Statistical Analysis Plan

NCT Number: NCT03746015

Title: An Open-Label, Phase 2 Trial to Investigate the Humoral and Cell-Mediated Immune Responses and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Flavivirus-Naïve and Dengue-Immune Healthy Adults

Study Number: DEN-210

Document Version and Date: Version 3.0, 29 March 2024

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STATISTICAL ANALYSIS PLAN

STUDY NUMBER: DEN-210

An Open-Label, Phase 2 Trial to Investigate the Humoral and Cell-Mediated Immune Responses and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Flavivirus-Naïve and Dengue-Immune Healthy Adults

Immunogenicity and Safety of TDV in Flavivirus-Naïve and Dengue-Immune Adults

PHASE 2

Version: Final, 3.0

Date: 29 March 2024

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Based on:

Protocol Version: 5.0

Protocol Date: 18 December 2020

1.1 Approval Signatures

Electronic signatures can be found on the last page of this document.

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3.0 LIST OF ABBREVIATIONS

AE	Adverse Event
CD	Cluster of Differentiation
COVID-19	Coronavirus Disease 2019
DENV	Wild Type Dengue Virus
DENV-1, -2, -3, -4	Wild Type Dengue Virus Serotypes 1, 2, 3 and 4
EC_{50}	Effective concentration 50
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set
GMT	Geometric Mean Titer
GSD	Geometric Standard Deviation
ICS	Intracellular Cytokine Staining
IFN-γ ELISpot	Interferon-gamma Enzyme-Linked Immunospot
IP	Investigational Product
LLOD	Lower Limit of Detection
LLOQ	Lower Limit of Quantification
M0, 1, 2, 3, 4, 5, 6, 9, 12	Month 0, 1, 2, 3, 4, 5, 6, 9, 12
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
MNT_{50}	Microneutralization Test 50%
NC	Negative Control
NS1	Non-Structural Protein 1
PBMC	Peripheral Blood Mononuclear Cells
PPS	Per-protocol Set
PT	Preferred Term
qRT-PCR	Quantitative Reverse Transcription-polymerase Chain Reaction
RU	Relative Units
RVP	Reporter Virus Particle
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SEM	Standard Error of the Mean
SFC	Spot Forming Cells
SOC	System Organ Class
TDV	Takeda's Tetravalent Dengue Vaccine Candidate Tetravalent Dengue Vaccine Candidate
TLF	Table, Listing and Figure
V1 - V19	Visit 1 - Visit 19
WHODrug	World Health Organization Drug Dictionary

4.0 OBJECTIVES

4.1 Primary Objective

- To assess the neutralizing antibody response (Geometric Mean Titers [GMT]) against each dengue serotype post-vaccination (Microneutralization Test 50% [MNT_{50}]).

4.2 Secondary Objectives

Immunogenicity

- To assess the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) and polyfunctionality (Intracellular Cytokine Staining [ICS]) of the T cell-mediated immune response post-vaccination.
- To assess vaccine viremia post-vaccination and the integrity of main attenuation mutations.

Safety

- To describe the safety of 2 doses of Tetravalent Dengue Vaccine Candidate (TDV) in healthy subjects aged 18 to 60 years (inclusive).

4.3 Exploratory Objectives

- To assess the neutralizing antibody response against each dengue serotype post-vaccination (dengue Reporter Virus Particle [RVP] test).
- To assess the B cell-mediated immune response post-vaccination (Quad-color FluoroSpot).
- To assess the anti-dengue non-structural protein 1 (NS1) antibody response post-vaccination (Enzyme-Linked ImmunoSorbent Assay [ELISA]).
- To map the epitopes of the dengue antibodies post-vaccination (IFN-γ ELISpot response).
- To assess the innate immune response post-vaccination.
- To characterize the relationships between TDV-specific innate, humoral and cellular immune responses on an individual level post-vaccination (MNT_{50}, dengue RVP, Quad color FluoroSpot, NS1 antibody ELISA, IFN- γ ELISpot, ICS, and exploratory assays).
- To characterize the relationships between vaccine viremia (quantitative Reverse Transcription-Polymerase Chain Reaction [qRT-PCR]) and TDV-specific innate, humoral and cellular immune responses on an individual level post-vaccination (MNT_{50}, dengue RVP, Quad color FluoroSpot, NS1 antibody ELISA, IFN-γ ELISpot, ICS, and exploratory assays).

4.4 Study Design

This is an open-label, phase 2 trial in 44 healthy adult subjects aged 18 to 60 years (inclusive) to investigate the immunogenicity and safety of subcutaneous administration of a 2-dose regimen of TDV.

Subjects will be enrolled in 2 trial groups based on results from serological testing performed either by the trial center prior to and outside the scope of this trial or through screening within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [Month 0 (M0)]):

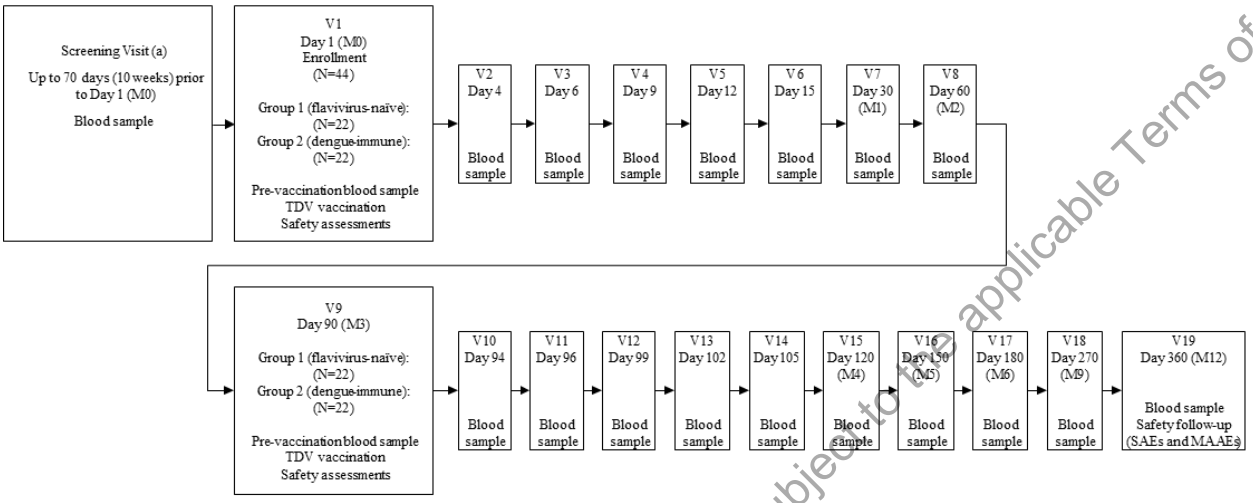
- Group 1: approximately 22 flavivirus-naïve subjects.
- Group 2: approximately 22 dengue-immune subjects (ie, subjects with serology consistent with primary infection either by wild type dengue virus [DENV]-1 or subjects with serology consistent with primary infection by DENV-3 defined as detectable neutralizing antibodies against DENV-1 or DENV-3 only, or titers for DENV-1 or DENV-3 ≥ 4-times higher than titers for the 2 other dengue serotypes).

Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.

TDV will be administered on Day 1 (M0) and on Day 90 (Month 3 [M3]) in both Groups 1 and 2.

All subjects will be followed-up for 9 months post second vaccination so the trial duration will be approximately 360 days (12 months) or 14.5 months for each subject depending on whether serological testing with regard to the inclusion criteria for the trial is performed outside or within the scope of this trial, respectively. A schematic of the trial design is included in Figure 4.a.

