.4)	0.4	5/1593 (0.3)	0.3	21.0 (-230.6, 81.1)
Baseline seropositive	1/457 (0.2)	0.2	4/941 (0.4)	0.4	-80.7 (-1517.3, 79.8)
Baseline seronegative	2/331 (0.6)	0.6	1/652 (0.2)	0.2	73.0 (-197.4, 97.6)
6-11 years	23/3453 (0.7)	0.7	12/6910 (0.2)	0.2	75.2 (50.2, 87.7)
Baseline seropositive	18/2401 (0.7)	0.8	5/4743 (0.1)	0.1	87.0 (65.0, 95.2)
Baseline seronegative	5/1051 (0.5)	0.5	7/2165 (0.3)	0.3	28.7 (-124.9, 77.4)
12-16 years	8/1960 (0.4)	0.4	4/3932 (0.1)	0.1	76.6 (22.3, 93.0)
Baseline seropositive	7/1644 (0.4)	0.5	3/3284 (<0.1)	<0.1	79.9 (22.1, 94.8)
Baseline seronegative	1/316 (0.3)	0.3	1/648 (0.2)	0.2	52.3 (-669.7, 97.0)
From 37 to 48 months post second vaccine dose (Year 4)					
4-5 years	3/781 (0.4)	0.4	0/1580 (0)	0.0	100.0 (NE, NE)
Baseline seropositive	2/454 (0.4)	0.5	0/936 (0)	0.0	100.0 (NE, NE)
Baseline seronegative	1/327 (0.3)	0.3	0/644 (0)	0.0	100.0 (NE, NE)
6-11 years	8/3427 (0.2)	0.3	1/6840 (<0.1)	<0.1	94.1 (52.8, 99.3)
Baseline seropositive	5/2381 (0.2)	0.2	1/4705 (<0.1)	<0.1	90.7 (20.6, 98.9)
Baseline seronegative	3/1045 (0.3)	0.3	0/2133 (0)	0.0	100.0 (NE, NE)
12-16 years	2/1895 (0.1)	0.1	0/3805 (0)	0.0	100.0 (NE, NE)
Baseline seropositive	1/1590 (<0.1)	<0.1	0/3179 (0)	0.0	100.0 (NE, NE)
Baseline seronegative	1/305 (0.3)	0.4	0/626 (0)	0.0	100.0 (NE, NE)

Source: DEN-301 54M CSR, Table 11.zz.

Abbreviations: CI, confidence interval; n, number of subjects with VCD fever; N, total number of evaluable subjects; NE, not estimable; VCD, virologically confirmed dengue.

(a) Number of VCD fever cases per 100 person-years.

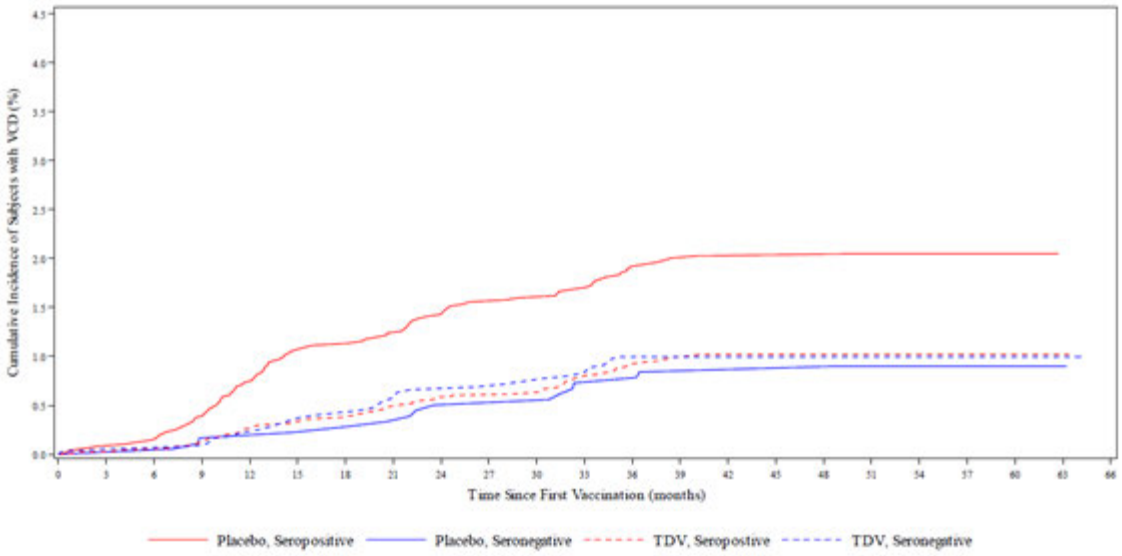
Additional data provided now by the applicant describe VE covering the period from 30 days to 54 months post second dose (PP set). It is shown that VE against VCD was high in the three age groups analysed, with the lowest VE estimate [41% (95%CI: 21%, 55%)] observed in the youngest age group (4-5 years of age). VE estimates were always positive when VE was calculated, for each age group, according to the baseline serostatus, and also when calculated, for each age group, according to the serotype of the infected Dengue virus. It is noted, however, that 95%CI of the VE estimates were wide, and in some cases crossed zero, due to the few cases that were observed in some of the subgroups analysed.

Similarly, VE estimates against hospitalised VCD were positive (with wide CI), for both baseline seropositive and seronegative subjects when each age group was analysed, from 30 days to 54 months post second dose (PP set), according to the baseline serostatus. Importantly, no evidence of increased hospitalised VCD for any age group was observed when the data were analysed by one-year intervals.

- ***Analyses of VCD Caused by DENV-3 or DENV-4 in Baseline Seronegative Subjects***

VCD Caused by DENV-3

The data up to 54 months post second dose did not suggest efficacy against VCD caused by DENV-3 in baseline seronegative subjects, both in the cumulative and the year-by-year analysis (see Figure 24). This is also reflected in the cumulative incidence curve (see Figure 30).



Source: DEN-301, Pt3 Figure 15.2.5.7.3.
Abbreviations: VCD, virologically confirmed dengue.

Figure 30: Trial DEN-301: Cumulative Incidence of Virologically Confirmed Dengue Fever Caused by DENV-3 Over Time from First Dose Until 54 Months Post Second Vaccine Dose, by Baseline Serostatus (Safety Set)

VCD Caused by DENV-4.

Up to 54 months post second dose, a total of 12 cases of VCD caused by DENV-4 were observed in the TDV group, of which 4 occurred during the last 18 months (see Figure 24). Importantly, none of these 12 cases led to hospitalisation, indicating no risk for severe disease. In comparison, there were 3 cases caused by DENV-4 observed in the placebo group, 1 of which occurred during the last 18 months and led to hospitalisation. It should be noted that this hospitalised VCD case in the placebo group was removed from the interval analysis (ie, Year 4 or last 18 months) because of the prespecified censoring rule as it was a second episode but is included in the cumulative analysis.

Of note, 3 of the 4 VCD cases in the last 18 months in the TDV group occurred in the last 6 months which was not uniform for all subjects; this may have introduced bias (as mentioned above).

Virologically confirmed dengue fever from First Dose to 54 Months Post Second Dose in Baseline Seropositive Subjects.

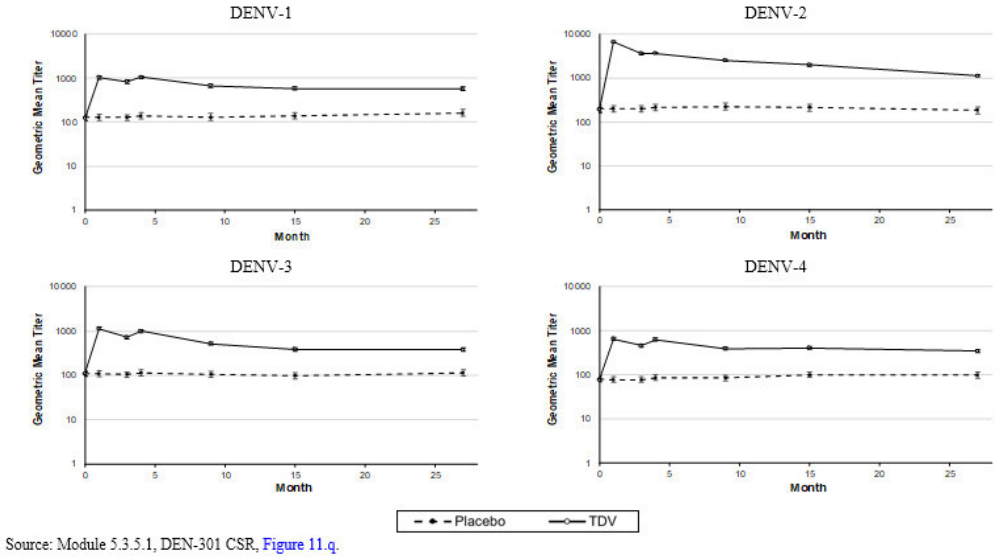
The VE against overall VCD from first dose to 54 months post second dose in baseline seropositive subjects was 64.2% (95% CI: 58.4%, 69.2%) and against VCD leading to hospitalisation 85.9% (95% CI: 78.7%, 90.7%). Analyses of overall VCD and VCD leading to hospitalisation by dengue serotype demonstrated the efficacy against each of the 4 dengue serotypes in baseline seropositive subjects.

During the last 18 months, the VE against overall VCD in baseline seropositive subjects was 59.6% (95% CI: 34.2%, 75.2%) and against VCD leading to hospitalisation 90.4% (95% CI: 56.4%, 97.9%). Year-by-year analyses showed continued VE in Year 4 post second vaccine dose for overall VCD (VE: 64.1%; 95% CI: 37.4%, 79.4%) and VCD leading to hospitalisation (VE: 94.0%; 95% CI: 52.2%, 99.3%).

Immunogenicity

Geometric Mean Titres of Neutralising Antibodies Against Dengue

GMTs in the TDV group of Trial DEN-301 corresponding to month 1 following the first vaccine dose, and second vaccine dose as well to Month 15 are shown in Figure 31. The magnitude of the response was comparable for DENV-1, DENV-3, and DENV-4, and was higher for DENV-2.



Source: Module 5.3.5.1, DEN-301 CSR, Figure 11.q.

Figure 31: Trial DEN-301: Geometric Mean Titres (95% Confidence Interval) of Neutralising Antibodies for Each Dengue Serotype After First Vaccination by Visit (Per-Protocol Set-Immunogenicity Subset)

Dengue Seropositivity Rates

For baseline seronegative subjects, in the TDV group, seropositivity rates for each serotype increased from baseline to 90.5% to 98.6% at Month 1. Rates continued to increase to Month 4 (≥99.5%) and remained high through Month 15 (≥92.7%) and Month 27 (≥90.4%). At Month 1, multivalent seropositivity rates were 96.5% (at least trivalent) and 85.3% (tetravalent), peaked at Month 4 (99.8% and 99.5%, respectively), and remained high at Month 27 (92.7% and 85.9%, respectively). In the placebo group, at Month 27, 16.5% of baseline seronegative subjects were seropositive against at least 1 dengue serotype and the at least trivalent and tetravalent seropositivity rates were 13.1% and 12.3%, respectively.

For baseline seropositive subjects, in both the placebo and TDV groups, seropositivity rates for each serotype were high at baseline (87.4% to 97.2% across trial groups and serotypes). For the TDV group, rates had increased at Month 1 to ≥99.5% for each serotype and remained high remained high (≥99.2%) at all subsequent time points. Most subjects in the TDV group showed at least trivalent or tetravalent seropositivity at baseline (87.5% and 83.5%, respectively), which increased in the TDV group to 99.8% (at least trivalent) and 99.1% (tetravalent) by Month 1 and remained high throughout

Exploratory Immunogenicity Analyses Based on 36-Month Data

At Month 39 (36 months post second vaccine dose), GMTs in the TDV group remained well above baseline with little change compared with the preceding time point at Month 27 for both baseline seronegative subjects and baseline seropositive subjects. Similarly, there were little changes in seropositivity rates in the TDV group at Month 39 compared with Month 27 in both subgroups by

baseline serostatus. At Month 39, the tetravalent seropositivity rate in the TDV group was 80.5% in baseline seronegative subjects and 98.4% in baseline seropositive subjects.

Correlate of Protection

In a descriptive approach to evaluate a potential correlate of protection, GMTs were compared between subjects who had VCD fever until end of Part 2 (18 months post second dose), referred to as "cases", and those who did not have VCD fever, referred to as controls, overall and by baseline serostatus. The correlate of protection subset of the PPS used for the correlate of protection analyses included 1404 subjects in the placebo group and 2603 subjects in the TDV group (see Table 45).

In general, and as expected, GMTs for both cases and controls were higher in the TDV group than in the placebo group, for each serotype and irrespective of baseline serostatus. In both trial groups, GMTs were higher for DENV-2 compared with the other 3 dengue serotypes. Overall, for each serotype and in both trial groups, antibody titres at Month 4 were higher for controls, ie, subjects who had no VCD fever, than for cases, ie, subjects who had VCD fever (see Table 45), indicating a potential association between the neutralising antibody titre and the efficacy outcome of preventing VCD fever. These differences between controls and cases were more pronounced in baseline seropositive subjects, while in baseline seronegative subjects, GMTs were similar or only marginally higher in controls compared with the cases. In general, there was considerable overlap in antibody titres between cases and controls, particularly in the seronegative group.

Table 45: Trial DEN-301: Geometric Mean Titres of Neutralising Antibodies in Dengue Cases and Controls at Month 4 (Per-Protocol Set-Correlate of Protection Subset)

Serostatus	Geometric Mean (Standard Deviation)			
	Placebo (N = 1404)		TDV (N = 2603)	
Serotype	Cases (Had VCD)	Controls (No VCD)	Cases (Had VCD)	Controls (No VCD)
Overall				
Number of subjects evaluated	196	1085	111	2234
DENV-1	46.0 (9.69)	140.1 (17.47)	371.8 (3.82)	1064.9 (6.33)
DENV-2	75.4 (10.46)	217.8 (18.02)	1958.6 (2.62)	3658.8 (3.10)
DENV-3	36.4 (7.76)	116.0 (15.17)	319.6 (3.22)	992.9 (4.85)
DENV-4	41.2 (8.49)	86.4 (12.12)	394.3 (4.27)	627.4 (4.28)
Baseline seropositive				
Number of subjects evaluated	144	787	73	1601
DENV-1	101.0 (8.65)	458.5 (11.37)	568.3 (3.60)	2135.7 (4.91)
DENV-2	190.8 (7.62)	778.0 (9.61)	2083.4 (2.68)	4916.3 (2.93)
DENV-3	73.5 (7.07)	360.3 (9.92)	459.9 (3.10)	1780.4 (3.98)
DENV-4	87.0 (7.55)	241.9 (8.38)	719.3 (3.70)	1124.3 (3.25)
Baseline seronegative				
Number of subjects evaluated	52	298	38	633
DENV-1	5.2 (1.33)	6.1 (2.27)	164.5 (2.87)	183.2 (3.13)
DENV-2	5.8 (1.80)	7.5 (3.16)	1739.5 (2.49)	1733.2 (2.46)
DENV-3	5.2 (1.30)	5.8 (2.25)	158.8 (2.48)	226.7 (2.62)
DENV-4	5.2 (1.30)	5.7 (2.08)	124.2 (2.52)	143.6 (2.61)

Source: Module 5.3.5.1, DEN-301 CSR, Table 11.ddd, Pt1+12m Table 15.2.6.1.2, and Table 15.2.6.1.3.
Abbreviations: N, number of subjects in the per-protocol set-correlate of protection subset; VCD, virologically confirmed dengue.
Cases had VCD fever (until end of Part 2) and blood sample taken (VCD cases from correlate of protection subset and non-subset subjects); controls had no VCD fever and blood sample (from subset subjects).

In preliminary exploratory analyses, various statistical models were applied to evaluate whether the neutralising antibody titre is a predictive factor for the VE observed. Results suggested that there could potentially be a correlate of protection for DENV-1, but not for DENV-2, DENV-3, and DENV-4. TDV elicits a broad range of immune responses which may also contribute to protection from infection or severe disease.

- **Ancillary analysis**

Summary of Immunogenicity Results From Other Studies Using the Final TDV Formulation

In addition to pivotal phase 3 Trial DEN-301, 6 further trials, phase 3 Trials DEN-304 and DEN-315, co-administration phase 3 Trials DEN-305 and DEN-314, and phase 2 Trials DEN-313 and DEN-204 provide immunogenicity data in support of TDV as vaccine for the prevention of dengue disease.

Phase 3 Trial DEN-304

Trial Design

This was a randomised, double-blind, placebo-controlled phase 3 trial with 923 healthy subjects aged 18 to 60 years, conducted in non-endemic regions of the United States to investigate lot-to-lot consistency of 3 consecutive TDV lots in terms of equivalence of the induced immunogenicity.

The primary objective was to demonstrate the lot-to-lot consistency of 3 consecutive TDV lots in terms of equivalence of immune responses at 1 month post second dose.

The secondary objectives pertaining to immunogenicity were:

- To describe the seropositivity (reciprocal neutralising titre ≥10) rates for all dengue serotypes at 1 month and 6 months post second TDV dose (ie, at Months 4 and 9 of the trial).
- To describe the persistence of the immune response at 6 months post second dose of TDV or placebo in subjects who were seronegative for dengue at baseline.

Subjects were randomised (2:2:2:1 ratio) to 1 of 4 trial groups to receive TDV (Groups 1 to 3 corresponding to Lots 1-3) or placebo, administered at Month 0 and Month 3 via SC injection. A subset of subjects was randomly selected for the immunogenicity evaluation (immunogenicity subset).

All subjects were followed-up for 6 months post second vaccine dose at Month 3 through Month 9.

Immunogenicity Endpoints

The primary endpoint was the GMT of neutralising antibodies (by MNT_{50}) for each of the 4 dengue serotypes at Month 4 in the immunogenicity subset.

Secondary immunogenicity endpoints included (DEN-304 CSR, Section 9.5.3):

- The seropositivity (defined as a reciprocal neutralising titre ≥10) rates for each of the 4 dengue serotypes at Month 4 and Month 9.
- The GMT of neutralising antibodies (by MNT_{50}) for each of the 4 dengue serotypes at Month 9.

Statistical Methods

Analyses were based on the PPS, which included all randomised subjects in the immunogenicity subset who received at least 1 dose of trial vaccine (TDV or placebo) and for whom a valid pre-dose and at least 1 valid post-dose blood sample was taken, and who had no major protocol violations.

The primary endpoint analysis evaluated the lot-to-lot consistency at Month 4. For each serotype, an analysis of variance (ANOVA) model on the natural logarithms of titres of dengue neutralising antibodies was fitted. The model included trial group as a factor, including data from subjects in Groups 1 to 3 (TDV vaccinated subjects) only; 95% CIs for the 3 pairwise comparisons were computed and equivalence of immune responses was established if the 95% CI for the ratio was contained between the equivalence margins of 0.5 and 2.0. Therefore, since TDV is a tetravalent vaccine it necessitated 12 pairwise comparisons to establish lot-to-lot consistency, which was concluded if equivalence of immune responses was established for all pairwise comparisons. The equivalence

margins were chosen to account for the variability inherent to both the live attenuated tetravalent vaccine TDV as well as the MNT_{50} assay used to determine immune response, and the variability inherent to individual subjects, as evidenced from immune response data in phase 1 and phase 2 TDV trials.

For the secondary endpoint of seropositivity, the difference in proportions between trial groups was estimated for each dengue serotype at each applicable visit.

Trial Results

Subject Disposition and Baseline Characteristics

A total of 923 subjects were randomised (placebo: 132 subjects; TDV Lot 1: 263 subjects; TDV Lot 2: 264 subjects; and TDV Lot 3: 264 subjects), and 615 subjects were included in the immunogenicity subset (placebo: 88 subjects; TDV Lot 1: 175 subjects; TDV Lot 2: 176 subjects; and TDV Lot 3: 176 subjects). More than 86% of subjects in any group completed the trial. The PPS used for immunogenicity analyses included a total of 442 subjects (71.9% of the immunogenicity subset).

Demographic and baseline characteristics were similar for all 4 groups. Overall, subjects had a mean age of 41.4 years, 45.9% were male, and 79.2% of subjects were white. Baseline seropositivity rates were relatively high for all trial groups (placebo: 16.7%; TDV Lot 1: 14.7%; TDV Lot 2: 13.8%; and TDV Lot 3: 16.3%), which was unexpected given the trial was based in a dengue non-endemic region where subjects are assumed to be predominantly dengue seronegative at baseline.

Immunogenicity

Lot-to-Lot Consistency

Based on the PPS that only included subjects who were dengue seronegative at baseline, the 95% CI of the GMT ratios was within pre-specified equivalence margins of 0.5 and 2.0 for all pairwise comparisons for DENV-2 and DENV-3, and for 1 comparison each for DENV-1 (TDV Lots 2 vs 3) and DENV-4 (TDV Lots 1 vs 3) (see Table 46). Therefore, lot-to-lot consistency in terms of equivalence of immune responses at 1 month post second vaccine dose (Month 4) could not be demonstrated statistically.

			Lot Comparison			
Serotype	TDV Lot Group	GMT	Pairwise Comparison	GMT Ratio	95% CI of GMT Ratio	Met Equivalence Condition [a]
Primary analysis based on the per-protocol set (baseline seronegative subjects; N = 379)						
DENV-1	TDV Lot 1	202.93	TDV Lot 1 vs TDV Lot 2	0.69	(0.46, 1.04)	NO
	TDV Lot 2	293.79	TDV Lot 2 vs TDV Lot 3	0.91	(0.60, 1.38)	YES
	TDV Lot 3	322.14	TDV Lot 1 vs TDV Lot 3	0.63	(0.41, 0.96)	NO
DENV-2	TDV Lot 1	3090.33	TDV Lot 1 vs TDV Lot 2	1.30	(0.98, 1.71)	YES
	TDV Lot 2	2386.05	TDV Lot 2 vs TDV Lot 3	0.67	(0.51, 0.88)	YES
	TDV Lot 3	3574.09	TDV Lot 1 vs TDV Lot 3	0.86	(0.65, 1.15)	YES
DENV-3	TDV Lot 1	149.46	TDV Lot 1 vs TDV Lot 2	1.27	(0.91, 1.78)	YES
	TDV Lot 2	117.30	TDV Lot 2 vs TDV Lot 3	0.95	(0.68, 1.33)	YES
	TDV Lot 3	122.84	TDV Lot 1 vs TDV Lot 3	1.22	(0.87, 1.71)	YES
DENV-4	TDV Lot 1	116.26	TDV Lot 1 vs TDV Lot 2	0.62	(0.46, 0.82)	NO
	TDV Lot 2	187.89	TDV Lot 2 vs TDV Lot 3	1.62	(1.22, 2.17)	NO
	TDV Lot 3	115.76	TDV Lot 1 vs TDV Lot 3	1.00	(0.75, 1.35)	YES
Supportive analysis based on the per-protocol set plus baseline seropositive subjects (N = 447)						
DENV-1	TDV Lot 1	229.67	TDV Lot 1 vs TDV Lot 2	0.74	(0.51, 1.09)	YES
	TDV Lot 2	308.51	TDV Lot 2 vs TDV Lot 3	0.99	(0.68, 1.45)	YES
	TDV Lot 3	311.59	TDV Lot 1 vs TDV Lot 3	0.74	(0.50, 1.09)	YES
DENV-2	TDV Lot 1	3030.67	TDV Lot 1 vs TDV Lot 2	1.27	(0.97, 1.65)	YES
	TDV Lot 2	2390.27	TDV Lot 2 vs TDV Lot 3	0.65	(0.50, 0.84)	YES
	TDV Lot 3	3686.92	TDV Lot 1 vs TDV Lot 3	0.82	(0.63, 1.07)	YES
DENV-3	TDV Lot 1	153.46	TDV Lot 1 vs TDV Lot 2	1.14	(0.84, 1.56)	YES
	TDV Lot 2	134.04	TDV Lot 2 vs TDV Lot 3	1.09	(0.80, 1.48)	YES
	TDV Lot 3	123.52	TDV Lot 1 vs TDV Lot 3	1.24	(0.91, 1.70)	YES
DENV-4	TDV Lot 1	126.66	TDV Lot 1 vs TDV Lot 2	0.64	(0.48, 0.85)	NO
	TDV Lot 2	199.23	TDV Lot 2 vs TDV Lot 3	1.55	(1.17, 2.07)	NO
	TDV Lot 3	128.33	TDV Lot 1 vs TDV Lot 3	0.99	(0.74, 1.32)	YES

Source: Module 5.3.5.1, DEN-304 CSR, Table 10.d, Table 11.a and Table 11.c.
Abbreviations: CI, confidence interval; GMT, geometric mean titer; N, number of subjects.
(a) 95% CI of the GMT ratio within pre-specified equivalence margins of 0.5 and 2.0.

Table 46: Trial DEN-304: Pairwise Comparison of Geometric Mean Titres for Each Dengue Serotype Between the 3 TDV Lots at Month 4 (Per-Protocol Set)

Geometric Mean Titres and Seropositivity Rates

Overall, an increase in GMTs of neutralising antibodies titres was observed between baseline and Month 4 for all serotypes across all TDV groups. Between Month 4 and Month 9, GMTs decreased for all serotypes for all 3 TDV lots but remained well above baseline values. GMTs were consistently higher for DENV-2 than for the other 3 serotypes.

There were no relevant differences in seropositivity rates between the 3 TDV lots. At Month 4, the percentage of seropositive subjects in the PPS had risen from 0% to between 94.6% and 100% of subjects for each serotype in the TDV lots.

Phase 3 Trial DEN-315

Trial Design

This was a randomised, double-blind, placebo-controlled, phase 3 trial planned in 400 healthy adolescent subjects aged 12 to 17 years in a dengue non-endemic region of Mexico (Mexico City) with 2 parallel groups to investigate the immunogenicity and safety of TDV.

The primary objective was to describe the neutralising antibody response against each dengue serotype at 1 month post second dose (Month 4) of TDV or placebo in adolescent subjects who were seronegative for dengue.

The secondary objectives pertaining to immunogenicity were:

-To describe the persistence of the immune response at 6 months post second dose (Month 9).

-To describe the seropositivity (reciprocal neutralising titre ≥10) rates for all dengue serotypes at 1 and 6 months post second dose.

Subjects were randomised (3:1 ratio) to receive either TDV or placebo, administered at Month 0 and Month 3 via SC injection. All subjects were followed-up for 6 months post second dose, ie, up to Month 9.

Immunogenicity Endpoints

The primary endpoint was the GMT of neutralising antibodies (by MNT_{50}) for each of the 4 dengue serotypes at 1 month post second dose (Month 4).

Secondary immunogenicity endpoints included:

-GMTs of neutralising antibodies (by MNT_{50}) for each of the 4 dengue serotypes at 6 months post second dose (Month 9).

-Seropositivity (defined as a reciprocal neutralising titre ≥10) rates for each of the 4 dengue serotypes and for multiple (2, 3, or 4) dengue serotypes at 1 month and 6 months post second dose (Month 4 and Month 9, respectively).

Statistical Methods

Analyses were based on the PPS, which included all enrolled subjects who received at least 1 dose of TDV or placebo, for whom a valid pre-dose measurement and at least 1 valid post-dose measurement was available for immunogenicity evaluation, who were not seropositive to any dengue serotype at baseline, and who had no major protocol violations.

For GMTs, descriptive statistics including 95% CIs were provided for each dengue serotype and for each applicable visit. Seropositivity rates were presented for each dengue serotype and multiple dengue serotypes, along with an exact 2-sided 95% CI based on the Clopper-Pearson method.

Trial Results

Subject Disposition and Baseline Characteristics

A total of 400 subjects were randomised, 300 into the TDV group and 100 into the placebo group. Of these, 296 (98.7%) in the TDV group and 95 (95.0%) in the placebo group completed the trial. The PPS used for analyses of immunogenicity included 271 subjects (90.3%) in the TDV group and 82 subjects (82.0%) in the placebo group. The most common reason for exclusion from the PPS was that the subject was seropositive to at least 1 dengue serotype at baseline, which affected 36 subjects in the TDV group and 12 in the placebo group.

Demographic and baseline characteristics were well balanced between the TDV and placebo groups of the PPS. In both groups, the mean age was 14.3 years with an age range of 12 to 17 years. More than half of the subjects were female (57.6% in the TDV group and 52.4% in the placebo group). All subjects were of Hispanic or Latino ethnicity and of either American Indian/Alaska Native. As per the definition of the PPS, all subjects were dengue seronegative at baseline.

Immunogenicity

TDV was shown to be immunogenic in baseline dengue seronegative adolescents aged 12 to 17 years. GMTs of neutralising antibodies (MNT_{50}) had increased to 327.9 against DENV-1, 1742.5 against DENV-2, 119.5 against DENV-3, and 142.7 against DENV-4 at 1 month post second dose of TDV (Month 4).

Six months post second dose of TDV (Month 9), GMTs had decreased compared with Month 4 but remained well above baseline level with values of 134.7, 740.9, 45.8, and 37.5, respectively.

At 1 month post second dose (Month 4), in the TDV group, the seropositivity rate increased from 0% at baseline to ≥99.6% for all serotypes. All subjects in the TDV group had at least trivalent seropositivity and almost all subjects (99.6%) had tetravalent seropositivity. At 6 months post second dose (Month 9), in the TDV group, the percentage of seropositive subjects was still high (≥89.4% across serotypes), with at least trivalent seropositivity reached by 94.5% of subjects and tetravalent seropositivity by 85.8% of subjects.

Phase 3 Co-Administration Trial DEN-305

The applicant also performed 2 co-administration studies (DEN-305 and DEN-314) in nonendemic countries with Yellow fever and Hepatitis A vaccines with the intention to study the possible interactions of TDV vaccine with the most common vaccines used for travellers. CHMP considers adequate the choice of these vaccines and that these studies will be included in the application (identical data submitted in parallel application for EU Marketing Authorisation).

Trial Design

This was a phase 3, observer-blind, randomised, multicentre trial in 900 healthy adults aged 18 to 60 years living in regions non-endemic for both dengue and YF, to investigate the immunogenicity and safety of TDV and a YF vaccine (YF-17D) administered concomitantly or sequentially. The trial was conducted in the United States.

Subjects were randomised (1:1:1) to 1 of 3 treatment groups:

- Group 1 (denoted as YF-17D/TDV/TDV): YF-17D vaccine + placebo concomitantly administered at Month 0, TDV administered at Months 3 and 6.
- Group 2 (denoted as TDV/TDV/YF-17D): first dose of TDV + placebo concomitantly administered at Month 0, second dose of TDV administered at Month 3, and YF-17D vaccine administered at Month 6.
- Group 3 (denoted as TDV + YF-17D/TDV/PBO): first dose of TDV + YF-17D vaccine concomitantly administered at Month 0, second dose of TDV administered at Month 3, and placebo administered at Month 6.

All subjects were followed-up for 6 months post third vaccination at Month 6 through Month 12.

The primary objective was to demonstrate non-inferiority of the YF seroprotection rate response to 1 dose of YF-17D vaccine, 1 month following concomitant administration of 1 dose of TDV, compared with placebo administered concomitantly with the YF-17D vaccine (Group 1 vs Group 3).

The secondary objectives pertaining to immunogenicity were:

- To demonstrate the non-inferiority of the GMT response to TDV for all dengue serotypes combined at 1 month post second dose of TDV following concomitant administration of the first dose of TDV with YF-17D vaccine or with placebo (Group 2 vs Group 3).
- To demonstrate the non-inferiority of the GMT response to 1 dose of YF-17D vaccine 1 month following concomitant administration with 1 dose of TDV compared with placebo administered concomitantly with the YF-17D vaccine (Group 1 vs Group 3).
- To describe seropositivity rates for all 4 dengue serotypes 1 month post second dose of TDV following concomitant administration of the first dose of TDV with YF-17D vaccine or placebo.

- To describe the GMTs of neutralising antibodies and the seropositivity rates for all 4 dengue serotypes 1 month post first dose of TDV administered concomitantly with YF-17D vaccine or placebo.
- To describe the GMT and seroprotection rate response to YF-17D vaccine 1 month after sequential administration of a 2-dose regimen of TDV at Months 0 and 3, followed by YF-17D vaccine 3 months later.
- To describe the GMTs of neutralising antibodies and the seropositivity rates for all 4 dengue serotypes, 1 month after sequential administration of YF-17D vaccine at Month 0, followed by a 2-dose regimen of TDV 3 months later at Months 3 and 6.

The trial was powered to meet the primary as well as the first 2 secondary objectives listed above.

Immunogenicity Endpoints

The primary endpoint was the proportion of subjects YF and dengue naive at baseline who were seroprotected against YF at Month 1 as measured by PRNT. YF seroprotection was defined as reciprocal anti-YF neutralising antibody titre ≥10. Immunological naivety to YF and dengue virus was defined as baseline reciprocal neutralising antibody titres <10 for YF and for the 4 dengue serotypes.

Secondary immunogenicity endpoints were:

- GMT of neutralising antibodies (by MNT_{50}) for each of the 4 dengue serotypes at pre-second and pre-third vaccine doses and 1 month post first, second, and third vaccine doses.
- GMTs of neutralising anti-YF antibodies (by PRNT) at 1 month post first and third vaccine doses.
- Seropositivity rates for each of the 4 dengue serotypes at pre-second and pre-third vaccine doses and 1 month post first, second, and third vaccine doses. Seropositivity was defined as a reciprocal neutralising antibody titre ≥10.
- Seropositivity rates for multiple dengue serotypes at pre-second and pre-third vaccine doses and 1 month post first, second, and third vaccine doses.
- Proportion of subjects YF and dengue naive at baseline who were seroprotected against YF at 1 month post third vaccination (PRNT).

Statistical Methods

Analyses were based on the PPS which included subjects of the FAS (all randomised subjects who received at least 1 dose of the trial vaccine and for whom a valid pre-dose [baseline] and at least 1 post-dose measurement were available for immunogenicity assessments) who had no major protocol violations. It was differentiated between the YF PPS for YF immunogenicity analyses and the PPS for TDV immunogenicity analyses. Per definition, all subjects in the YF PPS or PPS were not seroprotected against YF virus and were seronegative for any dengue serotype at baseline.

As primary analysis, the non-inferiority of the immune response to the YF-17D vaccine when concomitantly administered with TDV (Group 3) compared with concomitant administration of the YF-17D vaccine with placebo (Group 1) was assessed in terms of YF seroprotection rates at Month 1. Non-inferiority was concluded if the upper bound of the 95% CI for the seroprotection rate difference (Group 1 minus Group 3) was less than the non-inferiority margin of 5% as requested by the US FDA.

The non-inferiority of the immune response to TDV when concomitantly administered with YF-17D vaccine (Group 3) compared with concomitant administration of TDV with placebo (Group 2) was assessed in terms of the GMTs of neutralising antibodies for all 4 dengue serotypes at Month 4. Non-inferiority was concluded if the upper bound of the 95% CI for the GMT ratio (Group 2/Group 3) was

less than the non-inferiority margin of 2.0. The non-inferiority of the immune response to YF-17D when concomitantly administered with TDV (Group 3) compared with concomitant administration of YF-17D with placebo (Group 1) was assessed in terms of the GMTs of anti-YF neutralising antibodies at Month 1. Non-inferiority was concluded if the upper bound of the 95% CI for the GMT ratio (Group 1/Group 3) was less than the non-inferiority margin of 2.0. This non-inferiority margin aligns with the equivalence margin used in Trial DEN-304.

Trial Results

Subject Disposition and Baseline Characteristics

A total of 900 subjects were randomised, 300 subjects to each group. A total of 161 subjects (17.9%) discontinued the trial, with similar proportions across groups. The most frequent reason for trial discontinuation was loss to follow-up. Consequently, 739 of the 900 randomised subjects (82.1%) completed that trial. The YF PPS used for analyses of YF seroprotection rates and YF GMTs included 715 subjects (79.4% of randomised subjects). The most common reasons for exclusion from the YF PPS were seropositivity to dengue virus at baseline (82 subjects) and seroprotection to YF virus at baseline (78 subjects). Analyses of dengue seropositivity rates and GMTs of neutralising antibodies against dengue were based on the PPS, which included 589 subjects (65.4%) overall (192 subjects [64.0%] in Group 1, 208 [69.3%] in Group 2, 189 [63.0%] in Group 3). The most common reasons for exclusion from the PPS were not having received all 3 vaccinations (148 subjects [17.0% of the FAS]), seropositivity to dengue virus at baseline (84 [9.6%]), and seroprotection to YF virus at baseline (78 [9.0%]).

Overall, demographic and baseline characteristics for the YF PPS were well balanced across groups. The mean age was 41.9 years in Group 1, 40.0 years in Group 2, and 41.6 years in Group 3. The majority of subjects were female: 57.8% in Group 1, 58.4% in Group 2, and 60.8% in Group 3. There were more Black/African American subjects in Group 2 (34.5%) than in the other groups (25.6% and 27.0%, respectively).

Immunogenicity

Yellow Fever Seroprotection Rates and Yellow Fever Geometric Mean Titres

At Month 1, all but 1 subject in Group 1 (YF-17D/TDV/TDV) (99.5%) and all but 2 subjects in Group 3 (TDV + YF-17D/TDV/PBO) (99.1%) had achieved YF seropositivity. The primary objective of demonstrating the non-inferiority between YF seroprotection rates was met with a 95% CI for the seroprotection rate difference of (-1.85%, 2.69%).

Similarly, at Month 1, the YF GMT response in Group 3 (TDV + YF-17D/TDV/PBO) was non-inferior to that in Group 1 (YF-17D/TDV/TDV), with the upper bound of the 95% CI for the geometric mean ratio in MNT response being below the predefined non-inferiority margin of 2.0 (geometric mean ratio: 1.0; 95% CI: 0.77, 1.26).

At Month 6 (6 months after YF-17D vaccination in Group 1 and Group 3, a high YF seroprotection rate of ≥98.9% was seen in Groups 1 and 3, and the YF GMTs were 2712.2 and 1184.6, respectively. In Group 2 (TDV/TDV/YF-17D), where subjects had not yet received the YF-17D vaccination, the YF seroprotection rate was 12% and the YF GMT was close to the baseline level (6.0). At Month 7 (7 months after YF-17D vaccination in Group 1 and Group 3 and 1 month after YF-17D vaccination in Group 2, YF seroprotection rates were ≥98.4% across groups, and YF GMTs were 2341.6 for Group 1, 1089.1 for Group 3, and 3078.2 for Group 2.

Geometric Mean Titres of Neutralising Antibodies Against Dengue and Dengue Seropositivity Rates

At Month 4 (30 days after the second dose of TDV in Group 2 and Group 3), the non-inferiority in TDV GMTs between Groups 2 and 3 was shown for DENV-2 (GMTs of 2616.1 in Group 2 vs 1947.7 in Group 3), DENV-3 (131.4 vs 104.5), and DENV-4 (111.8 vs 97.7). For DENV-1 (GMTs of 297.1 in Group 2 vs 182.6 in Group 3), the upper bound of the 95% CI for the GMT ratio (Group 2/Group 3) was 2.22 and thus above the predefined non-inferiority margin of 2.0. Therefore, non-inferiority of the TDV GMT response between Group 3 and Group 2 at Month 4 was demonstrated for DENV-2, DENV-3, and DENV-4, but not for DENV-1.

At 1 month post first TDV dose (Month 4 for Group 1; Month 1 for Group 2 and Group 3), the seropositivity rate for at least 1 dengue serotype was 99.4% in Group 1, 99.0% in Group 2, and 96.2% in Group 3, and remained above 99% for the remainder of the trial. Seropositivity rates were generally highest for DENV-2 in all groups. Lowest seropositivity rates (<85%) post first TDV dose were seen in Group 2 (TDV/TDV/YF-17D) at Month 3 for DENV-3 and DENV-4 (84.6% each) and in Group 3 (TDV + YF-17D/TDV/PBO) at Month 1 for DENV-3 (80.5%) and DENV-4 (78.4%).

At 1 month post first TDV dose (Month 4 for Group 1; Month 1 for Group 2 and Group 3), the at least trivalent seropositivity rate was 99.4% in Group 1, 91.7% in Group 2, and 81.6% in Group 3, and the tetravalent seropositivity rates were 98.9%, 79.3%, and 65.9%, respectively. In Group 1 (YF-17D/TDV/TDV), these rates were at least 99% at subsequent time points. In Group 2 (TDV/TDV/YF-17D) and Group 3 (TDV + YF-17D/TDV/PBO), rates were in the range of 75.5% to 88.9% at Month 3 and at least 88% at Month 4 or later time points, ie, after subjects had received the second TDV dose.

Phase 3 Co-Administration Trial DEN-314

Trial Design

This was a phase 3, observer-blind, randomised trial in 900 healthy adult subjects aged 18 to 60 years living in regions non-endemic for both dengue and hepatitis A, to investigate the immunogenicity and safety of the co-administration of SC TDV and an intramuscular (IM) HAV vaccine. The trial was conducted in the United Kingdom.

At trial entry, subjects had to be HAV-naive (no prior HAV infection or vaccination) as well as DENV-naive, ie, seronegative for Hepatitis A and for dengue.

Subjects were randomised (1:1:1) to 1 of 3 treatment groups:

- Group 1 (denoted as HAV + placebo/placebo): HAV vaccine (IM) and placebo (SC) concomitantly administered at Month 0, placebo (SC) administered at Month 3.
- Group 2 (denoted as placebo + TDV/TDV): TDV (SC) and placebo (IM) concomitantly administered at Month 0, TDV (SC) administered at Month 3.
- Group 3 (denoted as HAV + TDV/TDV): TDV (SC) and HAV vaccine (IM) concomitantly administered at Month 0, TDV (SC) administered at Month 3.

All subjects were followed-up for 6 months post second vaccine dose at Month 3 through Month 9.

The primary objective was to demonstrate non-inferiority of the immune response to 1 dose of HAV vaccine in HAV/DENV-naive subjects, 1 month following co-administration with 1 dose of TDV (Group 3) compared with 1 dose of HAV vaccine co-administered with placebo (Group 1).

The secondary objectives pertaining to immunogenicity were:

-To describe the immune response to the HAV vaccine in HAV/DENV-naïve subjects 1 month following 1 dose of HAV vaccine co-administered with TDV (Group 3) or placebo (Group 1).

-To describe the immune response to TDV in HAV/DENV-naive subjects 1 month following a second dose of TDV given 3 months after a first dose of TDV co-administered with HAV vaccine (Group 3) or placebo (Group 2).

-To describe the immune response to TDV in HAV/DENV-naive subjects 1 month following a first dose of TDV co-administered with HAV vaccine (Group 3) or placebo (Group 2).

Immunogenicity Endpoints

The primary endpoint was the proportion of subjects HAV/DENV-naive at baseline who were seroprotected against HAV at Month 1 (seroprotection rate) in a subset of 120 subjects per group (immunogenicity subset).

Secondary immunogenicity endpoints were:

-The geometric mean concentrations (GMCs) of anti-HAV antibodies at Month 1 in subjects HAV/DENV-naive at baseline.

-GMT of neutralising antibodies (by MNT_{50}) for each of the 4 dengue serotypes at Month 1 and Month 4 in subjects HAV/DENV-naive at baseline.

-The proportion of subjects HAV/DENV-naive at baseline who were seropositive for each of the 4 dengue serotypes at Month 1 and Month 4 (seropositivity rate).

Statistical Methods

A subset of 360 subjects (120 in each group), the immunogenicity subset, was randomly selected for the immunogenicity analyses. Analyses were based on the PPS which included subjects of the FAS (all randomised subjects who received at least 1 dose of the trial vaccine and for whom a valid pre-dose [baseline] and at least 1 post-dose measurement were available for immunogenicity assessments) who had no major protocol violations. It was differentiated between the HAV-PPS for HAV immunogenicity analyses and the TDV-PPS for TDV immunogenicity analyses.

Descriptive statistics including 95% CIs for the primary and secondary endpoints, including seroprotection or seropositivity rates and GMCs or GMTs, were computed by trial group and visit for subjects in the immunogenicity subset for all available assays at all relevant time points. The 95% CIs were calculated by the exact (Clopper-Pearson) method.

The non-inferiority of the immune response to the HAV vaccine when co-administered with TDV, compared with its co-administration with placebo, was assessed in terms of seroprotection rates at Month 1. Non-inferiority of immunogenicity was concluded if the upper bound of the 95% CI for the seroprotection rate difference (Group 1 minus Group 3) was less than the non-inferiority margin of 10%. A non-inferiority margin of 10% is commonly used and accepted in vaccine co-administration trials and was selected for this trial based on other similar co-administration trials with HAV vaccines.

Trial Results

Subject Disposition and Baseline Characteristics

A total of 900 subjects were randomised, 300 subjects to each group. Of these, 897 subjects (99.7%) received at least 1 dose of trial vaccine and 778 subjects (86.7%) completed all trial visits. The immunogenicity subset included 359 subjects: 119 subjects in Group 1, 120 each in Groups 2 and 3. The HAV-PPS used for analyses of HAV seropositivity rates and HAV GMCs included 226 subjects (63.0% of the immunogenicity subset) overall (74 subjects [62.2%] in Group 1, 71 [59.2%] in

Group 2, and 81 [67.5%] in Group 3). The most common reasons for exclusion from the HAV-PPS were seroprotection against HAV at baseline (95 subjects) and seropositivity to dengue virus at baseline (27 subjects). The TDV-PPS used for analyses of TDV seropositivity rates and TDV GMTs included 196 subjects (54.6%) overall (66 subjects [55.5%] in Group 1, 63 [52.5%] in Group 2, and 67 [55.8%] in Group 3). The most common reason for exclusion from the TDV-PPS were seroprotection against HAV at baseline (97 [27.0% of the immunogenicity subset]), not having received both vaccine doses (37 [10.3%]), and seropositivity to dengue virus at baseline (27 [7.5%]).

