Title: An open label, Phase 2 Study to Investigate Cell-mediated Immunity and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Healthy Children Aged 4 to 16 Years

NCT Number: NCT02948829

SAP Approve Date: 14 May 2020

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STATISTICAL ANALYSIS PLAN

STUDY NUMBER: DEN-313

An open label, Phase 2 Study to Investigate Cell-mediated Immunity and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Healthy Children Aged 4 to 16 Years

Safety and Immunogenicity of Takeda's TDV in Healthy Children

PHASE 2

Version: Final, 2.0

Date: 14 May 2020

Prepared by:

PPD

Based on:

Protocol Version: 1.0

Protocol Date: 01 July 2016

1.1 Approval Signatures

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

2.0 TABLE OF CONTENTS

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3.0 LIST OF ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
CD	Cluster of Differentiation
COVID-19	Corona Virus Disease 2019
CRF	Case Report Form
DENV-1, 2, 3, 4	Dengue serotype 1, 2, 3, 4
ELISA	Enzyme-linked Immunosorbent Assay
ELISPOT	Enzyme-linked Immunospot
FAS	Full Analysis Set
GMT	Geometric Mean Titer
IA	Interim Analysis
ICH	International Conference on Harmonization
ICS	Intracellular Cytokine Staining
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IL-2	Interleukin-2
IP	Investigational Product
LAR	Legally Authorized Representative
LLOD	Lower Limit of Detection
LLOQ	Lower Limit of Quantification
M0, 1, 3, 4, 9	Month 0, 1, 3, 4, 9
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
MIP	Macrophage Inflammatory Protein
MNT_{50}	Microneutralization test 50%
NC	Negative control
NS1, 2, 3, 4, 5	Nonstructural protein 1, 2, 3, 4, 5
PBMCs	Peripheral Blood Mononuclear Cells
PPS	Per-protocol Set
PT	Preferred Term
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System

SC	Subcutaneous
SFC	Spot Forming Cells
SOC	System Organ Class
TDV	Tetravalent Dengue Vaccine candidate
TLFs	Tables, Listings and Figures
TNF-α	Tumor Necrosis Factor-alpha
VCD	Virologically Confirmed Dengue
WHO Drug	World Health Organization Drug Dictionary

4.0 OBJECTIVES

4.1 Primary Objective

- To assess the cellular immune responses to 2 doses of tetravalent dengue vaccine candidate (TDV) in healthy subjects aged 4 to 16 years at 1 month post second vaccination.

4.2 Secondary Objectives

- To assess cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years up to 3 years post second vaccination.
- To assess cellular immune responses to 2 doses of TDV in healthy subjects aged 4 to 16 years by region and dengue Baseline seropositivity status.
- To characterize phenotype of cellular immune responses to TDV by intracellular cytokine staining (ICS) in a subset of trial subjects.
- To assess the post-vaccination neutralizing antibody response against each dengue serotype.
- To assess the post-vaccination neutralizing antibody response against multiple dengue serotypes.
- To describe the safety of 2 doses of TDV in healthy subjects aged 4 to 16 years.

4.3 Exploratory Objectives

CCI [redacted]

4.4 Study Design

This is an open-label phase 2 trial with a single trial group. The target sample size is 200 healthy subjects (100 subjects aged 4 to 16 years in Latin America; 100 subjects aged 4 to 8 years in Asia). Each subject will receive a 2-dose schedule of TDV by subcutaneous (SC) injection into the upper arm at Day 1 (Month 0 [M0]) and at Day 90 (Month 3 [M3]). Subjects will be screened prior to vaccination to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each trial center. Any withdrawals or screen failures from enrolment until vaccination at Day 1 (M0) will be replaced.

Subjects will be followed up for immunogenicity and safety assessments for 3 years after the second vaccination at Day 90 (M3) including febrile illness surveillance to monitor any febrile illness with potential dengue etiology for long term safety.

Definition of febrile illness

The subjects and the subject's parent(s)/legally authorized representative (LAR), as applicable, will be asked to return to the trial center for dengue fever evaluation of the subjects by the investigator in case of febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days).

Febrile illness surveillance

The subjects or the subject's parent(s)/LAR, as applicable, will be contacted at least weekly (eg, phone calls, home visits, or school-based surveillance) to ensure robust identification of febrile illness by reminding subjects or the subject's parent(s)/LAR, as applicable, of their obligation to return to the site in case of febrile illness.