Figure 4.a Schematic of Trial Design



Note: SAEs and MAAEs are collected continuously throughout the trial.
Note: The actual number of subjects in Group 2 will be lower than the planned 22 subjects as it was decided to stop the recruitment of subjects in Group 2.
M=month, MAAE=medically attended adverse event, SAE=serious adverse event, V=visit
(a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.

Immunogenicity evaluations:

- Dengue neutralizing antibodies will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (Month 1 [M1]), and 60 (Month 2 [M2]); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (Month 4 [M4]), 150 (Month 5 [M5]), 180 (Month 6 [M6]), 270 (Month 9 [M9]), and 360 (Month 12 [M12]).
- T cell-mediated immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- B cell-mediated immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Day 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 180 (M6), 270 (M9), and 360 (M12).
- T cell epitopes will be mapped using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Anti-dengue NS1 antibodies will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).

- The innate immune response will be measured using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 4 and 6; pre second vaccination (Day 90 [M3]), and on Days 94 and 96.

Safety evaluations:

- Diary cards (paper or electronic) will be distributed for the recording of:
    - Solicited Adverse Events (AEs):
        - Solicited local (injection site) reactions for 7 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 6 days). These will include: injection site pain, injection site erythema, and injection site swelling.
        - Solicited systemic events for 14 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 13 days). These will include: fever, headache, asthenia, malaise, and myalgia.
    - Unsolicited AEs for 28 days following administration of each trial vaccine dose on Day 1 (M0) and Day 90 (M3) (day of administration + 27 days).
- Serious Adverse Events (SAEs), Medically Attended Adverse Events (MAAEs), and AEs leading to trial vaccine withdrawal or trial discontinuation will be collected from first trial vaccination at Day 1 (M0) until the end of the trial (Day 360 [M12]). MAAEs are defined as AEs leading to an unscheduled medical visit to or by a healthcare professional including visits to an emergency department, but not fulfilling seriousness criteria.

Vaccine viremia evaluation:

Vaccine viremia will be measured by quantitative reverse transcription-polymerase chain reaction (qRT- PCR) using blood samples collected from all subjects pre first vaccination (Day 1 [M0]) and on Days 6, 9, 12, 15, and 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 96, 99, 102, 105, and 120 (M4).

Data will be collected via electronic Case Report Form (eCRF).

5.0 ANALYSIS ENDPOINTS

5.1 Primary Endpoint

- GMT of neutralizing antibodies (by MNT50) for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).

5.2 Secondary Endpoints

- Frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 Peripheral Blood Mononuclear Cells [PBMC]) of IFN-γ ELISpot responses to TDV using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1) and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).

  Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared to baseline [Day 1 (M0)] and ≥5 SFC/10^6 PBMC. Note: Negative control (NC) [sample no peptide] will be subtracted from all reported values prior to calculating the response. In cases where the value corrected for the NC is negative (<0) this value will be set to "0".
- Phenotype characteristics of cellular immune responses to TDV by ICS using blood samples collected post first vaccination on Days 15, 30 (M1) and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). Markers will include Cluster of Differentiation (CD)4, CD8, IFN-γ, tumor necrosis factor-alpha and interleukin-2. This endpoint will be evaluated in a subset of subjects with IFN- γ ELISpot responses ≥50 SFC/10^6 cells and availability of sufficient cells.
- Incidence, duration, and level of vaccine viremia for each of the 4 dengue serotypes measured by qRT- PCR using blood samples collected from all subjects post first vaccination on Days 6, 9, 12, 15, and 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 96, 99, 102, 105, and 120 (M4).
- Frequency and percentage of subjects with solicited local (injection site) reactions for 7 days (day of administration + 6 days) and solicited systemic events for 14 days (day of administration + 13 days) following administration of each trial vaccine dose (Day 1 [M0] and Day 90 [M3]).
- Frequency and percentage of subjects with any unsolicited AEs for 28 days (day of administration + 27 days) following administration of each trial vaccine dose (Day 1 [M0] and Day 90 [M3]).
- Frequency and percentage of subjects with SAEs throughout the trial.
- Frequency and percentage of subjects with MAAEs throughout the trial.

5.3 Exploratory Endpoints

- Average Effective Concentration 50 ([EC_{50}], dengue RVP) of neutralizing antibodies for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Seropositivity rates (percentage of subjects) from dengue RVP for each of the 4 dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Seropositivity rates (percentage of subjects) from dengue RVP for multiple (2, 3 or 4) dengue serotypes using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Average number of memory B cells expressing type-specific and cross-reactive dengue-specific antibodies/10^6 PBMC measured by Quad-color FluoroSpot using blood samples collected from all subjects post first vaccination on Day 30 (M1); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 180 (M6), 270 (M9), and 360 (M12).
- Average concentration Relative Units/mL (RU/mL) of anti-dengue NS1 antibodies for each of the 4 dengue serotypes measured by ELISA using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Epitope mapping of the IFN-γ ELISpot responses to TDV on an individual level using blood samples collected from all subjects post first vaccination on Days 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12). This endpoint will be evaluated in a subset of subjects with IFN- γ ELISpot responses >50 SFC/10^6 cells and availability of sufficient cells.
- Gene expression profiles on an individual level using blood samples collected from all subjects post first vaccination on Days 4 and 6; pre second vaccination (Day 90 [M3]) and on Days 94 and 96.
- Type-specificity and cross-reactivity of neutralizing antibody response (average EC_{50}, dengue RVP) - to DENV-1, DENV-3, and DENV-4 serotypes post-depletion of DENV-2 neutralizing antibodies using blood samples collected from subjects post first vaccination on Days 1, 120 (M4), 270 (M9) and 360 (M12). This endpoint will be evaluated in a subset of subjects with a neutralizing antibody titer about a specific level (to be defined) and availability of sufficient serum for testing.
- Average concentration RU/mL of anti-dengue IgG antibodies for each of the 4 dengue serotypes measured by ELISA using blood samples collected from all subjects post first vaccination on Days 1, 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).
- Antigen-specific IgG avidity to dengue 1, 2, 3 and 4 Virus-Like Particles (VLPs), represented as a ratio of antibody response to the strength of antibody binding, or avidity index, measured by Octet-based assay using blood samples collected from all subjects post first vaccination

on Days 1, 15, 30 (M1), and 60 (M2); pre second vaccination (Day 90 [M3]) and on Days 105, 120 (M4), 150 (M5), 180 (M6), 270 (M9), and 360 (M12).

6.0 DETERMINATION OF SAMPLE SIZE

This trial is designed for a descriptive evaluation of the endpoints that does not require hypotheses testing. Therefore, the sample size was not determined based on formal statistical power calculations.

7.0 METHODS OF ANALYSIS AND PRESENTATION

7.1 General Principles

This Statistical Analysis Plan (SAP) was developed based on the information provided in Protocol DEN-210, Version 5.0, dated 18 December 2020 [1] and on International Conference on Harmonization E3 [2] and E9 [3] Guidelines.

All statistical outputs will be generated using the statistical analysis system SAS® version 9.4.

This document provides the details regarding the definition of analysis variables and analysis methodology to address all trial objectives. No inferential analyses will be performed for this trial, i.e. all analyses described in this SAP will be descriptive only.

Data reviews will be conducted prior to the database lock. These reviews will assess the accuracy and completeness of the trial database and subject evaluability.

As some trial laboratory procedures might not be carried out during the COVID-19 pandemic there is a possibility that not all pre-defined test results will be available prior to database lock. Thus, there is a risk for database unlock/relock and additional data may be added in CSR amendments or addenda to the final CSR.

7.1.1 Data Presentation

Summary tables for categorical variables will display both frequencies and percentages. For categorical variables with defined categories in the eCRF, all possible categories will be displayed, even if the subject count is zero. For any other categorical variables recorded (eg, category of AE or medication/vaccination), only categories with at least 1 subject count will be displayed. Percentages will be presented with 1 decimal place (eg, 80.3%).