Overall, the demographic and baseline characteristics of the HAV-PPS (the primary analysis set) were in line with the safety set; the mean age was 34.8 years and 96.5% of subjects were white. There was however an unexpected imbalance for gender across the groups in the HAV-PPS that was not observed to this extent in the safety set, with a higher proportion of females in Group 2 (42.3%) compared with Group 1 (31.1%) and Group 3 (21.0%). The demographic and baseline characteristics for the TDV-PPS were similar to those in the HAV-PPS.

Immunogenicity

Hepatitis A Virus Seroprotection Rates and Geometric Mean Concentrations of Anti-Hepatitis A Virus Antibodies

At Month 1, all but 2 subjects in Group 1 (HAV + placebo/placebo) (97.1%) and all but 1 subject in Group 3 (HAV + TDV/TDV) (98.7%) had achieved HAV seropositivity. The primary objective of demonstrating non-inferiority of the immune response following co-administration of HAV + TDV/TDV was met: in the primary analysis, the seroprotection rate difference (Group 1 minus Group 3) was -1.68 and the associated 95% CI was (-8.91%, 4.28%). The upper bound was less than the pre-defined non-inferiority margin of 10%, demonstrating non-inferiority of the immune response following co-administration of HAV + TDV/TDV (Group 1) versus administration of HAV + placebo/placebo (Group 3).

Geometric Mean Titres of Neutralising Antibodies Against Dengue and Dengue Seropositivity Rates

For all 4 dengue serotypes, an increase in GMTs was seen at 1 month post first TDV dose, ie, at Month 1, in Group 2 (placebo + TDV/TDV) and Group 3 (HAV + TDV/TDV). Overall, GMTs were slightly higher in Group 3 (HAV + TDV/TDV) than in Group 2 (placebo + TDV/TDV). The highest GMTs were observed for DENV-2 (2897.9 for Group 2 and 3960.0 for Group 3); GMTs were 108.2 and 152.5, respectively, for DENV-1, 95.4 and 140.5, respectively, for DENV-3, and 74.3 and 142.1, respectively, for DENV-4. Post second TDV dose, ie, at Month 4, for both TDV groups, GMTs of dengue neutralising antibodies for DENV-1 were higher compared with Month 1 (171.3 in Group 1, 173.7 in Group 2), whereas for the other 3 dengue serotypes they were slightly lower compared with Month 1 (DENV-2: 2064.1 and 1764.3, respectively; DENV-3: 83.8 and 92.6, respectively; DENV-4: 56.1 and 81.4, respectively), but well above baseline levels. In Group 1 (HAV + placebo/placebo), where no TDV was administered, GMTs of neutralising antibodies against dengue fever at Month 1 and Month 4 remained at baseline levels.

At Month 1, seropositivity rates for individual dengue serotypes varied between 85.0% and 91.7% in Group 2 and between 90.8% and 96.9% in Group 3. The at least trivalent seropositivity rate at Month 1 was 90.0% in Group 2 and 95.4% in Group 3, and the tetravalent seropositivity rates were 71.7% and 84.6%, respectively. At Month 4, 1 month post second TDV dose, seropositivity rates were consistently higher for all serotypes in both Group 2 (placebo + TDV/TDV) and Group 3 (HAV + TDV/TDV) compared with Month 1 (100% for DENV-1 and DENV-2 in both groups, >92% for DENV-3, and >96% for DENV-4), and the at least trivalent seropositivity rate was 98.2% in Group 2 (placebo + TDV/TDV) and 98.4% in Group 3 (HAV + TDV/TDV), and the tetravalent seropositivity rates were

90.9% and 96.8%, respectively. In Group 1 (HAV + placebo/placebo), at both Month 1 and Month 4, none of the subjects were seropositive for at least 3 dengue serotypes.

In summary, the primary objective of this trial was met, demonstrating that co-administration of the HAV vaccine with TDV does not have a negative impact on the immune response to the HAV vaccine.

- **Ancillary analyses- Immunogenicity Summary**

Trials Contributing to Immunogenicity

Data in support of the immunogenicity of TDV in its final formulation are available from the pivotal phase 3 Trial DEN-301 and from phase 3 and phase 2 trials that used the final clinical, lyophilised TDV formulation administered 3 months apart (DEN-304, DEN-315, DEN-305, DEN-314, DEN-313, and DEN-204). Trials conducted in endemic regions (DEN-301, DEN-313, and DEN-204) provide data for both baseline seropositive (reciprocal neutralising titre ≥10 for at least 1 dengue serotype) subjects and baseline seronegative (reciprocal neutralising titre <10 for all 4 dengue serotypes) subjects in the age group of 2 to 17 years. Trials conducted in non-endemic regions (DEN-304, DEN-315, and co-administration Trials DEN-305 and DEN-314) provide data for baseline seronegative subjects in the age groups of 12 to 17 years (DEN-315) and 18 to 60 years.

In this section, immunogenicity results across all trials are summarised for the endemic region and the non-endemic region. For Trials DEN-305, DEN-314, and DEN-204, the data from those trial groups in which TDV was administered 3 months apart (and without other concomitant vaccination) were used, ie, from Groups 2 in Trials DEN-305 and DEN-314 and from Group 1 of DEN-204.

By far the most subjects were included in pivotal phase 3 Trial DEN-301, where 13,380 subjects were vaccinated with TDV, 12,704 of whom were included in the PPS used for efficacy analyses and 2518 in the PPS used for immunogenicity analyses. Immunogenicity was assessed in all these trials, mostly in a randomly selected subset of subjects: 3717 subjects in total, 2796 in the endemic region and 921 subjects in the non-endemic region.

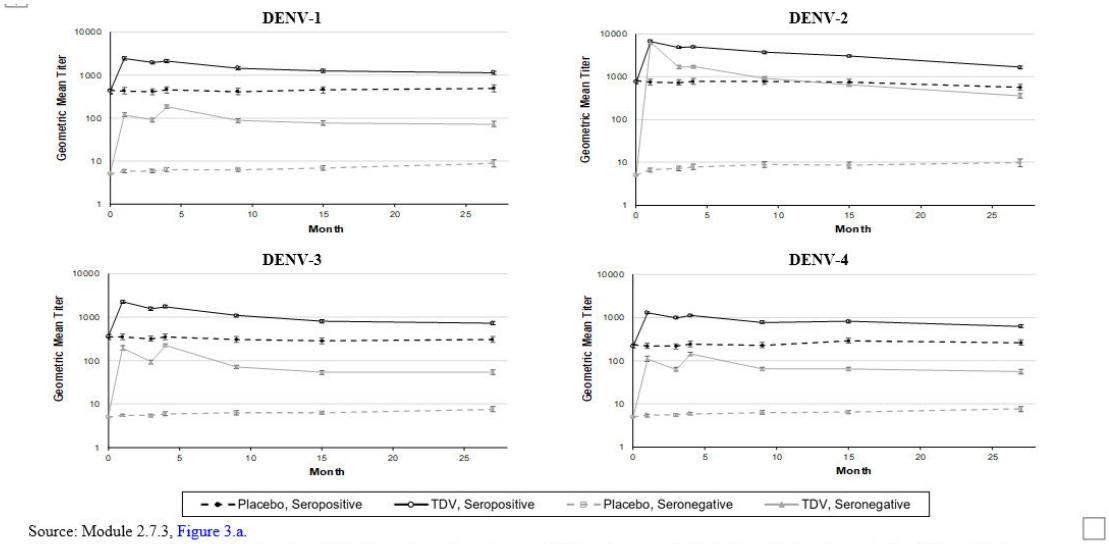
Immunogenicity Assessment Criteria

In each trial, vaccine immunogenicity endpoints included GMTs of neutralising antibodies measured by dengue MNT resulting in a titre reduction of at least 50% (MNT_{50}) for each dengue serotype and seropositivity rates for each of the 4 dengue serotypes and for multiple serotypes (eg, at least trivalent or tetravalent seropositivity). Seropositivity was defined as a reciprocal neutralising titre (determined by MNT_{50}) of ≥10 (for at least 1 dengue serotype), which is the lowest detectable level. Seropositivity rates in initially seronegative trial subjects were used to measure the proportion of responders to the vaccine, with tetravalent seropositivity providing a measure of response to all 4 dengue serotypes. For dengue fever, a seroprotective titre against dengue has not been established to date, but a titre of <10 is unlikely to be protective against dengue. In each trial, immunogenicity endpoints were summarised using descriptive statistics.

Immunogenicity Results

Endemic Regions

In general, the GMT profile of TDV administered in 2 doses 3 months apart was consistent between pivotal phase 3 Trial DEN-301 (subjects aged 4-16 years), phase 2 Trial DEN-313 (4-16 years), and phase 2 Trial DEN-204 (Trial Group 1; 2-17 years) conducted in endemic regions. The results from trial DEN-301, as a representative example, are shown in Figure 32. In both baseline seronegative and seropositive subjects of pivotal phase 3 Trial DEN-301, a gradual decline in GMTs was observed for DENV-2 from Month 9 onwards, whereas for the other dengue serotypes, GMTs remained relatively constant.



Source: Module 2.7.3, Figure 3.a.

Abbreviations: LLOD, lower limit of detection; LLOQ, lower limit of quantification; MNT_{50}, microneutralization test resulting in a titer reduction of at least 50%. For subjects with MNT_{50} titer <LLOD (LLOD = 10) the titer value is imputed with 5, thus curves start at the value of $\log_{10}(5) = 0.7$. For subjects with MNT_{50} titer between LLOD and LLOQ (LLOQ: 68 for DENV-1, 87 for DENV-2, 58 for DENV-3, and 20 for DENV-4), the titer value is imputed with the mid-point between the LLOD and LLOQ (39, 48.5, 34, and 15, respectively).

Figure 32: Trial DEN-301: Geometric Mean Titre (95% Confidence Interval) of Neutralising Antibodies for Each Dengue Serotype by Visit – Subgroup Analysis by Baseline Serostatus (Per-Protocol Set-Immunogenicity Subset)

Among baseline seronegative subjects in all 3 trials conducted in the endemic regions, after the second dose (administered at Month 3), rates were ≥97.6% across all serotypes, with the exception of DENV-4 in Trial DEN-204 (88.1%). At later time points up to Month 27 in Trial DEN-301 and Month 48 in Trial DEN-204, seropositivity rates remained high (DEN-301: ≥90.4% for all dengue serotypes; DEN-204 [Group 1]: ≥89.2% for DENV-1, DENV-2, DENV-3; ≥78.0% for DENV-4). Tetravalent seropositivity rates at the evaluated time points after the second dose were high (99.5% in DEN-301 and 98.7% in DEN-313 at Month 4 and 85.7% at Month 6 in DEN-204).

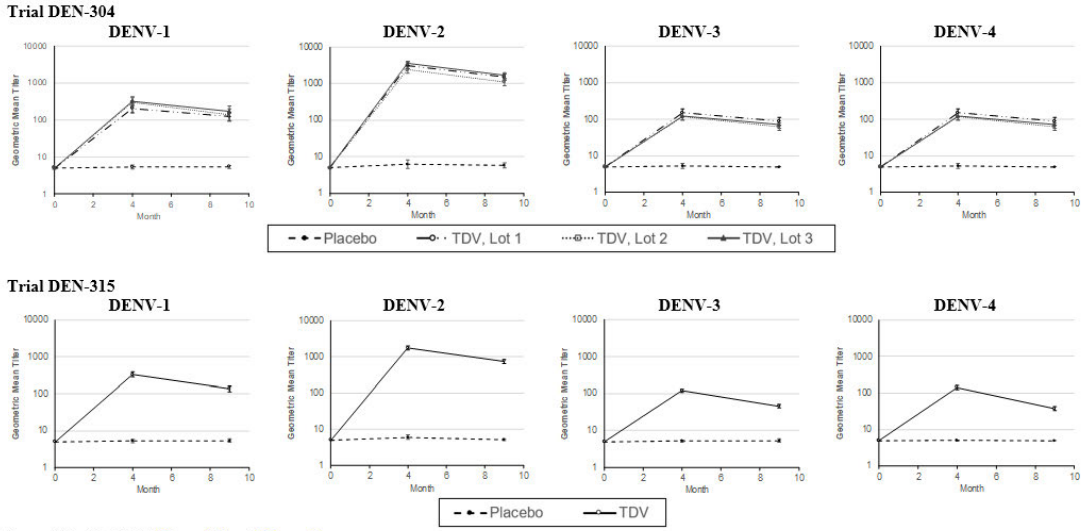
Among baseline seropositive subjects, seropositivity rates for each dengue serotype were high already at baseline, increased at Month 1, and remained high at all subsequent time points.

Non-Endemic Regions

In general, the GMT profile of TDV administered in 2 doses 3 months apart was consistent across the trials conducted in non-endemic regions, ie, Trials DEN-304 (subjects aged 18-60 years), DEN-315 (12-17 years), DEN-305 (Group 2; 18-60 years), and DEN-314 (Group 2; 18-60 years).

In both trials DEN-304 and DEN-315 GMT titres had increased at Month 4 from levels below the limit of detection at baseline and had slightly decreased at Month 9 compared with Month 4 but remained well above baseline levels showing immune persistence of antibody response) (see Figure 33).

The GMT profiles in these trials conducted in the non-endemic region were also similar to those seen in the baseline seronegative subjects of the trials conducted in the endemic region.



Source: Module 2.7.3, Figure 3.b and Figure 3.c.

Figure 33: Trials DEN-304 and DEN-315: Geometric Mean Titres (95% Confidence Interval) of Dengue Neutralising Antibody for Each Serotype (Per-Protocol Set)

In general, seropositivity rates following the administration of TDV were consistent across the trials conducted in non-endemic regions (DEN-304 and DEN-315). The patterns of seropositivity rates over time seen in these trials were also similar to those seen in the baseline seronegative subjects of the trials conducted in the endemic region.

In both Trials DEN-304 and DEN-315, at Month 4 (1 month post second vaccine dose), seropositivity rates had increased from baseline, mostly to levels well above 95%; tetravalent seropositivity was achieved in 95.6% of subjects in Trial DEN-304 and 99.6% in Trial DEN-315, demonstrating a high proportion of vaccine responders. At Month 9, seropositivity rates had slightly decreased compared with Month 4 but remained high, mostly above 90%. In Group 2 (TDV administered at Months 0 and 3) of Trials DEN-305 and DEN-314, at Month 4, 1 month post second TDV dose, seropositivity rates for individual dengue serotypes had increased to >92%.

In all 4 trials, seropositivity rates were generally higher for DENV-1 and DENV-2 than for DENV-3 and DENV-4.

Immunogenicity in Subgroups

Because of its importance and size with 20,071 treated subjects, subgroup analyses of immunogenicity data were only planned and performed in pivotal Trial DEN-301, which was conducted in different regions with different epidemiological settings and where the number of subjects within each of the prespecified subgroups was large enough to allow for interpretable results.

Results from subgroup analyses of immunogenicity by age group (4-5, 6-11, and 12-16 years) were dependent on the different proportions of baseline seropositive and seronegative subjects within each age group, as confirmed by exploratory analyses within each baseline serostatus subgroup that did not show any notable difference in GMTs or seropositivity rates between the 3 age groups. Results from the overall PPS showed that baseline GMTs were lowest in subjects aged 4 to 5 years and highest in those aged 12 to 16 years for each serotype, which is related to the lower proportion of seropositive subjects in the youngest subgroup (58.7%) compared with the older subgroups (68.9% and 83.7%, respectively). In each age group, the pattern over time was similar.

Trends seen in subgroup analyses of GMTs and seropositivity rates by region (Asia Pacific region vs Latin America) or by country were similar to those described overall, indicating no relevant difference in immune response between these subgroups. Similarly, previous vaccination against YF or JE did not appear to have a marked effect on the GMT response or seropositivity rates.

Immunobridging

TDV is developed for prevention of dengue disease caused by any dengue virus serotype in individuals 4 years and above. Pivotal Trial DEN-301 was conducted in subjects 4 to 16 years of age in endemic regions to allow for the inclusion of a reasonably large proportion of subjects who were seronegative at baseline, and for the collection of sufficient VCD cases for the various subgroup analyses. In endemic countries, the peak incidence of dengue fever tends to be in children and adolescents while the adult population tends to be pre-exposed to dengue. This limits the evaluation of VE particularly in a dengue naive adult population in a reasonably sized trial conducted in endemic regions. Therefore, the extension to the age range of >16 years is based on the available immunogenicity data through a prespecified immunobridging analysis.

The primary analysis for this extension of age range compared the immune response data in baseline seronegative subjects between Trials DEN-301 (paediatric subjects aged 4-16 years, endemic region) and DEN-304 (adult subjects aged 18-60 years, non-endemic region; data from the 3 TDV groups, corresponding to the 3 TDV lots, were combined). From both trials, immunogenicity data at 1 and 6 months post second vaccine dose, ie, at Months 4 and 9, were used.

The scientific principle of the applied analysis was to demonstrate similar levels of immune response, in terms of neutralising antibodies, elicited in both trial populations and thereby to conclude similar levels of expected protection.

The analysis population for the primary immunobridging evaluation was restricted to baseline seronegative subjects to minimise the influence of confounding factors. Dengue exposure increases with age in endemic areas and is known to positively influence subsequent immune response, both in magnitude and quality. The use of baseline seronegative subjects for this analysis is considered appropriate because it ensures that the 2 populations, ie, subjects aged 4 to 16 years (DEN-301) and subjects aged 18 to 60 years (DEN-304), are comparable except for the age factor. Additionally, the group of baseline seronegative subjects also represents a population that is universal across all regions, and thus independent of endemicity. It is reasonable to assume that the efficacy in baseline seropositive subjects will be at least the same as that in baseline seronegative subjects. In Trial DEN-301, the overall VE of TDV was shown to be similar between baseline seronegative and baseline seropositive subjects.

The number of subjects from each of Trials DEN-301 and DEN-304 included in the PPS used for immunobridging analyses were as follows:

-DEN-301 (4-16 years of age): 2518 subjects, of whom 1816 were seropositive and 702 seronegative at baseline.

-DEN-304 (18-60 years of age): 447 subjects, of whom 68 were seropositive and 379 seronegative at baseline. Overall, 109 subjects were 18 to 30 years, 153 subjects were 31 to 45 years, and 185 subjects were 46 to 60 years of age.

In the primary immunobridging analysis, differences in GMTs between the age range of 4 to 16 years (DEN-301) and the age range of 18 to 60 years (DEN-304) were estimated using an ANOVA model with natural logarithms of titres as a response variable and age group as factor. Non-inferiority was concluded for a given serotype if the upper bound of the respective 95% CI for the geometric mean ratio was below 2.0, which corresponds to the same criterion as was used for the lot-to-lot consistency

Trial DEN-304. This margin was chosen to account for the variability inherent to both the live attenuated tetravalent vaccine TDV as well as the MNT_{50} assay to determine immune response.

The non-inferiority criterion (upper bound of the 95% CI for the geometric mean ratio <2.0) for the comparison of GMTs at Month 4 was fulfilled for serotypes DENV-1, DENV-2, and DENV-4, but marginally missed for DENV-3 with an upper bound of the associated 95% CI of 2.04 (see Table 47). Thus, overall non-inferiority, ie, in all 4 serotypes, could not be shown statistically in the primary immunobridging analysis of immunogenicity data at Month 4. At Month 9, however, the non-inferiority criterion was fulfilled for all 4 dengue serotypes, with upper bounds of the 95% CI for the geometric mean ratios not having exceeded 1.18.

Table 47: Immunobridging Analysis: Adjusted Geometric Mean Titres of Dengue Neutralising Antibody for Each Serotype: Age 4 to 16 Years (DEN-301) Versus Age 18 to 60 Years (DEN-304) – Baseline Seronegative Subjects (Per-Protocol Set)

		DEN-301 (N = 702)		DEN-304 (N = 379)		
Dengue Serotype	Visit	n	Adjusted GMT (95% CI) [(a)]	n	Adjusted GMT (95% CI) [(a)]	GMR (95% CI) [(a)]
DENV-1	Month 4	641	184.2 (165.9, 204.6)	367	268.1 (233.5, 307.9)	0.69 (0.58, 0.82)
	Month 9	607	87.8 (77.8, 99.2)	353	141.7 (120.9, 166.1)	0.62 (0.51, 0.76)
DENV-2	Month 4	641	1730.2 (1603.1, 1867.3)	367	2956.9 (2673.4, 3270.4)	0.59 (0.52, 0.66)
	Month 9	607	929.4 (851.7, 1014.1)	355	1403.3 (1251.9, 1572.9)	0.66 (0.57, 0.76)
DENV-3	Month 4	641	228 (209.2, 248.5)	367	128.9 (115.0, 144.4)	1.77 (1.53, 2.04)
	Month 9	607	71.7 (65.4, 78.6)	355	73.1 (64.8, 82.4)	0.98 (0.84, 1.14)
DENV-4	Month 4	641	143.9 (132.8, 156.0)	367	137.4 (123.5, 152.9)	1.05 (0.92, 1.20)
	Month 9	607	64.0 (58.2, 70.4)	354	63.5 (56.0, 71.9)	1.01 (0.86, 1.18)

Source: Module 2.7.3, Table 3.g.

Abbreviations: CI, confidence interval; GMR, geometric mean ratio; GMT, geometric mean titer; MNT_{50}, microneutralization test resulting in a titer reduction of at least 50%; n, number of subjects with MNT_{50} results available; N, total number of subjects.

(a) Adjusted GMTs, GMRs, and corresponding 95% CIs are calculated using an analysis of (co-)variance model with comparison group as factor and baseline MNT_{50} titer as covariate, as appropriate.

Furthermore, similarity between the age groups of 4 to 16 years (DEN-301) and 18 to 60 years (DEN-304) was shown in the reverse cumulative distribution curves of dengue neutralising antibodies and the analysis of seropositivity rates with more than 95% of subjects having reached tetravalent seropositivity at Month 4, irrespective of age group.

- **Summary of main efficacy results**

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 48: Summary of efficacy for trial DEN-301 (24-month [Pt1, Pt2, and Pt1+12m])

Title: Phase III, Double-Blind, Randomised, Placebo-Controlled Trial to Investigate the Efficacy, Safety and Immunogenicity of a Tetravalent Dengue Vaccine (TDV) Administered Subcutaneously in Healthy Children Aged 4–16 Years Old	
Study identifier	Protocol number: DEN-301 EudraCT number: 2018-003979-34

	IND number: 014292 NCT number: NCT02747927	
Design	Phase 3, double-blind, randomised, placebo-controlled trial with 2 parallel groups conducted in 3 countries in Asia Pacific (Philippines, Sri Lanka, and Thailand) and 5 countries in Latin America (Brazil, Colombia, Dominican Republic, Nicaragua, and Panama). All subjects were randomised to receive two doses, 3 months apart, of either Takeda's tetravalent dengue vaccine candidate (TDV) or 0.9% normal saline placebo in a 2:1 ratio and a subgroup of subjects is to receive a booster dose according to the same randomisation for the first two doses. The planned sample size was 20,100 subjects (13,400 TDV:6700 placebo). Randomisation was stratified by region (Asia Pacific and Latin America) and age range (4-5 years, 6-11 years, and 12-16 years). The trial includes 5 time periods (Parts 1, 2, and 3 for all subjects and Parts 4 and 5 for subjects participating in the booster phase) for surveillance of febrile illness with potential dengue aetiology. Part 1 (completed) consisted of active surveillance until 120 dengue cases had been confirmed and a minimum duration of 12 months follow-up post-second vaccination had been completed. Part 2 (completed) consisted of an additional 6 months of active surveillance following completion of Part 1. Virologically confirmed cases of dengue fever (VCD) in Part 1 counted towards the primary efficacy objective, and VCD in Parts 1 and 2 contributed towards the secondary efficacy objectives. Part 3 is ongoing and consists of modified active surveillance, lasting approximately 3 years after completion of Part 2. Part 4 is planned and consists of modified active surveillance for 13 months following a booster dose of TDV or placebo (only in subjects from the per protocol set who were aged 4 to 11 years at the time of randomisation in Part 1). Part 5 is planned and consists of modified active surveillance for 1 year after the completion of Part 4.	
	Duration of main phase:	April 2016 to July 2018 (primary endpoint) (Part 1). Additional 6 months after end of Part 1 until end of Part 2 (January 2019). 12 months after end of Part 1: July 2019.
	Duration of run-in phase:	Up to 10 months prior to first vaccination (dry-run phase to test surveillance methodology and not applicable to all sites, depending on experience).
	Duration of extension phase:	Not applicable.
Hypothesis	To evaluate the superiority of 2 doses of TDV over placebo in preventing symptomatic dengue fever of any severity and due to any of the 4 dengue virus serotypes in 4- to 16-year-old subjects.	
Vaccine groups	TDV	Tetravalent dengue vaccine candidate. N=13,400 subjects. Two doses separated by 3 months (all subjects). Booster dose (planned: Parts 4 and 5) in subgroup of subjects from the per protocol set aged 4 to 11 years at the time of randomisation in Part 1.

	Placebo		Normal saline (0.9% solution). N=6700 subjects. Two doses separated by 3 months (all subjects). Booster dose (planned: Parts 4 and 5) in subgroup of subjects from the per protocol set aged 4 to 11 years at the time of randomisation in Part 1.
Endpoints and definitions	Primary endpoint	PE1	Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by any dengue serotype occurring from 30 days post-second vaccination (Day 120 [Month 4 (M4)]) until the end of Part 1, with VE defined as 1 - (λ_V/λ_C) (where λ_V and λ_C denote the hazard rates for the TDV and placebo arms, respectively).
	Key secondary endpoint	KE1	Vaccine efficacy of 2 doses of TDV in preventing hospitalisation due to VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2.
	Secondary endpoints	SE1	Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by each dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2.
		SE2	Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2 in subjects dengue seronegative at baseline.
		SE3	Vaccine efficacy of 2 doses of TDV in preventing VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2 in subjects dengue seropositive at baseline.
		SE4	Vaccine efficacy of 2 doses of TDV in preventing severe[a] VCD fever induced by any dengue serotype from 30 days post-second vaccination (Day 120 [M4]) until the end of Part 2. [a]Presented separately for assessment Dengue Case Adjudication Committee (DCAC) and Dengue Haemorrhagic Fever (DHF)
Database lock	Part 1: 15 November 2018 Part 2: 01 April 2019		

Results and Analysis

Analysis description	**Primary, Key Secondary, and Secondary Efficacy Endpoints**		
Analysis population and time point description	Per protocol set (all randomised subjects who received at least 1 dose of TDV or placebo who had no major protocol deviations). Time period is defined in each endpoint.		
Descriptive statistics and estimate variability	Treatment group	Placebo	TDV
	Number of subjects randomised	6698	13,401
	PE1 VCD cases, n/N[b] (%)	149/6316 (2.4)	61/12,700 (0.5)
	KE1 Hospitalised VCD cases, n/N[b] (%)	66/6316 (1.0)	13/12,700 (0.1)

	SE1 *DENV-1* VCD cases, n/N[b] (%)	62/6316 (1.0)	38/12,700 (0.3)
	DENV-2 VCD cases, n/N[b] (%)	80/6316 (1.3)	8/12,700 (<0.1)
	DENV-3 VCD cases, n/N[b] (%)	60/6316 (0.9)	63/12,700 (0.5)
	DENV-4 VCD cases, n/N[b] (%)	5/6316 (<0.1)	5/12,700 (<0.1)
	SE2 *Seronegative* VCD cases, n/N[b] (%)	56/1726 (3.2)	39/3531 (1.1)
	SE3 *Seropositive* VCD cases, n/N[b] (%)	150/4589 (3.3)	75/9167 (0.8)
	SE4 *Severe (DCAC)* VCD cases, n/N[b] (%)	1/6316 (<0.1)	2/12,700 (<0.1)
	DHF DHF Cases, n/N[b] (%)	7/6316 (0.1)	2/12,700 (<0.1)
	[b]n, number of cases; N, number of subjects evaluated (per protocol set)		
Effect estimate per comparison	Primary endpoint: PE1	Comparison groups	Placebo, TDV
		Vaccine efficacy	80.2%
		95% confidence interval	73.3, 85.3
		P-value	<0.001
		The primary efficacy objective was met since the lower bound of the 2-sided 95% CI for the VE was above 25%.	
	Key Secondary endpoint: KE1	Comparison groups	Placebo, TDV
		Vaccine efficacy	90.4%
		95% confidence interval	82.6, 94.7
		P-value	<0.001
		The key secondary efficacy endpoint was met since the lower bound of the 2-sided 95% CI for the VE was above 0%.	
	Secondary endpoints:		
	SE1 (DENV-1)	Comparison groups	Placebo, TDV
		Vaccine efficacy	69.8%
		95% confidence interval	54.8, 79.9
		P-value	Not applicable

		The secondary efficacy endpoint was met since the lower bound of the 2-sided 95% CI for the VE was above 0%.	
	SE1 (DENV-2)	Comparison groups	Placebo, TDV
		Vaccine efficacy	95.1%
		95% confidence interval	89.9, 97.6
		P-value	Not applicable
		The secondary efficacy endpoint was met since the lower bound of the 2-sided 95% CI for the VE was above 0%.	
	SE1 (DENV-3)	Comparison groups	Placebo, TDV
		Vaccine efficacy	48.9%
		95% confidence interval	27.2, 64.1
		P-value	Not applicable
		The secondary efficacy endpoint was met since the lower bound of the 2-sided 95% CI for the VE was above 0%.	
	SE1 (DENV-4)	Comparison groups	Placebo, TDV
		Vaccine efficacy	51.0%
		95% confidence interval	-69.4, 85.8
		P-value	Not applicable
		The secondary efficacy endpoint was not met since the lower bound of the 2-sided 95% CI for the VE was below 0%.	
	SE2 (Subjects dengue seronegative at baseline)	Comparison groups	Placebo, TDV
		Vaccine efficacy	66.2%
		95% confidence interval	49.1, 77.5
		P-value	Not applicable
		The secondary efficacy endpoint was met since the lower bound of the 2-sided 95% CI for the VE was above 0%.	
	SE3 (Subjects dengue seropositive at baseline)	Comparison groups	Placebo, TDV
		Vaccine efficacy	76.1%
		95% confidence interval	68.5, 81.9
		P-value	Not applicable
		The secondary efficacy endpoint was met since the lower bound of the 2-sided 95% CI for the VE was above 0%.	
	SE4 (Severe [DCAC])	Comparison groups	Placebo, TDV
		Vaccine efficacy	2.3%
		95% confidence interval	-977.5, 91.1
		P-value	Not applicable

		The secondary efficacy endpoint was not met since the lower bound of the 2-sided 95% CI for the VE was below 0%.	
	SE4 (DHF)	Comparison groups	Placebo, TDV
		Vaccine efficacy	85.9%
		95% confidence interval	31.9, 97.1
		P-value	Not applicable
		The secondary efficacy endpoint was met since the lower bound of the 2-sided 95% CI for the VE was above 0%.	
Notes	The primary and key secondary endpoints were met. The secondary efficacy endpoints were met except for DENV-4 and severe (DCAC) VCD which were not met due to the low number of VCD cases due to DENV-4 and the low number of severe (DCAC) VCD cases. For the primary endpoint, vaccine efficacy against VCD was 80.2%, and for the key secondary endpoint, vaccine efficacy against hospitalised VCD was 90.4%. For the secondary endpoints, vaccine efficacy against VCD was 69.8%, 95.1%, and 48.9% for DENV-1, DENV-2, and DENV-3, respectively. Vaccine efficacy against VCD was 66.2% in baseline seronegative subjects and 76.1% in baseline seropositive subjects. Vaccine efficacy against DHF was 85.9%.		

2.6.6. Discussion on clinical efficacy

Pivotal Phase 3 trial DEN-301

Design and conduct

Trial (DEN-301) was developed in two regions (Asia Pacific and Latin America). Results based on efficacy, immunogenicity, and safety data up to 24 months post second vaccine dose were initially submitted and they are summarised in detail for this submission. Moreover, data up to 54 months post second vaccine dose became available during assessment of this vaccine application and the results are also described.

Study DEN-301 is a Phase III, Double-Blind, Randomised, Placebo-Controlled Trial to investigate the efficacy, safety and immunogenicity of a Tetravalent Dengue Vaccine (TDV) administered subcutaneously in healthy children aged 4–16 years old. The trial is conducted in 3 countries of the Asia Pacific region (The Philippines, Sri Lanka, and Thailand) and in 5 countries of Latin America (Brazil, Colombia, The Dominican Republic, Nicaragua, and Panama). It is considered adequate to have chosen two different regions with 8 different countries to run this trial since this approach should allow getting an overall picture of vaccine efficacy against dengue disease, considering the varying and unpredictable epidemiology (in terms of attack rates and circulating Dengue serotypes) in different countries and regions.

Eligible subjects aged 4 to 16 years were randomised in a 2:1 ratio to receive either TDV or placebo (normal saline for injection) by a subcutaneous (SC) injection into the upper arm (planned number of subjects: 13,400 TDV; 6700 placebo). It is agreed with the applicant to have recruited subjects in the age range of 4 to 16 based on the epidemiology of dengue in the endemic Asia Pacific and Latin American countries. This approach allowed including sufficient subjects who were seronegative at baseline to allow an analysis of VE by serostatus. Moreover, it was anticipated that this population would include a relevant number of subjects who had passed only one dengue infection. This is

considered adequate as to assess vaccine efficacy since symptomatic dengue is most common during a second dengue infection.

The primary objective of the trial was to evaluate the efficacy of 2 doses of TDV in preventing symptomatic dengue fever of any severity and caused by any of the 4 dengue virus serotypes that occurred at least 30 days post-second vaccination. The key secondary objective of the trial was to assess the efficacy of TDV in preventing hospitalisation due to VCD fever. These two objectives were endorsed in the CHMP scientific advice. It was noted in the advice that it would have been unfeasible to ask for demonstrating VE against every serotype due to the varying and unpredictable epidemiology of dengue virus.

The trial comprises 5 consecutive trial parts: Part 1 was the primary analysis period, a 15-month period lasting until 12 months post second dose and included the primary efficacy analysis. This part started on the day of vaccination and ended once both of the following 2 criteria had been fulfilled: i) 120 cases of confirmed dengue fever; ii) minimum duration of subject follow-up of 12 months post-second vaccination. Part 2 was a 6-month period lasting until 18 months post second dose, at the end of which the secondary efficacy endpoints were analysed. Part 3 is a 2.5- to 3-year period for the assessment of long-term efficacy and safety; and Parts 4 and 5 comprise the booster phase of at least 25 months for the assessment of the efficacy, immunogenicity, and safety following an additional placebo or TDV booster dose administered 48 to 54 months (4-4.5 years) after the second vaccine dose. The trial protocol, which initially included Parts 1 to 3 only, has been amended to introduce a booster phase comprising Parts 4 and 5. The design of parts 1, 2 and 3 were agreed by CHMP in two scientific advice procedures. The duration of Part 3 is considered long enough to detect the possibility that in the long term, vaccination could induce the undesirable effect of more severe cases in vaccinated subjects as compared to non-vaccinated ones, as it has been observed in young baseline seronegative children that received the already licensed dengue vaccine (Hadinegoro et al, New Engl J Med. 2015;373 (13):1195-206). Parts 4 and 5 were not previously discussed by CHMP in scientific advice. In fact, as detailed in this report, parts 4 and 5, that would assess the impact of the administration of an additional booster dose, have been included after detecting that VE wanes (especially for some serotypes) two years after vaccination.

At baseline, blood samples were taken from all subjects to determine the dengue serostatus. During Parts 1 and 2, active surveillance (subjects/guardians were contacted at least weekly) was applied. Any subject with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) was asked to return to the site for dengue fever evaluation by the investigator. Febrile episodes were confirmed virologically by dengue detection RT-PCR to detect specific serotypes. During Part 3 and the booster phase (Parts 4 and 5), modified active surveillance is applied to detect dengue cases of any severity in a tiered approach based on the need for hospitalisation. The active surveillance was modified for Parts 3 to 5 to enable detection of symptomatic dengue. Subjects presenting with febrile illness not requiring hospitalisation are to be screened for dengue disease (by RT-PCR) unless there is an alternative laboratory confirmed aetiology (note: only occasionally an acute sample for RT-PCR was not taken because of an alternative laboratory confirmed diagnosis). The principal difference between the surveillance methods is that clinical chemistry, hematology, and dengue serology in acute or convalescent samples are not mandated by the protocol during Parts 3 to 5 unless the subject requires hospitalisation.

Parts 1, 2, and 3, have been completed; the remainder of the trial is still ongoing. Efficacy data up to 24 months post second vaccine dose are discussed in this MAA, and in addition, recent data up to 54 months post second vaccine dose are also discussed. It is noted that results of part 4 and 5 of the study (as amended to incorporate a booster doses) are not provided in this MAA.

The applicant detailed that for the first 18 months of Part 3, in only 3 of the 9792 febrile illness cases a sample for RT-PCR confirmation was not collected. Therefore, this situation had a negligible impact on the VE estimate for this part of the trial.

The clinical trial vaccine material used for the two doses (at Month 0 and Month 3) was intentionally formulated at a lower potency than that intended for commercial lots as to demonstrate clinical effectiveness of TDV at or near the predicted end of shelf-life of TDV. This approach was endorsed by CHMP in a scientific advice.

Subjects presenting with febrile illness (defined as temperature ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue during the dry-run, Parts 1 and 2 or requiring hospitalisation during Parts 3, 4, or 5 had/will have 2 blood samples taken to confirm dengue infection (the first or acute blood sample was taken during the acute phase of the disease -preferably within 5 days after the onset of fever- and the second or convalescent blood sample was taken 7-14 days after the acute sample. These samples were tested for analysis of dengue IgM and IgG (by ELISA), dengue NS1 antigen ELISA, dengue detection RT-PCR, haematocrit, platelet count, and liver function tests (LFTs [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)]). In addition to blood tests, clinical evaluation was performed for signs of haemorrhage or plasma leakage as well as any other abnormal signs or symptoms. The applicant states that "each local laboratory provided their reference ranges to the sponsor and centralised laboratory analyses were performed in validated laboratories designated by the sponsor". The efficacy objectives and endpoints are agreed upon.

The key secondary efficacy endpoint was determining VE in preventing hospitalisations due to VCD fever by any dengue serotype. Upon request the applicant clarified that hospitalisation criteria were not stipulated in the protocol of Trial DEN-301, due to operational difficulty to implement criteria in the absence of universally accepted hospitalisation criteria in the setting of a global trial in 8 countries across 26 sites.

The applicant has provided the clinical criteria to adjudicate a dengue case as severe, and a Dengue Case Adjudication Committee (DCAC) evaluated the severity of dengue cases. This approach is endorsed.

It is considered adequate to stratify by region (Asia Pacific and Latin America) and age range (children aged 4-5 years, 6-11 years, and 12-16 years) to ensure that each age range had the appropriate ratio of TDV to placebo in each region. The applicant states that "recruitment followed an enrolment plan to ensure representative enrolment across the age ranges and regions", and upon request, it was clarified that the planned and actual enrolment is very similar.

The population sets defined for efficacy analysis are considered adequate. The primary analysis of VE was to be made after the 2 criteria for the end of Part 1 had been fulfilled, ie: (1) at least 120 cases of VCD fever were accrued, and (2) the minimum duration of subject follow-up was at least 12 months post-second vaccination. The primary analysis method was based on a Cox proportional hazard model with trial vaccine as a factor, adjusted for age and stratified by region in the PPS. The analysis reported in the study report is conform with the analysis planned in the protocol and SAP. Multiplicity adjustment over the primary and key secondary endpoint using a hierarchical approach is endorsed. Further exploratory secondary endpoints were not controlled for multiplicity, which is considered acceptable. The adjustment for age and stratification for region in the primary analysis is considered acceptable. As agreed by CHMP in the letter of advice, the primary efficacy objective was considered to have been met if the lower bound of the 2-sided 95% CI for the VE was above 25%. VE was calculated from the HR obtained from the Cox model.

The statistical methods are generally acceptable. There were some issues which needed further clarification, such as inconsistencies in the time of collection for the primary endpoint (part 1),

unblinding and some methodological details on the randomisation procedures. These aspects were resolved on the assessment of the applicant's responses. It is noted that the protocol and study report mention the existence of a DMC Charter containing information on the adjudication of adverse events and severity of dengue cases, which has been submitted on request. Moreover, the applicant has provided additional information regarding handling of missing data, dropouts/withdrawals, and other intercurrent events with respect to the primary and key secondary endpoints.

The applicant has planned and initiated a part 4 and 5 of the study to evaluate the effect of a booster dose. An interim CSR (end of Part 4) is expected for Q4 2024, and the Final Report (Final CSR Parts 1, 2, 3, 4 and 5) for Q1 2026.

Efficacy data and additional analyses

The trial initiation date was 26 April 2016 and the data lock points were 15 November 2018 (Part 1), 1 April 2019 (Part 2), 25 October 2019 (12 months after end of Part 1), and 5 October 2020 (18 months after end of Part 2), 21 March 2022 (end of Part 3).

Overall, as planned, the 2:1 ratio for TDV: placebo was achieved (13,401:6698) and this ratio was also maintained in each age group and region as planned, although it varied slightly by age group. In general, there was good compliance in completing parts 1 and 2 of the trial since 97.4%, 96.9%, and 96.3% of subjects completed Part 1, Part 2, and until 12 months after end of Part 1, respectively. It is noted that discontinuations were equally distributed between TDV and Control groups. In total 6573 (placebo) and 13,164 (TDV) subjects received two vaccinations; 6519 (placebo), 13,033 (TDV) subjects completed Part 1; and 6487 (placebo) and 12,961 (TDV) subjects completed Part 2. Protocol deviations were also limited (7,4%) and with similar frequency in both TDV and the control group. The most common significant protocol deviations were study procedures and assessments, which therefore do not question the reliability of the trial.

There were no important differences in demographic data between the placebo and TDV groups. Overall, the mean age was 9.6 years; with most of the subjects being between 6 to 11 years (12.7% of subjects were aged 4 to 5 years, 55.2% were aged 6 to 11 years, and 32.1% were aged 12 to 16 years).

According to Baseline Dengue serostatus, 27.7% of subjects were seronegative (ie, seronegative against all 4 dengue serotypes) and 72.3% seropositive (ie, at least for one dengue serotypes) at baseline, with similar proportions per trial group. As expected, the proportion of baseline seropositive subjects was lower in subjects aged 4 to 5 years (58.7%) compared with those aged 6 to 11 years (68.9%) or 12 to 16 years (83.7%). As anticipated, considering the varying epidemiology of dengue virus, the proportions by baseline serostatus also varied by country. The history of prior vaccinations against JE or YF varied by region since prior vaccination against JE only occurred in the Asia Pacific region (where subjects are routinely vaccinated against JE) and prior vaccination against YF only occurred in Latin America.

The applicant did not determine the baseline serostatus regarding the closely related flavivirus Zika virus. Hence, the importance of such analysis was questioned since a recent publication (Katzelnick et al, Science 369, 1123-1128 (2020)) indicates that a previous Zika virus infection enhances future risk of severe dengue disease. The applicant has adequately indicated several limitations of the paper by Katzelnick et al, which questions the conclusions reached that indeed, a previous Zika infection may enhance dengue disease.

Importantly, 94.6% (19,021) of the randomised subjects were included in the PPS. Similarly, as planned, 4000 subjects (19.9%) were included in the immunogenicity subset and 98.9% (3954 subjects) were included in the safety set of immunogenicity.

Overall, from the first vaccination to the end of Part 2, acute samples were taken for 98.4% of febrile illness cases, and within 5 days for 93.6% of cases. The overall incidence of samples taken was similar in Asia Pacific (99.5%) and Latin America (97.1%), although the percentage taken within 5 days was higher in Asia Pacific (98.5%) than Latin America (88.0%). Percentages of samples taken from febrile illness cases were similar for TDV and placebo. These figures provide confidence on the results obtained from the vaccine efficacy analysis since acute sample were taken from practically all (around 98%) febrile illness cases, mostly during within five days (90%) as stated in the clinical trial protocol.

As expected from the varying dengue epidemiology, the incidence of VCD cases and the circulating dengue serotypes varied across regions and countries. In fact, a total of 390 cases were confirmed by serotype-specific RT-PCR to be VCD fever from first dose to 18 months post second vaccine dose (Parts 1 and 2 combined) and a higher proportion of VCD cases was observed in the Asia Pacific region (307/390 cases [78.7%]) than in Latin America (83/390 cases [21.3%]). Moreover, the serotype distribution for VCD fever cases in the placebo group, ie, unaffected by active vaccine administration, from the first placebo dose to 18 months post second placebo dose shows that DEN-2 caused 42.1 % of the cases followed by DEN-1 (30.1%), DEN-3 (25.1%) and DEN-4 (2.7%) was poorly represented among cases in the placebo group.