Handling of febrile illness cases (suspected dengue cases)

Subjects with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have 1 blood sample taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever) to confirm dengue infection. Testing will include dengue reverse transcriptase polymerase chain reaction (RT-PCR) and nonstructural protein 1 (NS1) enzyme-linked immunosorbent assay (ELISA). Additional blood samples may be taken for diagnosis and clinical management of the subject as per standard medical practice. RT-PCR and NS1 ELISA results from the central laboratory will not be available for real time case management.

A new episode of febrile illness as described above will require an interval of at least 14 days from a previous febrile illness episode (ie, counting from the first day of febrile illness).

Duration of febrile illness surveillance

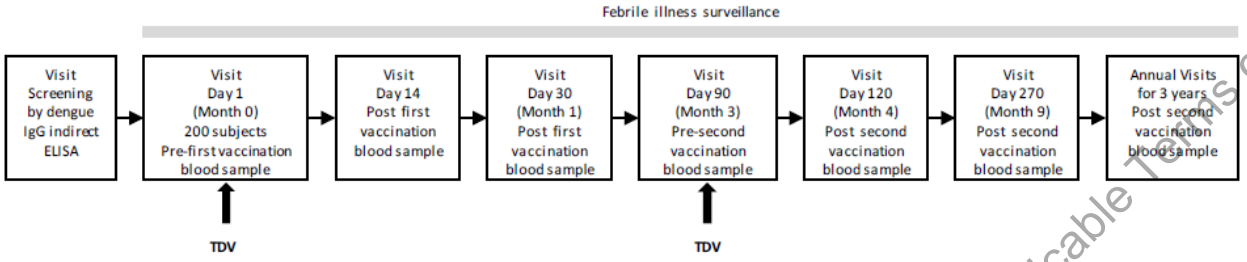
For each subject, surveillance for febrile illness will commence on the day of first vaccination (Day 1 [M0]) and will end 3 years after the second vaccination at Day 90 (M3).

Procedures

After informed consent/assent has been obtained, each subject will be assessed for eligibility to participate in the trial. A dengue immunoglobulin G (IgG) indirect ELISA will be performed during the screening period to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each trial center. Enrolment tracking of dengue naïve and dengue exposed subjects will be done using the interactive web response system. Subjects may proceed with vaccination in the trial prior to availability of the screening IgG indirect ELISA result until approximately 60% of subjects (ie, sample size at each trial center) are of the same dengue Baseline seropositivity status. Once this limit is reached, the dengue Baseline seropositivity status (ie, screening dengue IgG ELISA result) will be reviewed before vaccination. At Day 1 (M0), a pre-vaccination blood sample will be taken and vaccination will occur. A second vaccination will be administered at Day 90 (M3). Subjects will be followed-up for 3 years post second vaccination.

The schematic of the trial design is included as Figure 4.a. A schedule of trial procedures is provided in Appendix A.

Figure 4.a Schematic of Trial Design



ELISA=enzyme-linked immunosorbent assay, IgG=Immunoglobulin G, TDV=tetravalent dengue vaccine candidate
Notes:
(i) Approximately 40% or more subjects of either dengue Baseline seropositivity status at each study center.
(ii) Subjects presenting with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have 1 blood sample taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever).
(iii) Visits at Day 30 (Month 1) and Day 120 (Month 4) should occur at least 28 days after the first and second vaccination at Day 1 (Month 0) and Day 90 (Month 3), respectively. The window period (ie, -1/+7 days) for these visits will be calculated from the 30th day after each vaccination (ie, day of vaccination +29 days).
(iv) Visit at Day 14 is for subjects >10 years of age.
(v) For each subject, additional blood samples will be taken annually for 3 years post-second vaccination at Day 90 (Month 3).
(vi) For each subject, surveillance for febrile illness and safety follow-up will end 3 years after the second vaccination at Day 90 (Month 3).

Assessment of dengue Baseline seropositivity status

- A blood sample will be collected at Screening or pre-first vaccination at Day 1 (M0) if screening is performed the same day to determine dengue Baseline seropositivity status by dengue IgG indirect ELISA.

Immunogenicity evaluation

- Blood samples for assessment of cellular immune responses will be collected from all subjects at pre-first vaccination (Day 1 [M0]), 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 1 month and 6 months post second vaccination (Day 120 [Month 4 [M4]] and Day 270 [Month 9 [M9]], respectively), and annually for 3 years post second vaccination. One additional blood sample will be collected at Day 14 from subjects >10 years of age.
- Blood samples for assessment of dengue neutralizing antibodies (microneutralization test 50% [MNT_{50}]) will be collected from all subjects at pre-first vaccination (Day 1 [M0]), 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 1 month and 6 months post second vaccination (Day 120 [M4] and Day 270 [M9], respectively), and annually for 3 years post second vaccination.