Summary tables for continuous variables will display the number of subjects with non-missing values, means or geometric means, medians, standard deviations (SDs) or geometric standard deviations (GSDs), and minimum and maximum values. Minimum and maximum values will be presented with the same number of decimal places as the recorded data. Means, geometric means and medians will be presented with 1 more decimal place than the recorded data. SD will be presented with 2 more decimal places than the recorded data.

Summaries for selected immunogenicity and safety variables may also include the confidence interval (CI) around parameter estimates (means or percentages), and standard errors of the means (SEMs). The CI will be presented with the same number of decimal places as the parameter estimate itself. SEM will be presented with 2 more decimal places than the recorded data.

All collected data will be displayed in the listings sorted by trial group, by site number, by subject identification number, and by date/time of the recorded event if applicable (eg, date/time of vaccination, date/time of blood draw, date/time of AE). Screen failures data will be grouped and listed separately.

Trial groups will be labeled as Naïve, DENV positive [DENV-1 positive, DENV-3 positive] in all the outputs.

7.1.2 Study Day, Baseline and Analysis Visit Window Definitions

Study Day 1 (M0) is defined as the date of the first trial vaccination, as recorded on the eCRF vaccination form. Other Study Days are calculated relative to Study Day 1 (M0), with Day -1 being the day prior to Day 1 (M0).

Baseline is defined as the last non-missing measurement taken before the first trial vaccination. Where the time is available, the time of the measurement must be prior to the first trial vaccination time. Day 1 (M0) measurements taken after the first trial vaccination time are considered as post-baseline values.

A windowing convention for immunogenicity and safety (vital signs) data will be used to determine the analysis value of a variable at a given trial visit. Following the schedule of trial procedures (Appendix A), the analysis windows for each visit will be calculated relative to the day on which each trial dose was administered (Day 1 [M0] and Day 90 [M3]). If several measurements of a variable are obtained for a given subject within the same visit window, the measurement taken at the date that is closest to the scheduled visit date will be used. Both scheduled and unscheduled visits will be considered equally.

The analysis windows are displayed in the Table 7.a.

Table 7.a Analysis Visit Windows

Visit	Scheduled Vaccination	Day (Month) relative to Dose 1 [a]	Day (Month) relative to Dose 2 [a]	Analysis Visit Windows		
				Per-Protocol Set	Safety Set (Vital Signs) and Full Analysis Set	
					Relative to Dose 1[b]	Relative to Dose 2
V1	Dose 1[c]	Day 1 (M0)	NA	NA	NA	NA
V2		Day 4	NA	-1/+1 day[d]	Day 2 – Day 5[d]	NA
V3		Day 6	NA	-1/+1 day[d]	Day 5[d] – Day 7	NA
V4		Day 9	NA	-1/+1 day	Day 8 – Day 10	NA
V5		Day 12	NA	-1/+1 day	Day 11 – Day 13	NA
V6		Day 15	NA	-1/+2 days	Day 14 – Day 22	NA
V7		Day 30 (M1)	NA	-1/+7 days	Day 23 – Day 45	NA
V8		Day 60 (M2)	NA	-5/+5 days	Day 46 – Day 75	NA
V9	Dose 2[c]	Day 90 (M3)	NA	-4/+7 days	Day 76 – Day 92	NA
V10		Day 94	Day 4	-1/+1 day[d]	Day 93 – 95[d]	Day 2 – Day 5
V11		Day 96	Day 6	-1/+1 day[d]	Day 95[d] – 97	Day 5 – Day 7
V12		Day 99	Day 9	-1/+1 day	Day 98 – 100	Day 8 – Day 10
V13		Day 102	Day 12	-1/+1 day	Day 101 – 103	Day 11 – Day 13
V14		Day 105	Day 15	-1/+2 days	Day 104 – 113	Day 14 – Day 22
V15		Day 120 (M4)	Day 30 (M1)	-1/+7 days	Day 114 – Day 135	Day 23 – Day 45
V16		Day 150 (M5)	Day 60 (M2)	-5/+5 days	Day 136 – Day 165	Day 46 – Day 75
V17		Day 180 (M6)	Day 90 (M3)	-7/+7 days	Day 166 – Day 225	Day 76 – Day 135
V18		Day 270 (M9)	Day 180 (M6)	-7/+14 days	Day 226 – Day 315	Day 136 – Day 225
V19		Day 360 (M12)	Day 270 (M9)	-14/+14 days	≥Day 316	≥Day 226

M=month, V=visit, NA=Not applicable

(a) Study Day relative to Dose X is calculated as [Date of Dose X] – [Date of Visit] + 1 (day).

(b) Applies to subjects who missed the second vaccine dose at V9.

(c) Blood draw for immunogenicity assessments and assessment of vital signs must be prior to the vaccination scheduled for the same visit, and where time is available, the time of the blood/vital signs collection must be prior to the vaccination time. Day 1 (M0) measurements taken after the first trial vaccination time are considered post-baseline values.

(d) Vaccine viremia measurements on Day 5/Day 95 will be allocated to V3/V11; in case an innate immune response measurement is allocated to V2 (V10) and a measurement is obtained on Day 5 (Day 95), it will be allocated to V3 (V11). If however a innate immune response measurement has not been allocated to V2 (V10) then a measurement obtained on Day 5 (Day 95) will be allocated to V2 (V10). Among two innate immune response measurements obtained on the same day (Day 5 or Day 95), the visit label will be used to allocate each to V2 (V10) and V3 (V11), accordingly.

7.1.3 Handling of Missing Data

Data will be presented in the listings as reported. For the summaries and analyses, following conventions apply:

Missing Immunogenicity Data

Dengue neutralizing antibody titers (MNT_{50}) which are below the lower limit of detection (LLOD) of 10 will be imputed with a value of 5 (ie, half of the LLOD). If a reported value is greater or equal to the LLOD (LLOD ≥ 10) and below the lower limit of quantification (LLOQ), which differs between serotypes, this value will be imputed with the mid-point between the LLOD and LLOQ. For example, given a LLOQ of 18 for a serotype, all values greater or equal to 10 and below 18 will be imputed with a value of 14 for this serotype. EC_{50} values, from a dengue RVP-based microneutralization assay, which are below the LLOD of 63 will be similarly imputed with a value of 31.5 (ie, half of the LLOD).

No imputation methods will be used for missing immunogenicity data and all analyses will be based on complete records only.

Missing or Partial Dates of Unsolicited AE

Missing and partial unsolicited AE start dates may be imputed only to determine the temporal relationship between the start date of the event and the dosing date of the most appropriate vaccination that the AE should be temporally associated with (Vaccination 1 or 2). The following rules apply when determining the associated vaccination:

- If the AE start and end dates are both completely missing, the AE will be allocated with the first trial vaccination;
- If only the month and/or the year of the AE start is/are available, the AE will be allocated with the latest vaccination prior to the AE start date;
- If the AE start date is completely missing, or the available start date information is insufficient to distinguish between the 2 trial vaccinations, but a partial AE end date (ie, month and/or year) is available, the AE end date will be assessed and the AE will be allocated with the vaccination after which the event ends. This approach is based on the assumption that any AE starting after Vaccination 1 and ongoing on the day of Vaccination 2 would be identified during the clinical assessments that are performed before administration of the second dose of investigational product (IP). If partial end date information indicates possible allocation with both vaccinations, the AE will be allocated with the first trial vaccination.

Missing AE Severity or Relationship to IP

Missing AE severity (mild/moderate/severe) and missing AE relationship to IP (related/not related) will be handled using the following conservative approach:

- unsolicited AE with missing severity will be considered as 'severe',
- solicited systemic events or unsolicited AE with missing relationship will be considered as 'related'.