Primary endpoint

The primary objective to show efficacy in the prevention of VCD fever caused by any serotype (ie, all 4 serotypes combined) from 30 days to 12 months post second vaccine dose (until the end of Part 1; primary endpoint) was met since the pre-established criterion (the lower bound of the 2-sided 95% CI for the VE was above 25%) was fulfilled. In fact, VE was 80.2% (95% CI: 73.3%, 85.3%; p<0.001).

Three sensitivity analyses of the primary endpoint were performed (analysis using exact 95% CIs calculated as described by Breslow & Day on the PPS; analysis based on the FAS, and analysis in which cases of VCD fever were observed at any time post-second vaccination), and they supported the results from the primary analysis since the VE estimates determined were very close to that determined for the primary analysis.

The cumulative incidence of VCD fever shows a steady increase in the cumulative incidence in both groups, with a steeper slope in the placebo group and the separation between curves starting during the first month. It is noted that the curve of the TDV group is almost horizontal after one month of first dose (due to the accumulation of very few cases), and thus the presumed additional protection conferred by the second dose cannot be observed. It is also noted that the slope of the TDV group changes around month 9 suggesting that VE wanes from this time point onwards.

Key secondary endpoint

VE against the key secondary endpoint (Hospitalisation due to Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose) was very high: 90.4% (95% CI: 82.6%, 94.7%; p<0.001). The lower bound of the 2-sided 95% CI for the VE was above 0%, and thus the criterion to meet this key secondary objective was fulfilled. Secondary infections by a Dengue serotype different from that of the first infection are those with higher severity and that course with high rates of hospitalisation. Thus, prevention of hospitalisation is a very relevant clinical endpoint.

However, the high VE determined for prevention of hospitalised VCD is questioned as representative of the true VE for all four dengue serotypes. As stated by the applicant, the hospitalisation criteria were not stipulated in the protocol of Trial DEN-301, due to operational difficulty to implement criteria in the

absence of universally accepted hospitalisation criteria in the setting of a global trial in 8 countries across 26 sites. Thus, as it would have been expected the percent of hospitalised VCD Cases Among VCD Cases up to 18 months after the end of part 2 was very high in Sri Lanka (68%) as compared to the other 7 countries where the trial was developed (2.5 - 37%). In the placebo group data for 30 days post second dose to end of Part 2, Sri Lanka contributed with 44 hospitalised cases (66.6 % of total hospitalised cases), and it is noted that 41 of these 44 cases are due to DENV-2 and that VE against VCD fever was highest (95.1%) against DENV-2. Hence, the major contributor to the VE determined against hospitalised VCD are prevention of DENV-2 cases derived from only one country (Sri Lanka) and thus this does not necessarily reflect VE against the other Dengue serotypes.

It is also noted that in trial DEN-301, VE against hospitalised VCD caused by DENV-3 was negative [VE: -51 (-1356; 84)] in dengue seronegative subjects at baseline when analysed from 30 days to 18 months post second vaccination (further discussed below). The applicant provided analyses including and excluding Sri Lanka data. The VE estimates for the key secondary endpoint (prevention of hospitalised cases) were 90.4% (95%CI: 82 – 94) including Sri Lanka data, and 73.4% (46.3 -86.8%) excluding the data from this country.

Secondary endpoints

Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose, Overall and by Serostatus. VE in preventing VCD fever caused by any serotype (ie, all 4 serotypes combined) decreased slightly from 80.2% (95% CI: 73.3%, 85.3%) determined at 12 months post second dose (primary endpoint) to 73.3% (95% CI: 66.5%, 78.8%) when the period of observation was extended by 6 additional months (18 months post second vaccine dose). Similar high VE was observed in subgroups by baseline serostatus, although it was slightly lower in baseline seronegative subjects as compared to baseline seropositive ones. In fact, VE in baseline seropositive subjects was 76.1% (95% CI: 68.5%, 81.9%) and in baseline seronegative subjects was 66.2% (95% CI: 49.1%, 77.5%).

Virologically Confirmed Dengue Fever Caused by Each Individual Serotype From 30 Days to 18 Months Post Second Vaccine Dose. When assessing VE against VCD fever from 30 days to 18 months post second vaccine dose, there were varying VE of TDV against each individual dengue serotype. The highest VE was shown for DENV-2 (95.1%; 95% CI: 89.9%, 97.6%), and then for DENV-1 (VE of 69.8%; 95% CI: 54.8%, 79.9%), and DENV-3 (48.9%; 95% CI: 27.2%, 64.1%). The VE against VCD fever caused by DENV-4 was inconclusive (51.0% (95% CI: -69.4%, 85.8%) due to the lower incidence rates of cases due to this serotype. The high VE shown against DENV-2 is in line with the highest immune response (in terms of neutralising antibodies) being raised against this same serotype in vaccinated subjects. This result is also in accordance with previous studies indicating a positive trend between neutralising antibody titres and protection against clinical disease.

Severe Forms of Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose. There were only three cases that were adjudicated as severe by the DCAC whereas there were 9 cases meeting the DHF definition (7 in the placebo and 2 in the TDV group), and only one of the DHF cases was considered a severe case by DCAC. VE could not be demonstrated for DCAC-defined severe VCD fever due to the few numbers of cases. It is noted that DHF cases were determined using a programmed algorithm without applying medical judgment. On the other hand, the efficacy of TDV against DHF from 30 days to 18 months post second vaccine dose was shown with an associated VE of 85.9% (95% CI: 31.9%, 97.1%). The applicant provided details on case definitions for DHF and DCAC-defined severe cases. The difference between rate of hospitalisation and severe forms of dengue observed in DEN-301 trial was adequately addressed. References to published data from different sources are in line with the observed differences between hospitalised VCD, DHF as per the WHO 1997 criteria, and DCAC-defined severe dengue in Trial DEN-301.

Exploratory Efficacy Analyses

It is noted that these exploratory analyses have not been powered to draw any confirmatory conclusions, and results are descriptive only. Moreover, in several sub-analyses sample sizes and incidence rates became small, often precluding any robust conclusions due to the limited data.

Virologically Confirmed Dengue Fever From 30 Days to 24 Months Post Second Vaccine Dose. In terms of VE against VCD fever from 30 days to 24 months there was a slight decrease from 73.3% (95% CI: 66.5%, 78.8%) determined up to 18 months as compared to 70.2% 95% CI: 63.7%, 75.6%) up to 24 months. The same pattern is observed in both baseline seropositive and seronegative subjects and a similar conclusion was reached when the analysis was done in relation to preventing VCD leading to hospitalisation and in preventing DHF (VE: 81.4%; 95% CI: 30.0%, 95.1%).

An additional exploratory subgroup analysis estimated VE against VCD fever according to each dengue serotype by baseline serostatus. VE was found to be similar between baseline seropositive and seronegative subjects for both DENV-1 (66% and 63%) and DENV-2 (89% and 94%). The efficacy of TDV was also seen for VCD fever caused by DENV-3 in baseline seropositive subjects (62%). For the rest of the subgroups (DENV-4 baseline seropositive subjects; and DENV-3 and DENV-4 seronegative subjects) no conclusive evidence of VE was shown, since in all cases the 95% CI of the VE figures crossed zero. There were very few cases caused by DEN-4 (7 cases in the placebo and 5 cases in the TDV group) so that calculation of VE for this subgroup yielded inconclusive results. When analysing the VCD fever cases caused by DENV-3 in baseline seronegative subjects, there was a higher incidence of cases in the TDV (23/3531) than in the placebo (9/1726) group, and thus the VE determined was -29.0 (-178, 40.3). Although this negative VE figure cannot be taken as a robust result given the wide CI, it is worrisome since it indicates that TDV induces more cases of VCD fever than those that would occur in the absence of vaccination.

These observations made in the previous paragraph are also reflected in the incidence cumulative curves according to Dengue serotype and baseline serostatus. For cases caused by DENV-1 and DENV-2 the same overall pattern was observed, showing that both for placebo and TDV groups, higher VE was seen for seropositive than for seronegative subjects during the time period of analysis. It is also noted that VE against DENV-1 wanes faster than against DENV-2 since the curve for TDV starts converging with the placebo curve at a faster rate. In relation to DENV-3 it is clearly seen that the curve corresponding to placebo seronegative individuals accumulates less cases than the TDV seronegative ones over the whole period analysed, thus suggesting a negative effect of TDV in seronegative subjects over the whole period of time analysed. The applicant has now incorporated data in the SmPC describing VE along time.

The same situation is observed when VE against VCD leading to hospitalisation was calculated by dengue serotype and by baseline serostatus. The highest VE observed for hospitalisation was against cases caused by DENV-2 (100%) since there were no hospitalised cases in the TDV group (for both seropositive and seronegative subjects) vs 17 cases in placebo seronegative subjects and 33 in placebo seropositive individuals. Positive VE estimates were observed for hospitalised cases due to DENV-1 (seropositive and seronegative) and DENV-3 (seropositive) but with very wide CI intervals for DENV-1 seronegative (LL of 95%CI: 1.5%) and DENV-3 seropositive (LL of 95%CI: 5.9%) to conclude on a clear VE in these two cases. Again, although it was not statistically significant, it was worrying to observe negative vaccine efficacy estimate of -101% (-1706, 77) for hospitalisations due to VCD fever caused by DENV-3 in baseline seronegative subjects (there was 1 case in the placebo group and 4 cases in the TDV group). There was only one case of VCD caused by DENV-4 that led to hospitalisation (in a baseline seropositive subject in the placebo group), so no conclusion could be reached on prevention of hospitalisation caused by this dengue serotype.

In conclusion, analyses from data from 30 days up to 24 months post second vaccine dose, showed robust evidence of VE against VCD leading to hospitalisation only against cases due to DENV-2 (both seropositive and seronegative), and evidence of a significant VE (i.e, LL of 95%CI >20%) was obtained only for cases due to DENV-1 (seropositive at baseline). A worrying observation was the negative VE estimate (non statistically significant) for DENV-3 baseline seronegative subjects.

Virologically Confirmed Dengue Fever From 30 Days to 12 Months (Year 1) and From 13 to 24 Months (Year 2) Post Second Vaccine Dose. Compared with Year 1, a decline in efficacy of TDV was observed during Year 2. The VE for overall VCD decreased from 80.2% (95% CI: 73.3%, 85.3%) in Year 1 to 56.2% (95% CI: 42.3%, 66.8%) in Year 2. To some extent, a factor contributing to these changes between Year 1 and Year 2 was the varying serotype distribution across the VCD cases in the respective time period and the fact that the VE against each serotype was different. A decline was observed against each of the serotypes, both in seronegative and seropositive subjects although the extent of the decline was not uniform against each of the 4 dengue serotypes.

A similar trend was observed for VCD fever leading to hospitalisation, with lower VE in year 2 (VE: 76.1%; 95% CI: 50.8%, 88.4%) as compared to year 1 (VE: 95.4%; 95% CI: 88.4%, 98.2%). Subgroup analyses of VCD leading to hospitalisation by baseline serostatus showed VE decreasing both in the baseline seropositive and seronegative subjects. Incidences of severe forms of dengue were low, both during Year 1 (DCAC-defined VCD: 1 case per intervention arm; DHF: 4 cases in the placebo group vs 1 case in the TDV group) and Year 2 (DCAC-defined VCD: 1 case per intervention arm; DHF: 4 vs 2 cases), with associated VE estimates of >50% in both periods. With all the limitations due to the limited number of hospitalised VCD cases in Year 2, these data do not indicate a clear trend towards increased number of hospitalised/severe/DHF cases in seronegative subjects in the second year as compared to the first one.

Virologically Confirmed Dengue Fever From First Vaccine Dose Onwards. VE against VCD fever of 81.1% (95% CI: 64.1%, 90.0%) was determined during the 3 months between the first and second vaccine dose. This is indicative of a rapid onset of the effect of TDV from the first vaccine dose onwards. A similar trend is observed for VCD cases reported between the first and second vaccine dose leading to hospitalisation (6 in the placebo group and 2 in the TDV group) during this 3-month period. Therefore, these results do not indicate that the first dose, - due to raising a low immune response -, could predispose the recipients to a more severe dengue disease.

Characteristics of Virologically Confirmed Dengue Fever Based on Clinical Signs, Symptoms, and Laboratory Data. Overall, a trend for of more severe clinical signs were observed in the placebo group than in the TDV group, and these differences were more pronounced in baseline seropositive subjects than in baseline seronegative subjects.

Subgroup Analyses of Efficacy

Results from subgroup analyses by age group (4-5, 6-11, and 12-16 years), region (Asia Pacific region, Latin America), country, and prior vaccination against YF or JE are summarised.

Vaccine Efficacy by Age Group. Results from subgroup analyses by age group (4-5, 6-11, and 12-16 years), from 30 days to 24 months post second dose, showed that VE of TDV in preventing VCD fever was seen across all predefined age groups with VE lower values in subjects aged 4 to 5 years (51.0%), and higher values, in subjects aged 6 to 11 years (71.8%) and in subjects aged 12 to 16 years (79.3%). Further analyses by baseline serostatus showed the VE values of ≥67.0% across all subgroups by baseline serostatus within age group, with the only exception of baseline seronegative subjects in the youngest subgroup of 4 to 5 years (VE: 4.6%). Moreover, some decline in VE was seen from Year 1 to Year 2, with a higher persistence of efficacy suggested in the older age groups. Additional data provided by the applicant describe VE covering the period from 30 days to 54 months

post second dose (PP set). It is shown that VE against VCD was high in the three age groups analysed, with the lowest VE estimate [41% (95%CI: 21%, 55%)] observed in the youngest age group (4-5 yoa). VE estimates were always positive when VE was calculated, for each age group, according to the baseline serostatus, and also when calculated, for each age group, according to the serotype of the infected dengue virus. It is noted, however, that 95%CI of the VE estimates were wide, and in some cases crossed zero, due to the few cases that were observed in some of the subgroups analysed. These results provided support for the use of the vaccine in children from 4 to 16 years of age.

Vaccine Efficacy by Region and Country. Results from subgroup analyses by region and country are mainly driven by the respective epidemiological setting (serotypes that circulate) in the respective country and region and the different VE efficacy shown for the Dengue serotypes, particularly high for DENV-2 and the variable VE against DENV-3 by baseline serostatus.

The VE against VCD fever leading to hospitalisation (from 30 days after second injection up to 24 months) was high in both the Asia Pacific region (88.9%) and Latin America (94.5%). It is however noted that most of the hospitalised cases were from Asia Pacific (81 cases) vs Latin America (10 cases). In fact, when examined by country in the placebo group, 60%% of the hospitalised cases (45 out of 75) were from Sri Lanka. These data that cover the period from 30 days after second injection up to 24 months only included 11 additional cases of hospitalised VCD in the placebo group as compared to the previous analysis (from 30 days after second injection up to 18 months). In line with the previous data, 60% of hospitalised VCD cases were from Sri Lanka, which was the country with the highest rate of hospitalised VCD cases based on the number of VCD fever cases.

*Vaccine Efficacy by Prior Vaccination Against Yellow Fever or Japanese Encephalitis. Su*bgroup analyses by prior vaccination against YF or JE showed VE values of 86.1% in those subjects previously vaccinated against JE, 68.8% in those previously vaccinated against YF, and 60.6% in those not previously vaccinated against either JE or YF, suggesting an impact of prior JE or YF vaccination on the VE of TDV. However, subgroup analysis results by prior vaccination against JE or YF were highly confounded by differences between regions and countries and their underlying serotype distribution, and thus when examined in detail, overall, the data did not suggest a clear and consistent impact of prior JE or YF vaccination on the efficacy of TDV.

Virologically Confirmed Dengue Fever From First Dose to 36 Months Post Second Vaccine Dose. The overall VE in preventing VCD fever from 30 days post second vaccination up to 36 months was 59.6% (95%CI: 53.6%, 64.9%), which is lower than that observed from 30 days post second vaccination up to 24 months (VE: 70.2%; 95% CI: 63.7%, 75.6%).

The applicant shows also initial results based on the entire period from first dose until 36 months post second vaccine dose using the safety set instead of the PPS. The results are in line with those described before based on data up to 24 months post second dose. It is however noted that in the safety set (from first dose up to 39 months): i) for dengue seronegative subjects at baseline, there were more cases of VCD due to DENV-4 in the TDV group (8 cases) than in the control group (2 cases) which resulted in a negative VE although with very wide CI (VE -100%; 95%CI: -867; 56) and ii) the efficacy in terms of prevention of DHF and severe cases is only observed in dengue seropositive subjects at baseline and not in those seronegative. In line with previous results, a similar observation was made for DENV-3 (VE: -23.4; 95%CI: -125.3, 32.4).

The applicant provided analyses including and excluding Sri Lanka data. These analyses indicated that VE against VCD fever, from first dose to 36 months post second vaccine dose in baseline seronegative subjects (safety set), was shown clearly for cases due to DENV-1 and DENV-2 both when including and excluding Sri Lanka data. The evaluation of VE against VCD fever caused by DENV-3 yielded negative VE estimates both when including and excluding Sri Lanka data (-23% and -3%, respectively). Nonetheless, the CIs were wide and included 0, so the actual VE could not be determined. It is noted

that for cases due to DENV-4, there were 2 cases in the placebo group and 8 cases in the TDV group, both when including and excluding Sri Lanka data, thus the estimate of VE was negative in both cases [(VE: -105% (-867; 56) when including Sri Lanka and very similar (-107%) when excluding this country]. Thus, overall, Sri Lanka data did not appear to act as a confounding factor regarding estimation of VE for VCD fever cases. When considering VE against hospitalised VCD due to DENV-1, a similar estimate (around 77%) was observed both when including and excluding Sri Lanka data. However, for cases due to DENV-2, clear VE was only shown when Sri Lanka data were included, but no evidence of VE was obtained when these data were excluded (since in this case there were 2 cases in the placebo group vs zero in the TDV group). For DENV-3, both when Sri Lanka data were included or excluded, VE estimates against hospitalised VCD were negative (-183% and -28%, respectively) again with 95% CIs including zero. Nothing can be concluded about VE against hospitalised cases due to DENV-4, since no hospitalised cases occurred in trial DEN-301. Thus, regarding VCD hospitalised cases, Sri Lanka data only had an important effect when calculating VE for cases due to DENV-2.

Virologically Confirmed Dengue Fever From 25 to 36 Months (Year 3) Post Second Vaccine Dose. During Year 3 post second dose, the VE against overall VCD in the total PPS was 44.7% (95% CI: 32.5%, 54.7%) as well as in baseline seronegative subjects (VE: 35.5%; 95% CI: 7.3%, 55.1%) and seropositive subjects (VE: 48.3%; 95% CI: 34.2%, 59.3%). Compared with Year 2 (VE: 56.2% (95% CI: 42.3%, 66.8%), there was a decline in the efficacy against overall VCD. Efficacy against VCD leading to hospitalisation in Year 3 was 70.8% (95% CI: 49.6%, 83.0%) for the total PPS, 45.0% (95% CI: -42.6%, 78.8%) for baseline seronegative subjects, and 78.4% (95% CI: 57.1%, 89.1%) for baseline seropositive subjects. With regard to the efficacy against VCD (overall and leading to hospitalisation) caused by individual dengue serotypes and by baseline serostatus in Year 3 the results show a marginal VE against VCD fever caused by DENV-1 in baseline seronegative subjects (VE: 17.2%; 95% CI: -31.8%, 47.9%), while in DENV-1 baseline seropositive subjects, efficacy was observed although lower than in the previous year (VE: 45.4%; 95% CI: 24.5%, 60.6%). For any VCD caused by DENV-3, a marginally positive efficacy estimate was seen in both baseline seronegative subjects (VE: 9.5%; 95% CI: -144.7%, 66.5%) and seropositive subjects (VE: 15.2%; 95% CI: -46.1%, 50.8%). There were few VCD cases caused by DENV-4 to allow for meaningful conclusions. These results are indicative of waning of immune response. Importantly, for VCD caused by DENV-3 leading to hospitalisation during Year 3, in baseline seronegative subjects, an imbalance in the number of cases was seen in the TDV group (7 hospitalisations of 11 VCD cases) compared with the placebo group (1 hospitalisation out of 6 VCD cases) (the randomisation ratio of 2:1 [TDV: placebo] should be considered). This result appears to be linked to the high rate of hospitalisation of VCD cases in Sri Lanka as compared to other countries. The applicant discussed this worrying result that indicates that vaccinated seronegative subjects are at higher risk of hospitalisation if infected by DENV-3 (particularly in Year 3 after second vaccination) than those that received placebo. Moreover, considering that the trial DEN-301 was still ongoing, it was required to provide further data, up to 54 months post second vaccine dose, to re-examine the B/R in light of these new data (see below).

As already demonstrated for the period 30 days post second doses until end of part 2 (18 months post second dose), the situation is repeated when the period is extended to month 36, in that there is a large difference in the percent of VCD cases that are hospitalised, being again Sri Lanka the country with the highest percentage (72%). Again, most of hospitalised cases were due to DENV-2 (56%).

It was also considered that despite the observation that VE declines with time, it remains high for dengue seropositive subjects at baseline for at least three years and that there is no clear indication that it translates into an increased risk of severe disease for seronegative subjects.

Virologically Confirmed Dengue Fever From First Dose to 54 Months Post Second Vaccine Dose. The applicant has provided the final data from pivotal Trial DEN-301 after completion of the pre-booster

phase until 54 months post second dose (Parts 1, 2 and 3 of the trial), focusing on the benefit-risk profile in baseline seronegative subjects.

A substantial number of subjects (91.0%) of those in the safety set completed Part 3 of the trial and this percentage was similar in the 2 trial intervention groups. This is a remarkable result that provides reassurance on the obtained results. Moreover, it is also noted that more than 96% of the dengue seronegative subjects that were included in the analysis of Year 1 remained at the study at Year 4 (from 37 to 48 months post second dose). It is noted that in the overall population the incidence of febrile illnesses was lower during the last 18 months of Part 3 (15.2 cases per 100 person years in the placebo group and 13.4 cases in the TDV group) compared with the entire follow-up period since first dose (31.7 and 29.4 cases, respectively). The applicant argues that this is likely due to the fact that the trial period after 36 months post second vaccine dose mostly occurred during the coronavirus disease 2019 (COVID-19) pandemic.

Compared with the 36-month data in baseline seronegative subjects (268 cases of VCD; 51 cases leading to hospitalisation, 5 DHF cases and 2 DCAC-defined severe VCD), a total of 40 VCD cases occurred during the additional 18-month follow-up period, including 7 cases leading to hospitalisation, 1 case of DHF, and no DCAC-defined severe cases. The 7 cases of VCD leading to hospitalisation included 6 cases in the placebo group, 1 of which was the DHF case. It is noted that of the 7 additional cases leading to hospitalisation, 4 were caused by DENV-1, and 1 each caused by DENV-2, 3 and 4. The additional case of DHF was caused by DENV-1. Of note, 2 of the 7 hospitalised cases (1 DENV-4 case in the placebo group and 1 DENV-1 case in the TDV group) were not included in the yearly analyses because of the censoring rules as they were a second episode, but they are included in the cumulative analyses (safety set). It is noted that not all subjects completed the last 6 months following Year 4, as some were enrolled into the booster phase thus, the results based on these six last months have to be viewed with caution. It is noted that the new data do not help to completely address the MO raised at D180 due to the few cases caused by DENV-3 and DENV-4 in the 18-month follow-up period. In particular, 1 hospitalised VCD and no severe dengue cases caused by DENV-3 have been observed and only 1 case of hospitalised VCD (a second episode) and 5 cases of VCD caused by DENV-4 have been observed (4 of them in the last 6 months period).

The benefit of TDV against overall VCD in baseline seronegative subjects was confirmed in the analysis from first dose to 54 months post second vaccine dose with a VE estimate of 53.5% (95% CI: 41.6%, 62.9%. The VE against VCD leading to hospitalisation was 79.3% (95% CI: 63.5%, 88.2%). These VE estimates were very similar to those reached from first vaccine dose to 36 months post second vaccine dose.

VE against VCD fever, from first dose to 54 months post second vaccine dose in baseline seronegative subjects (safety set), was shown clearly for cases due to DENV-1 and DENV-2 both when including and excluding Sri Lanka data (VE estimates higher than 44%, with CI excluding zero).

The evaluation of VE against VCD fever caused by DENV-3 yielded VE estimates of -15% (95%CI: -108 , 36) and 3.4% (95%CI: -77 , 47), when including and excluding Sri Lanka data, respectively, and it is noted that in both cases, the 95%CI was very wide and included 0. Thus, these data do not suggest VE of TDV against VCD cases due to DENV-3, in baseline seronegative subjects.

Regarding cases due to DENV-4, the estimate of VE was negative when including Sri Lanka (VE: -105% (-628; 42) and very similar (-106%) when excluding this country. Considering the few VCD cases due to DENV-4, which resulted in a large 95%CI, VE against cases due to DENV-4 cannot be assessed, precluding a meaningful conclusion on the performance of TDV against this dengue serotype. Thus, overall, Sri Lanka data did not appear to act as a confounder regarding estimation of VE for VCD fever cases according to the different dengue serotypes.

Regarding VE against hospitalised VCD, identical VE estimates (78%) against hospitalised VCD due to DENV-1 were observed both when including and excluding Sri Lanka data (the LL of the 95%CI of VE were 63 and 28%, respectively). However, for cases due to DENV-2, VE was only shown when Sri Lanka data were included VE 100% [(23 vs 0 cases)], but no clear evidence of VE was obtained when Sri Lanka data were excluded (3 cases in the placebo group vs zero cases in the TDV group). However, it is pointed out that these results are not indicative of an increased risk of hospitalised VCD cases due to DENV-2 in baseline seronegative subjects.

For DENV-3, both when Sri Lanka data were included or excluded, VE estimates against hospitalised VCD were -87.9% and 15%, respectively; again, with wide 95% CIs that include zero. The applicant interpreted the negative VE estimate observed when Sri Lanka cases are included to be confounded by the high hospitalisation rate of VCD cases in Sri Lanka.

The interpretation of VE results in dengue baseline seronegative subjects by year and infecting dengue serotype is difficult due to the few numbers of cases to be compared for each analysis. No obvious pattern is observed that would indicate that hospitalised cases increase with time, a result that appears to rule out the possibility that waning of VE drives an increase in hospitalised cases. The clearest imbalance was observed in Y3 for cases due to DENV-3, where 1 vs 7 cases of hospitalised VCD were observed for the placebo and TDV groups, respectively. The applicant indicates that these data appear to be confounded by the high hospitalisation rates of Sri Lanka, since when the cases from this country are excluded, only 1 case each for the placebo and TDV groups are observed. Thus, it appears that the imbalance in hospitalisations in Year 3 cannot be reliably linked to waning of VE.

As indicated above, in dengue seronegative subjects there were no additional cases of severe Dengue (DHF and DCAC-defined severe VCD cases) caused by DENV-3. Thus, during the 54-month follow-up period, in baseline seronegative subjects infected by DENV-3 there was 1 severe case in the placebo group and 5 cases in the TDV group (that corresponded to 1 vs 4 cases of DHF, and 0 vs 2 cases of DCAC-defined severe VCD cases in the placebo and TDV groups, respectively). It is noted that one case was classified both as DHF and DCAC-defined severe VCD cases. The applicant indicated that DHF detection in Sri Lanka was almost 3 times the rate of other countries (5.8% vs 2.0% across serotypes) in the placebo group. This is likely because of the conservative hospitalisation criteria and the close monitoring with frequent platelet count evaluation and ultrasonography, a sensitive method which can detect subclinical plasma leakage. Notably, thrombocytopenia and plasma leakage are 2 of the 4 defining criteria of DHF, and thus the rate of DHF detection can be sensitive to frequency and nature of investigations. Moreover, it is noted that the 2:1 randomisation also predisposes an imbalance towards the TDV group. In conclusion, it is agreed with the applicant that the increased number of severe dengue cases due to DENV-3 in seronegative subjects is confounded by the small number of DCAC-defined severe and DHF cases, the higher rate of DHF detection in Sri Lanka, and additional statistical considerations, and thus there is no robust evidence of increased risk of severe dengue disease caused by DENV-3 in baseline seronegative subjects.

The benefit from TDV vaccination in baseline seropositive subjects, as demonstrated based on the data up to 18 months post second dose, is maintained. The VE against overall VCD from first dose to 54 months post second dose in baseline seropositive subjects was 64.2% (95% CI: 58.4%, 69.2%) and against VCD leading to hospitalisation 85.9% (95% CI: 78.7%, 90.7%).

Immunogenicity

Geometric Mean Titres of Neutralising Antibodies Against Dengue

Overall, GMTs in the TDV group increased for each serotype (independently of baseline serostatus) to Month 1 following the first vaccine dose, declined to the second vaccine dose (Month 3), increased again following the second vaccine dose to Month 4, and thereafter slightly declined to Month 15. The

magnitude of the response was comparable for DENV-1, DENV-3, and DENV-4, and was higher for DENV-2. The fact that the highest VE was found for DENV-2 fits with the GMTs against this serotype being also the highest induced by TDV, in accordance with general knowledge in that higher neutralising antibodies are associated with clinical protection.

For baseline seronegative subjects, in the TDV group, seropositivity rates for each serotype increased from baseline to 90.5% - 98.6% at Month 1, and rates continued high up to month 27 (≥90.4). At Month 39 (36 months post second vaccine dose), GMTs and seropositivity rates in the TDV showed little change compared with the preceding time point at Month 27 for both baseline seronegative subjects and baseline seropositive subjects. VE declined from 12 to 36 months from second dose (particularly during the last year), and despite that, the immunogenicity analysis indicates that in terms of GMTs and seropositivity rates that decline was not so evident. These results are indicative that other elements of the immune system, apart from neutralising antibodies, are important in maintaining long-term VE.

In a descriptive approach to evaluate a potential correlate of protection, it was found that GMTs for both cases and controls were higher in the TDV group than in the placebo group, for each serotype and irrespective of baseline serostatus. However, not specific cut-off titre was identified as predictive of clinical protection since in fact there was considerable overlap in antibody titres between cases and controls, particularly in the seronegative group.

Other Phase 3 trials

In addition to pivotal phase 3 Trial DEN-301, 6 further trials, phase 3 Trials DEN-304 and DEN-315, co-administration phase 3 Trials DEN-305 and DEN-314, and phase 2 Trials DEN-313 and DEN-204 provide immunogenicity data in support of TDV as vaccine for the prevention of dengue disease.

It is noted that the CHMP agreed, in a scientific advice letter, with the design of the lot-to-lot consistency study (study DEN-304), the 2 co-administration studies with Yellow fever and Hepatitis A vaccines (DEN-305 and DEN-314) in non-endemic countries, and with the design of the DEN-315 trial to investigate the immunogenicity and safety of 2 doses of TDV, in healthy adolescent subjects aged 12 to 17 years in a non-endemic area.

Study DEN-304. Phase 3 Trial DEN-304 was a randomised, double-blind, placebo-controlled phase 3 trial with 923 healthy subjects aged 18 to 60 years, conducted in non-endemic regions of the United States to investigate lot-to-lot consistency of 3 consecutive TDV lots in terms of equivalence of the induced immunogenicity. The primary endpoint analysis evaluated the lot-to-lot consistency at Month 4. Therefore, since TDV is a tetravalent vaccine, 12 pairwise comparisons were made. Lot-to-lot consistency could be demonstrated statistically for only 8 of the 12 pairwise comparisons, and thus, the primary endpoint was not met since it was required to show equivalence of immune responses for all pairwise comparisons. The applicant considered that the lower-than-expected size of the PPS, and high level of variability observed in the GMTs for DENV-1 could in fact have influenced the failure to meet the primary endpoint. As to complete the data of the trial, the applicant has provided reverse cumulative distribution curves of MNT_{50} titres by lot and serotype.

It is noted, however that the high levels of at least trivalent and tetravalent positivity rates (between 94.6% and 100% of subjects for each serotype in the TDV lots) were observed for up to 6 months after the second dose in all TDV lots for all dengue serotypes. Considering the explanations given by the applicant and the fact that lot-to-lot consistency is not currently (EMEA/CHMP/VWP/164653/05 Rev. 1) a requirement for approval, the lack of fulfilment of the primary endpoint for this trial is not raised as a major objection.

Study DEN-315. Trial DEN-315 was a randomised, double-blind, placebo-controlled, phase 3 trial planned in 400 healthy adolescent subjects aged 12 to 17 years in a dengue non-endemic region of Mexico (Mexico City) with 2 parallel groups to investigate the immunogenicity and safety of TDV in a dengue non-endemic area.

TDV was shown to be immunogenic in baseline dengue seronegative adolescents aged 12 to 17 years since GMTs of neutralising antibodies (MNT_{50}) increased to 327 against DENV-1, 1742 against DENV-2, 119 against DENV-3, and 142 against DENV-4 at 1 month post second dose of TDV (Month 4). Six months post second dose of TDV (Month 9), GMTs had decreased by around two-fold as compared with Month 4. In line with other studies the highest GMTs were reached against DENV-2. At 1 month post second dose (Month 4), in the TDV group, the seropositivity rate increased up to ≥99.6% for all serotypes, and a very similar figure was observed two months later.

In conclusion, this study showed that TDV was immunogenic both in terms of GMTs and seropositivity rates in adolescent (12 -17 years) seronegative population. Nonetheless, at six months post second dose the GMTs have decreased overall by more than 50%.

The applicant also performed 2 co-administration studies (DEN-305 and DEN-314) in non-endemic countries with Yellow fever and Hepatitis A vaccines with the intention to study the possible interactions of TDV vaccine with the most common vaccines used for travellers.

Study DEN-305. DEN-305 was a phase 3, observer-blind, randomised, multicentre trial in 900 healthy adults aged 18 to 60 years living in regions non-endemic for both dengue and YF, to investigate the immunogenicity and safety of TDV and a YF vaccine (YF-17D) administered concomitantly or sequentially. The trial was conducted in the United States. The primary objective was met since the difference between YF seroprotection rates 1 month after vaccination of subjects that received concomitant administration of 1 dose of TDV +YF vaccine, compared to those that received concomitantly YF vaccine +placebo was of 0.40 (-1.85%, 2.69%), being the upper bound of the 95% CI below the pre-established non-inferiority margin of 5%.

However, one of the secondary objectives aimed to demonstrate the non-inferiority of the GMT response to TDV for all dengue serotypes combined at 1 month post second dose of TDV following concomitant administration of the first dose of TDV with YF-17D vaccine or with placebo, was not met. In fact, the non-inferiority in TDV GMTs was shown for DENV-2, DENV-3, and DENV-4, but not for DENV-1 (GMTs of 297.1 vs 182.6). Moreover, lower seropositivity rates 1 month post first TDV dose were seen in the group that received TDV and YF concomitantly vs the group that received TDV and placebo for DENV-3 (80% vs 88%) and DENV-4 (78% vs 89%). This same pattern was maintained at month 7 (when the three doses of vaccines, 2 TDV and 1 YF, had been administered) since the seropositivity rates were 99% (for DENV-1 and DENV-2) and 93% (DENV-3) and 94% (DENV-4), respectively.

The applicant further discussed the data from study DEN-305 and showed that the GMT levels and the seropositivity rates in the group that received concomitant administration of TDV and YF-17D vaccine in study DEN-305 are comparable with the results obtained in the other trials. The applicant's justification is endorsed and thus it is considered that there is support for co-administration of TDV with 17D yellow fever vaccine.

Study DEN-314. DEN-314 was a phase 3, observer-blind, randomised trial in 900 healthy adult subjects aged 18 to 60 years living in regions non-endemic for both dengue and hepatitis A, to investigate the immunogenicity and safety of the co-administration of subcutaneous (SC) TDV and an intramuscular (IM) HAV vaccine. The trial was conducted in the United Kingdom. At trial entry, subjects had to be seronegative for Hepatitis A and for dengue. The primary objective of demonstrating non-inferiority of the immune response following co-administration of HAV + TDV/TDV as compared to

sequential administration was met. In terms of GMTs and seropositivity rates for the four Dengue virus serotypes, the co-administration of TDV with HAV did not show any negative impact as compared to subjects that received the two vaccines sequentially.

Other immunogenicity analysis across trials

The applicant prepared an immunogenicity report comparing humoral immunogenicity (measured by the MNT assay) in terms of GMTs and seropositivity rates, achieved in response to TDV from subjects from the seven trials in which TDV in its final formulation was administered as 2 SC doses 3 months apart. In total immunogenicity was assessed in 3717 subjects, 2796 in endemic regions and 921 subjects in the non-endemic regions. Trials conducted in non-endemic regions (DEN-304, DEN-315, and co-administration trials DEN-305 and DEN-314) provide data for baseline seronegative subjects in the age groups of 12 to 17 years (DEN-315) and 18 to 60 years.

Endemic Regions

In general, the GMT profile was consistent between trials (DEN-301, DEN-313 and DEN-204) performed in subjects from 2 to 17 years conducted in endemic regions. GMTs increased after first vaccine dose from baseline to Month 1 and decreased slightly from Month 1 to Month 3. One month after the second dose an increase was observed. At subsequent time points (the longest Month 48 in Trial DEN-204), GMTs remained well above baseline levels. In all 3 trials and in both subgroups by baseline serostatus, GMTs were generally lower for baseline seronegative subjects than for baseline seropositive subjects, and GMTs were highest (about 10-fold) for DENV-2 compared with the other serotypes. Although there is no antibody threshold to serve as a correlate of protection, the highest GMT titres observe against DENV-2 correlate well with the highest VE showed against VCD fever for this serotype in trial DEN-301. Overall, the same correlation is seen for Dengue serotypes 1, 2 and 3 in that higher titres and VE are observed in baseline seropositive subjects vs seronegative ones. No dramatic decrease in titres is observed from 24 to 36 moth post vaccination that would explain the clear reduction in VE in this period of time observed in trial DEN-301. This reflects that not only MNT titres but other elements of the immune system are involved in long-term VE.

Among baseline seronegative subjects after the second dose, seropositivity rates were generally high (≥97% across all serotypes). Similarly high values (around 10% lower) were observed up to Month 27 in Trial DEN-301 and up to Month 48 in Trial DEN-204. Among baseline seropositive subjects, seropositivity rates for each dengue serotype were high already at baseline, increased at Month 1, and remained high (around 99%) at all subsequent times.

All these data indicate a strong immune response in both seropositive and seronegative dengue subjects living in endemic areas following TDV administration. It should be noted, however, that the GMTS and seropositivity rates determined in both groups in the long term are most likely influenced by additional natural booster doses due to natural infection with Dengue viruses from of one or more different serotypes.

Non-Endemic Regions

In general, the GMT profile and seropositivity rates of TDV administered in 2 doses 3 months apart was consistent across the trials conducted in non-endemic regions (DEN-304, DEN-315 and DEN-314) which included subjects aged 18-60 years. No negative effect of age was observed.

Overall, GMT titres had increased at Month 4 and had slightly decreased at Month 9 compared with Month 4 but remained well above baseline levels showing immune persistence of antibody response. As observed in endemic areas about 10-fold higher GMTs were observed against DENV-2 than against the other 3 dengue serotypes.

In general, at Month 4 (1 month post second vaccine dose), seropositivity rates had increased from baseline, mostly to levels well above 95%. At Month 9, seropositivity rates had slightly decreased. No information on long-term (more than 9 months post second dose) persistence of antibody response is known from trials DEN-304, DEN-315 and DEN-314.

Immunobridging

The product will be indicated for prevention of dengue disease caused by any dengue virus serotype in individuals 4 years of age and above. In endemic countries the adult population tends to be pre-exposed to dengue, which makes it not feasible to evaluate VE in a dengue naive adult population. Thus, the applicant proposed the extension to the age range of >16 to 60 years based on the available immunogenicity data through a pre-specified immunobridging analysis. The primary analysis compared the immune response data in baseline seronegative subjects from Trials DEN-301 (702 paediatric subjects aged 4-16 years, endemic region) and DEN-304 (379 adult subjects aged 18-60 years, non-endemic region; data from the 3 TDV groups, corresponding to the 3 TDV lots, were combined).

From both trials, immunogenicity data (in terms of neutralising antibodies) at 1 and 6 months post second vaccine dose, ie, at Months 4 and 9, were used for the comparisons. The analysis population for the primary immunobridging evaluation was restricted to baseline seronegative subjects. The applicant used as the non-inferiority criterion for the inmunobridging analysis that the upper bound of the 95% CI for the geometric mean ratio <2.0. There is not much explanation on the rationale to choose this limit of 2 instead of a more restrictive one (1.5) used also in many immunobridging studies. The non-inferiority criterion (upper bound of the 95% CI for the geometric mean ratio <2.0) for the comparison of GMTs at Month 4 was fulfilled for serotypes DENV-1, DENV-2, and DENV-4, but marginally missed for DENV-3 with an upper bound of the associated 95% CI of 2.04. Thus, overall non-inferiority, ie, in all 4 serotypes, could not be shown statistically in the primary immunobridging analysis of immunogenicity data at Month 4. At Month 9, however, the non-inferiority criterion was fulfilled for all 4 dengue serotypes, with upper bounds of the 95% CI for the geometric mean ratios not having exceeded 1.18. It is noted that the analysis of seropositivity rates with more than 95% of subjects having reached tetravalent seropositivity at Month 4, irrespective of age group.

Considering the general variability in GMTs, these immunobridging results in their totality support the proposed indication of TDV to prevent dengue disease caused by any dengue virus serotype in individuals 4 years to 60 years of age.

Nonetheless, an important difference is to be made for seronegative subjects from 4 to 60 years of age living in endemic areas from those not living in endemic areas. On one hand, VE against VCD fever has been demonstrated in trial DEN-301 for subjects from 4-16 years of age living in endemic areas. Thus, taken into account the immunobridging exercise, it is considered that the VE observed in seronegative subjects from trial DEN-301 could be extrapolated to dengue seronegative subjects from 17-60 years of age and thus the indication can be extended from 17 to 60 years.

In addition, the CHMP concurred with the applicant that healthy subjects over 60 years of age could also benefit from vaccination with TDV and therefore agreed to remove the upper age limit of 60 years from the therapeutic indication. A detailed analysis of the safety and immunogenicity data collected in the development programme from subjects aged up to 60 years did not suggest relevant age effects; hence, a similarly favourable benefit-risk profile is anticipated for subjects aged>60 years.

Additional expert consultation

Q1. Concerning the lack of demonstration of vaccine efficacy, in baseline dengue seronegative subjects, against VCD fever in infections due to DENV-3 and the small increase

in the number of VCD hospitalised/severe cases in subjects vaccinated with TDV as compared to placebo recipients: Does the SAG consider that these limited set of data actually indicate a higher risk of hospitalised/severe dengue cases in TDV vaccinated seronegative subjects or that these results could be due to a confounding factor or be the consequence of random variability due to low number of hospitalised/severe dengue cases analysed?

The scientific advisory group (SAG) reached consensus that the existing data do not provide clear evidence on a higher risk of severe disease / hospitalisation in baseline seronegative vaccinees infected with DENV-3 serotype.

In view of non-conclusive data, the applicant's proposal to conduct a post-authorisation observational effectiveness Study (DEN-401), assessing the risk of hospitalisation associated with dengue infection by serotype in vaccinated baseline seronegative participants is supported.

Q2. During the DEN-301 clinical trial, there were very few cases due to DENV-4 to get any reliable measure of vaccine efficacy of TDV against this serotype. Taking into account the current knowledge of infections caused by DENV-4 and the immunogenicity data of the TDV clinical development programme, does the SAG expect a higher risk of VCD fever, hospitalised, or severe cases due to DENV-4 in dengue baseline seronegative recipients of TDV?

The SAG considered that there seems to be no clear increased risk. Nevertheless, the SAG considered the data to be limited to derive a definite conclusion on higher risk of VCD fever, hospitalised or severe diseases due to DENV-4 in dengue baseline seronegative recipient of Takeda's Tetravalent Dengue Vaccine (TDV). On reviewing immunological data, for DENV-4, geometric mean neutralising antibodies titres were at par with data for vaccine serotypes DENV-1 and DENV-3, but these data are not helpful if viewed against different clinical outcomes noted per serotype. On current knowledge of infection though, the SAG indicated that serotype DENV-4 is less prevalent and typically associated with milder disease. Observational study data (DEN-401) as proposed by the applicant will provide further assurance on absence of increased risk.

Q3. If in response to questions 1 and 2, the SAG considers that there is an increased risk of hospitalised/severe dengue cases in baseline dengue seronegative subjects: Does the SAG consider that limiting the use of the vaccine in baseline positive serostatus subjects could be an adequate strategy to minimise the risk of severe dengue occurring in vaccinated subjects?

In view of the advice provided for the above questions 1 & 2, the SAG concluded that the totality of data for TDV supports a broad indication, for both baseline seronegative and seropositive subjects. The assertion of the applicant that a broad indication is important for practical implementation of community vaccination and is in the interest of global public health, is acknowledged.

Q4. In relation to the use of TDV for travellers to endemic areas (taking into account that most travellers will be seronegative at baseline):

a) Does the SAG consider that TDV could be used in individuals living in non-endemic areas traveling to endemic areas?

b) Does the SAG have any specific considerations for those who travel frequently to dengue endemic areas as compared to those who travel only occasionally to these areas?

In both cases, are there any specific considerations or precautionary measures that should be taken into account for these individuals?