Safety evaluation

- Unsolicited adverse events (AE) during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be collected by interview and will be recorded for all subjects (ie, at Day 30 [M1] and Day 120 [M4]).
- Medically attended adverse events (MAAE) will be collected from first vaccination at Day 1 (M0) up to 6 months post second vaccination at Day 90 (M3) by interview and will be recorded (ie, at Day 30 [M1], Day 90 [M3], Day 120 [M4], and Day 270 [M9]). MAAEs are defined as AEs leading to a medical visit to or by a healthcare professional, including visits to an emergency department, but not fulfilling seriousness criteria.

- Collection of serious adverse events (SAE) and AEs leading to subject discontinuation or withdrawal for the entire trial duration.
- Identification of febrile episodes with potential dengue etiology for the entire trial duration.

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

Trial Duration

The trial duration for each subject will be approximately 3 years and 4 months including screening (up to 28 days prior to Day 1 [M0]), vaccination (Day 1 [M0] and Day 90 [M3]), and 3 years follow-up post second vaccination).

5.0 ANALYSIS ENDPOINTS

5.1 Primary Endpoint

- Frequency of cellular immune responses to 2 doses of TDV at 1 month post second vaccination (Day 120 [M4]).

  Cellular immune response is defined as an interferon-gamma enzyme-linked immunospot (IFN-γ ELISPOT) response that is >3 times higher compared to Baseline (Day 1 [M0]) and ≥5 spots per well.

5.2 Secondary Endpoints

- Magnitude of (IFN-γ ELISPOT) responses to 2 doses of TDV at 1 month post second vaccination (Day 120 [M4]).
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV at 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 6 months post second vaccination (Day 270 [M9]), and then annually for 3 years post second vaccination.
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV by region and dengue Baseline seropositivity status at 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 1 month and 6 months post second vaccination (Day 120 [M4] and Day 270 [M9], respectively), and then annually for 3 years post second vaccination.
- Frequency and magnitude of IFN-γ ELISPOT responses to TDV at Day 14 in subjects >10 years of age.
- Phenotype characterization of cellular immune responses to TDV in a subset of trial subjects by ICS at 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 1 month and 6 months post second vaccination (Day 120 [M4] and Day 270 [M9], respectively), and then annually for 3 years post second vaccination. Markers will include cluster of differentiation (CD4, CD8), IFN-γ, tumor necrosis factor-alpha (TNF-α) and interleukin-2 (IL-2). This subset of subjects will be selected from samples with IFN-γ ELISPOT responses >500 spot forming cells (SFC)/10^6 cells and availability of sufficient cells.
- Phenotype characterization of cellular immune responses to TDV by region and dengue Baseline seropositivity status in a subset of trial subjects by ICS at 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 1 month and 6 months post second vaccination (Day 120 [M4] and Day 270 [M9], respectively), and then annually for 3 years post second vaccination. 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 >500 SFC/10^6 cells and availability of sufficient cells.
- Geometric mean titer (GMT) of neutralizing antibodies for each of the 4 dengue serotypes.
- Seropositivity rates (% of subjects) for each of the 4 dengue serotypes.

- Seropositivity rates (% of subjects) for multiple (2, 3 or 4) dengue serotypes.

Note: Seropositivity is defined as a reciprocal neutralizing titer ≥10.

- Percentage of subjects experiencing non-serious unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccination.
- Percentage of subjects with MAAEs from first vaccination up to 6 months post second vaccination.
- Percentage of subjects experiencing SAEs throughout the trial.
- Percentage of subjects with virologically confirmed febrile illness with potential dengue etiology throughout the trial.

5.3 Exploratory Endpoints

CCI

[redacted]

6.0 DETERMINATION OF SAMPLE SIZE

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

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-313, Version 1.0 dated 01 July 2016 [1] and on International Conference on Harmonization (ICH) E3 [2] and E9 [3] Guidelines.

All statistical analyses will be generated using the statistical analysis system SAS® Version 9.4.

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

Data reviews will be conducted prior to the planned interim and final analysis. These reviews will assess the accuracy and completeness of the trial database, subject evaluability, and appropriateness of the planned statistical methods.