No other imputation for missing AE data will be implemented.

Missing or Partial Dates of Medications or Vaccinations

Missing and partial dates for medications or vaccinations will be assessed, only to determine the relationship between the end date of the medication or vaccination and the first dose of IP (ie, to distinguish if a medication or vaccine is prior or concomitant). A medication will be considered prior only if the partial end date indicates that it was stopped before the first trial vaccination. A vaccine will be considered prior only if the partial vaccination date indicates that it was given before the first trial vaccination. In all other cases medications or vaccinations will be considered concomitant.

Missing End Dates of Medical History/Concurrent Medical Conditions

In case the "End Date" or "End Date Unknown" fields are missing on the medical history/ concurrent medical conditions form of the eCRF and from the partial date it can't be concluded that the event is clearly a medical history, the event will be considered as a concurrent medical condition.

7.1.4 Implausible Values

Data outside the plausible ranges (according to the Table 7.b) will be excluded from analyses, but presented as recorded and flagged in data listings.

Table 7.b Plausible Data Ranges

	Parameter	Plausible range
Solicited AE	Swelling	≤500 mm
	Erythema	≤500 mm
	Body Temperature [(a)]	32 – 43°C
Vital Signs	Height	110 – 210 cm
	Weight	20 – 200 kg
	Heart Rate	40 – 200 beats/min
	Systolic Blood Pressure	70 – 180 mmHg
	Diastolic Blood Pressure	30 – 120 mmHg

(a) Also applicable to body temperature measurements collected as vital sign.

7.2 Analysis Sets

All Screened: All subjects who signed the informed consent, regardless of whether subjects were screen failures.

All Enrolled: All subjects who signed the informed consent and who were eligible for vaccination.

Safety Set: The Safety Set will consist of all subjects who received at least 1 dose of trial vaccine.

Full Analysis Set (FAS): The FAS will include all subjects who received at least 1 dose of trial vaccine and for whom a valid pre-dose and at least one valid post-dose measurement is available for immunogenicity.

Per–Protocol Set (PPS): The PPS will include all subjects in the FAS who have no major protocol violations. The major protocol violation criteria will be defined as part of the data reviews prior to database lock. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving prohibited therapies, (3) not receiving 2 doses of TDV, or receiving the second vaccination inadmissibly outside of the visit window, and (4) other major protocol violations that may be identified during data reviews prior to database lock.

Major protocol violations are defined as deviations from the protocol, which have potentially significant impact on the immunogenicity results of a given subject. These violations will be identified via programming and data reviews prior to database lock, using criteria described in Table 7.c.

ICS Subset: Subset of PPS subjects with IFN-γ ELISPOT responses >50 SFC/10^6 PBMCs and availability of sufficient cells.

Table 7.c Criteria for Exclusion of Subjects from PPS

Criteria for Subject Exclusion	Method of Identification
Not receiving at least 1 dose of trial vaccine [(a)]	Identified programmatically using dosing data.
Not having a pre-dose (Baseline) and at least 1 post-dose measurement.[(b)]	Identified programmatically using immunogenicity data
Not receiving both doses of trial vaccine	Identified programmatically using dosing data.
Receiving the second trial vaccination inadmissibly outside of the scheduled visit window (ie, outside Day 90 [-15/+25 days])	Identified programmatically using dosing data.
Product preparation error	Identified through protocol deviation review/medical review..
Subject meets any of the trial exclusion criteria: 6, 7, 9, 11, 12, 20, 21, 22	Identified through protocol deviation review/medical review, Identified programmatically using eCRF-recorded data.
Use of prohibited medications/vaccines	Identified by medical review of eCRF-recorded medication/vaccines data.

(a) Subjects with this protocol violation will be excluded from Safety Set, and thus also from FAS and PPS.
(b) Subjects with this protocol violation will be excluded from FAS, and thus also from PPS.

Other major protocol violations may be identified for the data listings and deviation logs throughout the trial, subject to medical review. Any changes to PPS exclusion criteria after approval of the SAP will be documented separately and approved prior to the database lock for final analysis.

Reasons for exclusions of subjects from analysis sets will be summarized by trial group for all enrolled subjects.

Analyses based on the Safety Set (except for AEs), FAS and PPS will include measurements obtained following the analysis visit windows defined in Table 7.a.

7.3 Disposition of Subjects

Trial information will be presented for all screened subjects including: the date the first subject signed the informed consent form, the date of the first subject's first visit, the date of the first subject's first vaccination, the date of the first subject's second vaccination, the date of the last subject's first vaccination, the date of the last subject's second vaccination, the date of the last subject's last visit, and the date of last subject's last trial procedure for collection of data for the primary endpoint. In addition, details will be provided for: versions of the Medical Dictionary for Regulatory Activities (MedDRA), World Health Organization Drug Dictionary (WHODrug), and the SAS® used for analyses.

Vaccination eligibility summary for all screened subjects will include: the number of screened subjects, the number of subjects eligible for vaccination, the number of subjects not eligible for vaccination and the primary reason(s) for ineligibility for vaccination. The number of screen failures and their demographic and baseline characteristics will also be summarized.

Disposition summaries for all enrolled subjects will include:

- Number of subjects vaccinated by trial site, for each trial group (Group 1 and 2) and by subgroups for Group 2 (DENV-1 positive and DENV-3 positive subjects),
- Number of subjects who completed the vaccination regimen/trial visits,
- Number of subjects who prematurely discontinued the vaccination regimen/trial (vaccine or trial withdrawals) including the primary reason(s) for premature discontinuation of the vaccination regimen/trial.

Additionally, significant protocol deviations will be summarized based on the Safety Set.

Number of subjects in analysis sets will also be provided as a separate summary.

An additional listing and summary table will be provided including all protocol deviations (significant and non-significant) related to the Corona Virus Disease 2019 (COVID-19) pandemic, if applicable.

7.4 Demographic and Other Baseline Characteristics

Age, gender, race, and other baseline characteristics will be summarized descriptively by trial group for the Safety Set, FAS, PPS and ICS Subset (based on the PPS).

7.5 Medical History and Concurrent Medical Conditions

A medical history is defined as any significant condition/disease that stopped at/or prior to administration of the first dose of trial vaccine. A concurrent medical condition is defined as any significant condition/disease that is ongoing at the time the first dose of trial vaccine is administered.

Medical history and concurrent medical conditions will be coded using the current version of the MedDRA coding system. Summary tables for each trial group will be provided by System Organ Class (SOC) and Preferred Term (PT) based on the Safety Set.

7.6 Medication History and Concomitant Medications

A prior medication/vaccination (history) is any medication/vaccination which administration was stopped before the first trial vaccination is administered. A concomitant medication/vaccine is any medication/vaccination regimen ongoing at the time of the first trial vaccination, or administered on or after the first trial vaccination.

Medication history, vaccination history, concomitant medications, and concomitant vaccinations will be coded using the WHODrug.

Summary tables for medication history and concomitant medications will be provided for each trial group by Anatomical Therapeutic Chemical class level 2 name and preferred medication name. Vaccination history and concomitant vaccinations will be summarized for each trial group using the vaccine type and name as recorded in the eCRF. Summary tables will be provided for the Safety Set.

7.7 Investigational Product Exposure and Compliance

The Investigator will record in the eCRF all trial vaccine injections that were given to the subject. Summary of trial vaccine compliance will be presented for the Safety Set. This summary will include: the number and percentage of subjects who received both doses of trial vaccine; and the number and percentage of subjects who only received the first dose of trial vaccine.