The SAG took various factors into account during the discussions, such as regional serotype specific endemicity, length of stay, frequency of visit to endemic areas and evidence of previous dengue infection. Although no specific guidance could be formulated, as a general recommendation, TDV could indeed be useful in individuals living in non-endemic areas travelling to endemic regions. The SAG expressed a strong recommendation for use of TDV in those travellers who previously suffered dengue (on history; laboratory confirmed). For all, the vaccination decision should be guided in concert with recommendations from their Public Health Authorities and their health care provider, taking account of individual circumstances (e.g. regions to visit, time spent at risk). The SAG agreed that there is no need to include the requirement to perform a pre-travel serostatus determination to be vaccinated.

Q5. The applicant proposes to carry out an observational study (a Nested Case-Control post-authorisation effectiveness study) post-authorisation. In this regard:

a) Does the SAG have any recommendations regarding the requirements of additional sponsored studies and or if the above-mentioned potential risks could be monitored adequately in post-approval vaccine effectiveness studies such as the proposed Nested Case-Control post authorisation effectiveness study?

b) Does the SAG consider feasible to assess the impact of TDV, on prevention of cases due to DENV-3 and DENV-4, by introducing TDV in the national vaccination programme of country where DEN-3 and DEN-4 are circulating widely and if so, would the SAG advice to include these countries in the post-marketing effectiveness study??

The SAG was in general agreement with the proposed nested case-control post-authorisation effectiveness study (DEN-401) as a means to monitor the potential risks and obtain vaccine effectiveness estimates DENV-3/DENV-4 serotypes. The SAG emphasised that the site selection should aim to maximise chance to include cases of DENV-3/DENV-4 serotypes, i.e. sites in South-East Asian countries with background circulation of DENV-3 and DENV-4. The importance of sampling of baseline serostatus in study, immediately prior to vaccination was stressed to assure there is no change of serostatus between sampling and vaccination.

In addition, particularly in reference to areas with circulating DENV-3/ DENV-4, the SAG advised to perform a phase 2 study with TDV, to be conducted in baseline seronegative individuals, allowing further characterisation of humoral and cellular immune responses, such as neutralising antibodies, binding antibodies to study the potential risk of antibody disease enhancement, B-cell memory and T-cell responses.

Q6. Does the SAG have any additional observations from the data on factors that might predispose to severe dengue after vaccination with TDV, e.g., with respect to the intended use in the paediatric or elderly population?

The SAG did not formulate specific recommendation on additional factors which might predispose to severe dengue after vaccination with TDV. It is however noted that the pivotal efficacy study was conducted in paediatric subjects from 4 years of age and above, whilst the burden of dengue is equally problematic in a younger age group. The SAG expressed the desirability for a future clinical investigation to be conducted in this regard, covering the youngest cohorts.

Q7. What is the SAG view - based on current knowledge - on the potential impact of TDV on the severity of disease caused by Zika and other flaviviruses, and vice-versa the impact of such flaviviruses on TDV protection from Dengue disease?

The SAG concluded that although interactions between flaviviruses are complex, there are insufficient data in the literature to assess the impact of TDV on severity of Zika and other flavivirus

infections. Additionally, there are no data to assess the negative impact of such flaviviruses on TDV protection from dengue.

Q8. Taking into account the frequently severe course after secondary dengue virus infection (by DENV-1 and DENV-2) without vaccination, would efficacy shown against serotype 1 and 2 outweighs the lack of efficacy, with the potential risk of disease enhancement, by serotype 3/possibly serotype 4, in seronegative subjects?

The SAG was of the view that efficacy against DENV-1 and DENV-2 (together causing most of dengue burden globally) outweighs any remaining uncertainty on lack of efficacy against DENV-3/4 and potential risks, in seronegative subjects.

Q9. Dengvaxia data suggest an increased risk of disease against serotype 2 that peaks around the third year after vaccination and declines thereafter. What is the scientific rationale for this temporal pattern, including the subsequent drop-off? Does the SAG see parallels for Qdenga regarding such a temporal pattern?"

The SAG commented that the temporal pattern of risk seen for Dengvaxia cannot easily be explained, but a similar pattern has not been observed for TDV quadrivalent vaccine.

Nevertheless, reference was made to the Kaplan Meier Curves demonstrating cumulative efficacy of TDV against [hospitalised] VCD presentation over 57 months. It was remarked that for vaccinees, a divergence of curves for baseline seropositives and seronegatives is noted around 33 months, providing rationale for booster administration around that period. In that regard, it was noted that the effect of booster vaccination is further assessed in ongoing phase 3 Trial DEN-301 (Parts 4 & 5) in the subset of approximately 10,500 subjects aged 4 to 11 years with booster given 4 to 4.5 years after the second dose of the primary vaccination in endemic regions.

Specific considerations on efficacy data for a non-EU vs. an EU context of use

In the context of a parallel EU MAA and EU-M4all application, the claimed indication is the same in principle for both applications. TDV has demonstrated efficacy in baseline seronegative subjects and hence can be used in individuals living in non-endemic areas and travelling to endemic areas.

For the parallel EU MA, it is stressed that its use should be guided in concert with recommendations from their Public Health Authorities and their health care provider, taking account of individual circumstances (SAG advice Q4; above). The proposed SmPC (section 4.4.) and Package leaflet (section 2) both include statements about the limitations of the TDV effectiveness profile and recommend continuing personal protection measures against mosquito bites after vaccination as precautionary measures.

2.6.7. Conclusions on the clinical efficacy

In relation to the Pivotal Phase 3 trial DEN-301, carried out in subjects from 4-16 years of age, the primary objective to show efficacy of Takeda Dengue Vaccine (TDV) in the prevention of Virologically Confirmed Dengue (VCD) fever caused by any serotype (i.e., all 4 serotypes combined) from 30 days to 12 months post second vaccine dose was met and the vaccine efficacy estimate determined was 80.2% (95% CI: 73.3%, 85.3%; $p<0.001$).

Similarly, VE against the key secondary endpoint (Hospitalisation due to Virologically Confirmed Dengue Fever From 30 Days to 18 Months Post Second Vaccine Dose) was very high: 90.4% (95% CI: 82.6%, 94.7%; $p<0.001$). VE against VCD fever decreased rapidly, so that in Year 2 post second dose

was 56.2% (95% CI: 42.3-66.8%) and in year 3 was 44.7% (95%CI: 32.5-54.7). This decrease was higher in dengue seronegative subjects at the time of vaccination as compared to those seropositive.

VE was also analysed according to the serotype of the infecting dengue virus. When assessing VE against VCD fever from 30 days to 18 months post second vaccine dose, there were varying VE of TDV against each individual dengue serotype. The highest VE was shown for DENV-2 (95.1%; 95% CI: 89.9%, 97.6%), and then for DENV-1 (VE of 69.8%; 95% CI: 54.8%, 79.9%), and DENV-3 (48.9%; 95% CI: 27.2%, 64.1%). The VE against VCD fever caused by DENV-4 was inconclusive (51.0% (95% CI: -69.4%, 85.8%) due to the lower incidence rates of cases due to this serotype. Importantly, significant VE estimates (higher than 42.8%, with the 95%CI always higher than 0), were reported against individual serotypes when VE was determined from 30 days to 54 months post second vaccine dose (PP set).

The benefit from TDV vaccination in baseline seropositive subjects, that was demonstrated based on the data up to 18 months post second dose, is maintained when analysed against VCD from first dose to 54 months post second dose [64.2% (95% CI: 58.4%, 69.2%)] and against VCD leading to hospitalisation [85.9% (95% CI: 78.7%, 90.7%)]

The benefit of TDV against overall VCD in baseline seronegative subjects (mainly derived from cases due to DENV-1 and DENV-2) was also confirmed in the analysis from first dose to 54 months post second vaccine dose with a VE estimate of 53.5% (95% CI: 41.6%, 62.9%. The VE against VCD leading to hospitalisation was 79.3% (95% CI: 63.5%, 88.2%).

A number of subgroup and exploratory analyses were performed to determine VE (against VCD and hospitalised VCD) according to Dengue serostatus and the infecting dengue serotype (in total 16 comparisons). It should be remarked that the trial was not powered to obtain robust estimates of VE from these analyses.

In subgroup analysis, it was noted that VE against VCD caused by DENV-3 in baseline dengue seronegative subjects was not demonstrated. In fact, the VE estimate was negative [VE: -51% (95%CI: -1356; 84)] when analysed from 30 days to 18 months post second vaccination. Moreover, VE against VCD fever caused by DENV-3, from first dose to 54 months post second vaccine dose in baseline seronegative subjects (safety set), yielded VE estimates of -15 % (95%CI: -108, 36) and it is noted that the 95%CI was very wide and included 0.

Regarding cases due to DENV-4, the estimate of VE was also negative (VE: -105 (-628; 42) when analysed in the time period from first dose to 54 months post second vaccine dose in baseline seronegative subjects (safety set). Considering the few VCD cases due to DENV-4, which resulted in a large 95%CI, VE against cases due to DENV-4 cannot be assessed, precluding a meaningful conclusion on the performance of TDV against this dengue serotype.

Other exploratory analysis showed that VE estimate against hospitalised VCD due to DENV-3 in seronegative subjects, form first doses to 54 months post second dose (safety set), was -87.9% (95%CI: -573%, 47.6%); however, VE was 15% (95%CI -254%, 79%) when data from Sri Lanka (the country with the highest hospitalisation rate of VCD cases) were excluded. These data are interpreted in that there is no clear evidence of higher hospitalisation of cases due to DENV-3 since the results are confounded by the high hospitalisation rate of VCD cases in Sri Lanka.

Only one case of hospitalised VCD caused by DENV-4 was observed in Baseline Seronegative Subjects from First Vaccine Dose to 36 and to 54 Months Post Second Dose, preventing obtaining a meaningful estimate of VE against hospitalised VCD caused by DENV-4.

The interpretation of VE results in dengue baseline seronegative subjects by year and infecting dengue serotype is difficult due to the few numbers of cases to be compared for each analysis. However, no

obvious pattern is observed that would indicate that hospitalised cases increase with time, a result that appears to rule out the possibility that waning of VE drives an increase in hospitalised cases.

Analysis of severe dengue cases (DHF and DCAC-defined severe VCD cases) identified an imbalance in baseline seronegative subjects infected by DENV-3. During the 54-month follow-up period, there was 1 severe case in the placebo group and 5 cases in the TDV group (that corresponded to 1 vs 4 cases of DHF, and 0 vs 2 cases of DCAC-defined severe VCD cases in the placebo and TDV group, respectively; it is noted that one case was classified both as DHF and DCAC-defined severe VCD cases). However, the applicant indicated that DHF detection in Sri Lanka was almost 3 times the rate in other countries (5.8% vs 2.0% across serotypes) in the placebo group. This is likely because of the conservative hospitalisation criteria and the close monitoring with frequent platelet count evaluation and ultrasonography, a sensitive method which can detect subclinical plasma leakage. Notably, thrombocytopenia and plasma leakage are 2 of the 4 defining criteria of DHF, and thus the rate of DHF detection can be sensitive to frequency and nature of investigations. Moreover, it is noted that the 2:1 randomisation also predisposes an imbalance towards the TDV group. Hence, it is agreed with the applicant that the increased number of severe dengue cases due to DENV-3 in seronegative subjects is confounded by the small number of DCAC-defined severe and DHF cases, the higher rate of DHF detection in Sri Lanka, and additional statistical considerations, and thus there is no robust evidence of increased risk of severe dengue disease caused by DENV-3 in baseline seronegative subjects.

In conclusion, it is considered that the results from primary and secondary analyses provide strong support of vaccine efficacy against VCD and hospitalised VCD. Nonetheless, it was not possible to conclude on the VE regarding DENV-4, due to the lack of cases due to this serotype in the pivotal trial, and the lack of demonstration of VE against VCD cases due to DENV-3 in seronegative subjects. It is noted, however, that VE against hospitalisation due to DENV-1 and DENV-2, in baseline seronegative subjects, was demonstrated. On the other hand, although VE against hospitalisation due to DENV-3 and DENV-4 could not be demonstrated, from the current data, although limited, there is no clear evidence of higher risk of severe disease/hospitalisation in baseline seronegative subjects infected with DENV-3 or DENV-4. From the efficacy point of view, there are no outstanding issues precluding a Scientific Opinion.

However, to better define the VE, particularly with reference to seronegative individuals, the CHMP considers the following measures necessary to address issues related to efficacy:

1. The applicant is requested to submit the final updated protocol as well as the interim status update reports and final CSR for study DEN-401.

2. The applicant is asked to provide yearly updates describing and discussing any effectiveness study published in scientific journals.

3.-The applicant is also asked to perform a study to investigate the potential benefit of an early booster dose (e.g. at about 12 months after dose 2) in seronegative individuals. [Trial DEN-301; Trial DEN-303].

2.6.8. Clinical safety

A total of 18 clinical trials (7 phase 3 trials, 6 phase 2 trials, and 5 phase 1 trials) including 27,573 subjects from dengue-endemic and non-endemic regions, covering an age range from 1.5 to 60 years were provided.

Indication is "for the prevention of dengue disease in individuals from 4years of age". The indication is irrespective of serostatus and geographical region of residence for the prevention of dengue disease caused by any dengue virus type. Cumulative safety data have been submitted up to an overall safety

cut-off date of 01 October 2020 (approximately 6 months prior to the first filling). Long-term safety data up to approximately 4.5after the second vaccine dose from the pivotal phase 3 trial (DEN-301) are available. From all subjects, 27,118 were aged 4 to 60 (target population). Of these, 19,589 subjects received at least 1 dose of any TDV, irrespective of the formulation, viral content, or regimen used, and irrespective of concomitant or subsequent co-administration of HAV or YF vaccine.

The pooled analysis of all phase 2 and phases 3 trials. The phase 2 and phase 3 trial data were used to create 2 different safety analysis pools:

- **Placebo-Controlled Safety Pool** (22,002 subjects total): Included placebo-controlled safety data from 5 randomised placebo-controlled clinical trials, 2 phase 2 (DEN-203 and DEN-204) and 3 phase 3 trials (DEN-301, DEN-304 and DEN-315)

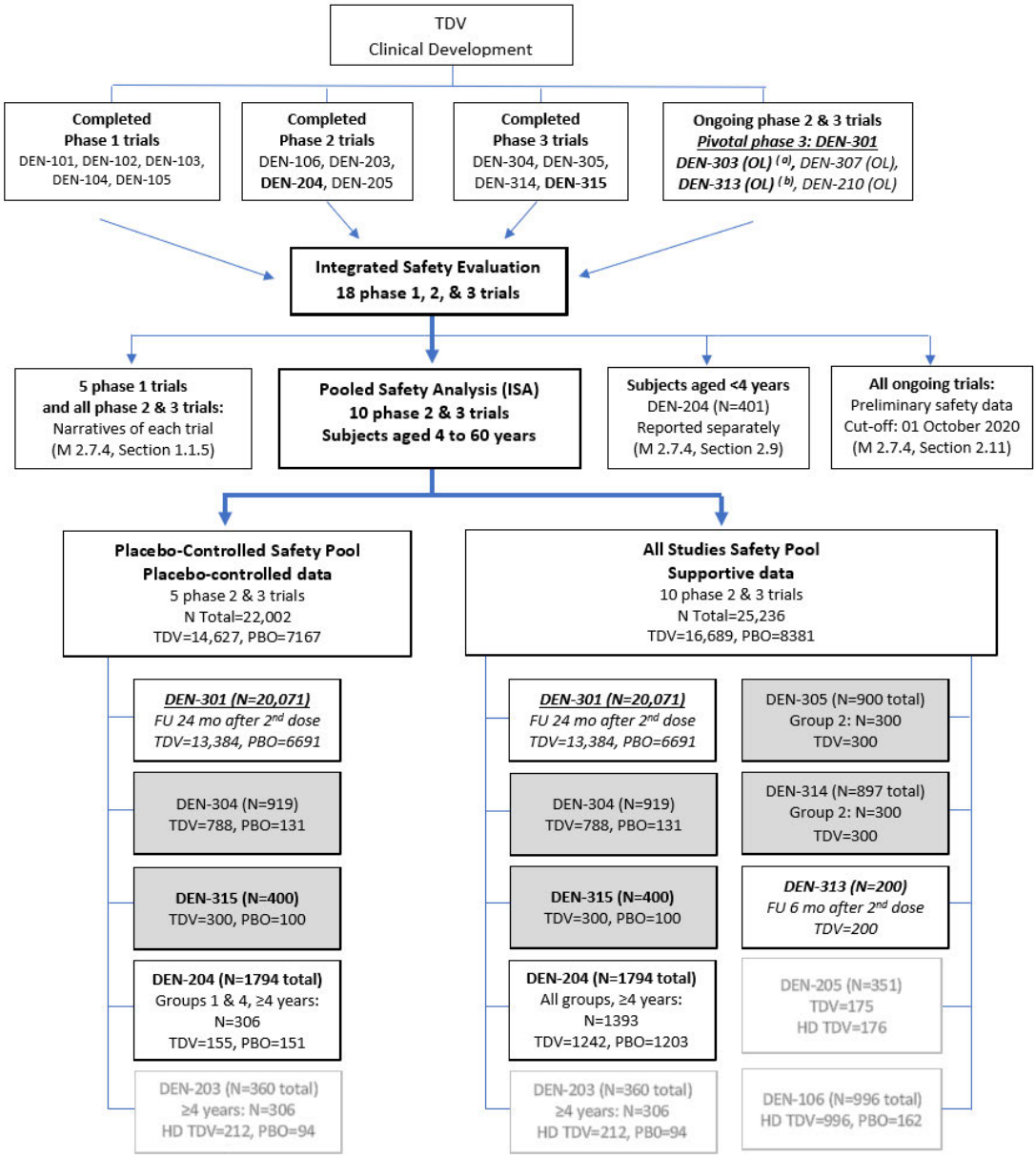
- **All Studies Safety Pool** (25,236 subjects total): Included safety data from 10 clinical trials, 5 phase 2 (DEN-203, DEN-204, DEN-205, DEN-106, DEN-313) and 5 phase 3 trials (DEN-301, DEN-304, DEN-305, DEN-314, DEN-315).

The data from 5 phase 1 trials in adults (455 subjects total) were not included in the pooled analysis because the trials were mainly designed to explore different doses, schedules, vaccine formulations, and methods or routes of administration. And data from subjects <4 years were not included in the pooled dataset because they were not part of the proposed age indication.

2.6.8.1. Patient exposure

The integrated evaluation of the safety profile of final formulation (TDV) was based primarily on the Placebo-Controlled Safety Pool (22,002 subjects total randomised 2:1; 14,627 subjects who received at least 1 dose of TDV, and 7,167 subjects who received at least 1 dose of placebo).

In All Studies Safety Pool, 16,689 subjects received at least 1 dose of TDV, and 8,381 subjects received at least 1 dose of placebo.



Abbreviations: FU, follow-up; HD TDV, high dose TDV (various formulations); ISA, Integrated Safety Analysis; M, module; mo, months; N, number of subjects; OL, open-label trial; PBO, subjects who received ≥1 dose of placebo.
Numbers in brackets refer to the total number of subjects in the safety set of the trial. All other numbers refer to the number of subjects included in the pooling (may be smaller). The number of subjects per trial group refers to subjects who received at least 1 dose of TDV, HD TDV, or placebo, and were included in the pooling. In Trials DEN-204 and DEN-106, subjects may have received both TDV (or HD TDV) and placebo at different time points. In Trial DEN-301, 4 subjects erroneously received 1 dose of TDV and 1 dose of placebo. For Trials DEN-314 and DEN-305, only subjects from Group 2 (received TDV at Month 0 and Month 3) were included in the pooling, to avoid confounding by co-administered yellow fever or hepatitis A virus vaccine.
Trials marked in bold include paediatric subjects.
Trials marked in italics have an ongoing safety follow-up.
Placebo-Controlled and All Studies Safety Pools: Trials marked in grey font used non-final TDV.
Placebo-Controlled and All Studies Safety Pools: Trials marked in grey shading were performed in non-endemic regions.
(a) Trial DEN-303 consists of an initial 15-month open-label follow-up phase, followed by a randomised, double-blind booster injection phase. All subjects enrolled in this trial already received at least 1 dose of TDV in parent Trials DEN-304 or DEN-315.
(b) Trial DEN-313: Follow-up is completed, final data analysis is ongoing.

Figure 34: Overview of Trials Used for Safety Evaluation

Methodology for collection of AEs

The pooled safety data were used to summarise the following:

- Solicited local and systemic adverse events (AEs) within the first 30 minutes after each vaccine dose (in clinic assessment).
- Solicited local AEs up to 7 days after each vaccine dose (diary-recorded).
- Solicited systemic AEs up to 14 days after each vaccine dose (diary-recorded).
- Unsolicited AEs up to 28 days after each vaccine dose.
- AEs leading to trial and/or vaccine discontinuation up to the end of trial.
- Medically attended AEs (MAAEs, defined as AEs leading to a medical consultation with healthcare professionals without fulfilling any seriousness criteria).
- Deaths and other serious AEs (SAEs).
- Vital signs data.

The evaluation of VCD, including severe dengue, vaccine viraemia, and clinical laboratory test were not part of the pooled safety analysis. The VCD was evaluated in pivotal trial DEN-301, the vaccine viraemia was evaluated mainly in DEN-205 and the reactogenicity in Phase 1 and 2 Phase 2 pooled, and laboratory findings in Phase 1 and 2 Phase 2 studies.

Demographic and baseline characteristics

In the overall population (4-60 years) no important differences between the TDV and placebo groups were observed regarding the demographic and baseline characteristics in the Placebo-Controlled Safety Pool. More than 90% of all subjects in the Placebo-controlled Safety Pool came from the pivotal Trial DEN-301 in children and adolescents aged 4 to 16 years (20,071 subjects, 91.2%). Placebo-controlled data in adults were available for a smaller set of 940 subjects enrolled in trials DEN-304 and DEN-203 and higher proportion of adults (18-60 years) was included in the TDV group than in the placebo group (5.4% vs 2.1%). In addition, >90% of all subjects in the Placebo-Controlled Safety Pool came from endemic regions, and the majority of subjects in both groups were seropositive at baseline. Moreover, a higher proportion of subjects from North America were included in the TDV group than in the placebo.

Table 49: Demographics and Other Baseline Characteristics, Target Population (4-60 Years) (Placebo-Controlled Safety Pool, Safety Set)

	TDV **N = 14,627**	**Placebo** **N = 7167**
Age, years		
Mean (SD)	11.4 (8.36)	10.3 (5.66)
Median (range)	10.0 (4.0-60.0)	10.0 (4.0-60.0)
Age categories, n (%)		
4-11 years	9210 (63.0)	4728 (66.0)
12-17 years	4629 (31.6)	2287 (31.9)
18-60 years	788 (5.4)	152 (2.1)
4-5 years	1741 (11.9)	898 (12.5)
6-60 years	12,886 (88.1)	6269 (87.5)
Gender, n (%)		
Male subjects	7295 (49.9)	3659 (51.1)

Female subjects	7332 (50.1)	3508 (48.9)
BMI, kg/m²		
N	14,617	7167
Mean (SD)	18.4 (4.49)	18.0 (3.96)
Median (range)	17.1 (8.5-64.8)	16.9 (8.8-42.1)
Race, n (%)		
Asian	6033 (41.2)	3069 (42.8)
American Indian or Alaska Native [(a)]	5469 (37.4)	2762 (38.5)
Black or African American	1643 (11.2)	819 (11.4)
White	937 (6.4)	253 (3.5)
Multiracial or other [(b)]	545 (3.7)	216 (3.0)
Unknown race	0	48 (0.7)
Region, n (%)		
Latin America	7807 (53.4)	3962 (55.3)
Asia Pacific	6032 (41.2)	3074 (42.9)
North America	788 (5.4)	131 (1.8)
Region endemic status, n (%)		
Endemic	13,539 (92.6)	6936 (96.8)
Non-endemic	1088 (7.4)	231 (3.2)
Baseline dengue serostatus, n (%)		
Seropositive	9808 (67.1)	4975 (69.4)
Seronegative	4472 (30.6)	2063 (28.8)
Unknown	347 (2.4)	129 (1.8)
Flavivirus vaccination status, n (%)		
Exposed (YF or JE)	6030 (41.2)	2995 (41.8)
Not exposed	8597 (58.8)	4172 (58.2)

Source: Module 5.3.5.3, ISA Report, Table 1.1.1.1.
Abbreviations: BMI, body mass index; HD TDV, high dose TDV; JE, Japanese encephalitis; N, number of subjects; n, number of subjects with event; YF, yellow fever.
Trials included in TDV and placebo groups: DEN-301, DEN-304, DEN-315, DEN-204 (Groups 1 and 4), and DEN-203 (placebo group only because the trial investigated HD TDV).
(a) Due to limited selection options on the case report form, this category was most frequently selected to describe the race of subjects in Latin America.
(b) Includes subjects of the category "Native Hawaiian or Other Pacific Islander".

Regarding the baseline demographics for the 2 paediatric age groups of children (4-11 years) and adolescents (12-17 years), exposure between the TDV and placebo groups were balanced. For the 4 to 11 years age group, no data are available for subjects from non-endemic regions.

In adults (18-60 years group), baseline demographics and disease characteristics were balanced in the TDV and placebo groups, except for the baseline serostatus (seropositive: 9.5% in TDV vs 20.4% in placebo and seronegative: 56.7% vs 48.7%, respectively) and the region of enrolment (100% from non-endemic region in TDV vs 86.2% from non-endemic region in placebo group).

2.6.8.2. Adverse events

Adverse events (solicited and unsolicited) were summarised for a subset in placebo-controlled trials. The results of All Studies Safety Pool were consistent with the standard analyses based on the Placebo-Controlled trials.

Solicited Adverse Events

All solicited local AEs were considered to be related to trial vaccine. The relatedness of solicited systemic AEs to trial vaccine was based on investigator assessment.

Solicited adverse events in target population (4-60 years)

Solicited AE data from placebo-controlled trials were available for a subset of 5,555 participants (3,830 subjects receiving TDV and 1,725 subjects receiving placebo). Overall, after any dose, the incidences of solicited local and systemic AEs were higher in the TDV group (43.4 % and 46.1%, respectively) than in the placebo group (25.7% and 40.1%, respectively). Solicited local and systemic AEs were generally less frequent after the second than after the first vaccine dose, both in the TDV and in the placebo groups (See Table 50).

Table 50: Solicited Adverse Events After the First, Second, and Any Vaccine Dose, Target Population (4-60 Years) (Placebo-Controlled Safety Pool, Safety Set)

	TDV N Total = 3830 [a]			Placebo N Total = 1725 [a]		
	N	n	(%)	N	n	(%)
Solicited local and/or systemic events within 30 minutes post vaccination (in clinic assessment)						
After first vaccination	3827	162	(4.2)	1724	56	(3.2)
After second vaccination	3707	116	(3.1)	1670	39	(2.3)
After any vaccination	3830	257	(6.7)	1725	90	(5.2)
Solicited local events within 7 days post vaccination (diary-recorded)						
After first vaccination	3747	1290	(34.4)	1690	327	(19.3)
After second vaccination	3654	1056	(28.9)	1647	222	(13.5)
After any vaccination	3782	1642	(43.4)	1703	437	(25.7)
Solicited systemic events within 14 days post vaccination, including fever (diary-recorded)						
After first vaccination	3752	1409	(37.6)	1691	545	(32.2)
After second vaccination	3653	981	(26.9)	1648	370	(22.5)
After any vaccination	3783	1743	(46.1)	1703	683	(40.1)
Solicited systemic events, related						
After first vaccination	3752	1063	(28.3)	1691	363	(21.5)
After second vaccination	3653	749	(20.5)	1648	260	(15.8)
After any vaccination	3783	1355	(35.8)	1703	481	(28.2)

Source: Module 5.3.5.3, ISA Report, Table 2.2.1.1.
Abbreviations: AE, adverse event; HD TDV, high dose TDV; N, number of subjects with data; n, number of subjects with event; N Total, total number of subjects (after any vaccination).
Trials included for TDV and placebo groups: DEN-301, DEN-304, DEN-315, DEN-204 (Group 1 and Group 4, data up to Month 12), and DEN-203 (placebo group only because the trial investigated HD TDV).
Solicited local AEs include injection site pain, erythema, and swelling.
Solicited systemic events may include headache, myalgia, malaise, and asthenia (adult/children panel) as well as irritability/fussiness, drowsiness, and loss of appetite (infant/toddler panel), and fever (both panels).
Related AEs: Solicited local AEs were considered to be related by default. For solicited systemic related AEs, the investigator assessed the event as related to trial vaccine. Note: Relatedness was not recorded in Trial DEN-203.
(a) In Trials DEN-301 and DEN-204, solicited AEs were collected in the randomly selected immunogenicity subset only.

Solicited Local AE in the Placebo-Controlled Safety Pool (4-60 years)

Within 30 minutes after any vaccination, a 5.7% of participants who received TDV and a 4.5% in placebo group reported any solicited local AEs. These AEs were lower after the second dose than after the first.

The most frequently solicited AE within 30 minutes reported after any vaccination was injection site pain (5.5% vs 4.5%), followed by erythema (0.2% vs 0) and swelling (0.1% vs 0). No severe solicited local AEs were reported within the first 30 minutes after any vaccine dose.

Up 7 days after any dose, the frequencies of solicited local AEs were 43.4% in TDV and 25.7% in placebo. The most frequently solicited local AEs reported was injection site pain in both groups (41.8% vs 25.4%), followed by erythema (7.1% vs 0.3%) and swelling (3.4% vs 0.6%). The majority of solicited local AEs (within 7 day) were mild. The frequency of severe (grade 3) solicited local AEs was 1.3% in TDV and 0.7% in placebo group, mainly due to injection site pain (1.3% vs 0.6%). The time to onset was, mainly, on day of vaccination in both groups and resolved with a median duration of 2-3 days in TDV groups and in 1 day in placebo group.

Overall, the solicited local AEs reported after the second dose (28.9% in TDV vs 13.5% in placebo) were lower than after the first dose (34.4% vs 19.3%, respectively). However, there was a trend of increased frequency of Grade 3 injection site pain after the second dose compared to the first one (0.8% after second dose, 0.3% after first dose, respectively), although these percentages are very low and probably not significant.

Solicited Systemic AE in the Placebo-Controlled Safety Pool (4-60 years)

Within 30 minutes after any vaccination, the solicited systemic AEs (excluding fever) were observed in 1.3% in the TDV and in 1.2% in the placebo. Headache was the most frequently reported (0.8% in TDV vs 1.0% in placebo), followed by myalgia and asthenia (0.3% each in TDV). No severe solicited systemic AEs were reported. Fever (≥38° C) within the first 30 minutes after any dose were reported by 0.1% of participants in TDV and <0.1% in placebo. No fever ≥39°C was reported.

Up to 14 days after any dose, the frequencies of solicited systemic AEs were 46.1% in TDV vs 40.1% in placebo. The solicited systemic AE after any dose most frequently reported was headache in both groups (33.8% vs 30.1%), followed by myalgia (28.0% vs 20.5%), malaise (22.9% vs 20.7%) and asthenia (19.7% vs 17.5%). Loss of appetite was reported by 17.0% and 12.4%, drowsiness by 13.2% and 12.4%, and irritability/fussiness by 12.4% and 9.6%. It should be noted that irritability/fussiness, drowsiness, and loss of appetite were part of the infant/toddler panel of AEs that was used for subjects <6 years only. The majority of the solicited systemic AEs (within 14 day) were considered related with TDV group (35.8% vs 28.2%) and mild in severity. The frequency of severe (grade 3) solicited systemic AEs was 4.1% in TDV and 3.5% in placebo group, mainly due to headache (2.8% vs 2.4%) and malaise (2% and 1.7%). The time to onset was, mainly, on the day or 1 day after the vaccination in both groups and resolved with a median duration of 3-4 days in TDV and 2-3 day in placebo groups.

The incidence of fever was lower in the TDV group (8.9%) than in the placebo group (10.5%). The incidence of severe fever (≥Grade 3) was 2.1% in TDV and 3% in placebo. Fever events generally resolved within a single day.

Upon request the applicant subsequently submitted a table summarising individual vaccine related solicited systemic AEs within 14 days after the first, the second, and any vaccine dose in the target population 4-60 years of age (overall population, Placebo-Controlled Safety Pool), together with corresponding references to source data in the dossier. In the Placebo-Controlled Safety Pool, the proportion of all subjects (4-60 years) reporting vaccine-related solicited systemic events (excluding fever) was higher in the TDV group compared with the placebo group (34.2% versus 27.9% of subjects). This numerical difference was mainly due to small differences for each systemic AE symptom, rather than due to a large difference for specific AE symptoms. After any vaccination, headache (24.4% vs 21.1% of subjects) and myalgia (22.8% vs 16.5% of subjects) were the most frequently reported related solicited systemic AEs in both groups. Vaccine related fever was not reported by a higher proportion of subjects in the vaccine group compared with the placebo group (5.4% versus 6.1% after any vaccination). The systemic AEs irritability/fussiness, drowsiness, and loss of appetite were part of the infant/toddler panel of AEs that was used for subjects <6 years only. The incidence of irritability/fussiness and loss of appetite were numerically only slightly higher in the vaccine group, while the incidence of drowsiness tended to be little higher in the placebo group (i.e. irritability/fussiness was reported for 8.6% versus 6.2% of subjects, drowsiness by 8.9% versus 9.6% of subjects, and loss of appetite by 11.2% versus 8.4% of subjects). Except for fever, vaccine-related solicited systemic AEs were consistently less frequent after the second than after the first vaccine dose. The majority of vaccine related systemic AES was mild to moderate. Severe solicited systemic ARs were reported for 3.0% of subjects in the vaccine and for 2.1% in the placebo group.

Overall, the solicited systemic AEs after the second dose (25.3% in TDV vs 19.7% in placebo) were less frequently reported than after the first dose (36.3% vs 30.7%, respectively). The incidence of Grade 3 events decreased from first to second vaccine dose in the TDV and placebo groups alike. The incidences in the TDV group after the first and second dose were 1.8% and 1.3% for headache, 1.2% and 1.0% for malaise, and 1.0% and 0.7% for asthenia, respectively. In addition, for the infant/toddler panel, the proportion of subjects with severe (Grade 3) events remained <1% for each individual event, and there was no clinically relevant change in severe (Grade 3) events from first to second vaccine dose in the TDV and placebo groups.

Solicited local and systemic prolonged AE in the Placebo-Controlled Safety Pool (4-60 years)

The incidence of prolonged solicited local (after 7 days) or systemic (after 14 days) AEs was higher in the TDV group than in the placebo group (prolonged local AEs: 3.8% in TDV vs 0.6% in Placebo; prolonged systemic AEs: 1.9% in TDV vs 1.5% in placebo after any dose). The events were mostly mild, and all subjects recovered from these events. Injection site erythema was the most frequent prolonged solicited local AE (2.8% vs 0.3%) and the most often recorded prolonged solicited systemic AE was headache (1.0% vs 0.9%).

In the TDV group, the incidence of prolonged solicited local and systemic AEs was lower after the second than after the first vaccine dose (prolonged local AEs: 3% after dose 1 and 1.4% after dose 2; prolonged systemic AEs: 1.1% after dose 1 and 0.8% after dose 2)

The majority of these events were mild or moderate in severity. In TDV group, 2 subjects (<0.1%) reported severe prolonged local AEs and 1 subject (<0.1%) in placebo. A total of 11 subjects (0.3%) reported severe prolonged systemic AEs, and 4 subjects (0.2%) in placebo. No severe events of prolonged fever were reported. The median duration of prolonged local ARs was 10 days in the TDV vaccine group.

Solicited adverse events in Children and Adolescents (4-17 years)

Solicited AE data from placebo-controlled trials were available for a subset of 2,882 participants aged 4-11 years (1,865 subjects receiving TDV and 1,017 subjects receiving placebo) and 1,733 participants aged 12-17 years (1,177 in TDV and 556 in placebo).

Overall, after any dose, the incidences of solicited local and systemic AEs were higher in the TDV group than in the placebo group in both paediatric groups. Higher frequencies of solicited local and systemic AEs in subjects aged 12-17 than in subjects aged 4-11 were observed. Additionally, in both age groups the incidence of any solicited local and systemic, after the second dose was lower than after the first dose (See Table 51).

The majority of the solicited local and systemic AEs in both age groups were mild. There were no clinically important differences regarding the onset or duration of these AEs between the 2 age groups, or between the TDV and placebo groups. Most of the AEs started on the day of vaccination or the day after and were resolved within 1 to 2 days for solicited local AEs, and within 1 to 3 days for solicited systemic AE in most of the subjects (no clinically important differences between TDV and placebo, or between the different paediatric age groups).

Table 51: Solicited Adverse Events After the First, Second, and Any Vaccine Dose, Children and Adolescents (4-17 Years) (Placebo-Controlled Safety Pool, Safety Set)

	4-11 Years						12-17 Years					
	TDV N Total = 1865 [a]			Placebo N Total = 1017 [a]			TDV N Total = 1177 [a]			Placebo N Total = 556 [a]		
	N	N	(%)	N	n	(%)	N	n	(%)	N	n	(%)
Solicited local and/or systemic events within 30 minutes post vaccination (in clinic assessment)												
After first vaccination	1862	77	(4.1)	1016	33	(3.2)	1177	67	(5.7)	556	23	(4.1)
After second vaccination	1821	36	(2.0)	993	19	(1.9)	1159	66	(5.7)	540	19	(3.5)
After any vaccination	1865	107	(5.7)	1017	50	(4.9)	1177	119	(10.1)	556	39	(7.0)
Solicited local events within 7 days post vaccination (diary-recorded)												
After first vaccination	1829	513	(28.0)	1000	196	(19.6)	1157	398	(34.4)	549	110	(20.0)
After second vaccination	1802	429	(23.8)	977	126	(12.9)	1143	350	(30.6)	534	80	(15.0)
After any vaccination	1840	671	(36.5)	1006	255	(25.3)	1168	535	(45.8)	550	154	(28.0)
Solicited systemic events within 14 days post vaccination, including fever (diary-recorded)												
After first vaccination	1831	555	(30.3)	1000	291	(29.1)	1159	532	(45.9)	549	201	(36.6)
After second vaccination	1800	397	(22.1)	978	183	(18.7)	1145	382	(33.4)	534	153	(28.7)
After any vaccination	1840	722	(39.2)	1006	366	(3i6.4)	1169	636	(54.4)	550	250	(45.5)
Solicited systemic events, related												
After first vaccination	1831	399	(21.8)	1000	179	(17.9)	1159	430	(37.1)	549	163	(29.7)
After second vaccination	1800	288	(16.0)	978	127	(13.0)	1145	315	(27.5)	534	114	(21.3)
After any vaccination	1840	527	(28.6)	1006	251	(25.0)	1169	530	(45.3)	550	197	(35.8)

Source: Module 5.3.5.3, ISA Report, Table 2.2.1.1.
Abbreviations: AE, adverse event; HD TDV, high dose TDV; N, number of subjects with data; n, number of subjects with event; N Total, total number of subjects (after any vaccination).
Trials included for TDV and placebo groups (children and adolescent subjects): DEN-301, DEN-315, DEN-204 (Group 1 and Group 4, data up to Month 12), and DEN-203 (placebo group only because the trial investigated HD TDV).
Solicited local AEs include injection site pain, erythema, and swelling.
Solicited systemic events may include headache, myalgia, malaise, and asthenia (adult/children panel) as well as irritability/fussiness, drowsiness, and loss of appetite (infant/toddler panel), and fever (both panels).
Related AEs: Solicited local AEs were considered to be related by default. For solicited systemic related AEs, the investigator assessed the event as related to trial vaccine. Note: Relatedness was not recorded in Trial DEN-203.
(a) In Trials DEN-301 and DEN-204, solicited AEs were recorded in the randomly selected immunogenicity subset only

Solicited Local AE in the Placebo-Controlled Safety Pool (4-17 years)

Within 30 minutes after any vaccination, the incidence of solicited local AEs observed was higher in the TDV group (5.1% in 4-11 years and 8.7% in 12-17 years) than in the placebo group (4.3% and 5.8%, respectively) and mainly were due to injection site pain (4-11: 5.0% vs 4.3% and 12-17: 8.5% vs 5.8%). The solicited local AEs were mostly mild in both age groups and there were no severe events within 30 minutes after any dose.

Up 7 days after any dose, the frequencies of solicited local AEs were 36.5% in TDV vs 25.3% in placebo in subjects aged 4-11 years and 45.8% vs 28.0% in 12-17 years, respectively. The most frequently solicited local AE was injection site pain in both 4-11 years and 12-17 years group (TDV: 36.1% and 45.4% vs placebo: 25% and 27.8%, respectively), followed by erythema and swelling. Severe solicited local AEs after any dose were reported by 1.0% in 4 to 11 years and 2.1% in 12 to 17 years in the TDV group, compared with 0.9% and 0.5% in the placebo group respectively

Solicited Systemic AE in the Placebo-Controlled Safety Pool (4-17 years)

Different panel of solicited systemic AEs was used for subjects <6 years (infant/toddler panel [irritability/fussiness, drowsiness, and loss of appetite]) and subjects aged ≥6 years (adult/children panel [headache, myalgia, malaise, and asthenia]). It should be noted, that for a total of 121 children aged 4 to <6 years in the Placebo-Controlled Safety Pool (122 in the All Studies Safety Pool) from Trials DEN-301, DEN-204, and DEN-203, solicited systemic AEs were recorded erroneously using the adult/children panel of symptoms.

Within 30 minutes after any vaccination, no clinically important differences between the TDV and placebo groups were observed (4-11: 0.9% vs 0.8% and 12-17: 2.0% vs 2.0%). These data were consistent with the reported in the overall population. Headache was reported most frequently in both age groups. All the solicited systemic AE within 30 minutes were mild or moderate, and non-severe immediate solicited systemic AEs were reported.

Up 14 days after any dose, the frequencies of solicited systemic AEs were 39.2% in TDV vs 36.4% in placebo in subjects aged 4-11 years and 54.4% vs 45.5% in 12-17 years, respectively.

In 4 to 11 years of age, the most frequent systemic AE for adult/children panel (subjects ≥6 years: 1,488 in TDV and 822 in placebo) was headache (27.4% vs 26.9%), followed by myalgia (22.7% vs 16.2%) and malaise (19.2% vs 17.3%). In the infant/toddler panel (subjects <6 years: 341 in TDV and 177 in placebo) loss of appetite (17% vs 12.4%) was the most frequently reported, followed by drowsiness (13.2% vs 12.4%) and irritability (12.4% vs 9.6%). Fever was reported by 10.5% and 11.6% in TDV and Placebo group, respectively. No case of fever ≥39°C was reported in subjects aged 4-11 years.

In 12 to 17 years of age, the most frequent systemic AE was headache (41.4% vs 34.2%), followed by myalgia (33.3% vs 27.3%) and malaise (29.8% vs 26.3%). Fever was reported by 10.6% and 10.1% of participants in TDV and Placebo group, respectively. No case of fever ≥39°C was reported in subjects aged 12-17 years.

Overall, the incidence of severe solicited systemic AEs in both age group was <2% for each individual event after the first or second dose of either TDV or placebo, with exception of headache. For 4-11 years group, the frequencies of severe headache were 1.3% (vs 1.3% for placebo) after the first vaccine dose and 1.1% (vs 0.7% for placebo) after the second vaccine dose and for 12-17years group, were 2.7% (vs 2.2% for placebo) and 1.9% (vs 1.9% for placebo), respectively.

Prolonged solicited local and systemic AEs in the Placebo-Controlled Safety Pool (4-17 years)

The incidence of prolonged AEs after any dose was higher in the 12 to 17 years age group (prolonged local AEs: 2.5% in TDV vs 0.5% in Placebo; prolonged systemic AEs: 2.3% in TDV vs 2.7% in placebo) than in the 4 to 11 years age group (prolonged local AEs: 0.9% in TDV vs 0.6% in Placebo; prolonged systemic AEs: 1.4% in TDV vs 0.8% in placebo). In both age groups, the incidence of prolonged solicited local and systemic AEs in the TDV groups was lower after the second than after the first vaccine dose.

In both paediatric age groups, the majority of the prolonged solicited local AEs were mild. Severe prolonged local AEs were observed in 2 subjects (prolonged injection site erythema in a 13-year-old subject in the TDV group and a 4-year-old subject in the placebo group).

The majority of the prolonged solicited systemic AEs were mild or moderate in severity. In the 4 to 11 years age group, 3 subjects in the TDV group (0.2%) and 0 in placebo had severe prolonged AEs. In the 12 to 17 years age group, 6 subjects in the TDV group (0.5%) and 4 subjects in the placebo group (0.7%) had severe prolonged AEs.

Solicited adverse events in Adults (18-60 years)

Solicited AE data from placebo-controlled trials were available for a subset of 940 participants (788 subjects receiving TDV and 152 subjects receiving placebo). The incidence of solicited local and systemic AEs, including solicited events within 30 minutes after vaccination, was higher in the TDV group than in the placebo group. The majority of the solicited local and systemic AEs were mild. Additionally, the incidence of any solicited local and systemic after the second dose was lower than after the first dose (See Table 52).

The solicited local AEs reported by adults started most frequently on the day of vaccination and the solicited systemic AEs started most frequently within 1 to 2 days. The median duration of solicited local AEs were longer in the TDV (4 days) than in placebo (1 day) group. In addition, the solicited systemic AEs were mainly resolved within 3 days for most of the adults.