7.1.1 Data Presentation

Immunogenicity and safety endpoints will be summarized descriptively (frequency and percent for categorical data; and number of subjects with non-missing observation, mean or geometric mean, SD or geometric SD, median, minimum and maximum for continuous data) by all relevant trial visits, if appropriate. In summary tables for categorical data for which categories are defined in the CRF, all categories will be presented as specified, even if the subject count within that category is zero. For other categorical data (eg, AEs and medications), only categories with at least one subject will be presented.

Minimum and maximum values will be presented using the same number of decimal places as the recorded data. Means, geometric means, and medians will be presented to 1 more decimal place than the recorded data. SD and geometric SD will be presented to 2 more decimal places than the recorded data. The CI about a parameter estimate will be presented using the same number of decimal places as the parameter estimate (ie, 1 more decimal place than the recorded data). Percentages will be presented to 1 decimal place (eg, 80.3%).

All data collected will be presented in listings, sorted by country, site number, subject number, and date/time of the finding, if applicable. If not stated otherwise, screen failure subjects will be grouped and listed at the end.

7.1.2 Study Day, Baseline and Analysis Window Definitions

Study Day 1 (M0) is defined as the date of the first trial vaccination, as recorded on the CRF vaccination form. Other study days are defined 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 time is available, the time of data collection must be prior to the first trial vaccination

time. Day 1 (M0) observations taken after the first trial vaccination are considered post-Baseline values.

A windowing convention for immunogenicity and safety data (vital signs) will be used to determine the analysis value of a variable for a given trial visit. Following the schedule of trial procedures (Appendix A), analysis visit windows will be calculated relatively to the days when trial vaccination was administered (Day 1 [M0] and Day 90 [M3]).

The analysis visit windows for each trial visit are displayed in Table 7.a.

Table 7.a Analysis Visit Windows

Visit	Scheduled Visit Day (Month/Year)	Scheduled Vaccination	Analysis Visit Window Full Analysis Set & Safety Set	Per-Protocol Set
V1	Day 1 (M0)	Dose 1	Prior [≤1 day] [(a)] to Dose 1	Prior [≤1 day] [(a)] to Dose 1
V2	Day 14		2 – 16 days [(b)] after Dose 1	12 – 16 days [(b)] after Dose 1
V3	Day 30 (M1)		17 – 60 days[(b)] after Dose 1	29 – 37 days [(b)] after Dose 1
V4	Day 90 (M3)	Dose 2	61 – 115 days [(b)] after Dose 1 and Prior [≤1 day] [(a)] to Dose 2	75 – 115 days [(b)] after Dose 1 and Prior [≤1 day] [(a)] to Dose 2
V5	Day 120 (M4)		2 – 105 days [(b)] after Dose 2 or 116 – 195 days [(b)] after Dose 1	29 – 37 days [(b)] after Dose 2
V6	Day 270 (M9)		106 – 272 days [(b)] after Dose 2 or 196 – 362 days [(b)] after Dose 1	159 – 201 days [(b)] after Dose 2
V7	Day 455 (Year 1)		273 – 547 days [(b)] after Dose 2 or 363 – 637 days [(b)] after Dose 1	320 – 410 days [(b)] after Dose 2
V8	Day 820 (Year 2)		548 – 912 days [(b)] after Dose 2 or 638 – 1002 days [(b)] after Dose 1	685 – 775 days [(b)] after Dose 2
V9	Day 1185 (Year 3)		≥913 days [(b)] after Dose 2 or ≥1003 days [(b)] after Dose 1	1050 – 1140 days [(b)] after Dose 2

(a) 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) observations taken after the first trial vaccination are considered post-Baseline values.
(b) Number of days after the visit is calculated with 1 day increment. For example, for V2 number of days after V1 is calculated as (Date of V2) – (Date of V1) + 1 day.

If more than one measurement for a variable is obtained for a subject within the same visit window, the measurement with the date closest to the scheduled visit date will be used. In the event that 2 measurements within a given visit window are equidistant to the scheduled visit

date, the later observation will be used. Only scheduled visits will be considered for the visit mapping.

7.1.3 Handling of Missing Data and Data Conventions

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

Immunogenicity data

Dengue neutralizing antibody titers (MNT_{50}) which are below the lower limit of detection (LLOD, 10) will be imputed with a value of 5 (half of the LLOD). If a reported value is greater or equal to the LLOD 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.

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

Adverse event data

Missing information regarding 'relationship to investigational product (IP)' (related/not related) and 'severity' (mild/moderate/severe) for unsolicited AEs will be handled using the worst-case approach. Thus, unsolicited AEs with missing severity will be considered as 'severe' and unsolicited AEs with missing relationship will be considered as 'related'.