Trial follow-up is defined as the time period between the first trial vaccination and the end of the trial, inclusive. Follow-up duration in days will be summarized by trial group for the Safety Set as a continuous variable (n, mean, median, SD, minimum and maximum), and also as a categorical variable (frequency, percentage) using the following intervals: 1 – 30 days, 31 – 90 days, 91 – 120 days, 121 – 180 days, 181- 270 days, 271 – 360 days, and >360 days. Additionally, the duration of follow-up after the second dose of trial vaccine (defined as the number of days from second vaccination to the end of the trial, inclusive) will be summarized in a similar way as a continuous variable and also as categorical variable for the following intervals: 1 – 30 days, 31 – 90 days, 91 – 180 days, 181 – 270 days, and >270 days.

7.8 Efficacy Analysis

Not applicable.

7.9 Pharmacokinetic/Pharmacodynamic Analysis

Not applicable.

7.10 Other Outcomes

7.10.1 Primary Immunogenicity Endpoint

For the GMTs of dengue neutralizing antibodies (derived from dengue MNT_{50} results), the number of subjects with non-missing assessment, geometric mean with 95% CI, geometric SD, median, minimum, and maximum will be presented for neutralizing antibody titers for each dengue serotype by visit. GMTs will be calculated as anti-logarithm of Σ(log transformed titer/n), where n is the number of subjects with titer information. The 95% CI for GMT will be calculated as the anti-log transformation of upper and lower limits for a 2-sided CI of the mean of the log-transformed titers (based on student's t-distribution).

GMTs (including 95% CIs) will be plotted over time (visit) for each dengue serotype. In addition, reverse cumulative distribution curves will be plotted by dengue serotype and visit (except for baseline).

The primary analysis will be based on the PPS. A supportive analysis will be provided based on the FAS.

Additional sensitivity analyses and/or summary tables may be provided to address the impact of the COVID-19 pandemic, if applicable. These may include, but not limited to, sensitivity analyses ignoring the analysis visit windows defined for the PPS for the trial visits and imputing missing value approaches, for subjects impacted by COVID-19.

7.10.2 Secondary Immunogenicity Endpoints

The frequency of cellular immune response (ie, % of subjects with cellular immune response (labelled as 'Cellular Immune Response' in the Tables, Listings and Figures [TLFs]) is defined as an IFN-γ ELISpot response that is >3 times higher compared to baseline [Day 1 (M0)] and ≥5 $SFC/10^6$ PBMC to 2 doses of TDV is one of the secondary endpoints in this trial. Note: NC (sample no peptide) will be subtracted from all reported values prior to calculating the cellular immune response. In cases where the value corrected for the NC is negative (<0) this value will be set to "0".

The percentage of subjects with cellular immune response to:

- Any of the tested peptide pools
- Each of the tested peptide pools
- Peptide pools matching a given dengue serotype (where at least 1 peptide pool for a given dengue serotype is positive)

will be analyzed by visit along with the exact 2-sided 95% CI. The exact 2-sided 95% CI will be calculated based on the Clopper-Pearson method [4]. A positive cellular immune response to any peptide pool for a given serotype constitutes a positive response to that serotype. Peptide pools analyzed for this study are still under evaluation and will be defined at a later time point.

For the magnitude (number of SFC/10^6 PBMCs) of IFN-γ ELISPOT responses, descriptive statistics (n, mean, SD, min, Q1, median, Q3, and maximum) will be presented for all subjects and for subjects with a cellular immune response only by visit (including changes from baseline or other relevant visits which may include absolute and/or mean fold changes).

The magnitude of the IFN-γ ELISPOT response of a serotype matching pool is calculated by adding all magnitude measures of each individual peptide within that peptide pool. The magnitude of the IFN-γ ELISPOT response for any peptide pool is calculated by adding all magnitude measures.

Before summarizing the magnitude of the IFN-γ ELISPOT responses the NC will be subtracted from all reported values. In cases where the value corrected for the NC is negative (<0) this value will be set to "0".

The magnitude of the IFN-γ ELISPOT responses will also be graphically presented including min, Q1, median, Q3 and max.

Phenotype characterization of cellular immune responses will be analyzed in a subset of trial subjects by ICS at all visits except for Baseline (Day 1 [M0]). Markers will include CD4, CD8, IFN-γ, TNF-α, and IL-2. This subset of subjects will be selected from samples with IFN-γ ELISPOT responses >50 SFC/10^6 PBMCs and availability of sufficient cells. Analyses will be done in a similar manner as for the IFN-γ ELISPOT responses described above. As for summaries of magnitude of the IFN- γ ELISPOT responses the NC needs to be subtracted from all reported values for cellular immune responses measure by ICS.

Selected cellular immune response data will be presented as figures as well.

Vaccine viremia summaries will be presented by vaccine received and visit for vaccine RNA levels (expressed as log10 [genome equivalents per mL]), overall based on the Safety Set as described for the seropositivity rates in this section.

The duration of vaccine viremia for each serotype = visit date when vaccine viremia is last detected (positive result) – visit date when vaccine viremia is first detected (positive result) + 1 day. For the number of subjects with non-missing assessment, mean, associated 2-sided 95% CI (based on student's t-distribution) and associated SD, median, minimum, and maximum will be presented. The number and percentage of subjects with different categories of vaccine viremia duration will also be presented. In addition, the number of days to onset of viremia will be summarized descriptively and categorized for each TDV strain.

Samples that are positive in the Dengue Vaccine Screening RT-PCR will undergo the vaccine confirmation sequencing assay to determine the nucleotide at three attenuation loci sites. Summaries for each TDV strain will include the following categories: No loci with possible reversion, 1 locus with possible reversion, 2 loci with possible reversion, and all loci with possible reversion.

Secondary immunogenicity endpoints will be analyzed based on the PPS, unless indicated otherwise. Supportive analyses may be provided based on the FAS. To assess the impact of the COVID-19 pandemic, similar analyses as per the primary endpoint may be performed.

7.10.3 Exploratory Immunogenicity Endpoints

Number of subjects with non-missing assessment, geometric mean with 95% CI, geometric SD, median, minimum and maximum will be presented for EC_{50} titers for each dengue serotype. GMTs will be calculated as anti-logarithm of $\sum(\log_{10}$ transformed $EC_{50}/n)$, where n is the number of subjects with EC_{50} results available. The 95% CI for GMTs will be calculated as the anti-log transformation of upper and lower limits for a 2-sided CI of the mean of the log-transformed EC_{50} (based on student's t-distribution). EC_{50} below the LLOD of 63 will be imputed with a value of 31.5 (ie, half the LLOD) in summary tables.

Seropositivity rates (% of subjects seropositive) along with exact 2-sided 95% CIs based on the Clopper-Pearson method [4] will be analyzed from dengue RVP for each of the 4 dengue serotypes by visit. Seropositivity derived from RVP-based microneutralization assay is defined as a reciprocal $EC_{50} \geq 63$ (the LLOD). This will be repeated for multiple serotypes, which will include the percentage of subjects with:

- monovalent seropositivity (seropositive for only 1 of the 4 dengue serotypes),
- bivalent seropositivity (seropositive for any 2 of the 4 dengue serotypes),
- trivalent seropositivity (seropositive for any 3 of the 4 dengue serotypes),
- tetravalent seropositivity (seropositive for all 4 dengue serotypes),
- at least bivalent seropositivity (seropositive for ≥2 dengue serotypes),
- at least trivalent seropositivity (seropositive for ≥3 dengue serotypes).

Seropositivity rates determined by MNT_{50} titers (seropositive [reciprocal neutralizing titer ≥10 for at least 1 dengue serotype] or seronegative [reciprocal neutralizing titer <10 for all dengue serotypes]) for each dengue serotype will be analyzed similarly to the seropositivity rates derived from RVP-based microneutralization assay, as described above. This will be repeated for multiple serotypes, as described above.