Table 52: Solicited Adverse Events After the First, Second, and Any Vaccine Dose, Adults (18-60 Years) (Placebo-Controlled Safety and All Studies Safety Pool, Safety Set)

	Placebo-Controlled Safety Pool						All Studies Safety Pool					
	TDV N Total = 788			Placebo N Total = 152			TDV N Total = 1562			Placebo N Total = 302		
	N	n	(%)	N	n	(%)	N	n	(%)	N	n	(%)
Solicited local and/or systemic events within 30 minutes post vaccination (in clinic assessment)												
After first vaccination	788	18	(2.3)	152	0	0	1562	57	(3.6)	152	0	0
After second vaccination	727	14	(1.9)	137	1	(0.7)	1268	35	(2.8)	137	1	(0.7)
After any vaccination	788	31	(3.9)	152	1	(0.7)	1562	89	(5.7)	152	1	(0.7)
Solicited local events within 7 days post vaccination (diary-recorded)												
After first vaccination	761	379	(49.8)	141	21	(14.9)	1513	765	(50.6)	141	21	(14.9)
After second vaccination	709	277	(39.1)	136	16	(11.8)	1229	469	(38.2)	290	36	(12.4)
After any vaccination	774	436	(56.3)	147	28	(19.0)	1530	866	(56.6)	301	48	(15.9)
Solicited systemic events within 14 days post vaccination, including fever (diary-recorded)												
After first vaccination	762	322	(42.3)	142	53	(37.3)	1514	689	(45.5)	142	53	(37.3)
After second vaccination	708	202	(28.5)	136	34	(25.0)	1227	361	(29.4)	291	80	(27.5)
After any vaccination	774	385	(49.7)	147	67	(45.6)	1530	802	(52.4)	302	113	(37.4)
Solicited systemic events, related												
After first vaccination	762	234	(30.7)	142	21	(14.8)	1514	537	(35.5)	142	21	(14.8)
After second vaccination	708	146	(20.6)	136	19	(14.0)	1227	278	(22.7)	291	19	(6.5)
After any vaccination	774	298	(38.5)	147	33	(22.4)	1530	648	(42.4)	302	33	(10.9)

Source: Module 5.3.5.3, ISA Report, Table 2.2.1.1 and Table 2.2.1.2.
Abbreviations: HD TDV, high dose TDV; N, number of subjects; n, number of subjects with event; N Total, total number of subjects (after any vaccination).
Trials included for TDV and placebo groups (adult subjects): Placebo-Controlled Safety Pool: DEN-304 and DEN-203 (placebo group only because the trial investigated HD TDV). All Studies Safety Pool: DEN-304, DEN-305 (Group 2 only), DEN-314 (Group 2 only), DEN-205 (TDV group), and DEN-203 (placebo group only because the trial investigated HD TDV). DEN-106 (although included in the All Studies Safety Pool) is not included since it investigated HD TDV (not TDV) and had no separate placebo arm.
Solicited local AEs include injection site pain, erythema, and swelling.
Solicited systemic events may include headache, myalgia, malaise, and asthenia (adult/children panel) as well as irritability/fussiness, drowsiness, and loss of appetite (infant/toddler panel), and fever (both panels).
Related AEs: Solicited local AEs were considered to be related by default. For solicited systemic related AEs, the investigator assessed the event as related to trial vaccine. Note: Relatedness was not recorded in Trial DEN-203.

Solicited Local AE in the Placebo-Controlled Safety Pool Adults (18-60 years)

Within 30 minutes after any dose, the incidence of any solicited local AEs was 2.5% in the TDV group and 0.7% in the placebo group, injection site pain being the most frequently reported in both groups (2.0% vs 0.7%, respectively).

Up 7 days after any dose, the frequencies of solicited local AEs were 56.3% in TDV and 19.0% in placebo. The most frequent solicited local AE after any dose reported was injection site pain (50% vs 18.4%), followed by erythema (26.5% vs 0.7) and swelling (8.9% vs 0.7%).

The solicited local AEs were mostly mild and transient. The frequency of severe local AEs was 0.8% in TDV (all for injection site pain) and 0 in placebo.

Solicited Systemic AE in the Placebo-Controlled Safety Pool (18-60 years)

Within 30 minutes after any dose, the incidence of any solicited systemic AEs was 1.4% in the TDV group; there were no events in the placebo group. Except for 1 headache AE of moderate severity, the other systemic AEs within 30 minutes reported were mild in severity. Fever was reported by 0.1% (1 subject) in TDV and no case in placebo group.

Up 14 days after any dose, the frequencies of solicited systemic AEs were 49.7% in TDV and 45.6% in placebo. Headache and myalgia were the most frequent solicited systemic AEs reported after any dose

in TDV (35% and 30.5%) and placebo (33.3% and 19.7%) group, followed by malaise (19.6 in TDV vs 17.5% in placebo) and asthenia (17.2% in TDV vs 14.3% in placebo). The majority of these events were mild and transient. The frequency of severe solicited systemic AEs were 4.0% in the TDV and 1.4% in the placebo group. Fever was reported by 2.8% in TDV and 4.8% in placebo. Of these, 0.5% in TDV and 2.7% in placebo reported fever ≥39°C.

Solicited local and systemic prolonged AEs in the Placebo-Controlled Safety Pool (18-60 years)

In adults, the incidence of prolonged solicited local and systemic AEs was higher in the TDV group than in the placebo group (prolonged solicited local: 12.6% in TDV vs 0.8% in placebo, prolonged systemic AEs: 2.3% vs 0.8% respectively). The incidence of prolonged solicited local and systemic AEs in adults in the TDV groups was lower after the second than after the first vaccine dose.

The prolonged solicited local and systemic AEs were mostly mild in severity. Only 1 subject in TDV group reported 1 severe solicited prolonged local injection site pain, and no prolonged fever was reported.

Unsolicited adverse events

Unsolicited adverse events in target population (4-60 years)

The incidence of unsolicited AEs, including any AEs and any SAEs reported up to 28 days after any vaccine dose, revealed no clinically important differences between the TDV and placebo groups (21.3% vs 22.8%, respectively). The frequencies of severe unsolicited AEs were 0.5% in TDV and 0.2% in placebo.

The majority of unsolicited AEs were considered as not related to the trial vaccine and most were mild or moderate in severity The incidence of related unsolicited AEs was higher in the TDV group (3.0%) than in the placebo group (1.7%). Of these, 0.1% (5 subjects) in TDV and <0.1% (1 subjects) were severe unsolicited related AE. No related unsolicited AEs (within 28 days after any vaccination) were considered as SAE.

The unsolicited AEs by SOC most frequently reported was infections and infestations in both groups (13.4% in TDV and 16.3% in placebo after any vaccine dose), followed by General disorders and administration site conditions (2.8% vs 1.7%) and Gastrointestinal disorders (2% and 1.5%).

At the PT level, the most frequently reported unsolicited AEs in both groups were nasopharyngitis (2.6% vs 3.2% of subjects) and upper respiratory tract infection (2.3% vs 3.4% of subjects). The only unsolicited AEs reported by ≥0.5% of subjects that were reported with ≥2-fold higher incidences in the TDV group than in the placebo group were injection site bruising (0.7% vs <0.1% of subjects) and injection site pruritus (0.7% of subjects vs no subjects). Of note, all of these events were reported by adult subjects (Table 53). A table containing all unsolicited AEs included into the placebo-controlled safety pool, irrespective of causality, did not reveal any safety signal. A numerical imbalance was observed for the AEs rhinitis (1 subject, <0.1% in the placebo, and 8 subjects, 0.2% in the TDV group), and abdominal pain (recorded by 1 subject, <0.1% and 8 subjects, 0.2%).

Table 53: Unsolicited Adverse Events (Preferred Terms) Reported by ⩾0.5% of Subjects in the TDV or Placebo Group Within 28 Days After Any Vaccine Dose, Target Population (4-60 Years) (Placebo-Controlled Safety Pool, Safety Set)

	TDV N = 3830 [(a)]		Placebo N = 1725 [(a)]	
	n	(%)	n	(%)
Any unsolicited AEs	814	(21.3)	394	(22.8)
Nasopharyngitis	98	(2.6)	56	(3.2)
Upper respiratory tract infection	90	(2.3)	58	(3.4)
Viral upper respiratory tract infection	38	(1.0)	14	(0.8)
Viral infection	30	(0.8)	14	(0.8)
Pyrexia	29	(0.8)	16	(0.9)
Gastroenteritis	27	(0.7)	20	(1.2)
Injection site bruising	27	(0.7)	1	(<0.1)
Injection site pruritus	26	(0.7)	0	0
Headache	22	(0.6)	21	(1.2)
Systemic viral infection	22	(0.6)	14	(0.8)
Pharyngitis	21	(0.5)	10	(0.6)
Pharyngotonsillitis	20	(0.5)	12	(0.7)
Influenza	19	(0.5)	9	(0.5)
Tonsillitis	19	(0.5)	5	(0.3)
Varicella	15	(0.4)	13	(0.8)

Source: Module 5.3.5.3, ISA Report, Table 4.1.1.1.1 and Table 4.1.6.1.1.
Abbreviations: AE, adverse event; HD TDV, high dose TDV; N, number of subjects; n, number of subjects with event.
Trials included for TDV and placebo groups: DEN-301, DEN-304, DEN-315, DEN-204 (Group 1 and Group 4, data up to Month 12), and DEN-203 (placebo group only because the trial investigated HD TDV).
(a) In Trials DEN-301 and DEN-204, unsolicited AEs up to 28 days after each vaccine dose were recorded in the randomly selected immunogenicity subset only.

Regarding related unsolicited AEs, injection site bruising and pruritus were the most frequently reported, followed by pyrexia (0.2% vs 0.2%) and systemic viral infection, fatigue and headache (<0.1% vs 0.2%) in all PT.

By PT, the grade 3 related unsolicited AEs in TDV were one case of myalgia, anhedonia, malaise, upper tract infection and pyrexia; in the placebo group the grade 3 related unsolicited AEs was pyrexia. There was 1 additional severe and related AE of severe vomiting in the TDV group of the All Studies Safety Pool.

Unsolicited adverse events in Children and Adolescents (4-17 years)

The incidence of unsolicited AEs showed no clinically important differences between the TDV and placebo groups and between the 2 age groups (4-11 and 12-17 years). In aged group 4 to 11 the incidence of unsolicited AEs was 19.1% in TDV and 23.2% in placebo. In the group aged 12 to 17 the incidences were 23.9% and 22.1%, respectively. The frequencies of severe (Grade 3) unsolicited AEs in subjects aged 4-11 were 0.4% in TDV vs <0.1% in placebo and in subjects aged 12-17, 0.4% in TDV and 0 in placebo.

A majority of these events were considered as not related to the trial vaccine (in 4-11: 1% in TDV vs 1% in placebo and in 12-17: 1.6% vs 2.3%, respectively were considered as related). Three related Severe AEs were reported by 3 subjects from the 4-11 years group (pyrexia and upper respiratory tract infection in the TDV group, and pyrexia in the placebo group). There were no severe and related

AEs reported in the 12 to 17 years age group. No related unsolicited AEs (within 28 days after any vaccination) were considered as SAE.

The majority of unsolicited AEs were reported by SOC infections and infestations in both age groups (in 4-11: 15.1% vs 17.9%, and in 12-17: 15.2% vs 15.3%), followed by gastrointestinal disorders (in 4-11: 1% vs 1.5%, and in 12-17: 3.1% vs 0.9%). The rest of unsolicited AEs by SOC were reported by ≤1.5% in both ages and vaccine groups.

At the PT level, the most frequently reported unsolicited AE in both age groups was nasopharyngitis, followed by upper respiratory tract infection (4-11 years) or viral upper respiratory tract infection (12-17 years), with no clinically important differences between the TDV and placebo groups. The only unsolicited AEs reported by ≥0.5% of subjects and with ≥2-fold higher incidences in the TDV group than in the placebo group were reported only in the 12 to 17 years age group: abdominal pain (0.6% vs 0), diarrhoea (0.6% vs 0), gastritis (0.5% vs 0), and vomiting (0.5% vs 0).

Table 54: Unsolicited Adverse Events (Preferred Terms) Reported by ⩾0.5% of Subjects in the TDV or Placebo Group Within 28 Days After Any Vaccine Dose, Children and Adolescents (4-17 Years) (Placebo-Controlled Safety Pool, Safety Set)

	4-11 Years				12-17 Years			
	TDV N = 1865 [a]		Placebo N = 1017 [a]		TDV N = 1177 [a]		Placebo N = 556 [a]	
	n	(%)	n	(%)	N	(%)	n	(%)
Any unsolicited AEs	356	(19.1)	236	(23.2)	281	(23.9)	123	(22.1)
Nasopharyngitis	60	(3.2)	42	(4.1)	33	(2.8)	14	(2.5)
Upper respiratory tract infection	50	(2.7)	45	(4.4)	21	(1.8)	8	(1.4)
Viral upper respiratory tract infection	9	(0.5)	4	(0.4)	27	(2.3)	10	(1.8)
Viral infection	26	(1.4)	12	(1.2)	3	(0.3)	2	(0.4)
Pyrexia	19	(1.0)	10	(1.0)	9	(0.8)	5	(0.9)
Gastroenteritis	17	(0.9)	10	(1.0)	7	(0.6)	10	(1.8)
Systemic viral infection	13	(0.7)	9	(0.9)	9	(0.8)	5	(0.9)
Pharyngitis	8	(0.4)	2	(0.2)	12	(1.0)	7	(1.3)
Pharyngotonsillitis	8	(0.4)	7	(0.7)	12	(1.0)	5	(0.9)
Influenza	13	(0.7)	5	(0.5)	6	(0.5)	4	(0.7)
Tonsillitis	16	(0.9)	4	(0.4)	2	(0.2)	1	(0.2)
Headache	9	(0.5)	11	(1.1)	8	(0.7)	7	(1.3)
Varicella	12	(0.6)	10	(1.0)	3	(0.3)	3	(0.5)
Vomiting	5	(0.3)	4	(0.4)	6	(0.5)	0	0
Diarrhoea	2	(0.1)	5	(0.5)	7	(0.6)	0	0
Abdominal pain	1	(<0.1)	1	(<0.1)	7	(0.6)	0	0
Gastritis	2	(0.1)	1	(<0.1)	6	(0.5)	0	0
Bronchitis	4	(0.2)	5	(0.5)	3	(0.3)	1	(0.2)
Lower respiratory tract infection	5	(0.3)	6	(0.6)	2	(0.2)	1	(0.2)
Viral pharyngitis	0	0	0	0	7	(0.6)	7	(1.3)
Influenza like illness	2	(0.1)	1	(<0.1)	4	(0.3)	3	(0.5)
Dysmenorrhoea	0	0	0	0	5	(0.4)	3	(0.5)
Urinary tract infection	3	(0.2)	5	(0.5)	2	(0.2)	2	(0.4)
Dermatitis allergic	3	(0.2)	0	0	0	0	3	(0.5)
Myalgia	1	(<0.1)	2	(0.2)	1	(<0.1)	3	(0.5)

Source: Module 5.3.5.3, ISA Report, Table 4.1.1.1.1 and Table 4.1.6.1.1.
Abbreviations: AE, adverse event; HD TDV, high dose TDV; N, number of subjects; n, number of subjects with event.
Trials included for TDV and placebo groups (children and adolescent subjects): DEN-301, DEN-315, DEN-204 (Group 1 and Group 4, data up to Month 12), and DEN-203 (placebo group only because the trial investigated HD TDV).
Preferred terms are sorted by decreasing frequency in the TDV group for both age groups combined.

(a) In Trials DEN-301 and DEN-204, unsolicited AEs up to 28 days after each vaccine dose were recorded for a randomly selected subset (immunogenicity subset) only.

Unsolicited adverse events in Adults (18-60 years)

The incidence of any unsolicited AEs after any vaccine dose showed no clinically important differences between the TDV and placebo group in adults (22.5% vs 23.0%) while the incidence of unsolicited AEs considered to be related to the trial vaccine was higher in the TDV group (9.6% vs 4.6% of subjects).

Most of these unsolicited AEs were mild or moderate in severity. The incidences of severe unsolicited AEs were 0.8% in TDV and 1.3% in placebo. Of these, a 0.4% in TDV was considered as related severe unsolicited AEs, but none were reported in the placebo group. No related unsolicited AE was considered SAE.

By SOC, the most frequently unsolicited AEs reported was general disorders and administration site conditions (8.4% vs 4.6%). The incidence of AEs in most other frequently reported SOC was numerically lower in the TDV group than in the placebo group (<7% in TDV vs <9.5% in placebo).

At the PT level, injection site bruising (3.4%), injection site pruritus (3.3%), and upper respiratory tract infection (2.4%) were the most frequently reported AEs in the TDV group compared with upper respiratory tract infection (3.3%) and fatigue (2.6%) in the placebo group.

Table 55: Unsolicited Adverse Events (Preferred Terms) Reported by ⩾1.0% of Subjects in the TDV Group or Placebo Group Within 28 Days After Any Vaccine Dose, Adults (18-60 Years) (Placebo-Controlled and All Studies Safety Pools, Safety Set)

	Placebo-Controlled Safety Pool				All Studies Safety Pool			
	TDV N = 788		Placebo N = 152		TDV N = 1563		Placebo N = 314	
	n	(%)	n	(%)	n	(%)	n	(%)
Any unsolicited AEs	177	(22.5)	35	(23.0)	396	(25.3)	60	(19.1)
Injection site bruising	27	(3.4)	1	(0.7)	35	(2.2)	1	(0.3)
Injection site pruritus	26	(3.3)	0	0	39	(2.5)	0	0
Upper respiratory tract infection	19	(2.4)	5	(3.3)	37	(2.4)	12	(3.8)
Arthralgia	7	(0.9)	2	(1.3)	14	(0.9)	2	(0.6)
Diarrhoea	7	(0.9)	2	(1.3)	17	(1.1)	2	(0.6)
Nausea	6	(0.8)	3	(2.0)	11	(0.7)	3	(1.0)
Headache	5	(0.6)	3	(2.0)	10	(0.6)	5	(1.6)
Nasopharyngitis	5	(0.6)	0	0	17	(1.1)	0	0
Myalgia	3	(0.4)	2	(1.3)	4	(0.3)	2	(0.6)
Dermatitis contact	2	(0.3)	2	(1.3)	4	(0.3)	2	(0.6)
Fatigue	2	(0.3)	4	(2.6)	5	(0.3)	4	(1.3)
Oropharyngeal pain	2	(0.3)	3	(2.0)	10	(0.6)	3	(1.0)
Rhinorrhoea	2	(0.3)	3	(2.0)	12	(0.8)	3	(1.0)
Back pain	1	(0.1)	2	(1.3)	7	(0.4)	3	(1.0)
Blood pressure increased	1	(0.1)	2	(1.3)	1	(<0.1)	2	(0.6)
Influenza like illness	1	(0.1)	2	(1.3)	3	(0.2)	3	(1.0)
Anaemia	0	0	2	(1.3)	0	0	2	(0.6)

Source: Module 5.3.5.3, ISA Report, Table 4.1.1.1.1, Table 4.1.1.1.2, Table 4.1.6.1.1, and Table 4.1.6.1.2.
Abbreviations: AE, adverse event; HD TDV, high dose TDV; N, number of subjects; n, number of subjects with event.
Trials included for TDV and placebo groups (adult subjects): Placebo-Controlled Safety Pool: DEN-304 and DEN-203 (placebo group only because the trial investigated HD TDV). All Studies Safety Pool: DEN-304, DEN-305 (Group 2 only), DEN-314 (Group 2 only), DEN-205 (TDV group), and DEN-203 (placebo group only because the trial investigated HD TDV). DEN-106 (although included in the All Studies Safety Pool) is not included since it investigated HD TDV (not TDV) and had no separate placebo arm.

2.6.8.3. Serious adverse event/deaths/other significant events

Deaths

Overall, 20 deaths occurred throughout the clinical programme, and none was related to vaccine administration. Of the 20 deaths, 12 deaths occurred up to database lock for the pooled analyses and 8 deaths occurred in Trial DEN 301 after database lock for the pooled analysis (during the period 25-36 months [5 deaths] and post 36 months [3 deaths]).

Of the 12 deaths, 6 deaths (0.04%) occurred in the TDV group and 4 (0.05%) in the placebo group. Two additional deaths from the TDV group were not included because 1 subject was aged <4 years (Trial DEN-204, death from septic shock) and 1 subject received a sequential dose of YF vaccine (Trial DEN-305, death from cardiac arrest).

Serious adverse events in the target population (4-60)

For both analysis pools, >95% of all SAEs occurred >30 days after the first or the second vaccine dose, and the majority resolved within 1 to 2 weeks. The overall incidence of SAEs (Placebo-Controlled Safety Pool) was lower in the TDV group than in the placebo group (4.60% in TDV vs 5.57% in placebo). Expressed as follow-up time adjusted SAE rates per 100 person-years (FTAR), the incidence rate was 2.84 in the TDV group and 3.26 in the placebo group after any vaccine dose. In the supportive All Studies Safety Pool, the corresponding incidence rates were consistent with those of the placebo-controlled pool.

In the TDV and placebo groups of both analysis pools, SAEs were most frequently reported from the SOC infections and infestations.

At the PT level, SAEs reported with a higher incidence in the TDV group than in the placebo group were gastroenteritis (0.25% vs 0.17%), asthma (0.12% vs 0.06%), cellulitis (0.11% vs 0.03%), and lower respiratory tract infection (0.10% vs 0.06%). None of these SAEs was considered to be related to TDV by the investigator. The results for the All Studies Safety Pool were consistent with those for the Placebo-Controlled Safety Pool.

There was a single, non-fatal related SAE in TDV (suspected dengue illness, not VCD), compared with 4 related SAE cases in the placebo group (hypersensitivity in 2 subjects, dengue fever, DHF). The related SAE in the TDV group (Trial DEN-301) was reported by a baseline dengue seropositive teenage female. The event started on Day 12 after the first vaccine dose, the subject was hospitalised, and was considered as related based on the temporal association with the expected time window of vaccine viraemia. The sponsor also considered the event as possibly related. The central laboratory RT PCR test was negative for wild-type dengue. However, because the subject was afebrile, no blood sample was collected to test for vaccine viraemia. The event resolved within 5 days. The case was not referred to the DCAC because this suspected dengue illness was not a VCD.

Serious adverse events in children and adolescents (4-17)

In both paediatric age groups, the incidences of any SAEs were lower in the TDV than in the placebo groups (4-11: 5.04% in TDV vs 5.75% in placebo and 12-17: 4.17% vs 5.38%).

No SAE was reported as related to TDV in age group 4- to 11 years. One non-fatal related SAE (suspected non-confirmed dengue illness) was reported in age group 12-17 years in TDV vs 4 in placebo.

At the PT level, SAEs in age group 4-11 that were reported with a ≥2-fold higher incidence in the TDV group than in the placebo group were asthma (0.17% vs 0.04%), lower respiratory tract infections (0.13% vs 0.06%), humerus fracture (0.14% vs 0.06%), cellulitis (0.13% vs 0.04%), lymphadenitis

(0.10% vs 0.02%), and road traffic accident (0.05% vs 0). In the 12 to 17 years age group, pneumonia (0.09% vs 0.04%), cellulitis (0.09% vs 0), road traffic accident (0.11% vs 0.04%), and lymphadenitis (0.06% vs 0) were reported with a ≥2-fold higher incidence in the TDV group than in the placebo group. For either age group, none of these SAEs with a notably higher incidence in the TDV group were considered to be related to TDV by the investigator.

Serious adverse events in adults (18-60)

In both, the Placebo-Controlled Safety Pool, and the All Studies Safety Pool, the overall incidence of any SAE irrespective of causality was comparable in the TDV and the placebo group. Slightly lower incidence of SAEs was reported in the TDV than in placebo group (2.03% vs 2.63%). The incidence of SAEs after any vaccine dose was 4.17 and 4.90 events per 100 person-years in the TDV and placebo group, respectively. No SAEs were reported that were considered to be related to trial vaccine.

Medically Attended Adverse Events

MAAE data are available from 1088 subjects who received TDV and from 231 subjects who received placebo aged 12-60 years (from DEN-304 and DEN-3015) for the Placebo-Controlled Safety Pool. No MAAE data is available from children in the 4 to 11 years age group.

The overall incidence of MAAEs showed no clinically important difference between the TDV and placebo groups. Expressed as MAAEs per 100 person-years to adjust for differences in duration of follow-up (FTAR), the overall incidence of MAAEs was 56.4 events per 100 person-years in the TDV group and 53.0 events per 100 person-years in the placebo group. Higher incidences were observed in adolescents than in adults in TDV group (110.0 versus 34.4 events per 100 person-years after any vaccine dose) and in placebo group (78.7 versus 32.1 events per 100 person-years after any vaccine dose.

In All Studies Safety Pool, the MAAEs per FTAR in TDV (70.4 per 100 person-year) was higher than in Placebo-Controlled Safety Pool (56.4 per 100 person-years). The imbalance could be explained by the fact that the 190 children aged 4 to 11 years in the TDV group of the All Studies Safety Pool had a high rate of 194.5 MAAEs per 100 person-years. The FATAR in adolescent and Adults were similar in both pools.

The majority of all MAAEs were not related to trial vaccine in both pools (related: ≤0.9% of subjects) and were mild or moderate in severity. The incidence of related MAAEs was similar between the TDV and placebo groups. However, the incidence of severe MAAEs was higher in the TDV group than in the placebo group in both analysis pools. None of these severe MAAEs was related to trial vaccine, and subjects fully recovered except for 4 subjects (back pain, pneumonia, hepatitis C, and wrist fracture who were still recovering at the time of database lock). Additionally, one MAAE (moderate pain in extremity) led to vaccine and trial discontinuation after the first TDV dose in DEN-304.

Virologically Confirmed Dengue Including Hospitalised VCD and Severe Forms of Dengue

The approach to assess severe forms of dengue varied across trials, and therefore, this evaluation was not part of the pooled safety analysis. The evaluation of VCD cases, including hospitalised VCD cases and severe forms of dengue, from the safety perspective was based primarily on the data from pivotal Trial DEN-301. Data from Trials DEN-204 and DEN-313 were considered as supportive because no predefined dengue severity criteria were used.

In the pivotal Trial DEN-301, all subjects presenting with febrile illness or presenting with clinical symptoms of dengue were centrally assessed for VCD by serotype-specific RT-PCR.

Among all cases of VCD, severe forms of dengue were determined in 2 different ways:

- All hospitalised VCD cases evaluated for classification as severe dengue by the blinded DCAC (using severe dengue DCAC predefined criteria). All non-hospitalised VCD cases were considered non-severe.
- All VCD cases meeting the WHO 1997 criteria for DHF, were identified using a programmed algorithm (without applying medical judgment) and including DHF of Grade I-IV.

Initially, the applicant submitted the data of VCD, including hospitalised VCD cases, and severe forms of dengue up to 12, 24 and 36 months after the second dose caused by all serotypes. However, the applicant submitted only the data of VCD, including hospitalised VCD caused by DEN-3. During assessment, the applicant submitted overall VCD data (all serotypes together) up to 54 months after the second dose and the cases of hospitalisations and severe forms of dengue caused by DENV-3 and DENV-4 in seronegative subjects at baseline. Also, the applicant submitted new summary tables from DEN-301 including data of VCD, hospitalised cases and severe forms up to 12, 24, 36 and 54 months after the second dose caused by all serotypes combined or by each serotype individually, in the overall population or by baseline serostatus.

Virologically confirmed dengue including hospitalised VCD cases, all serotypes combined

Overall, the incidences of VCD and of hospitalised VCD (all 4 serotypes combined) up to 12, 24, 36 and 54 months after the second vaccine dose were lower in the TDV group versus placebo. Same results were observed in the baseline seropositive and seronegative subgroups for all 4 serotypes combined.

For the overall population, the incidence of VCD were 0.6% in TDV and 3.0% in placebo up to 12 months after second dose; 1.3% vs 4.6% up to 24 months; 2.9% vs 7.4% up to 36 months; 3.3% vs 8.2% up to 54 months, respectively (RR of VCD were <0.5 up to 12, 24, 36 and 54 months after the second dose). And the incidence of Hospitalised VCD were <0.1% vs 1.0% up to 12 months after second dose; 0.1% vs 1.4% up to 24 months; 0.3% vs 1.9% up to 36 months; and 0.3% vs 2.1 up to 54 months, respectively (RR of hospitalised VCD were <0.2 up to 12, 24, 36 and 54 months after the second dose).

In baseline seropositive subjects, the incidence of VCD were 0.6% in TDV and 3.0% in placebo up to 12 months after second dose; 1.2% vs 4.7% up to 24 months; 2.7% vs 7.4% up to 36 months; 3.1% vs 8.1% up to 54 months, respectively (RR of VCD in seropositive subjects were <0.5 up to 12, 24, 36 and 54 months after the second dose). And the incidence of Hospitalised VCD were <0.1% vs 0.9% up to 12 months after second dose; 0.1% vs 1.3% up to 24 months; 0.3% vs 1.9% up to 36 months; and 0.3% vs 2.1% up to 54 months, respectively (RR of hospitalised VCD in seropositive subjects were <0.2 up to 12, 24, 36 and 54 months after the second dose).

In baseline seronegative subjects, the incidence of VCD were 0.6% in TDV and 2.9% in placebo up to 12 months after second dose; 1.5% vs 4.5% up to 24 months; 3.4% vs 7.4% up to 36 months; 3.96% vs 8.35% up to 54 months, respectively (RR of VCD in seronegative subjects were <0.5 up to 12, 24, 36 and 54 months after the second dose). And the incidence of Hospitalised VCD were <0.1% vs 1.1% up to 12 months after second dose; 0.2% vs 1.5% up to 24 months; 0.4% vs 1.9% up to 36 months; 0.46% vs 2.24% up to 54 months, respectively (RR of hospitalised VCD in seronegative subjects were ≤0.2 up to 12, 24, 36 and 54 months after the second dose).

Table 56: Trial DEN-301: Incidence of Virologically Confirmed Dengue (VCD) and Hospitalised VCD From First Dose Up to 12, 24, 36, and 54 Months After the Second Vaccine Dose, Overall and by Baseline Serostatus (Safety Set)

	12 Months		24 Months		36 Months		54 Months	
	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687
Overall								
VCD								
Number (%) of cases	78 (0.6)	199 (3.0)	175 (1.3)	310 (4.6)	390 (2.9)	494 (7.4)	442 (3.3)	547 (8.2)
Relative risk (95% CI)	0.20 (0.15, 0.25)		0.28 (0.23, 0.34)		0.39 (0.35, 0.45)		0.40 (0.36, 0.46)	
Hospitalised VCD								
Number (%) of cases	9 (<0.1)	67 (1.0)	20 (0.1)	91 (1.4)	42 (0.3)	126 (1.9)	46 (0.3)	142 (2.1)
Relative risk (95% CI)	0.07 (0.03, 0.13)		0.11 (0.07, 0.18)		0.17 (0.12, 0.24)		0.16 (0.12, 0.23)	
Baseline seropositive subjects								
N evaluated	9661	4852	9663	4854	9663	4854	9663	4854
VCD								
Number (%) of cases	55 (0.6)	146 (3.0)	119 (1.2)	227 (4.7)	262 (2.7)	358 (7.4)	295 (3.1)	394 (8.1)
Relative risk (95% CI)	0.19 (0.14, 0.26)		0.26 (0.21, 0.33)		0.37 (0.31, 0.43)		0.38 (0.32, 0.44)	
Hospitalised VCD								
Number (%) of cases	8 (<0.1)	46 (0.9)	13 (0.1)	64 (1.3)	26 (0.3)	91 (1.9)	29 (0.3)	101 (2.1)
Relative risk (95% CI)	0.09 (0.04, 0.18)		0.10 (0.06, 0.19)		0.14 (0.09, 0.22)		0.14 (0.10, 0.22)	
Baseline seronegative subjects								
N evaluated	3714	1832	3714	1832	3714	1832	3714	1832
VCD								
Number (%) of cases	23 (0.6)	53 (2.9)	56 (1.5)	83 (4.5)	128 (3.4)	136 (7.4)	147 (3.96)	153 (8.35)
Relative risk (95% CI)	0.21 (0.13, 0.35)		0.33 (0.24, 0.46)		0.46 (0.37, 0.59)		0.47 (0.38, 0.59)	
Hospitalised VCD								
Number (%) of cases	1 (<0.1)	21 (1.1)	7 (0.2)	27 (1.5)	16 (0.4)	35 (1.9)	17 (0.46)	41 (2.24)
Relative risk (95% CI)	0.02 (0.00, 0.17)		0.13 (0.06, 0.29)		0.23 (0.13, 0.41)		0.20 (0.12, 0.36)	

Source: Module 5.3.5.1, DEN-301 54M CSR, Pt1 Table 15.2.1.3.2, Pt1 Table 15.2.1.3.4, Pt1 Table 15.2.1.10.1, and Pt1 Table 15.2.1.10.4 (12 months), Pt1+12m Table 15.2.3.3.2, Pt1+12m Table 15.2.3.3.4, Pt1+12m Table 15.2.3.10.1, and Pt1+12m Table 15.2.3.10.4 (24 months), DEN-301, Pt2+18m Table 15.2.4.3.1, Pt2+18m Table 15.2.4.3.3, Pt2+18m Table 15.2.4.10.1, and Pt2+18m Table 15.2.4.10.3 (36 months), Pt3 Table 15.2.5.3.1, Pt3 Table 15.2.5.3.3, Pt3 Table 15.2.5.10.1, and Pt3 Table 15.2.5.10.3 (54 months).
Abbreviations: CI, confidence interval; N, number of subjects; VCD, virologically confirmed dengue.
The relative risk was calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.

Virologically confirmed dengue including hospitalised VCD cases by serotype

- **Virologically confirmed dengue including hospitalised VCD cases by DENV-1**

In the overall population, as in seropositive or seronegative subjects, the RR for VCD caused by DENV-1 up to 54 months was <1 (including upper limit). Same result was observed in Hospitalised VCD case, the RR was <1 (including upper limit) for the overall population, and for subjects seronegative and seropositive at baseline.

Table 57: Trial DEN-301: Virologically Confirmed Dengue, Hospitalised Dengue, DHF, and DCAC-Defined Severe Dengue Caused by Serotype DENV 1 From First Dose Up to 54 Months After the Second Vaccine Dose, Overall and by Baseline Serostatus (Safety Set)

	Overall		Baseline Seropositive		Baseline Seronegative	
	TDV N = 13,380	Placebo N = 6687	TDV N = 9663	Placebo N = 4854	TDV N = 3714	Placebo N = 1832
VCD Overall						
Number (%) of cases	222 (1.7)	230 (3.4)	133 (1.4)	151 (3.1)	89 (2.4)	79 (4.3)
Relative risk (95% CI)	0.48 (0.40, 0.58)		0.44 (0.35, 0.56)		0.56 (0.41, 0.75)	
Hospitalised VCD						
Number (%) of cases	22 (0.2)	38 (0.6)	16 (0.2)	24 (0.5)	6 (0.2)	14 (0.8)
Relative risk (95% CI)	0.29 (0.17, 0.49)		0.33 (0.18, 0.63)		0.21 (0.08, 0.55)	
DHF (per programmed algorithm, WHO 1997 criteria)						
Number (%) of cases	2 (<0.1)	4 (<0.1)	2 (<0.1)	3 (<0.1)	0	1 (<0.1)
Relative risk (95% CI)	0.25 (0.05, 1.36)		0.33 (0.06, 2.00)		0.00 (NE, NE)	
DCAC-defined severe dengue						
Number (%) of cases	0	1 (<0.1)	0	1 (<0.1)	0	0
Relative risk (95% CI)	0.00 (NE, NE)		0.00 (NE, NE)		NE	

Source: DEN-301 54M CSR, Pt3 Table 15.2.5.8.1 and Pt3 Table 15.2.5.8.2 (VCD), Pt3 Table 15.2.5.12.1 and 15.2.5.12.2 (hospitalised VCD), Pt3 Table 15.2.5.16.3 (DHF by baseline serostatus), and Pt3 Table 15.2.5.20.3 (DCAC-defined severe dengue by baseline serostatus), and ad-hoc tables Pt3 Table 15.2.5.16.3.2 (DHF overall) and Pt3 Table 15.2.5.20.3.2 (DCAC-defined severe dengue overall).
Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue haemorrhagic fever; N, number of subjects; NE, not evaluable; VCD, virologically confirmed dengue; WHO, World Health Organization.
The relative risk was calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.

- **Virologically confirmed dengue including hospitalised VCD cases by DENV-2**

In the overall population, and in both seropositive or seronegative subjects, the RR for VCD caused by DENV-2 up to 54 months was <1 (including upper limit). Similar result was observed in Hospitalised VCD case by DENV-2, the RR was <1 (including upper limit) for the overall population, and for seropositive subjects at baseline. In seronegative subjects, there were 23 hospitalised VCD cases in the placebo group and 0 in TDV group (the RR was 0; the confidence interval could not be calculated).

Table 58: Trial DEN-301: Virologically Confirmed Dengue, Hospitalised Dengue, DHF, and DCAC-Defined Severe Dengue Caused by Serotype DENV 2 From First Dose Up to 54 Months After the Second Vaccine Dose, Overall and by Baseline Serostatus (Safety Set)

	Overall		Baseline Seropositive		Baseline Seronegative	
	TDV N = 13,380	Placebo N = 6687	TDV N = 9663	Placebo N = 4854	TDV N = 3714	Placebo N = 1832
VCD Overall						
Number (%) of cases	68 (0.5)	193 (2.9)	54 (0.6)	135 (2.8)	14 (0.4)	58 (3.2)
Relative risk (95% CI)	0.18 (0.13, 0.23)		0.20 (0.15, 0.27)		0.12 (0.07, 0.21)	
Hospitalised VCD						
Number (%) of cases	5 (<0.1)	82 (1.2)	5 (<0.1)	59 (1.2)	0	23 (1.3)
Relative risk (95% CI)	0.03 (0.01, 0.08)		0.04 (0.02, 0.11)		0.00 (NE, NE)	
DHF (per programmed algorithm, WHO 1997 criteria)						

Number (%) of cases	0	7 (0.1)	0	7 (0.1)	0	0
Relative risk (95% CI)		0.00 (NE, NE)		0.00 (NE, NE)		NE
DCAC-defined severe dengue						
Number (%) of cases	0	1 (<0.1)	0	1 (<0.1)	0	0
Relative risk (95% CI)		0.00 (NE, NE)		0.00 (NE, NE)		NE

Source: DEN-301 54M CSR, Pt3 Table 15.2.5.8.1 and Pt3 Table 15.2.5.8.2 (VCD), Pt3 Table 15.2.5.12.1 and 15.2.5.12.2 (hospitalised VCD), Pt3 Table 15.2.5.16.3 (DHF by baseline serostatus), and Pt3 Table 15.2.5.20.3 (DCAC-defined severe dengue by baseline serostatus), and ad-hoc tables Pt3 Table 15.2.5.16.3.2 (DHF overall) and Pt3 Table 15.2.5.20.3.2 (DCAC-defined severe dengue overall).
Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue haemorrhagic fever; N, number of subjects; NE, not evaluable; VCD, virologically confirmed dengue; WHO, World Health Organization.
The relative risk was calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.

- **Virologically confirmed dengue including hospitalised VCD cases by DENV-3**

In the overall population, the RR for VCD and for hospitalised VCD by DENV-3 up to 54 months after second dose were <1 (including upper limit) in both cases. Depending on baseline serostatus, some differences were found. In seropositive subjects the RR for VCD and Hospitalised VCD by DENV-3 were <1 (including upper limit), in line with the overall population. However, in seronegative subjects the relative risk of VCD (TDV vs placebo, irrespective of hospitalisation) caused by DENV-3 was 1.11 (0.62, 1.99) through month 54. This result, there was also observed up to 12 months (1.60 [0.52, 4.91]), 24 months (1.37 [0.64, 2.93]) and 36 months after the second dose (1.18 [0.65, 2.16]).

Table 59: Trial DEN-301: Virologically Confirmed Dengue Caused by Serotype DENV-3 From Baseline Up to 12, 24, and 36 Months After the Second Vaccine Dose, Overall and by Baseline Serostatus (Safety Set)

	12 Months		24 Months		36 Months	
	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687
Overall						
N evaluated	13,380	6687	13,380	6687	13,380	6687
Number (%) of VCD cases, serotype DENV-3	45 (0.3)	56 (0.8)	82 (0.6)	83 (1.2)	130 (1.0)	110 (1.6)
Person-years at risk	16,382.0	8177.1	29,334.8	14,621.6	42,057.2	20,955.7
Number of cases per 100 person-years	0.3	0.7	0.3	0.6	0.3	0.5
Relative risk (95% CI)	0.40 (0.27, 0.59)		0.49 (0.36, 0.67)		0.59 (0.46, 0.76)	
Baseline seropositive subjects						
N evaluated	9661	4852	9663	4854	9663	4854
Number (%) of VCD cases, serotype DENV-3	32 (0.3)	52 (1.1)	57 (0.6)	74 (1.5)	94 (1.0)	95 (2.0)
Person-years at risk	11,819.2	5921.9	21,175.8	10,589.9	30,366.1	15,169.4
Number of cases per 100 person-years	0.3	0.9	0.3	0.7	0.3	0.6
Relative risk (95% CI)	0.31 (0.20, 0.48)		0.39 (0.27, 0.55)		0.50 (0.37, 0.66)	
Baseline seronegative subjects						
N evaluated	3714	1832	3714	1832	3714	1832
Number (%) of VCD cases, serotype DENV-3	13 (0.4)	4 (0.2)	25 (0.7)	9 (0.5)	36 (1.0)	15 (0.8)
Person-years at risk	4556.5	2251.5	8152.2	4029.4	11,681.4	5783.0
Number of cases per 100 person-years	0.3	0.2	0.3	0.3	0.3	0.3
Relative risk (95% CI)	1.60 (0.52, 4.91)		1.37 (0.64, 2.93)		1.18 (0.65, 2.16)	

Source: Module 2.7.4, Table 2.qqq.
Abbreviations: CI, confidence interval; N, number of subjects; VCD, virologically confirmed dengue.
The relative risk was calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.

Table 60: Trial DEN-301: Virologically Confirmed Dengue, Hospitalised Dengue, DHF, and DCAC-Defined Severe Dengue Caused by Serotype DENV 3 From First Dose Up to 54 Months After the Second Vaccine Dose, Overall and by Baseline Serostatus (Safety Set)

	Overall		Baseline Seropositive		Baseline Seronegative	
	TDV N = 13,380	Placebo N = 6687	TDV N = 9663	Placebo N = 4854	TDV N = 3714	Placebo N = 1832
VCD Overall						
Number (%) of cases	132 (1.0)	113 (1.7)	96 (1.0)	97 (2.0)	36 (1.0)	16 (0.9)
Relative risk (95% CI)	0.58 (0.45, 0.75)		0.50 (0.38, 0.66)		1.11 (0.62, 1.99)	
Hospitalised VCD						
Number (%) of cases	19 (0.1)	18 (0.3)	8 (<0.1)	15 (0.3)	11 (0.3)	3 (0.2)
Relative risk (95% CI)	0.53 (0.28, 1.00)		0.27 (0.11, 0.63)		1.81 (0.51, 6.48)	
DHF (per programmed algorithm, WHO 1997 criteria)						
Number (%) of cases	7 (<0.1) [a]	3 (<0.1) [b]	3 (<0.1) [c]	2 (<0.1)	4 (0.1) [c]	1 (<0.1)
Relative risk (95% CI)	1.17 (0.30, 4.51)		0.75 (0.13, 4.51)		1.97 (0.22, 17.64)	
DCAC-defined severe dengue						
Number (%) of cases	3 (<0.1) [a]	3 (<0.1) [b]	1 (<0.1) [c]	3 (<0.1)	2 (<0.1) [c]	0
Relative risk (95% CI)	0.50 (0.10, 2.48)		0.17 (0.02, 1.61)		NE	

Source: DEN-301 54M CSR, Table 11.ggg and Table 11.hhh; Pt3 Table 15.2.5.8.1 and Pt3 Table 15.2.5.8.2 (VCD), Pt3 Table 15.2.5.12.1 and 15.2.5.12.2 (hospitalised VCD), Pt3 Table 15.2.5.16.3 (DHF by baseline serostatus), and Pt3 Table 15.2.5.20.3 (DCAC-defined severe dengue by baseline serostatus), and ad-hoc tables Pt3 Table 15.2.5.16.3.2 (DHF overall) and Pt3 Table 15.2.5.20.3.2 (DCAC-defined severe dengue overall).
Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue haemorrhagic fever; N, number of subjects; NE, not evaluable; VCD, virologically confirmed dengue; WHO, World Health Organization.
The relative risk was calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.
(a) Two subjects in the TDV group had both DHF and DCAC-defined severe dengue.
(b) One subject in the placebo group had both DHF and DCAC-defined severe dengue.
(c) One subject in the TDV group had both DHF and DCAC-defined severe dengue

Regarding hospitalised VCD up to 54 months in seronegative subjects, there were 11 cases in the TDV group (0.3%) compared with 3 cases in placebo group (0.1%), with a relative risk of 1.81 (95% CI: 0.51, 6.48). These, data should be interpreted with caution, as there are confounding factors like different standards of care at different trial sites like "thresholds for hospitalisation" (Sri Lanka site had higher hospitalisation rates [67.4%] than Philippines [6.6%] and Thailand [44%] and "differences in assessment of clinical severity, including methods used to assess plasma leakage and thrombocytopenia." All hospitalised cases of VCD in Sri Lanka were considered mild forms of dengue by DCAC (Dengue Case Adjudication Committee) and were not severe based on the DCAC assessment.