Missing and partial AE start dates may be imputed only to determine the temporal relationship between the start date of the event and the dose date of the most appropriate vaccination that the AE should be associated with (ie, Vaccination 1 or Vaccination 2). An AE should be temporally allocated with the correct dose using the following rules:

- If the AE start and end dates are both completely missing, the AE will be allocated with the first trial vaccination;
- If at least month and/or the year of the AE start date 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 an AE end date or 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 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 trial vaccination. If partial end date information indicates possible association with both vaccinations, the AE will be allocated with the first trial vaccination.

Prior/concomitant medication/vaccination data

Missing and partial medication/vaccination dates will be assessed only to distinguish between a prior or a concomitant medication/vaccine. A medication will be considered prior only if the partial end date indicates that it was stopped before first trial vaccination. An unknown end date and a partial end date (if available) are insufficient to determine whether the medication is a prior or concomitant one; in this case, the tick box "Ended prior to informed consent" from the CRF should be used. Ie, if this box is ticked and the informed consent date is prior to the first vaccination date then the medication will be considered as prior medication. 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, the medication or vaccine will be considered concomitant.

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 CRF and from the partial date it can't be concluded that the event is clearly a medical history, the event will be considered concurrent medical condition.

7.1.4 Implausible Values

Data outside the plausible ranges as defined in Table 7.b will be excluded from respective analyses but presented as recorded in data listings including a flag that highlights implausible values.

Table 7.b Plausible Data Ranges

	Parameter	Applicable age range	Plausible range
Demographic/ Physical examination	Height	1.5 – 5 years	60 – 140 cm
		6 – 11 years	90 – 180 cm
		12 – 17 years	100 – 210 cm
	Weight	1.5 – 5 years	5 – 50 kg
		6 – 11 years	10 – 120 kg
		12 – 17 years	20 – 200 kg
Vital Signs	Heart Rate	All ages	40 – 200 beats/min
	Systolic Blood Pressure	All ages	70 – 180 mmHg
	Diastolic Blood Pressure	All ages	30 – 120 mmHg
	Body Temperature	All ages	32 – 43ºC

7.2 Analysis Sets

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

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

Safety Set: All enrolled subjects who received at least 1 dose of IP.

Full Analysis Set (FAS): All enrolled subjects who received at least 1 dose of IP and for whom a valid a pre-dose measurement and at least 1 valid post-dose measurement is available for immunogenicity.

Per-Protocol Set (PPS): All subjects from the FAS who have no major protocol violations as presented in Table 7.c.

ICS Subset: Subset selected among subjects from PPS who have IFN-γ ELISPOT responses >500 SFC per million PBMCs and availability of sufficient cells.

Table 7.c Criteria for Exclusion from the PPS

Criteria for Exclusion	Probable Method of Identification
Not receiving at least one dose of IP [(a)]	Identified programmatically using dosing data
Not having a valid pre-dose (Baseline) and at least 1 valid post-dose measurement [(b)]	Identified programmatically using immunogenicity data
Not receiving both doses of IP	Identified programmatically using dosing data
Receiving the second dose 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 log
Subject meets any of the following exclusion criteria: 2, 3, 4, 5	Identified programmatically using CRF-recorded data; Identified through protocol deviation review/medical review
Use of prohibited medications/vaccines	Identified through medical review

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

The major protocol violation criteria described in Table 7.c will be used to identify subjects who will be excluded from the PPS and will be defined as part of the data review prior to the planned interim analysis (IA). These criteria are considered to have a potentially significant impact on the immunogenicity results of the subject.

Other major protocol violations may be identified based on the data review and deviation logs throughout the trial, subject to medical review. Any changes to these criteria after approval of the SAP will be documented and approved prior to the planned IA.

Reasons for exclusion of subjects from the analysis sets will be summarized based on the Safety Set.

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 only.

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 first subject's first vaccination, the date of last

subject's first vaccination, the date of first subject's second vaccination, the date of last subject's second vaccination, the date of the first subject's first visit, the date of the last subject's last visit, the Medical Dictionary for Regulatory Activities (MedDRA) version, the World Health Organization Drug Dictionary (WHO Drug) version, and the SAS version used for analysis.

Disposition of all screened subjects will be summarized descriptively, including a summary of the number of screened subjects, the number of subjects eligible for vaccination, number of subjects not eligible for vaccination and the primary reason for not being eligible for vaccination. The number of screen failures and their characteristics will also be summarized.