Seropositivity rates will be graphically presented by visit for each of the 4 dengue serotypes, for at least trivalent, and for tetravalent seropositivity using bar graphs including the percentage of subjects seropositive and corresponding 95% CIs.

The following endpoints will be summarized descriptively by trial group, visit and for each dengue serotype, if applicable:

- Average number of memory B cells expressing type-specific and cross-reactive dengue-specific antibodies/10^6 PBMC measured by Quad-color FluoroSpot.
- Average concentration RU/mL (relative units/mL) of anti-dengue NS1 antibodies measured by ELISA.
- Cellular immune response specificity determined by epitope mapping as measured by ELISPOT in subset of subjects (IFN-γ ELISpot responses >50 SFC/10^6 cells and availability of sufficient cells).

- Average EC_{50} of DENV-1, -3 and -4-specific neutralizing antibody response, post-depletion of DENV-2 neutralizing antibodies.
- Dengue NS1 IgG concentration (RU/mL) and DENV Total Binding IgG concentration (RU/mL).
- Avidity index
- Complement fixing antibody titer (ELISA units/mL [EU/mL]).

Gene expression profiles will be summarized graphically over time and all collected data will be displayed in a listing sorted by trial group, by site number, by subject identification number, and by date/time of the recorded event, if applicable.

To assess the relationship between immunogenicity measures non-parametric correlation coefficients [5, 6] will be used, as appropriate.

Exploratory immunogenicity endpoints will be analyzed based on the PPS, or ICS subset, where applicable. Supportive analyses may be provided based on the FAS. To assess the impact of the COVID-19 pandemic, similar analyses as per the primary/secondary endpoints may be performed.

7.11 Safety Analysis

All summaries of safety data will be provided for the Safety Set.

7.11.1 Adverse Events

AE data will be summarized by trial group after each and any vaccination.

Solicited local (injection site) reactions and systemic events are collected for at least 30 min after each vaccination at the site (in-clinic assessment) and then using diary cards that are provided to the subject. Unsolicited AEs are collected by interview. Subjects will be evaluated for solicited local (injection site) reactions for 7 days (day of vaccination + 6 days), solicited systemic events for 14 days (day of vaccination + 13 days), and unsolicited Aes for 28 days (day of vaccination + 27 days), following each vaccination. MAAEs, AEs leading to trial vaccine withdrawal or trial discontinuation, and SAEs will be collected throughout the trial from first vaccination (Day 1 [M0]) until the end of the trial (Day 360 [M12]).

Reactogenicity (Solicited AE)

Solicited local (injection site) reactions include injection site pain, injection site erythema, and injection site swelling; for erythema and swelling, the subject will record the greatest surface diameter in mm but for the summaries and listings these data will be converted to cm. The intensity of erythema and swelling will be derived from the recorded diameters.

Solicited systemic events include headache, asthenia, malaise, myalgia, and fever (defined as a body temperature ≥38°C). The subject can record the body temperature in either °F or °C. However, body temperature measured in °F will be converted to °C for the summaries and listings. Fever will be derived from the recorded temperature measurements and presented using

the proposed temperature increments published by the Brighton Collaboration Fever Working Group [7].

Severity grades for solicited safety parameters are defined in the Appendix B.

For each solicited AE, the number and percentage of subjects reporting an event will be summarized by event severity for the following time intervals:

- 30 minutes after each vaccination (in-clinic, assessed by investigator);
- Days 1 – 7 (overall, for local [injection site] reactions) or Days 1 – 14 (overall, for systemic events) following each vaccination;
- Days 1 – 7 (daily, for local [injection site] reactions) or Days 1 – 14 (daily, for systemic events) following each vaccination;
- Days 1 – 3, Days 4 – 7 (overall, for local [injection site] reactions) or Days 1 – 7, Days 8 – 14 (overall, for systemic events) following each vaccination.

Percentages will be calculated based on the number of subjects who received the respective dose of trial vaccine and provided at least 1 record (none, mild, moderate or severe) for this AE in the relevant time interval. For example, subjects reporting solicited AEs (at least 1 non-missing record) for Days 1 – 3 will only be included in denominator for the Days 1 – 3 and Days 1 – 7 summaries, but will be excluded from denominator for Days 4 – 7 summaries. For subjects with more than 1 episode of the same event, the maximum severity will be used in summaries.

All solicited local (injection site) reactions are considered as related to trial vaccine. For solicited systemic events, relationship to IP is assessed by the investigator.

The number and percentage of subjects with solicited systemic events will also be summarized by relationship to IP for the following time intervals:

- 30 minutes after each vaccination;
- Days 1 – 14 (overall) following each vaccination.

If a subject reported more than one 1 episode for the same event, then the strongest relationship will be included in the summaries.

An overview table for solicited AEs will be provided. This will include:

- 30 minutes post-vaccination events (solicited local [injection site] and systemic events combined);
- Solicited AEs (solicited local [injection site] and systemic events combined);
- Solicited local (injection site) reactions;
- Solicited systemic events (overall and by relationship to IP);
- Prolonged solicited AEs (overall and for solicited local [injection site] and systemic events separately).

A summary of the first day of onset for each solicited AE and, the number of days the subject reports experiencing the AE will be presented for each vaccination. The number of days a subject reports each event is calculated as the total number of days the subject reports this event, regardless of whether the event was reported on consecutive days.

Persistent/prolonged solicited local (injection site) or systemic events continuing on Day 8 and Day 15, respectively, following each trial vaccination will be captured as an AE recorded in the Adverse Event eCRF. These Aes will not be included in the summaries of unsolicited Aes, and will be presented in separate listings. Any solicited local (injection site) or systemic events that resolved before 8 days and 15 days, respectively, following each trial vaccination, but recurring at a later time (ie, discontinued), will be recorded as an unsolicited AE on the Adverse Event eCRF.

Unsolicited AE

Unsolicited AEs will be assessed for 28 days following each vaccination (day of vaccination + 27 days). MAAEs, AEs leading to trial vaccine withdrawal or trial discontinuation, and all SAEs will be collected for the duration of the trial: from Day 1 (M0) through Day 360 (M12).

All unsolicited AEs, including MAAEs, SAEs and AEs leading to trial vaccine withdrawal or trial discontinuation will be coded using the current version of MedDRA. Summary tables of unsolicited AEs will include the number of events and the number and percentage of subjects who experienced events. Percentages will be calculated based on the number of subjects in the Safety Set who received the respective dose of the trial vaccine. Subjects who report more than 1 occurrence for a particular MedDRA term (level) will only be counted once in the summaries. Where relationship or severity is concerned, the AE with the most closely related occurrence or the highest known severity will be counted, following conservative approach.

All unsolicited AEs collected up to 28-days post-vaccination will be summarized as follows:

- By SOC and PT;
- By SOC and PT including events with a frequency greater than 2% in any trial group;
- By SOC and PT for trial vaccine related AEs;
- By SOC and PT including events with a frequency greater that 2% in any trial group for trial vaccine related AEs;
- By SOC, PT, and severity (mild, moderate, severe);
- By SOC, PT, and severity (mild, moderate, severe) for trial vaccine related AEs.

MAAEs, SAEs and AEs leading to trial vaccine withdrawal or trial discontinuation will be summarized for the duration of the trial as follows:

- By SOC and PT;
- By SOC and PT for IP related AEs;
- By SOC, PT, and severity (mild, moderate, severe) – for MAAE only.