The incidence of VCD and/or hospitalised VCD by DENV-3 was impacted by local epidemiology and hospitalisation practices. Overall, incidences of VCD (including Sri Lanka) caused by DENV-3 in seronegative subjects were 1% in TDV and 0.9% in placebo with RR of 1.11 (95% CI 0.62, 1.99). However, incidence of VCD (excluding Sri Lanka) by DENV-3 in seronegative subjects were 0.9% vs 1% with RR of 0.92 (95% CI 0.50, 1.69), in TDV and placebo respectively.

In addition, no new hospitalised cases caused by DENV-3 occurred in the TDV group since the last interim analysis at the end of Year 3 (36 months post second dose). The incidences of hospitalised VCD caused by DENV-3 in baseline seronegative subjects (including Sri Lanka) up 54 months after second dose were 11 cases in the TDV group and 3 cases in the placebo group with RR of 1.81 (95% CI 0.51, 6.48). However, the incidences of hospitalised VCD caused by DENV-3 in baseline seronegative subjects (excluding Sri Lanka) were 5 cases in the TDV and 3 cases in the placebo with RR of 0.82 (95% CI 0.20, 3.42).

Table 61: Virologically Confirmed Dengue, Hospitalised Dengue, DHF, and DCAC-Defined Severe Dengue Caused by Serotype DENV-3 in Baseline Seronegative Subjects from First Dose Up to 54 Months Post Second Dose, Including and Excluding Data from Sri Lanka (Safety Set; 2:1 randomisation [TDV:placebo] to be considered)

	Overall Including Sri Lanka		Excluding Sri Lanka	
	TDV N=3714	Placebo N=1832	TDV N=3181	Placebo N=1564
VCD overall				
Number (%) of cases	36 (1.0)	16 (0.9)	30 (0.9)	16 (1.0)
Relative risk (95% CI)	1.11 (0.62, 1.99)		0.92 (0.50, 1.69)	
VCD leading to hospitalisation				
Number (%) of cases	11 (0.3)	3 (0.2)	5 (0.2)	3 (0.2)
Relative risk (95% CI)	1.81 (0.51, 6.48)		0.82 (0.20, 3.42)	
DHF (per programmed algorithm, WHO 1997 criteria)				
Number (%) of cases	4 (0.1) [a]	1 (<0.1)	2 (<0.1) [a]	1 (<0.1)
Relative risk (95% CI)	1.97 (0.22, 17.64)		0.98 (0.09, 10.84)	
DCAC-defined severe dengue				
Number (%) of cases	2 (<0.1) [a]	0	2 (<0.1) [a]	0
Relative risk (95% CI)	NE (NE, NE)		NE (NE, NE)	

- **Virologically confirmed dengue including hospitalised VCD cases by DENV-4**

In the overall population and in seropositive subjects, the RR for VCD by DENV-4 was <1 (including upper limit) up to 54 months after second dose. However, in seronegative subjects, the RR for VCD caused by DENV-4 up to 54 months (TDV vs placebo, irrespective of hospitalisation) was 1.97 (95% CI: 0.56, 6.98) with 12 cases (0.3%) in TDV and 3 cases (0.2%) in placebo). Importantly, 0 of 12 VCD cases in the TDV group resulted in hospitalisation while 1 of 3 VCD cases in the placebo group was hospitalised, so the Confidence Intervals for RR risk of hospitalised VCD by DENV4 cannot be calculated.

Table 62: Trial DEN-301: Virologically Confirmed Dengue, Hospitalised Dengue, DHF, and DCAC-Defined Severe Dengue Caused by Serotype DENV 4 From First Dose Up to 54 Months After the Second Vaccine Dose, Overall and by Baseline Serostatus (Safety Set)

	Overall		Baseline Seropositive		Baseline Seronegative	
	TDV N = 13,380	Placebo N = 6687	TDV N = 9663	Placebo N = 4854	TDV N = 3714	Placebo N = 1832
VCD Overall						
Number (%) of cases	24 (0.2)	23 (0.3)	12 (0.1)	20 (0.4)	12 (0.3)	3 (0.2)
Relative risk (95% CI)	0.52 (0.29, 0.92)		0.30 (0.15, 0.62)		1.97 (0.56, 6.98)	
Hospitalised VCD						
Number (%) of cases	0	4 (<0.1)	0	3 (<0.1)	0	1 (<0.1)
Relative risk (95% CI)	0.00 (NE, NE)		0.00 (NE, NE)		0.00 (NE, NE)	
DHF (per programmed algorithm, WHO 1997 criteria)						
Number (%) of cases	0	1 (<0.1)	0	1 (<0.1)	0	0
Relative risk (95% CI)	0.00 (NE, NE)		0.00 (NE, NE)		NE	
DCAC-defined severe dengue						
Number (%) of cases	0	0	0	0	0	0
Relative risk (95% CI)	NE		NE		NE	

Source: DEN-301 54M CSR, Pt3 Table 15.2.5.8.1 and Pt3 Table 15.2.5.8.2 (VCD), Pt3 Table 15.2.5.12.1 and 15.2.5.12.2 (hospitalised VCD), Pt3 Table 15.2.5.16.3 (DHF by baseline serostatus), and Pt3 Table 15.2.5.20.3 (DCAC-defined severe dengue by baseline serostatus), and ad-hoc tables Pt3 Table 15.2.5.16.3.2 (DHF overall) and Pt3 Table 15.2.5.20.3.2 (DCAC-defined severe dengue overall).
Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue haemorrhagic fever; N, number of subjects; NE, not evaluable; VCD, virologically confirmed dengue; WHO, World Health Organization.
The relative risk was calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group

Severe Forms of Dengue (DCAC-Defined Severe Dengue and DHF)

In the overall population up to 12 months post second vaccination in the TDV group there were 2 cases (0.01%) of DCAC-defined severe Dengue and DHF (1 each) vs 5 cases (0.07%) in placebo (1 DCAC-defined severe dengue and 4 DHF). Up to 24 months, there were 4 cases (0.03%) in TDV (1 DCAC-defined severe dengue, 2 DHF and 1 was considered both DCAC-defined severe dengue and DHF) vs 11 cases (0.16%) in placebo (3 DCAC-defined severe dengue and 8 DHF). Up to 36 months, there were 10 cases (0.07%) in TDV (1 DCAC-defined severe dengue, 7 DHF and 2 considered both) and 17 cases (0.25%) in placebo (4 DCAC-defined severe dengue, 12 DHF and 1 considered both).

Finally, up to 54 months, there were no additional cases of DCAC-defined severe Dengue and DHF in the TDV. However, two new cases (both DHF) were reported in placebo group. Therefore, up to 54 months, there were 10 cases of DCAC-defined severe Dengue and DHF (0.07%) in TDV and 19 cases (0.28%) in placebo.

Table 63: Trial DEN-301: Incidence of Severe Forms of Dengue (DCAC-Defined Severe Dengue and DHF) From First Dose Up 12, 24, 36, and 54 Months After the Second Vaccine Dose, Overall and by Baseline Serostatus (Safety Set)

	12 Months		24 Months		36 Months		54 Months	
	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687	TDV N = 13,380	Placebo N = 6687
Overall								
DCAC-defined severe dengue								
Number (%) of cases	1 (0.01)	1 (0.01)	2 (0.01) [a]	3 (0.04)	3 (0.02) [b]	5 (0.07) [c]	3 (0.02) [b]	5 (0.07) [c]
Relative risk (95% CI)	0.50 (0.03, 7.99)		0.33 (0.06, 1.99)		0.30 (0.07, 1.25)		0.30 (0.07, 1.25)	
DHF (per programmed algorithm, WHO 1997 criteria)								
Number (%) of cases	1 (0.01)	4 (0.06)	3 (0.02) [a]	8 (0.12)	9 (0.07) [b]	13 (0.19) [c]	9 (0.07) [b]	15 (0.22) [c]
Relative risk (95% CI)	0.12 (0.01, 1.12)		0.19 (0.05, 0.71)		0.35 (0.15, 0.81)		0.30 (0.13, 0.68)	
DCAC-defined severe dengue and/or DHF								
Number (%) of cases	2 (0.01)	5 (0.07)	4 (0.03)	11 (0.16)	10 (0.07)	17 (0.25)	10 (0.07)	19 (0.28)
Baseline seropositive subjects								
N evaluated	9661	4852	9663	4854	9663	4854	9663	4854
DCAC-defined severe dengue								
Number (%) of cases	0	1 (0.02)	0	3 (0.06)	1 (0.01)	5 (0.10)	1 (0.01)	5 (0.10)
Relative risk (95% CI)	0.00 (NE, NE)		0.00 (NE, NE)		0.10 (0.01, 0.86)		0.10 (0.01, 0.86)	
DHF (per programmed algorithm, WHO 1997 criteria)								
Number (%) of cases	1 (0.01)	3 (0.06)	1 (0.01)	7 (0.14)	5 (0.05)	12 (0.25)	5 (0.05)	13 (0.27)
Relative risk (95% CI)	0.17 (0.02, 1.61)		0.07 (0.01, 0.58)		0.21 (0.07, 0.59)		0.19 (0.07, 0.54)	
DCAC-defined severe dengue and/or DHF								
Number (%) of cases	1 (0.01)	4 (0.08)	1 (0.01)	10 (0.21)	5 (0.05)	16 (0.33)	5 (0.05)	17 (0.35)
Baseline seronegative subjects								
N evaluated	3714	1832	3714	1832	3714	1832	3714	1832
DCAC-defined severe dengue								
Number (%) of cases	1 (0.03)	0	2 (0.05) [a]	0	2 (0.05) [a]	0	2 (0.05) [a]	0
Relative risk (95% CI)	NE		NE		NE		NE	
DHF (per programmed algorithm, WHO 1997 criteria)								
Number (%) of cases	0	1 (0.05)	2 (0.05) [a]	1 (0.05)	4 (0.11) [a]	1 (0.05)	4 (0.11) [a]	2 (0.11)
Relative risk (95% CI)	0.00 (NE, NE)		0.99 (0.09, 10.87)		1.97 (0.22, 17.64)		0.99 (0.18, 5.38)	
DCAC-defined severe dengue and/or DHF								
Number (%) of cases	1 (0.03)	1 (0.05)	3 (0.08) [a]	1 (0.05)	5 (0.13) [a]	1 (0.05)	5 (0.13) [a]	2 (0.11)

Source: Module 5.3.5.1, DEN-301 54M CSR, Table 11.ggg and Table 11.hhh; Pt1 Table 15.2.1.14.1, Pt1 Table 15.2.1.14.3, Pt1 Table 15.2.1.18.1, and Pt1 Table 15.2.1.18.3 (12 months); Table Pt1+12m Table 15.2.3.14.1, Table Pt1+12m Table 15.2.3.14.3, Pt1+12m Table 15.2.3.18.1, and Pt1+12m Table 15.2.3.18.3 (24 months); Pt2+18m Table 15.2.4.14.1, Pt2+18m Table 15.2.4.14.3, Pt2+18m Table 15.2.4.18.1, and Pt2+18m Table 15.2.4.18.3 (36 months); Pt3 Table 15.2.5.14.1, Pt3 Table 15.2.5.14.3, Pt3 Table 15.2.5.18.1, and Pt3 Table 15.2.5.18.3 (54 months).
Abbreviations: CI, confidence interval; DCAC, Dengue Case Adjudication Committee; DHF, dengue haemorrhagic fever; N, number of subjects; NE, not evaluable; WHO, World Health Organization.
The relative risk was calculated as the number of events divided by the number of subjects evaluated in the TDV group, over the number of events divided by the number of subjects evaluated in the placebo group.
(a) One baseline seronegative subject in the TDV group had both DCAC-defined severe dengue and DHF (refer to DEN-301 54M CSR, Table 11.ggg and Table 11.hhh).
(b) Two of the 9 subjects in the TDV group had both DCAC-defined severe dengue and DHF (refer to DEN-301 54M CSR, Table 11.ggg and Table 11.hhh).
(c) One of the 13 subjects in the placebo group had both DCAC-defined severe dengue and DHF (refer to DEN-301 54M CSR, Table 11.ggg and Table 11.hhh).

Up to 54 months, the 3 of the 10 severe cases in TDV group were DCAC-defined severe dengue, all of them were caused by DENV-3. Two of the 3 cases were in seronegative subjects and one in seropositive. In addition, the 5 of the 19 severe cases in placebo group were DCAC-defined severe dengue, all of them were in seropositive subjects at baseline (1 case by DENV-1 and DENV-2 and 3 cases by DENV-3).

Additionally, of the 9 cases of DHF in TDV group, 4 cases occurred in seronegative subjects (all caused by DENV-3) and 5 in seropositive (2 cases by DENV-1 and 3 cases by DENV-3). In comparison, in the placebo group 13 of 15 cases were in seropositive subjects (3 cases by DENV-1, 7 cases by DENV-2, 2 cases by DENV-3 and 1 case by DENV-4) and 2 in seronegative (1 case by DENV-1 and 1 case by DENV-3).

Specifically for the serotype DENV-3, the incidences of DHF (including Sri Lanka) or severe dengue caused remained very low at the end of Part 3 (4 versus 1 DHF and 2 versus 0 severe dengue cases in the TDV and placebo group, respectively). Considering the impact of dengue case management practices in Sri Lanka and the absence of any new hospitalised VCD, DHF and/or severe dengue cases by DENV-3 during the last 1.5 years of follow-up in the trial, the data do not indicate worsening or increased severity over time. The two cases in TDV recipients that were assessed as severe by the DCAC occurred early in the trial during Parts 1 and 2 (i.e., before 18 months post second dose).

Moreover, no cases of DHF and/or severe dengue were caused by DENV-4 throughout the trial up to 54 months post second dose

Supportive information from **DEN-204** (1596 subjects in TDV and 198 subjects in placebo groups) showed a total of 50 cases of VCD up 48 months after the first dose (2.3% in TDV vs 6.6% in placebo, with RR 0.35 [95%IC: 0.19-0.65]). No significant difference of incidence was identified between seropositive and seronegative subjects. Of the 50 cases, only 3 were considered a SAE by the sponsor (2 in TDV groups and 1 in placebo). Additionally, in 200 subjects received TDV, in the single-arm Trial **DEN-313**, no cases of VCD requiring hospitalisation or meeting the WHO 1997 criteria for DHF have been reported up to 9 months after the first dose.

Long-Term Safety Follow-Up

The long-term safety follow-up data focused on AEs; VCD long-term follow-up data are described in the previous section.

The long-term SAE data from DEN-301 (up to 36 months after second dose) was available for a total of 20,071 subjects aged 4 to 16 years. There were 13 deaths reported (10 in TDV and 3 in placebo) and none was related to trial vaccine. SAEs were reported by 4.8% in TDV and 4.8% in placebo up to 18 months (single SAE in TDV and 5 SAEs in 4 subjects in Placebo were considered as related) and 2.9% vs 3.5% during the period 19-36 month (none considered related SAEs).

The long-term SAE data from DEN-204 (up to 48 months after first dose) was available for a total of 1794 subjects aged 2 to 17 years. SAEs were reported by 5.83% in TDV and 5.05% in placebo. None of these SAEs was considered to be related to trial vaccine, and the majority of events resolved completely. Additionally, no clinically important differences between seropositive and seronegative subjects of the TDV groups were observed.

Vaccine Viraemia

The vaccine viraemia was evaluated at predefined time points on 775 subjects from 6 different clinical trials receiving different vaccine formulations (DEN-101, DEN-102, DEN-103, DEN 104, DEN-203 [Part 1], and DEN 205). Serum samples were collected at 2-to-3-day intervals after each vaccine dose for approximately 3 weeks, depending on the trial design. Moreover, the trials used different routes of administration, age ranges, and different grading for severity assessment of AEs. All analyses in every single trial were post-hoc, exploratory, and descriptive. Since the integrated (pooled) analyses are based on different methods of data collection, post-hoc and descriptive, they should be interpreted with caution.

The majority of subjects in the pooled were therefore dengue seronegative at baseline (495 baseline seronegative, 237 baseline seropositive, and 23 subjects' baseline serostatus unknown).

The trial DEN-205 was the only trail analysed for vaccine viraemia where one group receiving final TDV formulation compared to HD TDV. Vaccine viraemia was observed in 54 of 175 subjects (30.9%) receiving final TDV formulation and in 110 of 176 subjects (62.5%) receiving HD TDV. In both groups, vaccine viraemia mainly occurred for vaccine strain TDV-2. Viraemia peaked at Day 11 and the mean duration of TDV-2 viraemia was similar (4.0 days in TDV vs 4.8 days in HD TDV). Independent of HD TDV or TDV use, the incidence of vaccine viraemia was higher in baseline seronegative than in baseline seropositive subjects and the mean duration was also longer for seronegative than for seropositive subjects in each vaccine group. No difference of reversion of attenuation loci were observed in TDV or HD TDV (14.8% and 13.6%, respectively).

In Trials DEN-101, DEN-102, DEN-103, DEN 104, and DEN 203 (Part 1), sequencing of the viral genomes was performed for all 129 subjects with replication-competent vaccine virus; 44 subjects had single reversions. Except for a single partial reversion in the NS1 locus in a subject from Trial DEN-104 (presence of both attenuated and reverted nucleotide), all reversions affected the 5'NCR attenuation locus.

Adverse Events in Subjects With and Without Vaccine Viraemia

Vaccine viraemia occurred most frequently during the period between 7 and 13 days after the first TDV dose and was rarely detected after the second vaccine dose; the incidence of solicited and unsolicited AEs in subjects with vaccine viraemia, replication-competent vaccine virus, or reversion of attenuation loci was analysed descriptively after the first vaccine dose in 775 subjects with vaccine viraemia and 418 subjects without vaccine viraemia.

Regarding solicited AEs, higher incidence of injection site pain, injection site erythema, rash, arthralgia, fatigue and fever in viraemic participants (47.4%, 24.4%, 10.2%, 11.4%, 35.8% and 6.2%, respectively) than non-viraemic (41.7%, 18.5%, 5.9%, 8.2%, 29.4% and 4.8%, respectively) receiving different vaccine formulations of TDV. No differences were observed in other solicited AEs between viraemic and non-viraemic subjects. Of note, most episodes of rash, fever, and arthralgia in viraemic subjects showed a temporal relationship with viraemia.

Additionally, in viraemic subjects there was difference in the incidence in some solicited AEs between with/without replication-competent vaccine virus. Solicited AEs with a ≥2-fold higher incidence in

viraemic subjects with replication-competent vaccine virus than in subjects without replication-competent vaccine virus included fatigue (23.4% vs 11.1%), myalgia (13.3% vs 4.8%), arthralgia (10.2% vs 4.8%), nausea (7.8% vs 2.4%), and photophobia (7.8% vs 3.2%)

Regarding subjects with or without attenuation locus, solicited AEs with a ≥2-fold higher incidence in subjects with reversion in attenuation locus compared with those without reversion included pain at the injection site (7 of 43 subjects [16.3%] vs 4 of 85 subjects [4.7%]), arthralgia (7 of 43 subjects [16.3%] vs 6 of 85 subjects [7.1%]), fever (3 of 43 subjects [7.0%] vs 3 of 85 subjects [3.5%]), nausea (6 of 43 subjects [14.0%] vs 4 of 85 subjects [4.7%]), and vomiting (1 of 43 subjects [2.3%] vs none of 85 subjects).

Regarding unsolicited AEs, higher incidence was observed in viraemic participants (62.0%) than in non-viraemic (52.1%). Unsolicited AEs reported by ≥5% of subjects and with approximately ≥2-fold higher incidences in subjects with than without vaccine viraemia were headache (20.1% vs 10.9%), fatigue (11.2% vs 3.1%), myalgia (8.9% vs 3.1%), arthralgia (6.0% vs 2.0%), nausea (6.0% vs 3.4%), and upper respiratory tract infection (5.0% vs 3.4%). In addition, most episodes of rash (75.0%) and all episodes (100%) of rash erythematous, rash papular, rash maculo-papular, rash generalised, rash macular, and rash pruritic showed a temporal relationship with vaccine viraemia. The incidence of rash (PT) was 3.8% vs 1.4% in subjects with and without viraemia.

Higher incidence of unsolicited AEs in subjects with replication-competent vaccine virus or with reversion in attenuation locus than in subjects without replication-competent vaccine virus or without reversion in attenuation locus. Unsolicited AEs with a ≥2-fold higher incidence were fatigue (in both), myalgia (in replication-competent vaccine virus) and arthralgia (in reversion in attenuation locus).

Vaccine Viraemia and Febrile Illness

In Trial DEN-301, subjects presenting with febrile illness within 30 days after each dose of trial vaccine were assessed for the presence of vaccine viraemia.

Vaccine viraemia was detected in 34 of 479 febrile illness subjects (7.1%) after the first TDV dose and in 1 of 503 subjects (0.2%) after the second vaccine dose. Most of viraemic case occur between 7 and 13 days after the first vaccine dose. Subjects with febrile illness and with vaccine viraemia detected showed a similar pattern of symptoms as those with febrile illness but without vaccine viraemia. In addition, the incidence of vaccine viraemia with febrile illness was higher in baseline seronegative than in baseline seropositive subjects (8.9% vs 6.5%).

2.6.8.4. Laboratory findings

Routine laboratory safety data (haematology, serum chemistry, urinalysis) were recorded in the 5 phase 1 trials and in 2 phase 2 trials, DEN-203 and DEN-106. Of note, none of these trials used the final formulation TDV intended for commercial lots.

The assessment of changes from baseline, individual subjects' shift data, and clinically significant abnormalities revealed no clinically important changes of laboratory parameters from baseline and no clinically important differences between the various TDV formulations assessed and placebo. Abnormal test results for parameters of special relevance in the context of the symptomatology associated with dengue fever, such as platelet count, haematocrit, or liver function tests, were infrequently reported, with no differences between any TDV and placebo, and did not reveal any relevant trends or potential safety risks.

Because these data did not indicate any consistent pattern of vaccine-related abnormalities, safety objectives related to clinical laboratory analyses were not included in the subsequent phase 2 and phase 3 trials, and no pooling of clinical laboratory data of the 5 phase 1 and 2 phase 2 trials was performed.

2.6.8.5. Safety in special populations

Pregnancy and Lactation

TDV has not been studied in pregnant women because pregnancy was an exclusion criterion in all clinical trials. However, there were limited data of vaccine administration in women who were pregnant or who became pregnant shortly after vaccination.

As of 01 October 2020, a total of 405 pregnancies were reported by 374 women who participated in clinical phase 2 and phase 3 trials. The majority of these pregnancies (84.2%) occurred in subjects from pivotal Trial DEN 301 and the 78.8% of pregnancies were in seropositive at baseline women.

Most of the pregnancies were considered as non-exposed (89.1%), i.e., the last menstrual period had occurred ≥6 weeks after a dose of trial vaccine and thus beyond the typical period for vaccine viraemia. There were 44 pregnancies receiving vaccine at any time between 6 weeks before the last menstrual period up to the outcome of pregnancy. Most had a normal outcome (79.4% in any TDV group and 50.0% in placebo). Spontaneous abortions were reported in 11.8% of pregnancies, and no differences in frequencies were observed between women exposed to any TDV and placebo.

In addition, there were 8 women who became pregnant during the trial and had births with neonatal deaths, all from Trial DEN-301 (including the 4 women who discontinued the trial due to pregnancy). One neonatal death (TDV group) occurred after an exposed pregnancy, and the remaining 7 cases (TDV group 1, placebo group 3; vaccination group still blinded for 3 cases) occurred after non-exposed pregnancies. None of the neonatal deaths were assessed as causally related to TDV.

It is unknown whether TDV is excreted in human milk. There are limited data on the excretion of wild-type dengue via breast milk. In a small study, dengue virus was detected in breast milk samples from 9 (75%) of 12 infected breastfeeding mothers

Intrinsic Factors

Age group

The data for the different age groups within the proposed target population (4-60 years) are presented in detail in the previous section.

Additional data for 401 subjects aged <4 years from Trial DEN-204 did no shown important safety finding and overall safety profile of TDV was considered satisfactory. High variability of the incidence of solicited AEs was observed in different vaccination regimen. The majority of solicited and unsolicited AEs were mild or moderate in severity. SAEs were reported by 6.5% participants receiving at least one dose of TDV and 1 death in placebo group. None of these events was considered related to trail vaccine.

Baseline serostatus

In Placebo-Controlled Safety Pool baseline serostatus information was available for 21,318 subjects. Of these, 14,783 were seropositive subjects (9,808 received TDV and 4,975 received placebo) and 6,535 seronegative subjects (4,472 TDV and 2,063 placebo).

Overall solicited AEs, including AEs within 30 minutes and prolonged solicited AEs, and unsolicited AEs in TDV were more frequently in higher in seronegative than in seropositive. The frequencies of solicited local AEs were 51.9% in seronegative and 36.2% in seropositive; the frequencies of solicited systemic were 50.1% in seronegative and 42.6% in seropositive and the frequencies of unsolicited AEs were 23.7% in seronegative and 19.0% in seropositive (of theses 0.7% and 0.3% were considered related, respectively).

The difference observed of solicited local and unsolicited AEs were most pronounced in adolescent group. The results observed are driven by adolescents from Trial DEN 315 who predominantly were seronegative (Mexico City) and generally reported higher rate of solicited local AEs and unsolicited than the adolescent subjects from pivotal Trial DEN-301.

Gender

In the Placebo-Controlled Safety Pool, 10,954 subjects were male (7,295 in TDV and 3,659 in placebo) and 10,840 were female (7,332 in TDV and 3,508 in placebo).

Overall solicited AEs, including AEs within 30 minutes and prolonged solicited AEs, and unsolicited AEs were more commonly experienced by female subjects. The frequencies of solicited local AEs in TDV were 52.3% in female and 34.0% in male; the frequencies of solicited systemic were 51.4% in female and 40.5% in male and the frequencies of unsolicited AEs were 23.2% in female and 19.1% in male (of theses 4.2% and 1.7% were considered related, respectively).

The incidences of solicited and unsolicited AEs in male and female subjects followed the pattern observed in the overall target population.

Race

No clinically important differences were noted between the AE profile observed in Asian, American Indian or Alaska Native, Black or African American, and White subjects. The only notable difference was that solicited local AEs (pain, erythema, swelling) were reported more frequently by White subjects (mainly adults from the US and the United Kingdom) than by the other race subgroups.

The other race subgroups (Native Hawaiian or Other Pacific Islander, Multiracial or Other, and Unknown) were too small to reach any conclusion.

Extrinsic Factors

Endemic and non-endemic

The solicited local AEs in TDV appeared to be more frequently reported in subjects from non-endemic regions (60.4%/59.5% after any dose) than by subjects from endemic regions (36.7%/36.8% after any dose). However, the underlying reasons for this finding data are probably the different age group distributions in both subgroups (endemic subgroup dominated by children and adolescents from Trial DEN-301 and non-endemic subgroup comprised adult subjects from Trial DEN-304 and adolescents from Trial DEN-315). In contrast, the evaluation of unsolicited AEs and SAE revealed no clinically important differences between the TDV in the subjects from endemic and non-endemic regions. Unsolicited AEs of injection site bruising and injection site pruritus were reported mainly in subjects from non-endemic regions

Region and countries

In Placebo-Controlled Safety Pool there were 919 participants from North America (all US), 11,769 from Latin America and 7,036 from Asia Pacific. The age distribution by region was different, in North America there were only adults, and in Latin America and Asia Pacific were mainly children and

adolescents (range of 65.7%-68.3% aged 4-11 and range of 31.4%-34.3% aged 11-17) with only <0.5% adult participants in placebo group.

The incidence of solicited local AES was higher in TDV participants from North America (56.3%) than from Latin America (43.2%) and Asia Pacific (35.5%). The incidence of related unsolicited AEs was also higher in participants from North America (9.6%) than from Latin America (1.1%) and Asia Pacific (1.4%). No clinically important differences were identified regarding the safety profile of TDV in the different regions.

Of note, participants from Europe were in All Studies Safety Pool (300 adult subjects from United Kingdom only) and reported solicited local AEs similar to the US subjects.

Previous Flavivirus vaccination

In Placebo-controlled Safety Pool, approximately 40% of subjects had documented previous vaccination against YF or JE (6,060 in TDV and 2,995 in placebo) compared to approximately 60% no previously vaccinated (8,597 and 4,172, respectively). The age distribution by previous flavivirus vaccination was different, adult participants were limited and only in not-previous vaccinated (9.2% in TDV vs 3.6% in placebo).

No clinically important differences in TDV were identified regarding the incidence of solicited local (41.5% in previously vaccinated and 44.3% not previously vaccinated) and systemic AEs (46.5% and 45.9%, respectively) in subjects with and without previous flavivirus vaccinations. However, differences of frequencies of unsolicited AEs were observed in subjects with and without previous flavivirus vaccinations (16.4% and 23.5%, respectively).

2.6.8.6. Immunological events

For either of the analysis pools, no vaccine-related anaphylactic reactions or anaphylactic shock events were reported within 28 days after vaccine dosing (there was 1 unrelated, serious, and severe anaphylactic reaction after ingestion of mefenamic acid starting 18 days after the second TDV dose).

For both analysis pools, the incidence of hypersensitivity reactions was ≤0.1% for both TDV and placebo recipients. None of these hypersensitivity events was serious or severe or considered to be related to trial vaccine.

2.6.8.7. Safety related to drug-drug interactions and other interactions

Co-administration of TDV with YF and HAV vaccines was evaluated in 2 phase 3 trials, DEN-305 and DEN-314.

In DEN-305, solicited local AEs after the first dose in the co-administration group (TDV plus YF) were reported by 160 of 282 participants (56.7%) compared to 140 of 185 participants (49.1%) receiving TDV plus placebo or 53 of 289 participants (18.3%) receiving YF vaccine plus placebo. Similar pattern was observed for solicited systemic AEs, although group differences were less pronounced (52.1%, 51.2% and 43.6%, respectively). And no clinically important differences between groups were noted regarding the incidence and profile of unsolicited AEs. The proportion of subjects with any SAEs was low and none was considered as related.

In DEN-314, solicited local AEs after the first dose in the co-administration group (TDV plus HAV) were reported by 196 of 258 participants (68.8%) compared to 152 of 292 participants receiving TDV plus placebo (52.1%) or 141 of 289 participants receiving HAV vaccine plus placebo (48.8%). Similar pattern was observed in solicited systemic AEs (49.5%, 45.2% and 48.1%, respectively). And no

clinically important differences between groups were noted regarding the incidence and profile of unsolicited AEs. The proportion of subjects with any SAEs was low and none was considered as related.

2.6.8.8. Discontinuation due to adverse events

In the Placebo-Controlled Safety Pool, the incidence of AEs leading to vaccine and/or trial discontinuation was ≤0.20% in both groups (TDV 0.16% or 0.12%, placebo 0.13% or 0.11%). Results for the supportive All Studies Safety Pool were consistent with the results based on the Placebo-Controlled Safety Pool. Most events occurred in 1 subject only and were considered as not related to trial vaccine.

In TDV, 6 subjects had 8 related AEs leading to vaccine discontinuation (of these, 3 subjects discontinued the trial) compared with 2 subjects with 3 events in placebo (of these, 1 discontinued the trial). The incidence of AEs leading to vaccine and/or trial discontinuation that were considered to be related by the investigator was <0.1% for both groups. The majority of these AE were no serious or severe, except for 2 SAEs of hypersensitivity in the placebo group and 1 non-serious but severe AE of myalgia in the TDV group.

The following AEs in the TDV group leading to trial or vaccine discontinuation were considered being vaccine related by the investigator: Rash pruritic, angioedema, arthralgia, injection site pruritus, myalgia, and urticaria.

2.6.8.9. Post marketing experience

No post-marketing data were available for this application.

2.6.9. Discussion on clinical safety

Eighteen trials (7 phase 3 trials, 6 phase 2 trials, and 5 phase 1 trials) with overall more than 27,000 subjects from dengue-endemic and non-endemic regions, covering an age range from 1.5 to 60 years, were used for the overall safety evaluation of TDV. Nonetheless, only phase 2 and 3 trials were pooled for safety assessment, as the time points and main safety endpoints remained similar in these trials. Two different safety analysis pools were created from phase 2/3 trials: "Placebo-Controlled Safety Pool" from 5 randomised placebo-controlled clinical trials and supportive "All Studies Safety Pool" from 10 clinical trials.

The integrated evaluation of the safety profile of TDV in the Placebo-Controlled Safety Pool was based on 22,002 subjects (mainly from pivotal Phase 3 trial DEN-301, randomised 2:1, aged 4-60) (14,627 subject who received at least 1 dose of TDV and 7167 subjects who received at least 1 dose of placebo). A total of 25,236 subjects aged 4-60, were evaluated in the All Studies Safety Pool as supportive safety data. The analysis of solicited and unsolicited AEs recorded for TDV compared with placebo within the placebo-controlled safety pool has been performed only in a subset of 5,555 participants (3,830 subjects receiving TDV and 1,725 subjects receiving placebo).

The methodology for safety analysis was descriptive; safety parameters pooled in the assessment were solicited AEs, including immediate AEs and prolonged solicited AEs, unsolicited AEs, SAEs, MAAES, and AEs Leading to Vaccine and/or Trial Discontinuation. The pooling method is deemed appropriate to allow consistent evaluation of AEs and the safety profile of TDV in the target population**.** The applicant submitted an overview and summary of the different severity assessment definitions used for recording the solicited local adverse events and the different panels for assessment of solicited systemic AEs in toddlers and young children (<6 years) versus older children (≥6 years) and adults. The rational for

adapting the severity assessment criteria for the two age ranges is appropriate and it could be agreed. Moreover, it can be concurred that the adapted diameter criteria erythema and swelling for the <6-year-old age group is conservative and any bias that might be introduced would not favor TDV. The severity criteria used to assess solicited AEs during the TDV trials paralleled the criteria used in clinical trials of the approved CYD-TDV dengue vaccine, although different age cut-off ranges were used in the CYD-TDV dengue vaccine. In summary, the explanation and assessment of the applicant is comprehensive.

The evaluation of VCD, including severe dengue, vaccine viraemia, and clinical laboratory tests were not part of the pooled safety analysis. The VCD was evaluated in pivotal trial DEN-301, the vaccine viraemia was evaluated mainly in DEN-205 and the reactogenicity was evaluated in a small pool of one phase 1 and two phase 2 trials. In addition, the laboratory findings were evaluated in pooled data of one phase 1 and two phase 2 trials.

Demographic and baseline characteristics

In the overall population in the Placebo-Controlled Safety Pool, more than 90% of all subjects came from the pivotal Trial DEN-301 and were children and adolescents aged 4 to 16 years (20,071 subjects, 91.2%). There were no important demographic differences between the TDV and placebo groups. Only, in the TDV group there was a higher proportion of adults (5.4% vs 2.1%), a higher proportion of white participants (6.4% vs 3.5%), a higher proportion of participants from North America (5.4% vs 1.8%) and a higher proportion of participants from non-endemic region (7.4% vs 3.2%) than in the placebo group. The supportive All Studies Safety Set enlarged the adult sample size; it included 1563 and 314 adult subjects in the vaccine and the placebo group. Though dominated by paediatric subjects the safety data base is deemed adequate for the overall target population.

Regarding the baseline demographics by age group in the Placebo-Controlled Safety Pool, in the 2 age groups of children (4-11 years) and adolescents (12-17) there were no important differences between the TDV and placebo groups. Of note, there were no participants from non-endemic regions in the 4-11 years group and there was a higher proportion of participants from non-endemic regions in TDV than in placebo group in subjects aged 12-17 years (6.5% vs 4.4%).

In adults (18-60 years group), there were no important differences between the TDV and placebo groups except for region and baseline serostatus. In TDV group all adult participants came from North America (non-endemic region), while in placebo group 86.2% come from North America and 13.8% came from Asia Pacific and Latin America (endemic regions). In addition, there were 9.5% of participants seropositive in TDV compared to 20.4% in the placebo group.

Solicited Adverse events

Solicited AEs data in overall population (4-60 years were available for a subset of 5,555 participants (3,830 subjects receiving TDV and 1,725 subjects receiving placebo):

Overall, solicited local and systemic AEs, including solicited within 30 minutes and prolonged solicited AEs reported after the second dose were lower than after the first dose and the majority of them were mild in severity.

Solicited AEs within 30 minutes were higher in the TDV group than in the placebo group (6.7% vs 5.2%). No severe reactions were reported.

The most frequently solicited local AE within 30 minutes reported after any vaccination was injection site pain, and the most frequently solicited systemic immediate AEs reported was headache, followed by myalgia and asthenia. Fever (≥38 ºC) was reported by 0.1% of participants in TDV and <0.1% in placebo.

After any dose, as expected, the incidences of solicited local AE (within 7 days) were higher in the TDV group than in the placebo group (43.4% vs 25.7%, respectively), including severe solicited local AEs (1.3% vs 0.6%). The most frequently solicited local AE reported after any dose was injection site pain in both, followed by erythema and swelling. The grade 3 solicited local AEs were mainly due to injection site pain. The time to onset was, mainly, on day of vaccination in both groups and resolved with a median duration of 2-3 days in TDV groups and in 1 day in placebo group. The local adverse reaction of itching is included in the PL (frequency uncommon) and SmPC section 4.8 Table (pruritis). There was a trend of increasing frequency of Grade 3 injection pain after the second dose compared to the first one, although these percentages are very low and probably not significant.

After any dose, as expected, the incidences of solicited systemic AEs (within 14 days) were reported to be higher in the TDV than in the placebo group (46.1% and 40.1%, respectively) including severe forms (4.1% vs 3.5%). The most frequently solicited systemic AE reported after any dose was headache in both groups, followed by myalgia, malaise and asthenia. Lower frequencies were reported of loss of appetite, drowsiness and irritability/fussiness. It should be noted that irritability/fussiness, drowsiness, and loss of appetite were part of the infant/toddler panel of AEs that was used for subjects <6 years only. The Grade 3 solicited systemic AEs were mainly due to headache and malaise. Fever and severe fever were less frequently reported in the TDV group than in the placebo group. The time to onset of all solicited systemic AEs was, mainly, on the day or 1 day after vaccination in both groups and resolved with a median duration of 3-4 days in the TDV and 2-3 day in the placebo groups (with the exception of fever with a median duration of one day).

The applicant reported the incidence of grade 3 solicited systemic AEs after one or a second dose. The incidence of Grade 3 events decreased from first to second vaccine dose in the TDV and placebo groups alike. The incidences in the TDV group after the first and second dose were 1.8% and 1.3% for headache, 1.2% and 1.0% for malaise, and 1.0% and 0.7% for asthenia, respectively. In addition, for the infant/toddler panel, the proportion of subjects with severe (Grade 3) events remained <1% for each individual event, and there was no clinically relevant change in severe (Grade 3) events from first to second vaccine dose in the TDV and placebo groups. A table containing safety data for solicited systemic AEs considered being vaccine related could not be found in the ISA or elsewhere. The applicant provided a table summarising individual vaccine related solicited systemic AEs within 14 days after the first, the second, and any vaccine dose in the target population 4-60 years of age (overall population, Placebo-Controlled Safety Pool), together with corresponding references to source data in the dossier. In the Placebo-Controlled Safety Pool, the proportion of all subjects (4-60 years) reporting vaccine-related solicited systemic events (excluding fever) was higher in the TDV group compared with the placebo group (34.2% versus 27.9% of subjects). This numerical difference was mainly due to small differences for each systemic AE symptom, rather than due to a large difference for specific AE symptoms. After any vaccination, headache (24.4% vs 21.1% of subjects) and myalgia (22.8% vs 16.5% of subjects) were the most frequently reported related solicited systemic AEs in both groups. Vaccine related fever was not reported by a higher proportion of subjects in the vaccine group compared with the placebo group (5.4% versus 6.1% after any vaccination). The systemic AEs irritability/fussiness, drowsiness, and loss of appetite were part of the infant/toddler panel of AEs that was used for subjects <6 years only. The incidence of irritability/fussiness and loss of appetite were numerically only slightly higher in the vaccine group, while the incidence of drowsiness tended to be little higher in the placebo group (i.e. irritability/fussiness was reported for 8.6% versus 6.2% of subjects, drowsiness by 8.9% versus 9.6% of subjects, and loss of appetite by 11.2% versus 8.4% of subjects). Except for fever, vaccine-related solicited systemic AEs were consistently less frequent after the second than after the first vaccine dose. The majority of vaccine related systemic AES was mild to moderate. Severe solicited systemic adverse reactions were reported for 3.0% of subjects in the vaccine and for 2.1% in the placebo group.

In Trial DEN-203, additional solicited systemic AEs were recorded (muscle pain, joint pain, eye pain, sensitivity to light, tiredness, rash, nausea, vomiting-number of episodes) that were not recorded in any of the other trials. Tiredness (fatigue) and nausea have been added to Table of Section 4.8 of the SmPC. The AEs muscle pain (myalgia), joint pain (arthralgia), rash, and vomiting were already adequately covered in the SmPC. Sensitivity to light (no events of photophobia in placebo-controlled trials) and eye pain (no related events in TDV in placebo-controlled trials) are not included. The incidence of prolonged solicited local (after 7 days) or systemic (after 14 days) AEs was higher in the TDV group than in the placebo group (prolonged local AEs: 3.8% in TDV vs 0.6% in Placebo; prolonged systemic AEs: 1.9% in TDV vs 1.5% in placebo after any dose).

Solicited AEs data by age groups (Placebo-controlled trials) were available for 2,882 subjects aged 4-11 years (1,865 in TDV and 1,017 in placebo); 1,733 subjects aged 12-17 years (1,177 in TDV and 556 in placebo) and 940 subjects aged 18-60 years (788 in TDV and 152 in placebo).

Overall, the incidence of solicited local and systemic AEs, including solicited events within 30 minutes after vaccination and prolonged solicited AEs, were higher in the TDV than in placebo group in all age groups and the incidence was lower after the second dose than after the first one, in line with the results observed in the overall population.

Solicited AEs within 30 minutes after any dose were higher reported in the adolescent group than in children or adults. The most frequently solicited local AE within 30 minutes reported after any vaccination was injection site pain in all the age groups, followed erythema and swelling. The most frequently solicited systemic AE after any dose was headache. Fever was reported by ≤0.1% of participants in TDV group.

The incidence of solicited local AEs (within 7 days) increased with age but was overall recorded within the same frequency range in all 3 age cohorts (i.e. 4-11, 12-17, 18-60 years of age). The most frequently solicited local AE reported after any dose was injection site pain in all groups, followed by erythema and swelling. Severe solicited local AEs after any dose were reported with higher frequency in the adolescent group aged 12-17 than in the paediatric group aged 4-11. Lower frequencies of severe solicited local AEs were observed in adults.

The time of onset of local solicited AEs in TDV participants was, mostly, on the day of vaccination in all age groups and had a median duration of 1-2 days in paediatric groups and 4 days in adults.

The incidences of solicited systemic AEs (within 14 days) were lower in children than in adolescents and adults but occurred overall within the same frequency range. The most frequently solicited systemic AE after any dose reported in subjects >6 years of age was headache in all age groups, followed by myalgia, malaise and asthenia. Fever was reported with similar frequencies in TDV children and adolescent group and higher than in adults group. There were no important differences in the time to onset and median duration of solicited systemic AEs by age group.

Frequencies of solicited systemic AEs graded by severity in both paediatric groups were also provided. Overall, in both paediatric age groups, the majority of solicited systemic AEs after the first and second dose of either TDV or placebo were mild (Grade 1). The incidence of severe solicited systemic AEs in both age groups was <2% for each individual event after the first or second dose of either TDV or placebo, with the exception of headache (1.3% after first dose and 1.1% after second doses in 4-11 years group and 2.7% after first doses and 1.9% after second dose in 12-17years group). Additionally, in both age groups, Grade 3 solicited systemic were in general reported with the same incidence after the first and second dose of TDV or placebo.

The incidence of prolonged AEs after any dose was higher in adults than in the 12 to 17 years age group and it was higher than in the 4-11 years age group, mainly due to local AEs. The incidence of prolonged solicited local and systemic AEs was generally higher in the subjects receiving TDV versus

those receiving placebo. During procedure the applicant reported the most frequent prolonged solicited local and systemic AEs for the overall target population (4-60 years) and for each age sub-group (4-11 years, 12-17 years, and 18-60 years) to justify the large difference on incidence of prolonged solicited local AEs in adults in the TDV group (12,6%) compared to the placebo (0,8%). In adult group, the majority of these prolonged solicited AEs were erythema (11.2% vs 0.8%). All of these erythema events were mild-moderate in severity.

With regard to the safety profile description and calculation of ARs/AEs in section 4.8 in the SmPC**,** the applicant compared the frequency (TDV, after any vaccination) for all terms listed in Table 1 of Section 4.8 of the SmPC between the overall target population (4-60 years) and the adult (18-60 years) and paediatric (4-17 years) subpopulations. Adverse Reactions with higher frequency category in any age subgroup compared to the overall target population 4 to 60 years of age were summarised. To account for frequency differences between the adult and the paediatric subpopulations, conservatively the highest frequency category was chosen, and the Product Information was updated accordingly. In addition, footnotes and a summarising text have been added to indicate differences in frequency categories between the age groups.