Disposition categories for all subjects in the Safety Set will include:

- Number of subjects vaccinated by country, site, dengue screening IgG status and Baseline seropositivity status.
- Number of subjects enrolled, number of subjects in the Safety Set, FAS and PPS.
- Number of subjects who completed the IP regimen/trial.
- Number of subjects who prematurely discontinued the IP regimen/trial, overall, and by country.
- Primary reason for premature discontinuation of the IP regimen/trial, overall, and by country.

Significant protocol deviations will be summarized based on the Safety Set.

For the final analysis 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.

7.4 Demographic and Other Baseline Characteristics

Demographic and Baseline characteristics will include age, age group (4-5 years/6-11 years/12-16 years), gender, ethnicity, race, country, weight, height, body mass index ([BMI] calculated based on weight and height measurements at Baseline) and for Baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes). Seropositivity at Baseline is defined as a reciprocal neutralizing titer ≥10 for one or more dengue serotypes.

Summary statistics (number of subjects [n], mean, SD, median, minimum, and maximum) will be generated for continuous variables (age, height, weight, and BMI), and the number and percentage of subjects within each category will be presented for categorical variables (gender, ethnicity, race, country, and Baseline seropositivity status) based on the Safety Set, PPS, FAS and ICS Subset (based on the PPS).

In addition, the demographic and Baseline characteristics will also be summarized by country (Philippines/Panama), Baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes), age group (4-5 years/6-11 years/12-16 years) and combination of Baseline seropositivity status and age group and combination of Baseline

seropositivity status and country. Note: Summary tables by subgroup/combination of subgroups will only include those subgroups/combination of subgroups with at least 3 subjects present.

7.5 Medical History and Concurrent Medical Conditions

Medical history and concurrent medical conditions will be coded using the current version of the MedDRA coding system.

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

Summary tables will be provided by system organ class (SOC) and preferred term (PT) based on the Safety Set.

7.6 Medication History and Concomitant Medications

Medication history, vaccination history, concomitant medications, and concomitant vaccinations will be coded using the current version of WHO Drug.

A prior medication/vaccine (history) is any medication/vaccine which intake was stopped before the first dose of IP. A concomitant medication/vaccine is any medication/vaccine ongoing at the time the first dose of IP is administered, or taken/administered on/after the first dose of IP.

Summary tables for medication history and concomitant medications will be provided by Anatomical Therapeutic Chemical class level 2 and preferred medication name. Vaccination history and concomitant vaccinations will be summarized by vaccine type and name as recorded in the CRF. Summaries will be provided based on the Safety Set.

7.7 Investigational Product Exposure and Compliance

The Investigator records all injections of the IP given to the subject in the CRF.

Investigational product compliance will be summarized overall, by country and Baseline seropositivity status for the Safety Set presenting the number and percentage of subjects receiving:

- Both vaccinations;
- First vaccination only.

The duration of follow-up (calculated as end of the trial – date of first or second vaccination + 1 day) will be summarized for the Safety Set as a continuous variable (n, mean, SD, median, minimum, and maximum), and also as categorical variable (number and percentage of subjects) for the following intervals: 1 – 30 days, 31 – 180 days, 181 – 360 days, 361 – 540 days, etc. (continued in 6 months intervals until end of trial).

7.8 Efficacy Analysis

Not applicable.

7.9 Pharmacokinetic/Pharmacodynamic Analysis

Not applicable.

7.10 Other Outcomes

7.10.1 Primary Immunogenicity Endpoint

The frequency of cellular immune responses (ie, % of subjects with cellular immune response to any of the tested peptide pools) to 2 doses of TDV at Day 120 [M4] is the primary endpoint in this trial. Cellular immune response is defined as an IFN-γ ELISPOT response that is >3 times higher compared to Baseline (Day 1 [M0]) and ≥5 spots per well (labelled as 'Cellular Immune Response Definition 1' in the tables, listings and figures [TLFs]).

The percentage of subjects with cellular immune response to any of the tested peptide pools along with exact 2-sided 95% CI will be presented. The exact 2-sided 95% CI will be calculated based on the Clopper-Pearson method [4].

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

7.10.2 Secondary Immunogenicity Endpoints

Blood samples from all subjects for assessment of cellular immune responses and of dengue neutralizing antibodies will be collected at pre-first vaccination (Day 1 [M0]), 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 1 month post second vaccination (Day 120 [M4], 6 months post second vaccination (Day 270 [M9]), and annually for 3 years post second vaccination. One additional blood sample for assessment of cellular immune responses only will be collected at Day 14 from subjects >10 years of age.