In addition, overview tables by trial group will be generated for all unsolicited AEs collected up to 28 days post-vaccination, MAAEs, SAEs and AEs leading to trial vaccine withdrawal or trial discontinuation and will include the variables as outlined in Table 7.d

Table 7.d Overview of Unsolicited Adverse Events

	All AEs (within 28 days post-vaccination)	SAEs	MAAEs	AEs leading to trial vaccine withdrawal or trial discontinuation
Relationship to the trial vaccine	✓	✓	✓	✓
Relationship to the trial procedure	✓	✓	✓	✓
Severity	✓	✓	✓	✓
AEs leading to trial vaccine withdrawal and/or trial discontinuation	✓	✓	✓	
AEs leading to trial vaccine withdrawal	✓	✓	✓	✓
AEs leading to trial discontinuation	✓	✓	✓	✓
MAAEs	✓			✓
SAEs and non-serious AEs	✓			✓
Deaths	✓	✓		✓

Subject mappings – list of subject identification numbers in each category of SOC and PT and each trial group – will be provided for unsolicited AEs, SAEs, MAAEs and AEs leading to trial vaccine withdrawal or trial discontinuation.

For disclosure of trial results an additional AE table by SOC and PT including PT events with a frequency greater than 2% in any trial group will be provided for all non-serious unsolicited events up to 28 days post-vaccination, and for all non-serious events leading to IP withdrawal and/or trial discontinuation during the entire trial duration.

7.11.2 Clinical Laboratory Evaluations

Not applicable.

7.11.3 Vital Signs

Vital signs will be measured on Day 1 (M0) and all subsequent visits. Summary statistics (number of subjects, mean, SD, median, minimum, and maximum) will be calculated for all observed vital signs and for each vital sign change from Baseline. Summaries will be prepared for each trial group and each trial visit.

7.11.4 12-Lead Electrocardiograms

Not applicable.

7.11.5 Other Observations Related to Safety

Not applicable.

7.12 Interim Analysis

No interim analysis is planned.

7.13 Changes in the Statistical Analysis Plan

Seropositivity rates determined by MNT_{50} titers was added as an exploratory endpoint for consistency with phase 3 TDV trials. In addition, exploratory endpoints on assays included in the clinical development plan were added. Specifically, average EC_{50} of DENV-1, -3 and -4-specific neutralizing antibody response, post-depletion of DENV-2 neutralizing antibodies, dengue NS1 IgG concentration (RU/mL), avidity index, DENV total binding IgG concentration (RU/mL) and complement fixing antibody titer (EU/mL). Finally, the SAP also describes additional analyses/summaries that may be provided to assess the impact of the COVID-19 pandemic, as compared to the protocol.

7.13.1 Amendment History

Date	Amendment Number
02 Jul 2020	Initial Analysis Plan
09 Mar 2021	1
29 Mar 2024	2

7.13.2 Summary of Changes

This section describes major changes to previous SAP versions.

Final Version	Section	Description of Change
3.0	General	The main rationale for this amended SAP was to change the cellular immune response and ICS subset definitions, to align with the most recent definitions used in other Takeda Vaccines studies.
	5.2, 7.10.2	Update the definition of cellular immune response definition from "*Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared* ***with background (no peptide) and ≥50 spots per 10^6 PBMC***" to: "*Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared* ***to baseline [Day 1 (M0)] and ≥5 SFC/10^6 PBMC***". In addition, a note was added that NC (sample no peptide) will be subtracted from all reported values prior to calculating the response and in cases where the value corrected for the NC is negative (<0) that value will be set to "0".
	5.2, 5.3, 7.2, 7.10.2, 7.10.3	Update the definition of ICS subset from "*…evaluated in a subset of subjects with IFN- γ ELISpot responses* ***>500 SFC/10^6*** *cells and availability of sufficient cells*" to: "*…evaluated in a subset of subjects with IFN- γ ELISpot responses* ***>50 SFC/10^6*** *cells and availability of sufficient cells*".
	7.10.2	Deleted statements: "*Positive cellular immune response definitions for ICS are still under evaluation and may be defined at a later time point*" and "*Analyses may be repeated with different definitions of cellular immune response. This is still under evaluation and (if needed) will be defined at a later time point*", as no alternative definitions will be evaluated.

Final Version	Section	Description of Change
2.0	General	The main rationale for this amended SAP was the Amendment to Protocol Version 4.0, dated 11 Mar 2020 to remove the planned interim analysis due to the short time period between the projected dates for the database lock for the interim analysis (March 2021) and final analysis (August 2021). The interim analysis would have provided safety and immunogenicity data when all subjects have completed the Day 120 (M4) visit. Given the anticipated timing of the database lock for both analyses, it was considered that the interim analysis was no longer needed
	7.1	Updated the study protocol version and date. Updated to specify that data reviews will be conducted prior to database lock, and not the interim analysis also, as this is now not applicable. Additional text added about the impact of COVID-19 on laboratory assessments. Risks of database unlocks/relocks and additional data to be added to future CSR amendments or addenda to the final CSR was also included
	7.2	Updated to specify that any changes to PPS exclusion criteria will be documented separately and approved prior to the database lock
	7.7	Updated the follow-up duration intervals to include 271 – 360 days and >360 days after first vaccination, and 181 – 270 days and >270 days after second vaccination
	7.10.3	Addition that seropositivity rates from dengue RVP will also be analyzed for multiple serotypes
	7.12	Updated to state that no interim analysis will be performed
	Appendix A	Updated to add a note to Table 8.a.

8.0 REFERENCES

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Appendix A Schedule of Trial Procedures

Table 8.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 (M12)])

	Screening visit [a]	V1	V2	V3	V4	V5	V6	V7	V8	V9
		D1 M0	D4	D6	D9	D12	D15	D30 M1	D60 M2	D90 M3
Visit window (days)	Up to 70 days (10 weeks) prior to D1 (M0)	1 day (±NA)	4 days (±1) after V1	6 days (±1) after V1	9 days (±1) after V1	12 days (±1) after V1	15 days (-1/+2) after V1	30 days (-1/+7) after V1	60 days (±5) after V1	90 days (-4/+7) after V1
Informed consent	X	X [b]								
Assessment of eligibility criteria[c]	X	X								
Trial group assignment (flavivirus-naïve or dengue-immune)[d]		X								
Demographics	X	X [e]								
Medical history	X	X								
Prior medication/vaccination	X	X								
Concomitant medications/vaccinations[f]	X	X	X	X	X	X	X	X	X	X
Review of systems		X								X
Complete physical examination[g]	X	X								X
Targeted physical examination[h]								X		
Vital signs[i]		X	X	X	X	X	X	X	X	X
Pregnancy test[j]	X	X								X
Pregnancy avoidance guidance[k]		X	X	X	X	X	X	X	X	X
Blood sampling for serological testing (2.5 mL)	X									
Blood sample for humoral immune response (20 mL)[l, m]		X					X	X	X	X

Footnotes are on last table page.

Table 8.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12]) (continued)

		Screening visit [a]	V1	V2	V3	V4	V5	V6	V7	V8	V9
			D1 M0	D4	D6	D9	D12	D15	D30 M1	D60 M2	D90 M3
Visit window (days)		Up to 70 days (10 weeks) prior to D1 (M0)	1 day (±NA)	4 days (±1) after V1	6 days (±1) after V1	9 days (±1) after V1	12 days (±1) after V1	15 days (-1/+2) after V1	30 days (-1/+7) after V1	60 days (±5) after V1	90 days (-4/+7) after V1
Blood sample for T cell-mediated immune response (60 mL)[l]			X					X	X	X	X
Blood sample for B cell-mediated immune response (30 mL)[l]			X						X		X
Blood sample for innate immune response (5 mL)[l]			X	X	X						X
Blood sample for vaccine viremia (5 mL)[l]			X		X	X	X	X	X		X
Check criteria for delay of trial vaccine administration			X								X
Check contraindications for trial vaccine administration			X								X
Trial vaccine administration[n]			X								X
Injection site evaluation[o]			X								X
Diary card [p]	Distribution		X								X
	Review/collection of solicited and unsolicited AEs			X	X	X	X	X	X		
AEs leading to trial vaccine withdrawal or trial discontinuation, SAEs, MAAEs[q]							X				

Footnotes are on last table page.