Unsolicited adverse events

No difference was observed regarding the frequency of unsolicited AEs between participants who received TDV or placebo in the overall population (21.3% vs 22.8%). The frequency of severe unsolicited AEs was slightly higher in TDV than in placebo (0.5% vs 0.2%), although the numbers are very low and probably not significant.

The majority of unsolicited AEs were considered as not related to the trial vaccine. The incidence of related unsolicited AEs was higher in the TDV group (3.0%) than in the placebo group (1.7%). Of these, 0.1% (5 subjects) in TDV and <0.1% (1 subjects) were severe and related unsolicited AE. None of the related unsolicited AEs were considered as SAE.

The most frequently reported (≥1%) unsolicited AEs by PT in both groups were nasopharyngitis (2.6% vs 3.2% of subjects), upper respiratory tract infection (2.3% vs 3.4% of subjects) and viral upper respiratory tract infection (1.0% vs 0.8%).

A table containing all unsolicited AEs included into the placebo-controlled safety pool, irrespective of causality, has been submitted within the ISA report. This table does not reveal any safety signal. However, a numerical imbalance was observed for the AEs rhinitis (1 subject, <0.1% in the placebo, and 8 subjects, 0.2% in the TDV group), and abdominal pain (recorded by 1 subject, <0.1% and 8 subjects, 0.2%). Both are listed in section 4.8 of the SmPC.

The unsolicited AEs considered as related most reported in TDV were injection site bruising and pruritus by 22 and 25 participants respectively (all adults), compared to no cases reported in placebo group. Both ADR (injection site bruising and injection site pruritus) have been added to SmPC. The applicant included a footnote indicating "collected in adults in clinical trials" in the SmPC.

The other unsolicited related AEs were pyrexia and systemic viral infection, fatigue and headache in TDV. Pyrexia and headache are included in the SmPC as were reported as solicited AEs. In addition, fatigue is included in SmPC with "uncommon" frequency. In contrast, the non-inclusion of "viral infection" in table of the SmPC is justified because there were no imbalances in the incidence of subjects experiencing systemic viral infection between the TDV and placebo groups and there were also no imbalances in the number of events assessed as related by the investigator.

Additionally, the applicant reassessed the events of injection site discoloration, chills, and dizziness with regard to causality and it is agreed that injection site discoloration and dizziness are added to SmPC.

Severe unsolicited AEs reported in TDV were one case of myalgia, anhedonia, malaise, upper tract infection and pyrexia. None of these events was serious and all events resolved completely. All but one are included into the SmPC, section 4.8.

The incidence of unsolicited AEs, including SAEs, showed no clinically important differences between the TDV and placebo groups by age groups. However, a significant difference was observed regarding the related unsolicited AEs by age groups. Higher incidences were reported in adults (9.6% vs 4.6% of subjects) than in paediatrics groups (1.0% vs 1.0% in 4-11 and 1.6% vs 2.3% in 11-17). No difference about incidence of severe related unsolicited by age were observed.

At the PT level there was some difference between age groups. In the paediatric groups, the most frequently reported unsolicited AE was nasopharyngitis, followed by upper respiratory tract infection in 4-11 years or by viral upper respiratory tract infection in 12-17 years (no clinically important differences between the TDV and placebo groups). In adults the most frequently reported unsolicited AEs were injection site bruising and pruritus, followed by upper respiratory tract infection.

The only unsolicited AEs reported by ≥0.5% of subjects and with ≥2-fold higher incidences in the TDV group than in the placebo group were reported in the 12 to 17 years age group: abdominal pain (0.6% vs 0), diarrhoea (0.6% vs 0), gastritis (0.5% vs 0), and vomiting (0.5% vs 0), and in adults: injection site bruising (3.4% vs 0.7%), injection site pruritus (3.3% vs 0) and nasopharyngitis (0.6% vs 0). All these ADR were included in SmPC, with exception of gastritis.

Serious adverse events and deaths

Overall, 20 deaths occurred throughout the clinical programme, and none was related to vaccine administration. Of these, 12 deaths occurred up to database lock for the pooled analyses. Ten deaths were included in the pooled analysis: 6 subjects (0.04%) in the TDV group and 4 subjects in the placebo group (0.05%). Two subjects from the TDV group were excluded, 1 subject <4 years from Trial DEN-204, death from septic shock and 1 subject who had received a sequential dose of YF vaccine from Trial DEN 305, death from cardiac arrest.

In the Placebo-Controlled Safety Pool, the incidence of SAEs in the overall target population 4-60 Years was lower in the TDV group than in the placebo group (4.60% vs 5.57% of subjects), owing to fewer events reported as SAEs of dengue fever, DHF, and viral infections in the TDV group.

At the PT level, SAEs with incidences in ≥0.1% of subjects that were reported with a notably higher incidence in the TDV group than in the placebo group were gastroenteritis, asthma, cellulitis and lower respiratory tract infection. None of these SAEs with a notably higher incidence in the TDV group was considered to be related to TDV by the investigator.

For both analysis pools, unrelated cases of appendicitis, gastroenteritis, dengue fever, and viral infections were the most frequently reported SAEs in the TDV group. In the placebo group, SAEs of dengue fever, appendicitis, DHF, and unrelated viral infections were most frequently reported.

For both analysis pools, >95% of all SAEs occurred >30 days after the first or the second vaccine dose, and the majority resolved within 1 to 2 weeks. Of the SAEs that occurred within 30 days after the first or second vaccine dose, renal and urinary disorders (SOC) after the first dose were reported only in the TDV group (4 subjects in All Studies Safety Pool [0.02%]). None of these events considered as related to trial vaccine by the Investigator or the applicant.

A single SAE of suspected dengue illness was considered to be related to TDV, compared with 5 SAEs (in 4 subjects) related to placebo. The related SAE in the TDV group in Trial DEN-301 was reported by a baseline dengue seropositive teenage female. The event started on Day 12 after the first vaccine dose, symptoms included rash, and thrombocytopenia and led to hospitalisation. The RT-PCR test was negative for wild-type dengue. The SAE was considered as possibly related since occurred within the

expected time window of vaccine viraemia although a test for vaccine viraemia was not performed. The event resolved within 5 days.

An additional SAE of polyarthritis (PT) was considered to be related to TDV in All Studies Safety Pool Trial DEN 205). This SAE was reported by an adult seronegative female that started on Day 16 after a single dose of HD TDV and resolved within 8 days. The subject also reported non-serious rash and prolonged solicited systemic AEs of asthenia, malaise, and myalgia. The subject had vaccine viraemia at the time of the SAE.

The related SAEs in the placebo group included hypersensitivity (3 SAEs), dengue fever (1 SAE), and DHF (1 SAE).

In all age groups, the incidences of any SAEs were slightly lower in the TDV than in the placebo groups. No SAE was reported as related to TDV in age group 4 to 11 years and 17 to 60 years. One non-fatal related SAE (suspected dengue illness) was reported in age group 12-17 years in TDV (described above) vs 4 in placebo.

At the PT level, SAEs in age group 4-11 that were reported with a ≥2-fold higher incidence in the TDV group than in the placebo group were asthma, lower respiratory tract infections, humerus fracture cellulitis, lymphadenitis and road traffic accident. In the 12 to 17 years age group, pneumonia, cellulitis, road traffic accident, and lymphadenitis were reported with a ≥2-fold higher incidence in the TDV group than in the placebo group. In adults, the only SAEs reported by >1 subject were major depression (2 subjects) and sciatica (2 subjects).

For either age group, none of these SAEs with a higher incidence in the TDV group were considered to be related to TDV by the investigator. The majority of SAE (>95%) in both age groups occurred >30 days after the first or the second vaccine dose, and the majority resolved within 1 to 2 weeks.

Medically Attended Adverse Events (MAAEs)

For the Placebo-Controlled Safety Pool, MAAE data were available from 1088 subjects who received TDV and from 231 subjects who received placebo, aged 12-60 years. No MAAE data is available for children in the 4 to 11 years age group. No remarkable difference was observed between the TDV and placebo groups although higher incidences were observed in adolescents than in adults.

In All Studies Safety Pool, (1888 subjects in TDV and 231 in placebo) the MAAEs per FTAR in TDV was higher than in Placebo-Controlled Safety Pool (70.4 per 100 person-year vs 56.4 per 100 person-years respectively). This imbalance is probably due to the fact that 190 children aged 4 to 11 years in the TDV group of the All Studies Safety Pool had a high rate of 194.5 MAAEs per 100 person-years. The applicant was requested to explain, in the first RSI, the high rate of MAAEs per FTAR in children aged 4-11 years in TDV. In all three age groups (4-11; 12-17; 18-60 years), the most frequently reported MAAEs were infections and infestation events. The most likely explanation for the high follow-up time-adjusted adverse event rate (FTAR) for MAAEs (and also the high number of MAAEs per subject) observed in the 4-11 years group (all of whom received TDV) is that it reflects young children who generally experience a high rate of infections that require medical attention, notably in tropical countries such as the Philippines or Panama (countries where the clinical trial was conducted).

In both pools the incidence of related MAAEs was low and similar between the TDV and placebo groups (≤0.9%) and events were mild or moderate in severity. None of the severe MAAEs was related to trial vaccine.

Virologically Confirmed Dengue Including Hospitalised VCD and Severe Forms of Dengue

The safety evaluation of VCD cases, including hospitalised VCD cases and severe forms of dengue, was based primarily on the data from pivotal trial DEN-301 where all subjects presenting with febrile illness

or with clinical symptoms of dengue were centrally assessed for VCD by serotype-specific RT-PCR. Among all cases of VCD, severe forms of dengue were determined in 2 different ways:

-All hospitalised VCD cases evaluated for classification as severe dengue by the blinded DCAC, using predefined criteria for severe dengue (DCAC-defined severe dengue). All non-hospitalised VCD cases were considered non-severe.

-All VCD cases meeting the WHO 1997 criteria for DHF were identified using a programmed algorithm, i.e., without applying medical judgment (DHF of any grade (DHF Grade I-IV)

Data were provided from DEN-301 of VCD including hospitalised VCD and severe forms of Dengue up to 12, 24 and 36 months after the second dose. In addition, the data of VCD including hospitalised VCD by DENV-3 were also submitted up to 12, 24 and 36 months after the second dose. Moreover, final data from pivotal Trial DEN-301 until 54 months post second dose, focus on the benefit-risk profile in baseline seronegative subjects, mainly by DENV-3 and DENV-4. New summary tables from DEN-301 including data regarding VCD, hospitalised cases and severe forms up to 12, 24, 36 and 54 months after the second dose caused by all serotypes together or by each serotype, in overall population or by baseline serostatus were submitted.

Data from Trials DEN-204 and DEN-313 were considered as supportive as not pre-specified criteria for dengue severity were defined.

Trial DEN-301

The incidences of VCD (all serotypes combined) were lower in TDV group than in placebo up to 12 months (0.6% vs 3.0%), up to 24 months (0.1% vs 1.4%), up to 36 months (2.9% vs 7.4%) and up to 54 months (2.5% vs 5.8%) after the second dose. The relative risk of VCD in the overall population and in both baseline seropositive and seronegative subgroups were <0.5 (with superior limit <1).

Moreover, the incidences of hospitalised VCD up to 12, 24, 36 and 54 months after the second vaccine dose (all 4 serotypes combined) were lower in the TDV group versus placebo, with relative risks <0.5 in the overall population and in the baseline seropositive and seronegative subgroups.

In seropositive subjects the relative risk of VCD and hospitalised VCD up to 12, 24, 36 and 54 months after the second dose were <0.5 and <0.2, respectively. In addition, the relative risk of VCD and hospitalised VCD by DENV-1, DENV-2 and DENV-3 up to 54 months after the second dose were <0.5. For DENV-4, the RR of VCD was also <0.5; the RR was 0 for hospitalised VCD and the 95%CI cannot be calculated (0 cases in TDV vs 3 in placebo through 54 months)

In seronegative subjects the relative risk of VCD and hospitalised VCD up to 12, 24, 36 and 54 months after the second dose were <0.5 and ≤0.2, respectively. For DENV-1 and DENV-2, the relative risk of VCD and hospitalised VCD in seronegative subjects were <1 (including upper limit). For serotype DENV-3 the relative risk of VCD was 1.60 (0.52, 4.91), 1.37 (0.64, 2.93), 1.18 (0.65, 2.16) and 1.11 (0.62, 1.99) up to 12, 24, 36 and 54 months after the second vaccine dose respectively. This imbalance was noted in hospitalised VCD caused by DENV-3 with 11 cases in the TDV group (N=3,714, 0.3%) compared with 2 cases in placebo group (N=1,832, 0.1%) up to 36 months after the second dose resulting in a relative risk of 2.71 (95% CI: 0.60, 12.23) for seronegative individuals receiving TDV. No additional hospitalised cases caused by DENV-3 occurred in the TDV group up to 54 months after the second dose, resulting in a RR of 1.81 (95% CI 0.51, 6.48).

Imbalance observed in DENV-3´s VCD hospitalised cases in seronegative subjects is explained by the different standard of care at a single trial site and other confounding factors. If hospitalised VCD cases from Sri Lanka were excluded, the incidences of hospitalised VCD caused by DENV-3 in baseline seronegative subjects up 54 months after the second dose would be 5 cases in the TDV and 3 cases in the placebo with a RR of 0.82 (95% CI 0.20, 3.42). Importantly, the RR in DENV-3 seronegative

subjects was >1 including Sri Lanka or <1 excluding Sri Lanka, but in both cases with an upper limit of CI greater than 1, indicating that the potential safety risk of TDV by DENV-3 in seronegative individuals could not be excluded. For this reason, *"Severe and/or hospitalized dengue following vaccination caused by dengue virus serotype 3 in individuals not previously infected by dengue virus"* as an important potential safety risk in the RMP (See Safety Specifications in RMP).

For DENV-4, in seronegative subjects, the number of cases was small. The relative risk of VCD by DENV-4 up to 36 months was 1.97 (95% CI: 0.42, 9.28) with 8 cases (0.2%) in TDV and 2 cases (0.1%) in placebo. Up to 54 months, the RR was 1.97 (95%IC 0.56, 6.98) with 12 cases in TDV (0.3%) and 3 cases in placebo (0.2%). Regarding hospitalised VDC by DENV-4 in seronegative subjects, there were no cases in the TDV group and there was only 1 subject in placebo group, therefore a firm conclusion cannot be drawn.

Regarding the VE in the seronegative population up to 36 and 54 months against VCD for DEN-4 it could not be demonstrated either. A higher incidence of DENV-4 VCD was observed in the TDV group than in the placebo. However, given the small number of DENV-4 cases in this trial a potential signal towards disease enhancement by DENV-4 in seronegative individuals cannot be conclusively confirmed or excluded. In the RMP "*Severe and/or hospitalized dengue following vaccination caused by dengue virus serotype 4 in individuals not previously infected by dengue virus*" has been included as an important potential safety risk (See Safety Specifications in the RMP).

Of the overall 29 cases of DCAC-defined severe dengue or DHF reported up to 54 months, 10 cases were in the TDV group and 19 cases in the placebo group.

In the TDV group, 3 cases (0.02%) were considered DCAC-defined severe dengue and 9 cases (0.07%) of DHF, since two cases were considered both severe and DHF. In the placebo group, there were 5 cases (0.07%) of DCAC-defined severe dengue and 15 cases (0.19%) of DHF; one case in placebo was considered both severe and DHF. It should be noted that this evaluation is exploratory and based on a very low number of severe cases.

Of the 3 overall cases of DCAC-defined severe dengue in the TDV group, 2 cases occurred in seronegative subjects (both were caused by DENV-3) and one in seropositive (caused by DENV-3). In placebo group, all 5 DCAC-defined severe dengue cases were seropositive at baseline (1 case by DENV-1 and DENV-2 and 3 cases by DENV-3).

Additionally, of the 9 overall cases of DHF in TDV group, 4 cases occurred in seronegative subjects (all caused by DENV-3) and 5 in seropositive (2 cases by DENV-1 and 3 cases by DENV-3). In comparison, in the placebo group 12 of 13 overall cases were in seropositive subjects (3 cases by DENV-1, 6 cases by DENV-2, 2 cases by DENV-3 and 1 case by DENv-4) and 1 in seronegative.

Up to 54 months, the incidences of DHF or severe dengue caused by DENV-3 in both groups remained very low (4 versus 1 DHF, and 2 versus 0 severe dengue cases in the TDV and placebo group with a 2:1 randomisation ratio, respectively). The two cases in TDV recipients that were assessed as severe by the DCAC occurred early in the trial during Parts 1 and 2 (i.e., before 18 months post second dose).

In addition, no cases of DHF and/or severe dengue were caused by DENV-4 throughout the trial up to 54 months post second dose.

Vaccine Viraemia

Methods of vaccine viraemia evaluation, trial design, dose and route of administration differ in the single trials contributing to the pooled analysis of vaccine viraemia. Results are descriptive and assessed post-hoc. It is agreed with the applicant, that pooled results should therefore be interpreted with caution. The data are however supported by (though only few) non-pooled cases of vaccine viraemia from 2 other trials.

Vaccine viraemia occurred mainly after the first TDV dose, peaked overall at 10 to 12 days after vaccine dosing, and was mostly of vaccine strain TDV-2.

The result of vaccine viraemia in the Trial DEN-205 analysed in final TDV formulation and HD TDV groups showed that the vaccine viraemia was higher in HD TDV (62.5%) than in TDV (30.9%). Additionally, independent of HD TDV or TDV, the viraemia mainly occurred for vaccine strain TDV-2, peaked at Day 11, the mean duration of viraemia was similar for TDV-2 and other serotypes. No difference of reversion of attenuation loci were observed in TDV or HD TDV (14.8% and 13.6%, respectively).

The incidence of vaccine viraemia was higher in baseline seronegative (48.8%) than in baseline seropositive (15.8%) subjects and the mean duration was also longer for seronegative than for seropositive subjects.

The incidence of solicited and unsolicited AEs in subjects with vaccine viraemia, replication-competent vaccine virus, or reversion of attenuation loci was analysed only after the first dose because vaccine viraemia was rarely detected after the second dose.

Overall, there was higher incidence of some solicited and unsolicited AEs in viremic than in non-viremic subjects and in viremic subjects with or without replication-competent vaccine virus.

Of note, the number of evaluated ADRs for viremic subjects was larger than those listed in tables - Solicited Local AE within 7 day or Systemic within 14 days After First, Second, and Any Vaccine Dose, Target Population [4-60 Years] -, as in early clinical development phase, an extended panel of solicited AEs was collected, as well as events that are known to occur in the scope of wild type dengue, whilst in contrast during the majority of the Phase 2 and 3, the panel of solicited AEs was focused on the characterisation of reactogenicity to the vaccine

Regarding solicited AEs, higher incidences of injection site pain, injection site erythema, rash, arthralgia, fatigue and fever were observed in viremic than in non-viremic subjects. Furthermore, in subjects with viraemia, a higher incidence of systemic AEs such as fatigue, myalgia, arthralgia, nausea, and photophobia were observed than in those without replication-competent vaccine virus. Likewise, a higher incidence of pain at the injection site, arthralgia, fever, nausea, and vomiting was observed in subjects with attenuation locus than in those without it. Most of these ADR, including fatigue and nausea were included in SmPC. In addition, the non-inclusion of "photophobia" is justified because the photophobia events reported in viremic subjects were collected in early phases clinical trials (using different formulations, vaccination schedules and routes of administration) and no events of photophobia were reported in the TDV group in placebo-controlled trials. Therefore, a causal relationship between TDV and photophobia could not be established.

Regarding unsolicited AEs, higher incidence of headache, fatigue, myalgia, arthralgia, nausea and upper respiratory tract infection were observed in with than without viremic subjects. In addition, most episodes of rash (75.0%) and all episodes (100%) of rash erythematous, rash papular, rash maculo-papular, rash generalised, rash macular, and rash pruritic showed a temporal relationship with vaccine viraemia. In the SmPC, is added a footnote in the table 1 in section 4.8 in the ADR of rash with "Rash includes rash, viral rash, rash maculopapular, rash pruritic".

Additionally, in DEN-301, the relationship/association of acute febrile illness (within 30 days) and viraemia was assessed. Of note, most febrile illness cases (within 30 days) were not associated with detection of vaccine viraemia. Vaccine viraemia was detected in 34 of 479 febrile illness subjects (7.1%) after the first TDV dose and in 1 of 503 subjects (0.2%) after the second vaccine dose.

Subjects with febrile illness and vaccine viraemia showed a similar pattern of symptoms as those with febrile illness but without vaccine viraemia. Additionally, the incidence of febrile illness was higher in viremic (TDV-2) seronegative than seropositive subjects.

Due to the limited number of subjects with febrile illness and vaccine viraemia, the pattern on AEs is inconclusive.

Laboratory findings

Laboratory clinical parameters (haematology, serum chemistry, urinalysis) were analysed approximately in 1800 subjects from the 5 phase 1 trials and in 2 phase 2 trials (DEN-203 and DEN-106). The results did not show any clinical safety concern. In addition, the results for parameters of special relevance in the context of the symptomatology associated with dengue fever did not reveal differences between any TDV and placebo.

No pooling of clinical laboratory data was performed they were not evaluated in the rest of clinical trials, including phase 3 pivotal trial.

Safety in special populations

Pregnancy or lactation:

TDV has not been studied in pregnant or lactating women. Pregnancy was an exclusion criterion for all trials in the TDV clinical development programme. As other live viral vaccines TDV is contraindicated during pregnancy (see SmPC, section 4.6) and the exposure of TDV in pregnant and lactating women is considered as missing information on the safety specification on the RMP. Nevertheless, there is limited data of vaccine administration in women who were pregnant or who became pregnant shortly after vaccination.

As of 01 October 2020, a total of 405 pregnancies were reported and most then were considered as non-exposed (89.1%). Only 44 of 405 were considered exposed. Of these, 79.4% in any TDV group and 50.0% in placebo had a normal outcome and no major differences in frequencies of spontaneous abortion were observed between women exposed to any TDV or placebo.

In addition, there were 8 women who became pregnant during the trial and had births with neonatal deaths (all from Trial DEN-301). Only one neonatal death (TDV group) occurred after an exposed pregnancy, and the remaining 7 cases occurred after non-exposed pregnancies. None of the neonatal deaths were assessed as causally related to TDV.

Intrinsic and extrinsic factors:

The safety of TDV in target population was evaluated by intrinsic and extrinsic factors. The safety profile by age group has been described previously, in addition, data for subjects aged <4 years were provided, and no safety concern was observed. However, due to the limited number of participants the high variability of the incidence of solicited AEs makes it difficult to reach a conclusion.

In general, in the analyses of the different subgroups, the incidence of solicited and unsolicited AEs was higher in TDV group than in placebo and they were observed less frequently after the second dose than after the first, in line with the profile observed in the overall population. In addition, no clinically important differences of the incidence of SAE by subgroups were observed.

By *intrinsic factor* (serostatus, gender and race) no significant demographic differences were observed by the different subgroups of TDV and placebo with exception of baseline serological status serostatus in which the majority of adults were seronegative.

The incidence of solicited AEs, including prolonged AEs, events within 30 minutes and unsolicited AEs was higher in seronegative than in seropositive subjects and in female than in male. The biggest

difference was in the local AEs requested. In addition, the differences of solicited and unsolicited AEs by serostatus were most pronounced in adolescent age group.

No clinically important differences were noted between the AE profile observed by race (Asian, American Indian or Alaska Native, Black or African American, and White subjects). The only notable difference was that solicited local AEs were reported more frequently by White subjects than by the other race subgroups.

No safety data are available for individuals with underlying disease and the frail population. No data are available for individuals with immunosuppression (missing information). Like other live viral vaccines, the use of TDV is contraindicated for individuals with congenital or acquired immune deficiency, including immunosuppressive therapies, and for individuals with symptomatic and asymptomatic HIV infection.

The safety profile by *extrinsic factor* (endemic/non-endemic region, region/countries and previous flavivirus vaccination) is inconclusive because there are underlying reasons that confound the observed data, such as important differences by age or baseline serostatus.

The subgroup of subjects from endemic regions (Latin America and Asia Pacific) is dominated by children and adolescents and the non-endemic subgroup (North America) comprised only adults. Solicited local AEs, mainly consisting of mild and transient injection site pain, appeared to be more frequently experienced by subjects from non-endemic regions than by subjects from endemic regions. The same but less pronounced was observed for the incidence of solicited systemic AEs (overall and considered to be related). The evaluation of unsolicited AEs reported up to 28 days after vaccine dosing for both analysis pools revealed no clinically important differences between the TDV and placebo groups in the subjects from endemic and non-endemic regions. Though data are limited for the use of TDV in non-endemic regions they did not raise concerns for the use in these regions.

With respect to previous flavivirus vaccination, the adult participants were limited and only in previous unvaccinated subjects. The Placebo-Controlled Safety Pool comprised 14,627 subjects exposed to at least 1 dose of TDV and 7167 subjects exposed to at least 1 dose of placebo. In the TDV and placebo groups alike, approximately 40% of subjects in the Placebo-Controlled Safety Pool and approximately 36% of subjects in the supportive. For both analysis pools, no clinically important differences were identified regarding the incidence of solicited and unsolicited AEs in subjects with and without previous flavivirus vaccinations. The incidence of unsolicited AEs (related and unrelated) was not higher in the previous vaccinated group compared with the not previously vaccinated group. No SAE in the previously vaccinated group was considered being vaccine related. No safety concern for the use of TDV in previously flavivirus vaccinated individuals (YF and JE) could be raised.

Immunological events

No vaccine-related anaphylactic reactions/anaphylactic shock events were reported within 28 days after any dose for either safety pools. There was only 1 unrelated anaphylactic shock event after the second dose in a TDV subject after ingestion of mefenamic acid. Additionally, the incidence of hypersensitivity reactions was ≤0.1% for both TDV and placebo recipients (both pools) and none of these hypersensitivity events was serious or severe. From the initial submitted information it remains unclear, whether hypersensitivity reactions considered being vaccine related were reported or not. The applicant has performed a post-hoc analysis to identify any hypersensitivity events that were considered vaccine related. In the Placebo-Controlled Safety Pool, the overall incidence of vaccine-related hypersensitivity events reported within the first 28 days after a TDV dose was low (12 events in 11 of 14,627 subjects [0.08%]) and showed no difference compared with the placebo group (8 events in 6 of 7,167 subjects [0.8%]. In the supportive updated All Studies Safety Pool, the incidence was higher in the TDV group than in the placebo group (0.14% vs 0.07% of subjects). This is due to higher

reported rates of skin reactions (rash, rash pruritic, pruritus, urticaria) in the TDV group. A minority of vaccine-related hypersensitivity reactions reported in the TDV group occurred within the first 4 days after vaccination, i.e. 5 of 12 events in the Placebo-Controlled Safety Pool, and 15 of 27 events in the All Studies Safety Pool. The majority of events identified by the SMQ search were skin reactions such as rash, pruritus, or urticaria. These types of hypersensitivity reactions are included in the SmPC as ADRs.

Only one additional notable vaccine-related hypersensitivity reaction was reported within 28 days after TDV vaccination (Placebo-Controlled Safety Pool). This was a single event of angioedema in a male under age 10 (Trial DEN-301). The child discontinued the trial after receiving the first TDV dose due to a non-serious AE of mild angioedema affecting both eyes. The event started on day 1 and was treated with systemic antihistamines. The event resolved on the following day. No other AEs and no medical history events were recorded for the subject. Applicant and investigator concluded that a causal relationship cannot be excluded for this event in the absence of alternative causes. The AE of angioedema has been included in SmPC section 4.8 accordingly.

Co-Administration With Yellow Fever or Hepatitis A virus Vaccine

Co-administration of TDV with YF and HAV vaccine has been evaluated in trials DEN 305 and DEN-314, respectively. In both trials, the incidences of solicited AEs were higher in the co-administration groups (TDV+YF or TDV+HAV) than in YF or HAV and placebo but occurred in general in a comparable frequency range. No clinically important differences between groups were noted regarding unsolicited AEs and SAEs. Generally, the co-administration was well tolerated and no safety concern was identified.

Discontinuation due to AEs

The incidence of AEs leading to vaccine and/or trial discontinuation was ≤0.20% in both groups and incidence of AEs considered to be related by the investigator was <0.1% for both groups (6 subjects in TDV and 2 subjects in placebo).

One event of mild angioedema in the region of both eyes was recorded for a male under age 10. The event occurred on the day of the first TDV dose and resolved within 2 days. The event was considered being vaccine related by the investigator and listed in the SmPC (see above).

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Additional expert consultations

Refer to "Additional expert consultations" stated in Efficacy section of this report.

Specific considerations on safety data for a non-EU vs. an EU context of use

In the context of a parallel EU-M4all and EU MAA application, it is considered that the majority (>90%) of all subjects in the TDV and placebo groups of the Placebo-Controlled Safety Pool were from endemic regions. Subjects from non-endemic regions in this analysis pool comprised 919 adults from North America (US) enrolled in Trial DEN-304 and 400 adolescents from Mexico City enrolled in Trial DEN-315.

The analysis of the submitted safety data (excluded long-term follow-up data for VCD, hospitalised VCD, DCAC-defined dengue, and DHF) revealed no clinically important differences between the TDV and placebo groups in the subjects from endemic and non-endemic regions. Though data are limited for the use of TDV in non-endemic regions they did not raise concerns for the use in these regions. The data from the US trials are deemed appropriate for extrapolation to the EU population, supporting the separate EU Marketing Authorisation.

2.6.10. Conclusions on the clinical safety

The method for describing and evaluating the safety profile of TDV for the target population (≥4 years of age), is general deemed acceptable. The safety data base (27,118 subjects from dengue-endemic (92.6%) and non-endemic regions) is, though dominated by the paediatric population, also acceptable.

Solicited AEs and unsolicited AEs in target population was evaluated in a subset of 5,555 participants randomised 2:1 (TDV: placebo).

As it can be expected after any dose the incidences of solicited local AE and solicited systemic AEs were higher in the TDV than in the placebo group although a majority of them were mild in severity. The most frequently solicited local AEs reported were injection site pain, followed by erythema and swelling. The most frequently reported solicited systemic AE was headache, followed by myalgia, malaise and asthenia. Fever and severe fever were less frequently reported in the TDV group than in the placebo group. The time to onset of majority of solicited local and systemic AEs was on the day or 1 day after the vaccination and resolved with a median duration of 2-3 days for solicited local AEs and 3-4 days for solicited systemic AEs (with exception of fever with a median duration of one day).

No difference was observed regarding the frequency of unsolicited AEs between participants who received TDV or placebo in the overall population. However, the incidence of unsolicited AEs considered related were higher in TDV than in placebo.

Overall, the incidence of solicited and unsolicited AEs was lower after the second dose than after the first one and there were higher frequencies in female vs males and in seronegative vs seropositive after any dose. Additionally, there was an increasing trend of solicited and unsolicited AEs with age but occurred within the same frequency range.

Only limited data for the use of TDV in pregnant women is available. These data do not allow a conclusion on the safety profile of TDV when administered to pregnant women in any state of pregnancy. The use of TDV is contraindicated for pregnant and lactating women and it is considered a missing information on the safety specification on the RMP.

In all age groups, the incidences of any SAEs were slightly lower in the TDV than in the placebo group owing to fewer events reported as SAEs of dengue fever, DHF, and viral infections in the TDV group. Related SAEs were also lower in the TDV group.

Vaccine viraemia was mainly detected after the first dose and there was higher in baseline seronegative than in baseline seropositive subjects. No relationship/association of acute febrile illness (within 30 days) and viraemia was detected. However, higher incidence of some solicited and unsolicited AEs was observed in viraemic than in non-viraemic subjects and in viraemic subjects with replication-competent vaccine virus than without it.

Importantly, in seronegative subjects the RR of VCD, including hospitalised VCD, caused by DENV-3 was >1 up to 12, 24, 36 and 54 months after the second vaccine dose. Different standards of care and other confounding factors may explain why the RR in DENV-3 seronegative subjects was >1 if Sri Lanka data were included or <1 if excluding Sri Lanka data, albeit with an upper limit of CI greater than 1 in both cases, indicating that a safety concern regarding DENV-3 infection in TDV vaccinated seronegative individuals cannot be completely excluded.

Again, the incidence of VCD by DENV-4 in seronegative individuals was low in both groups. However, the RR of VCD by DENV-4 in seronegative subjects up to 54 months after the second dose was >1, indicating that a risk of enhanced dengue disease caused by DENV-4 in seronegative subjects cannot be conclusively ruled out.

Finally, the incidence of DCAC-defined severe dengue and/or DHF was low in seronegative subjects at baseline. However, there was an imbalance with 5 cases in the TDV group (all caused by DENV-3) compared to 2 cases in placebo group (caused by DENV-1 and DEN-3) at month 54 after the second vaccination

In conclusion, the risk of severe VCD caused by DENV-3 and DENV-4 in seronegative subjects are considered a safety concern in the Safety specification on the RMP.

2.7. Risk Management Plan

2.7.1. Safety concerns

Table SVIII.1: Summary of safety concerns

Summary of safety concerns	
Important identified risks	None
Important potential risks	Anaphylaxis including anaphylactic shock Dengue disease due to waning protection against dengue over time Severe and/or hospitalised dengue following vaccination caused by dengue virus serotype 3 or 4 in individuals not previously infected by dengue virus
Missing information	Safety profile of inadvertent use in pregnant or lactating women Safety and immunogenicity in immunocompromised individuals Safety and immunogenicity of concomitant administration with other vaccines other than HAV and YF Safety and reactogenicity of a booster dose

2.7.2. Pharmacovigilance plan

Table Part III.1: On-going and planned additional pharmacovigilance activities

Study Status	**Summary of objectives**	**Safety concerns addressed**	**Milestones**	**Due dates**
Category 3 - Required additional pharmacovigilance activities				
Efficacy, Safety and Immunogenicity of TDV in Trial DEN-301 (Part 4 and 5) Study status: ongoing	To evaluate the efficacy, immunogenicity and safety of a TDV booster dose	Dengue disease due to waning protection against dengue over time Severe and/or hospitalised dengue following vaccination caused by dengue virus serotype 3 or 4 in individuals not previously infected by dengue virus Safety and reactogenicity of a booster dose	Interim report: End Part 4 CSR	Q4 2024 (planned)
			Final Report (Final CSR Parts 1, 2, 3, 4 and 5)	Q1 2026 (planned)

Study Status	Summary of objectives	Safety concerns addressed	Milestones	Due dates
Category 3 - Required additional pharmacovigilance activities				
Long-term safety and antibody persistence of TDV and the impact of a booster dose (Trial DEN-303) Study status: ongoing	To assess the immunogenicity and safety of a TDV booster dose in Healthy Adolescents and Adults.	Dengue disease due to waning protection against dengue over time Severe and/or hospitalised dengue following vaccination caused by dengue virus serotype 3 or 4 in individuals not previously infected by dengue virus Safety and reactogenicity of a booster dose	Final CSR	Q1 2025
Immunogenicity and safety of TDV and 9vHPV in subjects aged ≥9 to <15 years (Trial DEN-308) Study status: ongoing	To investigate the immunogenicity and safety of the co-administration of a subcutaneous dengue tetravalent vaccine and intramuscular recombinant 9-valent human papillomavirus (9vHPV) vaccine in subjects aged ≥9 to <15 years in endemic country for dengue	Safety and immunogenicity of concomitant administration with other vaccines other than HAV and YF	Final CSR	Q4 2023

2.7.3. Risk minimisation measures

Table Part V.3: Summary table of pharmacovigilance activities and risk minimisation activities by safety concern

Safety concern	Risk minimisation measures	Pharmacovigilance activities
Anaphylaxis including anaphylactic shock	**Routine risk minimisation measures:** SmPC Section 4.3 and Section 4.4 PL Section 2 **No additional risk minimisation measures**	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** Cumulative review **No additional pharmacovigilance activities**
Dengue disease due to waning protection against dengue over time	**Routine risk minimisation measures:** SmPC Section 4.4 PL Section 2 **No additional risk minimisation measures**	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** Cumulative review **Additional pharmacovigilance activities:**

Safety concern	Risk minimisation measures	Pharmacovigilance activities
		Efficacy, Safety and Immunogenicity of TDV in Trial DEN-301 (includes administration of a booster dose) DEN-303 – Long-term immunogenicity trial (includes administration of a booster dose)
Severe and/or hospitalised dengue following vaccination caused by dengue virus serotype 3 or 4 in individuals not previously infected by dengue virus	**Routine risk minimisation measures:** SmPC Section 4.4 PL Section 2 **No additional risk minimisation measures**	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** Cumulative review **Additional pharmacovigilance activities:** Efficacy, Safety and Immunogenicity of TDV in Trial DEN-301 (includes administration of a booster dose) DEN-303 – Long-term immunogenicity trial (includes administration of a booster dose)
Safety profile of inadvertent use in pregnant or lactating women	**Routine risk minimisation measures:** SmPC Section 4.3, Section 4.4 and Section 4.6 PL Section 2 **No additional risk minimisation measures**	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** Cumulative review **No additional pharmacovigilance activities**
Safety and immunogenicity in immunocompromised individuals	**Routine risk minimisation measures:** SmPC Section 4.3 and Section 4.5 PL Section 2 **No additional risk minimisation measures**	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** Cumulative review **No additional pharmacovigilance activities**
Safety and immunogenicity of concomitant administration with other vaccines other than HAV and YF	**Routine risk minimisation measures:** SmPC Section 4.5 PL Section 2 **No additional risk minimisation measures**	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:** Cumulative review **Additional pharmacovigilance activities:** Coadministration with 9vHPV vaccine Trial (DEN-308)
Safety and reactogenicity of a booster dose	**Routine risk minimisation measures:** SmPC Section 4.2	**Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:**

Safety concern	Risk minimisation measures	Pharmacovigilance activities
	No additional risk minimisation measures	None **Additional pharmacovigilance activities:** Efficacy, Safety and Immunogenicity of TDV in Trial DEN-301 (includes administration of a booster dose) DEN-303 – Long-term immunogenicity trial (includes administration of a booster dose)

No additional risk minimisation measures have been proposed.

The proposed routine risk minimisation measures are deemed sufficient.

Since this is a parallel EU-M4all and an EU MAA application, it is reminded that the same pharmacovigilance requirements apply to both applications, i.e. as for the EU Marketing Authorisation, although the pharmacovigilance system has to be adapted to the patients and to the health systems of the countries where the medicinal product is intended to be authorised. The opinion holder should record all serious adverse reactions and submit them to EMA within the timeframes, submit PSURs, and manage safety signals. The opinion holder should also submit to EMA any information that may affect the medicine's benefit-risk balance. EMA's requirements are additional to the PhV requirements of the regulatory authorities where the medicine is authorised.

2.7.4. Conclusion

The CHMP considers the risk management plan version 1.0 acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant does fulfil the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The submission of PSURs for this medicinal product will follow the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The holder of the scientific opinion shall submit the first PSUR for this product within 6 months following the scientific opinion.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

3.1. Therapeutic Context

3.1.1. Disease or condition

Dengue is a severe, flu-like illness that affects infants, young children and adults, but seldom causes death. Symptoms usually last for 2–7 days, after an incubation period of 4–10 days after the bite from an infected mosquito. The World Health Organization classifies dengue into 2 major categories: dengue (with / without warning signs) and severe dengue.

-Dengue should be suspected when a high fever (40°C/104°F) is accompanied by 2 of the following symptoms during the febrile phase: severe headache; pain behind the eyes; muscle and joint pains; nausea; vomiting; swollen glands, or rash.

-Severe dengue: A patient enters what is called the critical phase normally about 3-7 days after illness onset. It is at this time, when the fever is dropping (below 38°C/100°F) in the patient, that warning signs associated with severe dengue can manifest. Severe dengue is a potentially fatal complication, due to plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment. Warning signs that doctors should look for include severe abdominal pain; persistent vomiting; rapid breathing; bleeding gums; fatigue; restlessness or blood in vomit. The most severe forms of dengue infection – dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) – are life threatening.

Primary infection with any of the 4 dengue serotypes is thought to result in decades of protection from re-infection by the same serotype but may not protect against a secondary infection by 1 or more of the other 3 dengue serotypes and may lead to an increased risk of severe disease over the course of secondary infection (DHF/DSS).

Dengue viruses are transmitted from human to human by mosquitoes (primarily by *Aedes aegypti* but also by *Aedes albopictus*). There are an estimated 390 million dengue infections per year worldwide, of which 100 million are symptomatic. Every year, around 500,000 cases of dengue haemorrhagic fever (DHF) require hospitalisation with an estimated death rate of 2.5%, primarily in children. It is estimated that 3.9 billion people are at risk of dengue infection, with an estimated death rate of approximately 20,000 to 25,000 per year, primarily in children.

The incidence of dengue has grown dramatically around the world in recent decades. This increase has been associated with societal changes such as population growth and increasing urbanisation, particularly in tropical cities with poor waste and water management, leading to proliferation of the domestic and peridomestic mosquito species (*Aedes aegypti* and *Aedes albopictus)*. Human migration and international trade and travel are constantly introducing new vectors and pathogens into novel geographic areas.

Before 1970, only 9 countries had experienced severe dengue epidemics. The disease is now endemic in more than 100 countries in the World Health Organization (WHO) regions of Africa, the Americas, the Eastern Mediterranean, South-East Asia and the Western Pacific. The Americas, South-East Asia and Western Pacific regions are the most seriously affected, with Asia representing approximately 70% of the global burden of disease. Not only is the number of cases increasing as the disease spreads to areas including Europe, but outbreaks are occurring. The threat of a possible outbreak of dengue now exists in Europe; local transmission was reported for the first time in France and Croatia in 2010 and imported cases were detected in 3 other European countries. In 2012, an outbreak of dengue on the

Madeira islands of Portugal resulted in over 2000 cases and imported cases were detected in mainland Portugal and 10 other countries in Europe. Autochthonous cases are now observed on an almost annual basis in many European countries.

Among travellers returning from low- and middle-income countries, dengue is the second most diagnosed cause of fever after malaria. A secondary dengue vector in Asia, *Aedes albopictus*, has spread to more than 32 states in the United States (US) and more than 25 countries in the European Region.

3.1.2. Available therapies and unmet medical need

Treatment of dengue fever is based solely on the clinical signs and symptoms, with fluid replacement required for haemorrhagic or shock cases. An antiviral therapy for dengue virus infection is not available. Most of the current preventive measures that rely on mosquito control and individual protection are of limited efficacy, complex to implement, and questionable in terms of cost-effectiveness. While the malaria transmitting Anopheles mosquitoes predominantly feed during the night, the dengue transmitting Aedes mosquitoes feed predominantly at dusk and bed nets are therefore not effective. Dengue continues to spread despite the use of vector control measures. New technologies under development appear to be effective at stopping local dengue transmission, such as where mosquitoes infected with Wolbachia (which reduces a mosquito's ability to transmit human viruses) are released into the environment.

Vaccine development has assumed the need for tetravalent vaccines against all 4 serotypes to avoid any potential risk of vaccine induced immune enhancement, as has been well documented with natural (wild type) infection. A first tetravalent dengue vaccine (chimeric Yellow Fever [YF] virus-Dengue virus Tetravalent Dengue Vaccine) has been approved since 2015 in several Asian and Latin American countries as well as in the US and in the European Union (EU). This vaccine was initially approved for use in vaccine recipients ≥9 years of age because clinical data indicated an unfavourable risk benefit profile for children <9 years of age. More recent analyses found that individuals who were dengue seronegative before vaccination had a higher risk of getting severe disease and/or getting hospitalised when they were infected by dengue virus after vaccination than individuals who were already seropositive. In a revised recommendation from April 2018, the Scientific Advisory Group for Emergencies (SAGE) concluded that for countries considering vaccination as part of their dengue control programme, a "pre vaccination screening strategy" would be the preferred option, and only dengue seropositive individuals should be vaccinated.

Hence, considering the epidemiology of dengue, the lack of available antiviral treatments and the limitations of already available vaccine, there is a continued unmet public health need for a safe and effective vaccine that will protect populations not covered by the available option (children aged <9 years and individuals with no previous exposure to dengue virus) against dengue infection.

The WHO has defined and reconfirmed dengue vaccines as high priority vaccines for WHO prequalification, i.e., dengue is included in the list of High Priority Vaccines in the WHO vaccines prequalification priority list 2018-2020.

To address this unmet medical need, the applicant has developed a tetravalent vaccine that aims to protect against dengue irrespective of baseline dengue serostatus and that can be administered to children as young as 4 years of age.

3.1.3. Main clinical studies

The applicant is seeking a Scientific Opinion for TDV with an indication for the prevention of dengue disease caused by any dengue virus serotype in individuals from 4 years of age and above. The vaccine is to be administered following a two-dose (0 and 3 months) schedule.

Data from a total of 7 phase 3, 6 phase 2, and 5 phase 1 trials, comprising more than 27,000 subjects from dengue-endemic and non-endemic regions and covering an age range from 1.5 to 60 years, are presented in this submission in support of the proposed indication for TDV.

The benefit-risk evaluation is based primarily on the efficacy results from pivotal phase 3 Trial DEN-301, the immunogenicity results from the seven phase 2 and phase 3 trials with available immunogenicity data that included TDV in its final lyophilised formulation administered as 2 doses 3 months apart, and the available safety data pooled across 10 phase 2 and phase 3 trials, with the focus on placebo-controlled trials. An assessment of immunogenicity when TDV is co-administered with a vaccine against the yellow fever (YF) virus or hepatitis A virus are also included in this application.