Secondary immunogenicity endpoints will be analyzed based on the PPS. Supportive analyses may be provided based on the FAS.

Unless otherwise specified, analyses (including the primary immunogenicity endpoint) will be repeated by country (Philippines/Panama) and by Baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes). Additional subgroup analysis may be performed by age group (4-5 years, 6-11 years and 12-16 years) and combination of subgroups including Baseline seropositivity status, age group and country.

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 as described for the analysis of the primary endpoint in Section 7.10.1.

Analyses will be repeated using the following definition for cellular immune response: an IFN-γ ELISPOT response that is ≥4 times higher compared to a negative control, and ≥50 SFC/10^6 PBMCs (labelled as 'Cellular Immune Response Definition 2' in the TLFs).

A positive cellular immune response to any peptide pool for a given serotype constitutes a positive response to that serotype. Peptide pools are shown in Table 7.d.

Table 7.d Peptide Pools

Proteome Region	Abbreviation	Description	Serotype-matched
NS1	NS1-2	NS1 pool for DENV-2	DENV-2
NS3	NS3-1	NS3 pool for DENV-1	DENV-1
	NS3-2	NS3 pool for DENV-2	DENV-2
	NS3-3	NS3 pool for DENV-3	DENV-3
	NS3-4	NS3 pool for DENV-4	DENV-4
NS5	NS5-1	NS5 pool for DENV-1	DENV-1
	NS5-2	NS5 pool for DENV-2	DENV-2
	NS5-3	NS5 pool for DENV-3	DENV-3
	NS5-4	NS5 pool for DENV-4	DENV-4

NS = non-structural proteine, DENV = dengue serotype.

For the magnitude (number of SFC per million PBMCs) of IFN-γ ELISPOT responses to TDV, descriptive statistics (n, mean, SD, min, Q1, median, Q3, and maximum) will be presented by visit (including changes from Baseline or other relevant visits which may include absolute and/or mean fold changes) for all subjects and for responders only (i.e. subjects with a positive cellular immune response) separately for both cellular immune response definitions.

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 negative control (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.

For subjects >10 years of age, the frequency and magnitude of IFN-γ ELISPOT responses to TDV at Day 14 will be presented in analogy as described for the other visits above.

Phenotype characterization of cellular immune responses to TDV will be analyzed in a subset of trial subjects by ICS. This subset of subjects will be selected from samples with IFN-γ ELISPOT responses >500 SFC per million PBMCs and availability of sufficient cells. Markers will include CD4, CD8, IFN-γ, TNF-α, and IL-2. Descriptive statistics will be presented 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. Positive cellular immune response definitions for ICS are still under evaluation and may be defined and analyzed at a later time point (if applicable).

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

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 ∑(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). Data will be presented for all subjects as well as by Baseline seropositivity status.

Seropositivity rates (% of subjects seropositive) along with exact 2-sided 95% CIs based on the Clopper-Pearson method [4] will be analyzed for each of the 4 dengue serotypes by visit. Seropositivity is defined as a reciprocal neutralizing titer ≥10.

Seropositivity rates for multiple dengue serotypes will be analyzed in analogy to the seropositivity rates for each dengue serotype, as described above, and 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 will be graphically presented (overall and by Baseline seropositivity status) 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.

Additional analyses and/or summary tables may be performed for the final analysis to assess the impact of the COVID-19 pandemic. Those may include sensitivity analyses ignoring the analysis visit window defined for the PPS for the last trial visit (Year 3) for subjects impacted by COVID-19.

7.10.3 Exploratory Immunogenicity Endpoints

CCI

[redacted]

7.11 Safety Analysis

All summaries of safety data will be based on subjects in the Safety Set.

7.11.1 Adverse Events

Unsolicited AEs will be assessed for 28 days following vaccination (day of vaccination + 27 subsequent days). MAAEs will be collected from first vaccination (Day 1 [M0]) up to 6 months post second vaccination (Day 270 [M9]). SAEs and AEs leading to IP or trial discontinuation will be collected from first vaccination until the end of the trial. Unsolicited AEs, MAAEs, SAEs, and AEs leading to IP withdrawal or trial discontinuation will be coded according to the current version of MedDRA and summarized by SOC and PT.

In general, the number of events, number of subjects, and the percentage of subjects will be tabulated at each of the following levels: overall summary (any AEs/subjects with any AEs) and by SOC and PT. Subjects reporting more than 1 occurrence for the term (level) being summarized will be counted only once in the number/percentage of subjects. Summaries will be provided post first vaccination, post second vaccination and post any vaccination. Percentages are based on the number of subjects in the Safety Set who received the respective trial dose.