Table 8.a Schedule of Trial Procedures (Screening Visit, Visits 1 to 9 [Day 1 (M0) to Day 90 (M3)] and Visits 10 to 19 [Day 94 to Day 360 [M12]) (continued)

	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19
	D94	D96	D99	D102	D105	D120 M4	D150 M5	D180 M6	D270 M9	D360 [r] M12
Visit window (days)	4 days (±1) after V9	6 days (±1) after V9	9 days (±1) after V9	12 days (±1) after V9	15 days (-1/+2) after V9	30 days (-1/+7) after V9	60 days (±5) after V9	90 days (±7) after V9	180 days (-7/+14) after V9	270 days (±14) after V9
Concomitant medications/vaccinations[e]	X	X	X	X	X	X	X	X	X	X
Targeted physical examination[g]						X				X
Vital signs[h]	X	X	X	X	X	X	X	X	X	X
Pregnancy avoidance guidance[j]	X	X	X	X	X	X				
Blood sample for humoral immune response (20 mL)[l]					X	X	X	X	X	X
Blood sample for T cell-mediated immune response (60 mL)					X	X	X	X	X	X
Blood sample for B cell-mediated immune response (30 mL)					X	X		X	X	X
Blood sample for innate immune response (5 mL)	X	X								
Blood sample for vaccine viremia (5 mL)		X	X	X	X	X				
Diary card[o] Review/collection of solicited and unsolicited AEs	X	X	X	X	X	X				
AEs leading to trial vaccine withdrawal or trial discontinuation, SAEs, MAAEs[p]						X				

AEs=Adverse Events, D=Day, M=Month, MAAEs=Medically Attended Adverse Events, NA=Not Applicable, SAEs= Serious Adverse Events, V=Visit
Note: When a site visit cannot be carried out due to the COVID-19 pandemic, telephone contacts will be made for subjects who are still under monitoring for safety reporting.
(a) A screening visit is only applicable if serological testing with regard to the inclusion criteria for the trial is performed within the scope of this trial.

(b) Up to 28 days prior to Day 1 (Month 0 [M0]).
(c) After informed consent has been obtained, eligibility of the subject will be assessed by review of inclusion/exclusion criteria at Screening or prior to TDV administration on Day 1 (M0), as applicable.
(d) Subjects will be enrolled in Group 1 (flavivirus-naïve subjects) or Group 2 (dengue-immune subjects with serology consistent with primary infection with either wild type dengue virus [DENV]-1 or DENV-3) based on serological testing performed either by the trial center prior to and outside the scope of this trial or within the scope of this trial (up to 70 days [10 weeks] prior to Day 1 [M0]).
(e) Not applicable if a Screening visit has been performed.
(f) All medications and vaccine history from 1 month (minimum 28 days) prior to administration of each trial vaccine dose up to 1 month (minimum 28 days) thereafter, steroids and immunostimulants within 60 days prior to Day 1 (M0), immunoglobulins and blood products within 3 months prior to Day 1 (M0), and immunosuppressive therapy within 6 months prior to Day 1 (M0). Concomitant medication/vaccination will be collected throughout the trial.
(g) Physical examination including measurement of weight and height; Body Mass Index (BMI) will be calculated. Measurement of height is only required at Screening or at Day 1 (M0), as applicable.
(h) Subjects may undergo a targeted symptom-directed physical examination. Clinically significant changes from the Baseline examination should be recorded in the subject's source documents and electronic Case Report Form (eCRF).
(i) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature.
(j) Pregnancy testing (serum or urine) for females of childbearing potential. Results must be confirmed and documented as negative prior to each trial dose administration. Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator.
(k) Females of childbearing potential who are sexually active will be reminded during trial visits to adhere to acceptable contraceptive methods up to 6 weeks after the last dose of TDV (Day 90 [Month 3] (M3]) + 6 weeks). Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy and donation of ova. During the course of the trial, subjects of childbearing potential will receive continued guidance with respect to the avoidance of pregnancy.
(l) All blood samples on days of vaccination (Day 1 [M0] and Day 90 [M3]) should be taken prior to administration of TDV.
(m) Dengue neutralizing antibodies and anti-dengue Non-Structural protein 1 (NS1) antibodies for all subjects, and T cell epitope mapping in a subset of subjects with Interferon-gamma Enzyme-Linked Immunospot (IFN-γ ELISpot) responses >50 Spot Forming Cells/106 cells and availability of sufficient cells).
(n) Subjects will receive TDV by subcutaneous injection.
(o) Injection site pain, erythema, and swelling assessed by trial staff for 30 minutes post-vaccination.
(p) Diary cards (paper or electronic) will be distributed for the recording of 1) solicited AEs including solicited local (injection site) reactions for 7 days (day of administration + 6 days) and solicited systemic events for 14 days (day of administration + 13 days) following administration of each trial vaccine dose, and 2) unsolicited AE for 28 days (day of administration + 27 days) following administration of each trial vaccine dose. The investigator will categorize events by severity (mild, moderate or severe) and will assess causality to vaccine administration ("related" or "not related"). For persistent/prolonged solicited local (injection site) reactions or systemic events observed as continuing on Day 8 or 15, respectively, following each trial vaccination, the end date will be captured on the "Adverse Event" eCRF to permit a separate analysis from the unsolicited AEs. Any solicited local (injection site) reaction or systemic event that resolves before 8 or 15 days, respectively, following each trial vaccination, but recurs at a later time (ie, if discontinues), should be recorded as an unsolicited AE on the "Adverse Event" eCRF.
(q) MAAEs and SAEs will be collected for the trial duration.
(r) The Final Visit will be performed on Day 360 (Month 12 [M12]). If a subject terminates trial participation earlier, Day 360 (M12) procedures should be

performed at their last trial visit, if possible.

Appendix B Solicited Local (Injection Site) and Systemic Adverse Events and Severity

Table 8.b Solicited Local (Injection Site) Reactions and Systemic Events

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

(a) Fever is defined as a body temperature ≥38°C (100.4°F) regardless of the method used [7].

Table 8.c Severity of Solicited Safety Parameters

Adverse Event	Severity grade	Severity
Pain at injection site	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity with or without treatment
	3	Severe: Prevents daily activity with or without treatment
Erythema at injection site [(a)]	0	< 25 mm
	1	Mild: 25 - ≤ 50 mm
	2	Moderate: > 50 - ≤ 100 mm
	3	Severe: > 100 mm
Swelling at injection site [(a)]	0	< 25 mm
	1	Mild: 25 - ≤ 50 mm
	2	Moderate: > 50 - ≤ 100 mm
	3	Severe: > 100 mm
Headache	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity with or without treatment
	3	Severe: Prevents normal daily activity with or without treatment
Asthenia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents normal daily activity
Malaise	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents normal daily activity
Myalgia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents normal daily activity
Fever [(b)]	NA	None
	NA	38.0-<38.5°C
	NA	38.5-<39.0°C
	NA	39.0-<39.5°C
	NA	39.5-<40.0°C
	NA	40.0-<40.5°C
	NA	40.5-<41.0°C
	NA	≥41.0°C

NA = not applicable
(a) Subjects are to record greatest surface diameter in mm on the diary card.
(b) Fever is defined as a body temperature ≥38°C (100.4°F) regardless of the method used [7].