Study DEN-301 is a Phase III, Double-Blind, Randomised, Placebo-Controlled Trial to Investigate the Efficacy, Safety and Immunogenicity of a Tetravalent Dengue Vaccine (TDV) Administered Subcutaneously in Healthy Children Aged 4–16 Years Old. This trial was developed in 8 countries from two regions (Asia Pacific and Latin America) where Dengue is endemic. Eligible subjects (aapproximately 20,000) were randomised in a 2:1 ratio to receive either TDV or placebo (normal saline for injection) by a subcutaneous (SC) injection into the upper arm.

The trial comprises 5 consecutive trial parts: Part 1 was the primary analysis period, a 15-month period lasting until 12 months post second dose and included the primary efficacy analysis. Virologically confirmed dengue cases in Part 1 counted towards the primary efficacy objective if they occurred at least 30 days post-second vaccination. Part 2 was a 6-month period lasting until 18 months post second dose, at the end of which the secondary efficacy endpoints were analysed. Part 3 is a 2.5- to 3-year period for the assessment of long-term efficacy and safety; and Parts 4 and 5 comprise the booster phase of at least 25 months for the assessment of the efficacy, immunogenicity, and safety following an additional placebo or TDV booster dose administered 48 to 54 months (4-4.5 years) after the second vaccine dose.

Parts 1, 2 and 3 have been completed; the remainder of the trial (parts 4 and 5) is still ongoing. In this a application for a Scientific Opinion, results based on efficacy, immunogenicity, and safety data up to 24 months post second vaccine dose were initially submitted and are summarised in detail in this assessment report. Moreover, data up to 54 months post second dose became available during assessment and the key results are also discussed. It is noted that results of part 4 and 5 of the study (as amended to incorporate a booster dose) are not provided in this application.

3.2. Favourable effects

Several favourable effects for TDV were shown from the pivotal trial DEN-301.

The primary endpoint for efficacy in the prevention of VCD fever caused by any serotype (i.e., all 4 serotypes combined) from 30 days to 12 months post second vaccine dose was met since the pre-established criterion (the lower bound of the 2 sided 95% CI for the VE was above 25%) was fulfilled. In fact, VE was 80.2% (95% CI: 73.3%, 85.3%; p<0.001).

The efficacy of TDV for the key secondary endpoint of hospitalisation due to VCD caused by all serotypes combined from 30 days to 18 months post second vaccine dose was confirmed with a VE of 90.4% (95% CI: 82.6%, 94.7%).

The results from other secondary endpoints, that measured VE from 30 days to 18 months post second dose showed:

-VE against VCD fever in dengue baseline seropositive subjects was 76.1% (95% CI: 68.5%, 81.9%) and in baseline seronegative subjects was 66.2% (95% CI: 49.1%, 77.5%).

-The VE of TDV against VCD fever was analysed for cases due to different dengue serotypes. The highest VE was shown for prevention of DENV-2 infections (95.1%; 95% CI: 89.9%, 97.6%), and then for DENV-1 (VE of 69.8%; 95% CI: 54.8%, 79.9%), and DENV-3 (48.9%; 95% CI: 27.2%, 64.1%). The VE determined against VCD fever caused by DENV-4 was inconclusive (51.0%; 95% CI: -69.4%, 85.8%) due to the lower incidence rates of cases due to this serotype.

In addition to determine VE against hospitalised VCD, the applicant also performed in trial DEN-301 other relevant analyses (from 30 days to 18 months post second vaccine dose) in relation to preventing severe dengue disease. In particular, VE was determined against:

DHF *(meeting the WHO 1997 criteria for DHF and dengue shock syndrome). DHF cases were identified using a programmed algorithm, i.e., without applying medical judgment. Broadly, the criteria include presence of fever lasting 2 to 7 days, haemorrhagic tendencies, thrombocytopenia, and evidence of plasma leakage".*

Severe cases *as determined by an independent "Dengue Case Adjudication Committee" (DCAC) based on the WHO 2009 guidelines. The DCAC assessed all hospitalized VCD cases utilizing agreed predefined criteria which included an assessment of bleeding abnormality, plasma leakage, liver function, renal function, cardiac function, the central nervous system, and shock."*

-There were only three cases (1 case in the placebo and 2 cases in the TDV group) that were adjudicated as severe by an independent adjudication committee (DCAC) that assessed all hospitalised cases. All of these 3 cases were caused by DENV-3. Due to these low rates, VE could not be demonstrated for DCAC-defined severe VCD fever (VE: 2.3%; 95% CI: -977.5%, 91.1%).

-There were 9 cases meeting the DHF definition (7 in the placebo and 2 in the TDV group), and only one of the DHF cases was considered a severe case by DCAC. The vaccine efficacy of TDV against DHF was 85.9% (95% CI: 31.9%, 97.1%). The 2 cases in the TDV group were both caused by DENV.

When VE in prevention of hospitalised VCD was analysed according to the infecting serotype, the estimates of VE calculated were: 77.3 % (95% CI: 40; 91), 100% (46 vs 0 cases); 42.9 % (95% CI: -69; 80) and undetermined (due to the few casas observed in the trial - only one case in placebo and zero in TDV), for Dengue virus serotypes 1, 2, 3 and 4, respectively.

Exploratory analyses included determining VE for longer periods of time (up to 24, 36 or 54 months counted one month post second dose):

-The overall VE in preventing VCD fever from 30 days post second vaccination up to 36 months was 59.6% (95%CI: 53.6, 64.9).

-VE against prevention of VCD fever decreased from 80.2% (95% CI: 73.3%, 85.3%), to 56.2% (95% CI: 2.3, 66.8) and to 44.7% (95% CI: 32.5,54.7), from 30 Days to 12 Months (Year 1) and from 13 to 24 Months (Year 2) and from 25 to 36 months (Year 3) post second vaccine dose, respectively. A similar trend was observed for VE against VCD fever leading to hospitalisation for the same periods of time with VE estimates of 95.4% (95% CI: 88.4%; 98.2%), 76.1% (95% CI: 50.8; 88.4) and VE: 70.8% (95% CI: 49.6; 83.0). Similarly, VE against DHF were for the same periods of time: 87.3%

(95% CI: -13.5%, 98.6%), 75.9% (95% CI: -31.3%-95.6%) and 65.4% (19.9-85.2%) VE against severe Dengue cases according to the DCAC committee was inconclusive even when counting from 1 month to 36 months after second dose [VE: 70.2% (-24.7; 92.9%)], since there were very few cases (5 cases in the placebo and 3 cases in the TDV group).

-When analysed from 30 days to 24 months post second dose, VE against VCD fever was found to be similar between baseline seropositive and seronegative subjects for cases due to both DENV-1 [66% (47;78) and 63% (37;78)]) and DENV-2 [89% (80;94) and 94% (82;98)]. For cases caused by DENV-3, VE in seropositive subjects was 62.7% (46.6; 74.0). However, when analyzing the VCD fever cases caused by DENV-3 in baseline seronegative subjects, there was a higher incidence of cases in the TDV (23/3531) that in the placebo (9/1726) group, and thus the VE determined was -29.0 (-178.8; 40.3). There were too few cases of VCD fever caused by DENV-4 (8 cases in the placebo and 8 cases in the TDV group), and thus the VE results were inconclusive.

-When analysed from 30 days to 24 months post second dose, positive VE estimates were observed for hospitalised cases due to DENV-1 (seropositive and seropositive) and DENV-3 (seropositive) but with very wide CI for DENV-1 seronegative (VE: 75.4%; 95% CI: 1.5; 93.8) and DENV-3 seropositive (VE: 72.5%;95% CI: 5.9; 91.9). In relation to hospitalisations due to VCD fever caused by DENV-3 in baseline seronegative subjects, there were 1 case in the placebo group and 4 cases in the TDV group, which resulted in a VE of -101% (95% CI: -1706; 77). There was only one case of VCD caused by DENV-4 that led to hospitalization (in a baseline seropositive subject in the placebo group), so no conclusion could be reached on prevention of hospitalisation caused by this dengue serotype. In line with the previous mentioned results regarding cases due to DENV-3, during Year 3, in baseline seronegative subjects, there were more hospitalised cases in the TDV group (7 hospitalisations of 11 VCD cases) compared with the placebo group (1 hospitalisation out of 6 VCD cases) (the randomisation ratio of 2:1 [TDV: placebo] should be considered).

-VE of TDV against of DCAC-defined severe and DHF is shown when the analysis is made on the safety set: a total of 8 cases of DCAC-defined severe VCD were reported in the safety set, 5 cases in the placebo group and 3 in the TDV group (VE: 70.2%; 95% CI: -24.7%, 92.9%). In the same period, 13 cases of DHF occurred in the placebo group and 9 cases in the TDV group (VE: 65.4%; 95% CI: 19.0%, 85.2%), including 3 cases (1 in the placebo group, 2 in the TDV group) that were classified as both DCAC-defined severe and DHF. Thus, taken together, there were 17 cases of DCAC-defined severe VCD or DHF in the placebo group and 10 such cases in the TDV group (the randomisation ratio of 1:2 placebo: TDV should be considered).

-Exploratory analyses included determining VE from first dose up to 54 months post second dose. The VE determined in baseline seropositive subjects was 64.2% (95% CI: 58.4%, 69.2%) and against VCD leading to hospitalisation 85.9% (95% CI: 78.7%, 90.7%). The benefit of TDV against overall VCD in baseline seronegative subjects was also confirmed in the analysis from first dose to 54 months post second vaccine dose with a VE estimate of 53.5% (95% CI: 41.6%, 62.9%. The VE against VCD leading to hospitalisation was 79.3% (95% CI: 63.5%, 88.2%).

Other exploratory analyses indicated:

-VE against VCD fever of 81.1% (95% CI: 64.1%, 90.0% was determined during the 3 months between the first and second vaccine dose. This is indicative of a rapid onset of the effect of TDV from the first vaccine dose onwards. A similar trend is observed for VCD cases reported between the first and second vaccine dose leading to hospitalisation (6 in the placebo group and 2 in the TDV group) during this 3-month period. Therefore, these results do not indicate that the first dose, due to raising a low immune response, could predispose the recipients to a more severe dengue disease.

-The cumulative incidence curves for the period up to 36 months from first vaccination indicates that VE against DENV-1 and DENV-3 wanes faster than against DENV-2. In relation to cases due to DENV-3, it is observed that the curve corresponding to placebo seronegative individuals accumulates fewer cases than the TDV seronegative ones over the whole period analysed.

-Results from subgroup analyses by age group (4-5, 6-11, and 12-16 years), from 30 days to 24 months post second dose, showed that VE of TDV in preventing VCD fever was seen across all predefined age groups with VE lower values in subjects aged 4 to 5 years (51.0%), and higher values, in subjects aged 6 to 11 years (71.8%), and in subjects aged 12 to 16 years (79.3%). Further analyses by baseline serostatus showed the VE values of ≥67.0% across all subgroups by baseline serostatus within age group, with the only exception of baseline seronegative subjects in the youngest subgroup of 4 to 5 years (VE: 4.6%). Moreover, additional data provided by the applicant describe VE covering the period from 30 days to 54 months post second dose (PP set). It is shown that VE against VCD was high in the three age groups analysed, with the lowest VE estimate [41% (95%CI: 21%, 55%)] observed in the youngest age group (4-5 years of age). VE estimates were always positive when VE was calculated, for each age group, according to the baseline serostatus, and when calculated for each age group, according to the serotype of the infected Dengue virus. It is noted, however, that 95%CI of the VE estimates were wide, and in some cases crossed zero, due to the few cases that were observed in some of the subgroups analysed. These results provided support for the use of the vaccine in children from 4 to 16 year of age.

-Subgroup analyses by prior vaccination against YF or JE showed, for the period from 30 days to 24 months after second dose, VE values of 86.1% (95% CI: 78; 91) in those subjects previously vaccinated against JE, 68.8% (95% CI:42 ; 83) in those previously vaccinated against YF, and 60.6% (95% CI: 49 ; 69) in those not previously vaccinated against either JE or YF, suggesting an impact of prior JE or YF vaccination on the VE of TDV.

Other favorable effects were determined from other Clinical trials:

-Coadministration of TDV and Hepatitis A vaccine in adults 18 to 60 years has been analysed (trial DEN-305) in seronegative subjects to Dengue and Hepatitis A. In terms of immune response, no interference against any of the two vaccines was shown when the vaccines were administered concomitantly.

-Overall, immune response to TDV was high and consistent in several Phase 2 and 3 trials. GMTs of neutralising antibodies in the TDV group increased for each serotype (independently of baseline serostatus) to Month 1 following the first vaccine dose, declined to the second vaccine dose (Month 3), increased again following the second vaccine dose to Month 4, and thereafter slightly declined to Month 15. The magnitude of the response was comparable for DENV-1, DENV-3, and DENV-4, and was higher for DENV-2. Similarly, for baseline seronegative subjects, in the TDV group, seropositivity rates for each serotype reached were high (≥99.5%) at Month 4 and remained high through Month 15 (≥92.7%) and Month 27 (≥90.4) and were maintained at similar levels at month 39 (36 months post second vaccine dose).

-The extension of the indication from subjects from 17 to 60 years was made based on the comparison of immune response (in terms of GMTs and seroconversion rates) in baseline seronegative subjects from Trials DEN-301 (702 paediatric subjects aged 4-16 years, endemic region) and DEN-304 (379 adult subjects aged 18-60 years, non-endemic region). In addition, available safety, efficacy, and immunogenicity data for TDV do not lead to any reason that would require restriction of the maximum age.

3.3. Uncertainties and limitations about favourable effects

The few VCD cases due to DENV-4 observed in pivotal trial DEN-301, which resulted in VE estimates with very wide 95%CI, precluded a meaningful conclusion on the performance of TDV against this dengue serotype.

A number of subgroup, exploratory analyses were performed to determine VE (against VCD and hospitalised VCD) according to Dengue serostatus and the infecting serotype (in total 16 comparisons). It should be emphasised that the trial was not powered to obtain robust estimates of VE from these analyses. It was noted that VE against VCD caused by DENV-3 in baseline dengue seronegative subjects was not demonstrated. In fact, the VE estimate was negative [VE: -51% (95%CI: -1356; 84)] when analysed from 30 days to 18 months post second vaccination. Moreover, VE against VCD fever caused by DENV-3, from first dose to 54 months post second vaccine dose in baseline seronegative subjects (safety set), yielded VE estimates of -15 % (95%CI: -108; 36) and it is noted that the 95%CI was very wide and included 0. Other exploratory analysis showed that VE estimate against hospitalised VCD due to DENV-3 in seronegative subjects, from first doses to 54 months post second dose (safety set), was -87.9% (95%CI: -573%, 40%); however, the VE was 15% (95%CI -254%, 79%) when data from Sri Lanka (the country with the highest hospitalisation rate of VCD cases) were excluded. These data indicate that there is no clear evidence of higher hospitalisation of cases due to DENV-3 since the results are confounded by the high hospitalisation rate of VCD cases in Sri Lanka.

Analysis of severe dengue cases (DHF and DCAC-defined severe VCD cases) identified an imbalance in baseline seronegative subjects infected by DENV-3. During the 54 months follow-up period, there was 1 severe case in the placebo group and 5 cases in the TDV group (that corresponded to 0 vs 4 cases of DHF, and 1 vs 4 cases of DCAC-defined severe VCD cases; it is observed that one case was classified both as DHF and DCAC-defined severe VCD cases). However, the applicant indicated that DHF detection in Sri Lanka was almost 3 times the rate in other countries (5.8% vs 2.0% across serotypes) in the placebo group. This is likely because of the conservative hospitalisation criteria and the close monitoring with frequent platelet count evaluation and ultrasonography, a sensitive method which can detect subclinical plasma leakage. Notably, thrombocytopenia and plasma leakage are 2 of the 4 defining criteria of DHF, and thus the rate of DHF detection can be sensitive to frequency and nature of investigations. Moreover, it is noted that the 2:1 randomisation also predisposes an imbalance towards the TDV group. In conclusion, it is interpreted that the increased number of severe dengue cases due to DENV-3 in seronegative subjects is confounded by the small number of DCAC-defined severe and DHF cases, the higher rate of DHF detection in Sri Lanka, and additional statistical considerations. It is thus considered that there is no robust evidence of increased risk of severe dengue disease caused by DENV-3 in baseline seronegative subjects.

It is acknowledged that in the few VCD hospitalised cases and severe forms of dengue observed over the four years of the trial, no clear pattern is observed on whether these cases increase over time. Thus, a clear conclusion cannot be reached to indicate that waning of VE drives an increase on hospitalised cases.

Another important limitation of TDV is that VE wanes rapidly (by two 2–fold) from year 1 (counted from one month after second dose) to year 3. The same trend (around 25% reduction) was seen when VE is determined against hospitalised and DHF cases. It is also observed that this decline is not homogeneous for all dengue serotypes since the cumulative incidence curves suggest that VE against DENV-1 and DENV-3 wanes faster than against DENV-2. Therefore, this aspect together with the different VE of TDV against different serotypes indicates that if this vaccine were used in a mass vaccination campaign in one country, the effectiveness results would be highly influenced by the dengue serotype(s) circulating in that area. It was considered that despite the observation that VE declines with time, it remains high for dengue seropositive subjects at baseline for at least three years

and that there is no clear indication that it translates into an increased risk of severe disease for seronegative subjects.

Although the results obtained indicate that prior vaccination against YF or JE has a favorable effect on VE against VCD fever, this interpretation is questioned since these results were highly confounded by differences between regions and countries and their underlying serotype distribution. Thus, it is unclear whether the impact of prior JE or YF vaccination on the efficacy of TDV.

Coadministration of TDV and Hepatitis A vaccine in baseline seronegative subjects 18 to 60 years has been supported from the results of trial DEN-314. It remains to be known whether these results can be extrapolated to younger subjects both dengue seropositive and dengue seronegative at baseline.

The immunogenicity results in terms of neutralising antibodies showed a good immune response induced by TDV. Nonetheless, these data could not be directly correlated with VE results since there is not an established correlate for protection. In fact, while it is observed in trial DEN-301 that VE wanes rapidly from year 1 to year 3, a similar strong decline is not seen in terms of GMT titres and seroprotection rates. These results are indicative that other elements of the immune system, apart from neutralising antibodies, are important in maintaining long-term VE.

Trial DEN-305 investigated the immunogenicity and safety of TDV and a YF vaccine (YF-17D) administered concomitantly or sequentially in healthy adults aged 18 to 60 years seronegative to both viruses. Although coadministration of the two vaccines did not affect the response to YF, the immune response to TDV was diminished when co-administered. The clinical significance of this finding is unknown.

Coadministration with other vaccines has only been analysed for the hepatitis A and Yellow Fever vaccine.

3.4. Unfavourable effects

The integrated evaluation of the safety profile of TDV in the Placebo-Controlled Safety Pool was based on 22,002 subjects, aged 4-60 randomised 2:1 (14,627 subject who received at least 1 dose of TDV and 7167 subjects who received at least 1 dose of placebo). The reactogenicity analyses were available for a subset of 5,555 participants (3,830 subjects receiving TDV and 1,725 subjects receiving placebo).

In the population 4-60 years of age solicited local and systemic AEs were reported more frequently in TDV than in placebo group and the difference was more pronounced for local reactogenicity (43.4% vs 25.7% within the first 7 days following any dose of TDV or placebo for solicited local AEs and 46.1% vs 40.1% within 14 days for solicited systemic AEs, respectively).

The most frequently reported solicited local AEs after any dose of TDV was injection site pain (41.8% vs 25.4%), followed by erythema (7.1% vs 0.3%) and swelling (3.4% vs 0.6%). Most of the local AEs were mild or moderate in intensity, but a 1.3% of subjects experienced grade 3 local AEs, mainly due to injection site pain. The time to onset was, mainly, on day of vaccination and resolved with a median duration of 2-3 days.

The most frequently reported solicited systemic AEs after any dose of TDV was headache (33.8% vs 30.1%), followed by myalgia (28.0% vs 20.5%), malaise (22.9% vs 20.7%) and asthenia (19.7% vs 17.5%). However, a 4.1% of subjects experienced grade 3 systemic AEs, being headache and malaise the most frequently grade 3 solicited systemic AE reported. The time to onset was, mainly, on the day or 1 day after the vaccination and resolved with a median duration of 3-4 days.

Pyrexia was reported in 8.9% of participants who received any dose of TDV (vs 10.5% in placebo) and the incidence of severe (≥ Grade 3) fever was 2.1%. Fever events generally resolved within a single day.

In addition to solicited AEs, the incidence of immediate solicited AE (within 30 minutes) or prolonged solicited AEs (after 7 days for solicited local AEs and after 14 days for solicited systemic AEs) were higher in TDV group than in placebo (6.7% vs 5.2% for any solicited AEs within 30 minutes; 3.8% vs 0.6% of prolonged local AEs and 1.9% vs 1.5% of prolonged systemic AEs in TDV or placebo, respectively). No severe solicited AEs within 30 minutes have been reported and most of prolonged solicited AEs were mild. Only 2 subjects (<0.1%) and 11 subjects (0.2%) who received TDV reported severe prolonged local and systemic AEs, respectively.

Overall, the incidence of solicited (local and systemic), including prolonged AEs and events within 30 minutes were lower after the second dose than after the first one and there were higher frequencies in female than in male subjects and in seronegative than in seropositive subjects at baseline. It was observed that the frequency of each of the solicited local AEs increased with age and that the incidence of solicited systemic AEs was lower in children than in adolescents and in these than in adults.

Any unsolicited AEs after any dose were reported with the same incidence in TDV and placebo (21.3% vs 22.8%). However, the incidence of unsolicited AEs considered related were higher in TDV than in placebo (3.0% vs 1.7%). Of these, 0.1% (5 subjects) in TDV and <0.1% (1 subjects) were severe unsolicited related AEs. No related unsolicited AEs (within 28 days after any vaccination) were considered as SAE.

There was no imbalance observed in the frequency of unsolicited AEs reported by SOC between TDV and placebo. The unsolicited AEs after any dose by SOC most frequently reported were infections and infestations (13.4% vs 16.3%), followed by general disorders and administration site conditions (2.8% vs 1.7%) and gastrointestinal disorders (2% and 1.5%).

The most frequently reported unsolicited AEs were nasopharyngitis (2.6%) and upper respiratory tract infection (2.3%). Injection site bruising and pruritus were the most frequently related unsolicited AEs reported (0.7% each), followed by pyrexia (0.2%) and systemic viral infection, fatigue and headache (<0.1% each). The grade 3 related unsolicited AEs in TDV were one case each of myalgia, anhedonia, malaise, upper tract infection and pyrexia.

Overall, the incidence of unsolicited AEs was lower after the second dose than after the first one. A higher incidence of unsolicited, including related, AEs was observed in seronegative than in seropositive subjects, in female than in males and in adults than in children and adolescents.

The incidence of SAEs was generally low and lower in the TDV group than in the placebo group (4.60% vs 5.57%), including related SAEs (a single case in TDV [suspected dengue illness] compared to four cases in placebo [of hypersensitivity (with 1 subject experiencing 2 events), dengue fev2 subjects reported related SAEs er and DHF]). By SOC, the SAE most frequently reported were Infections and infestations (2.64% vs 3.74%), the rest were reported <1% in both groups. None of the SAEs by PT reported with higher incidence in TDV than in placebo was considered as related (gastroenteritis, asthma, cellulitis and lower respiratory tract infection). No clinically important differences in the incidence of SAEs by serostatus or gender were reported. However, lower incidence of SAEs was observed in adults (2.03%) than in children (5.04%) and adolescents (4.17%).

Similar and low frequencies of participants experienced AEs leading to vaccine and/or trial discontinuation in both groups.

No clinically important differences were found of MAAEs between the TDV and placebo groups although higher incidences were observed in adolescents than in adults (no MAAE data from the children 4-11 years group is available).

In pivotal DEN-301, overall, the incidences of VCD and hospitalised VCD (all serotypes combined) were lower in the TDV group than in placebo up to 12, 24, 36 and 54 months after the second dose in the overall population and in the baseline seropositive and seronegative subgroups.

However, there was a numerical imbalance in the incidences of hospitalisation and severe forms of dengue caused by DENV-3 in baseline seronegative subjects (RR of VCD by serotype DENV-3 was 1.11 (95% CI 0.62, 1.99) and RR of hospitalised VCD caused by DENV-3 was 1.81 (95% CI 0.51, 6.48) up to 54 months after the second vaccine dose).

In addition, few cases of VCD caused by DENV-4 in seronegative subjects. Up to 54 months after the second vaccine dose were reported, there were 12 cases (0.3%) in TDV and 3 cases (0.2%) in placebo, with a RR 1.97 (0.56, 6.98). There were no cases of hospitalised VCD by DENV-4 in the TDV group and there was only 1 subject in placebo group, therefore the potential increase in severe / hospitalised cases by DENV-4 in seronegative subjects is currently unknown.

Although preliminary an additional safety concern is that in seronegative subjects a higher incidence of severe forms of dengue (DCAC-defined severe dengue and/or DHF) from baseline up to 36 months after the second dose were reported in TDV (5 cases [0.13%]) than in placebo group (1 case [0.05%]). The incidence of DHF or severe dengue caused by DENV-3 up to 54 months after the second dose remained very low in both groups (4 versus 1 DHF, and 2 versus 0 severe dengue cases in seronegative TDV and placebo group respectively, with a 2:1 randomisation ratio) and no cases of DHF and/or severe dengue were caused by DENV-4 throughout the trial up to 54 months post second dose.

Vaccine viraemia was observed in 30.9% (54 of 175 participants) of participants who received final formulation of TDV in the trial DEN-205. The viraemia mainly occurred for vaccine strain TDV-2 and peaked at Day 11. Of 54 participants with vaccine viraemia, the 14.8% presented single reversion. Vaccine viraemia was rarely detected after the second dose and it was higher in baseline seronegative than in baseline seropositive subjects. Overall, there was a higher incidence of some solicited (injection site pain, injection site erythema, rash, arthralgia, fatigue and fever) and unsolicited AEs (headache, fatigue, myalgia, arthralgia, nausea and upper respiratory tract infection) in viraemic than in non-viraemic subjects and in viraemic subjects with replication-competent vaccine virus than without it.

The relationship/association of acute febrile illness (within 30 days) and viraemia was assessed in trial DEN-301 and most febrile illness cases were not associated with detection of vaccine viraemia. Vaccine viraemia was detected in 34 of 479 febrile illness subjects (7.1%) after the first TDV dose and in 1 of 503 subjects (0.2%) after the second vaccine dose. Subjects with febrile illness and vaccine viraemia showed a similar pattern of symptoms as those with febrile illness but without vaccine viraemia. Additionally, the incidence of febrile illness was higher in viraemic (TDV-2) seronegative than seropositive subjects.

3.5. Uncertainties and limitations about unfavourable effects

In the Placebo-Controlled Safety Pool (Safety Set) the majority of participants (63.0%) was 4 to 11 years of age (11.9% between 4 and 5 years and 51.1% between 6 to 11 years), followed by 31.6% of participants 12 to 17 years of age. Both age groups come from the Latin America and Asia Pacific regions; therefore, there were limited safety data on the paediatric population from non-endemic areas.

The proportion of adults was limited, only the 5.4% of participants who received TDV were 18-60 years of age and all were from North America (US), therefore there are no data in adults from non-endemic areas in the Safety Set. There were no data in subjects >60 years of age.

The proportion of seropositive subjects in the TDV group was 67.1% and the proportion of seronegative subjects was 30.6%. However, the age distribution was not homogeneous by baseline serostatus. Seropositive participants were aged 4 to 17 years, and seronegative participants were aged 4 to 60 years.

The safety profile by extrinsic factors (endemic/non-endemic region, region/countries and previous flavivirus vaccination) is inconclusive because there are underlying reasons that confound the observed data, such as important differences of age or baseline serostatus. The subgroup of subjects from endemic regions (Latin America and Asia Pacific) is dominated by children and adolescents and the non-endemic subgroup (USA) comprised only adults. Regarding previous flavivirus vaccination, the number of adult participants was limited, and they were all non-vaccinated.

TDV safety regarding virologically confirmed dengue (VCD) including hospitalised VCD and severe forms of dengue was based primarily on the data from pivotal Trial DEN-301 (4 to 16 years). Additionally, data from Trials DEN-204 (2 to 17 years) and DEN-313 (4 to 15 years) were considered as supportive because no predefined dengue severity criteria were used. Consequently, no data of VCD including hospitalised VCD and severe forms of dengue was available from subjects ≥18 years of age.

The imbalance in the incidences of VCD, including hospitalised VCD caused by DENV-3 in seronegative individuals, observed in trial DEN-301, is a concern regarding potential disease enhancement by DENV-3 in seronegative subjects vaccinated with TDV. However, the increased number of severe dengue cases due to DENV-3 in seronegative subjects is confounded by the small number of DCAC-defined severe and DHF cases, the higher rate of DHF detection in Sri Lanka, and additional statistical considerations, and thus there is no robust evidence of increased risk of severe dengue disease caused by DENV-3 in baseline seronegative subjects. In addition, the limited data on VCD, including hospitalisation and clinically severe forms by DENV-4 in seronegative subjects, makes not possible to draw a robust conclusion on the possibility of enhanced disease severity by DENV-4 in that population.

Therefore, from a safety perspective, the occurrence of DHF and clinically severe forms of dengue caused by DENV-3 and DENV-4 in seronegative individuals remains uncertain. Due to these uncertainties, DHF and clinically severe forms of dengue by DENV-3 and DENV-4 in TDV vaccinated seronegative individuals is included as important potential risks in the RMP.

Data from supportive trial DEN-204 showed a RR of VCD (all serotypes included) up to 48 months <1 (with 95% CI superior limit <1). The participant's baseline serostatus was available only for a subset of trial participants, with no data regarding serotype or serostatus at baseline have been provided for these VCD cases. This makes data interpretation difficult. No relevant information from DEN-313 was obtained, the follow up was only of 9 months after the first dose and there were no cases of VCD requiring hospitalisation or meeting the WHO 1997 criteria for DHF.

Pregnant/breastfeeding women and immunocompromised participants were excluded from the studies. Safety data regarding these populations are lacking and both safety concerns are considered as missing information in the Safety Specifications of the RMP.

3.6. Effects Table

Table 64: Effects Table for Dengue Tetravalent Vaccine (data cut-off: 01 October 2020)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
Randomised 2:1 **Primary endpoint**: First dengue occurrence induced by any dengue serotype from 30 days post-second dose until the end of Part 1 Database lock Part 1: 15 November 2018		**VE%** Vaccine Efficacy **(95% CI)** Confidence Interval	**TDV N=13,401**	**Placebo (saline) N=6698**		
Overall (any dengue serotype)		**80.2%** (73.3, 85.3)	**61**/12,700	**149**/6316		
Key secondary and other secondary endpoints (30 days post-second dose until end of part 2) Database lock Part 2: 01 April 2019						
Hospitalisations		**90.4%** (82.6, 94.7)	**13**/12,700	**66**/6316		
DENV-1		**69.8%** (54.8, 79.9)	**38**/12,700	**62**/6316		
DENV-2		**95.1%** (89.9, 97.6)	**8**/12,700	**80**/6316		
DENV-3		**48.9%** (27.2, 64.1)	**63**/12,700	**60**/6316		
DENV-4		**51.0%** (-69.4, 85.8)	**5**/12,700	**5**/6316		
Any dengue serotype in seropositive subjects at baseline		**76.1%** (68.5, 81.9)	**75**/9167	**150**/4589		
Any dengue serotype in seronegative subjects at baseline		**66.2%** (49.1, 77.5)	**39**/3531	**56**/1726		
Severe VCD fever caused by any dengue serotype		**2.3%** (-977.5, 91.1)	**2**/12,700	**1**/6316		
Dengue Haemorrhagic Fever (DHF)		**85.9%** (31.9, 97.1)	**2**/12,700	**7**/6316		
Unfavourable Effects						
	Incidence after any dose		TDV	Placebo		

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Solicited Local AE (1)	Pain	%	41.8	25.4	Most of the local AEs were mild or moderate in intensity. The frequency of solicited local AEs was higher after the first dose than after the second dose in TDV and placebo treatment The solicited local AEs increased with age in subjects receiving any TDV Higher frequencies of solicited local AEs were observed in participants seronegative than in seropositive subjects and in female than in male.	Table 2.u from 2.7.4 (Table 3.3.1.1 from ISA Report)
	Erythema	%	7.1	0.3		
	Swelling	%	3.4	0.6		
Solicited systemic AE (1)	Headache (2)	%	33.8	30.1	Most of the systemic AEs following TDV were mild or moderate. The frequency of solicited systemic AEs was higher after the first dose than the second dose in TDV and placebo treatment. The incidence of solicited systemic AEs was lower in children than in	Table 2.v from 2.7.4 (Table 3.4.1.1 from ISA Report)
	Myalgia (2)	%	28.0	20.5		
	Malaise (2)	%	22.9	20.7		
	Asthenia (2)	%	19.7	17.5		
	Irritability/fussiness (3)	%	12.4	9.6		
	Drowsiness (3)	%	13.2	12.4		
	Loss of appetite (3)	%	17.0	12.4		

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Fever [4]	%	8.9	10.5	adolescents and adult. Higher frequencies of solicited systemic AEs were observed in participants seronegative than in seropositive subjects and in female than in male.	
Unsolicited AE within 28 day [1]	Incidence of any unsolicited AEs after any dose	%	21.3	22.8	Most of the unsolicited AEs following TDV were mild or moderate. The frequency of unsolicited AEs was higher after the first dose than the second dose in TDV and placebo treatment. Higher frequencies of unsolicited AEs were observed in participants seronegative than in seropositive subjects and in female than in male.	Table 2.e from 2.7.4 (Source : Module 5.3.5.3, ISA Report, Table 2.2.1.1 and Table 4.1.9.1. 1.)
	Incidence of any related unsolicited AE s after any dose	%	3.0	1.7		
SAE from day 0 to last day the follow-up [1]	Incidence of any SAE	%	4.6	5.57		Table 2.g from 2.7.4 (Source : Module 5.3.5.3, ISA Report, Table 2.2.1.1, Table 4.3.1.1. 1, Table 4.3.3.1. 1, Table
	Incidence of related SAE	%	<0.01	0.06		
AEs leading to death [1]	Incidence of any AEs leading to death	%	0.04	0.04		
	Incidence of any AEs leading to related death	%	0	0		
Any AEs leading to vaccine discontinuation [1]	Incidence of Any AEs leading to vaccine discontinuation	%	0.16	0.13		

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Incidence of Any related AEs leading to vaccine discontinuation	%	0.04	0.03		4.3.5.1.1, Table 4.4.1.1, and Table 4.4.2.1.)
Any AEs leading to trial discontinuation (1)	Incidence of Any AEs leading to trial discontinuation	%	0.12	0.11		
	Incidence of Any related AEs leading to trial discontinuation	%	0.02	0.01		
MAAEs from day 0 to last day the follow-up (1)	FTAR of any MAAEs	Events per 100 person-years	56.4	53.0		Section 2.5.2 from 2.7.4 and table 2.kkk from 2.7.4
	FATR of related MAAEs	Events 100 person-years	0.4	-		
VCD (5)	Incidence of VCD up to 12 months	% (overall population)	0.6	3.0	RR for VCD was 0.20 (95%CI:0.15, 0.25)	Table 5.f and Table 5.g from 2.7.4. For seronegative participants the RR of VCD (including hospitalised VCD) by different serotypes: Table 15.2.4.12.1; Table 15.2.4.12.2; Table 15.2.4.8.1 and Table
		% (seropositive)	0.6	3.0	RR for VCD was 0.19 (95%CI:0.14, 0.26)	
		% (seronegative)	0.6	2.9	RR for VCD was 0.21 (95%CI:0.13, 0.35) The RR for VCD by DENV-3 up to 12 was 1.60 (95% CI 0.52, 4.91).	
	Incidence of VCD up to 24 months	% (overall population)	1.3	4.6	RR 0.28 (95% CI:0.23, 0.34)	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
		% (seropositive)	1.2	4.7	RR 0.26 (95% CI:0.21, 0.33)	15.2.4.8.2 And Table 2.a and Table 2.b (responses to CHMP D180 LoOI-clinical aspects
		% (seronegative)	1.5	4.5	RR 0.33 (95% CI:0.24, 0.46) The RR for VCD by DENV-3 up to 24 was 1.37 (95% CI 0.64, 2.93).	
	Incidence of VCD up to 36 months	% (overall population)	2.9	7.4	RR 0.39 (0.35, 0.45)	
		% (seropositive)	2.7	7.4	RR 0.37 (0.31, 0.43)	
		% (seronegative)	3.4	7.4	RR 0.46 (0.37, 0.59) The RR for VCD by DENV-3 up to 36 was 1.18 (95% CI 0.65, 2.16). The RR for VCD by DENV-4 up to 36 was 1.97 (95% CI: 0.42, 9.28) with 8 cases in TDV and 2 cases in placebo.	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Incidence of VCD up to 54 months	% (overall population)	2.5	5.8	RR 0.40 (95% CI 0.36, 0.46)	
		% (seropositive)	2.2	5.9	RR <0.5	
		% (seronegative)	3.3	5.6	RR <0.5 The RR for VCD by DENV-3 up to 54 was 1.11 (95% CI: 0.62, 1.99) including Sri Lanka or 0.92 (95%CI 0.5, 1.69) excluding Sri Lanka. The RR for VCD by DENV-4 up to 54 was 1.97 (95% CI: 0.56, 6.98) with 12 cases in TDV and 3 cases in placebo.	
Hospitalised VCD (5)	Incidence of VCD up to 12 months	% (overall population)	<0.1	1.0	RR 0.07 (95%CI: 0.03, 0.13)	
		% (seropositive)	<0.1	0.9	RR 0.09 (95%CI: 0.04, 0.18)	
		% (seronegative)	<0.1	1.1	RR 0.02 (0.00, 0.17)	
	Incidence of VCD up to 24 months	% (overall population)	0.1	1.4	RR 0.11 (0.07, 0.18)	
		% (seropositive)	0.1	1.3	RR 0.10 (0.06, 0.19)	
		% (seronegative)	0.2	1.5	RR 0.13 (0.06, 0.29)	
	Incidence of VCD up to 36 months	% (overall population)	0.3	1.9	RR 0.17 (0.12, 0.24)	
		% (seropositive)	0.3	1.9	RR 0.14 (0.09, 0.22) No case of hospitalised VCD by DENV-4 in TDV vs 3 cases in placebo (<0.1%)	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
		% (seronegative)	0.4	1.9	RR 0.23 (0.13, 0.41) The RR for VCD by DENV-3 up to 36 was 2.71 (95% CI: 0.60, 12.23) with 11 cases in TDV and 2 cases in placebo. No case of hospitalised VCD by DENV-4 in TDV and placebo	
	Incidence of VCD up to 54 months	% (overall population)	0.3%	1.4%	RR 0.16 (95% CI 0.12, 0.23)	Table 2.a and Table 2.b (responses to CHMP D180 LoOI-clinical aspects)
		% (seronegative)	0.5	2.2	RR <0.5 The RR for VCD by DENV-3 up to 54 was 1.81 (95% CI: 0.51, 6.48) including Sri Lanka or 0.82 (95%CI 0.2, 3.42) excluding Sri Lanka 1 case of hospitalised VCD by DENV-4 in placebo group	
DCAC-defined severe dengue and/or DHF [5]	Incidence of severe VCD up to 12 months	% (overall population)	0.01	0.07	Relative risk of DCAC-defined severe dengue (95% CI) was 0.50 (0.03, 7.99) Relative risk of DHF (95% CI) was 0.12 (0.01, 1.12)	Table 2.rrr and Table 2.sss from 2.7.4
	Incidence of severe VCD up to 24 months	% (overall population)	0.03	0.16	Relative risk of DCAC-defined severe dengue (95% CI) was 0.33 (0.06, 1.99) Relative risk of DHF (95% CI) was 0.19 (0.05, 0.71)	

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Incidence of severe VCD up to 36 months	% (overall population)	0.07	0.25	Relative risk of DCAC-defined severe dengue (95% CI) was 0.30 (0.07, 1.25) Relative risk of DHF (95% CI) was 0.35 (0.15, 0.81)	
		% (seropositive)	0.05	0.33	Relative risk of DCAC-defined severe dengue (95% CI) was 0.10 (0.01, 0.86) Relative risk of DHF (95% CI) was 0.21 (0.07, 0.59)	
		%(seronegative)	0.13	0.05	Relative risk of DCAC-defined severe dengue (95% CI) was no evaluable (2 cases vs 0) Relative risk of DHF (95% CI) was 1.97 (0.22, 17.64)	
Vaccine viraemia (6)	Incidence of vaccine viraemia	%	30.9 (54 of 175 subjects)	-	The incidence of vaccine viraemia was lower in participants who received TDV (final formulation) than HD TDV (62.5%). The viraemia mainly occurred for vaccine strain TDV-2, peaked at Day 11 Higher incidence of some solicited and unsolicited AEs in viraemic than in non-viraemic subjects and in viraemic subjects between with or without replication-competent vaccine virus.	Section 2.7 from 2.7.4.

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
	Incidence of reversion of attenuation loci in subjects with vaccine viraemia	%	14.8 (8 of 54 subjects)	-	Similar result was observed in participants who received HD TDV (13.6%)	

Abbreviations:
Notes: (1) Placebo Controlled Safety Pool, Safety Set. Pooled data from DEN-203, DEN-204, DEN-301, DEN-304 and DEN-315; (2) solicited systemic AEs panel for participants ≥6 years; (3) solicited systemic AEs panel for participants <6 years; (4) body temperature ≥38° C measured in all participants (4 to 60 years); (5) in DEN-301; (6) in DEN-205

Benefit-risk assessment and discussion

3.6.1. Importance of favourable and unfavourable effects

The high VE against VCD fever (primary objective) and hospitalised VCD cases (key secondary objective) due to any dengue serotype, independently of dengue serostatus at baseline, is an important benefit. It is noted however, that vaccine efficacy was different for each dengue serotype, and thus effectiveness results would be highly dependent on the dengue serotype(s) circulating in that country. Although VE wanes with time, still significant VE is shown for the period from first dose up to 54 months post-second dose.

It is noted that serotype DENV-4 is less prevalent and typically associated with milder disease. Similarly, that VE against VCD caused by DENV-3 in baseline dengue seronegative subjects was not demonstrated. However, it should be mentioned that there is no robust evidence in TDV recipients of increased risk of hospitalised/ severe dengue disease caused by DENV-4 nor by DENV-3 in baseline seronegative subjects. Efficacy was shown overall and in baseline seropositive subjects.

In agreement with the consulted experts (Scientific Advisory Group on Vaccines), it is considered that efficacy against DENV-1 and DENV-2 (together causing most of dengue burden globally) outweighs any remaining uncertainty on lack of efficacy against DENV-3/4 and potential risks, in seronegative subjects.

Further data to complement the current analyses will be derived from post-marketing data, in particular from a large prospective (nested case-control) observational post-authorisation effectiveness study (DEN-401) (Post-Authorisation measure- [REC]). This will address the important potential risks around DENV-3 and DENV-4 in baseline seronegative subjects.

Short term safety is well described and covered from the data provided by the Safety Set. Reactogenicity is generally mild to moderate and likewise to other vaccines. The most frequent AEs after any dose were injection site pain and headache, followed by myalgia and malaise. The incidence of fever was generally low, and lower in TDV than in placebo. Fever events, generally, resolved within a single day. Overall, the reactogenicity was lower after the second dose than after the first.

The incidence of SAEs (excluding VCD data) was low and lower in TDV in than placebo. There was only one SAE considered related to TDV vaccine by the applicant (suspected dengue illness, not confirmed by PCR).

The safety database lacks data for pregnant and breast-feeding women, and immunocompromised individuals.

In agreement with the Expert Group (SAG), it is considered that a broad indication, for both baseline seropositive and seronegative subjects, will be important for practical implementation of community vaccination and is in the interest of global public health.

The CHMP also agreed that available safety, efficacy, and immunogenicity data for TDV do not lead to any reason that would require restriction of the maximum age for vaccine administration.

3.6.2. Balance of benefits and risks

The high VE against VCD fever and hospitalised VCD caused by any dengue serotype, independently of dengue serostatus at baseline, is an important benefit. In addition, in terms of reactogenicity the vaccine is well tolerated, and no evidence of SAEs directly related to the vaccine components has been shown.

3.7. Conclusions

The overall benefit/risk balance of Dengue Tetravalent Vaccine (Live, Attenuated) Takeda is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP adopt by consensus a scientific opinion as the benefit-risk balance of Dengue Tetravalent Vaccine (Live, Attenuated) Takeda:

> "Indicated for the prevention of dengue disease in individuals from 4 years of age.
>
> The use of Dengue Tetravalent Vaccine (Live, Attenuated) Takeda should be in accordance with official recommendations."

is favourable.

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Official batch release

The CHMP recommends that batch compliance control of individual batches be performed before release on the market in third countries.

Other conditions and requirements of the scientific opinion

- **Periodic Safety Update Reports**

The submission of PSURs for this medicinal product will follow the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

The holder of the scientific opinion shall submit the first PSUR for this product within 6 months following the scientific opinion.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The Scientific opinion Holder (SOH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the scientific opinion application and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.