Unsolicited AEs up to 28 days post-vaccination will be summarized as follows:

- By SOC and PT (all subjects and by Baseline seropositivity status);
- By SOC and PT including events with frequency greater than 2% (all subjects and by Baseline seropositivity status);
- By SOC and PT including non-serious events with frequency greater than 2% (all subjects);
- By SOC and PT for IP related events (all subjects and by Baseline seropositivity status);
- By SOC and PT for IP related events with frequency greater than 2% (all subjects and by Baseline seropositivity status);

- By SOC, PT, and severity (mild, moderate, severe) (all subjects and by Baseline seropositivity status).

MAAEs up to 6 months post second vaccination (Day 270 [M9]) will be summarized as follows:

- By SOC and PT (all subjects and by Baseline seropositivity status);
- By SOC and PT for IP related events (all subjects and by Baseline seropositivity status);
- By SOC, PT, and severity (mild, moderate, severe) (all subjects and by Baseline seropositivity status).

SAEs post-vaccination throughout the trial duration will be summarized as follows:

- By SOC and PT (all subjects and by Baseline seropositivity status);
- By SOC and PT for IP related events (all subjects).

AEs leading to IP withdrawal or trial discontinuation post-vaccination throughout the trial duration will be summarized as follows:

- By SOC and PT (all subjects);
- By SOC and PT for IP related events (all subjects).

In addition, overview tables will be generated for unsolicited AEs, SAEs, MAAEs and AEs leading to IP withdrawal or trial discontinuation including the variables as outlined in Table 7.e.

Table 7.e Overview of Unsolicited Adverse Events

	All AEs (28 days post-vaccination)	SAEs	MAAEs (up to 6 months post-second vaccination)	AEs leading to IP withdrawal and/or trial discontinuation
Relationship to IP	✓	✓	✓	✓
Relationship to trial procedure	✓	✓	✓	✓
Severity	✓	✓	✓	✓
AEs leading to IP withdrawal and/or trial discontinuation	✓	✓	✓	
AEs leading to IP withdrawal	✓	✓	✓	✓
AEs leading to trial discontinuation	✓	✓	✓	✓
MAAEs	✓			✓
SAEs and Non-serious AEs	✓			✓
Deaths	✓	✓		✓

Overview tables for unsolicited AEs, MAAEs and SAE will also be provided by Baseline seropositivity status.

Subject mappings (ie, list of subject numbers in each category of SOC and PT) will be provided for unsolicited AEs, SAEs, MAAEs and AEs leading to IP withdrawal or trial discontinuation.

7.11.2 Clinical Laboratory Evaluations

Not applicable.

7.11.3 Vital Signs

The vital signs collected in the trial include systolic and diastolic blood pressure, heart rate, body temperature, height, weight, and BMI. Summary statistics (n, mean, SD, median, minimum, and maximum) will be presented for each scheduled visit (observed and changes from Baseline).

7.11.4 12-Lead ECGs

Not applicable.

7.11.5 Other Observations Related to Safety

For subjects with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue, the following data will be summarized descriptively (overall and by country):

- Total number of febrile illness cases;
- Number of febrile illness cases positive for NS1 ELISA;
- Percentage of subjects with virologically confirmed dengue (VCD) defined as febrile illness with a positive serotype-specific RT-PCR (ie, positive dengue detection RT-PCR), overall and by serotype.

A listing will be provided including viremia data obtained from subjects with febrile illness or clinically suspected dengue within 30 days after each vaccination.

7.12 Interim Analysis

An IA is planned on clean immunogenicity and safety data when all subjects have completed Day 270 (M9). This analysis will not be used to alter the trial conduct but to provide data to support the planning and execution of other studies in the development plan of Takeda's TDV.

The methodology for the IA will be as described in the respective sections of the SAP, with the exception that only data through the time of the interim data cut (individual cut-off for each subject) will be included. The IA will include descriptive summary tables for demographic and Baseline characteristics, safety data as well as descriptive summary tables and figures for immunogenicity based on availability of respective data at the time of the IA. The PPS will be defined prior to the Month 9 IA and will be kept unchanged for the final analysis at the end of

the trial. An IA CSR will be written including all available data up to the Day 270 (M9) IA data cut.

At the end of the trial a full CSR will be provided. All TLFs will be re-run including additional and/or final data. Any major changes from the IA will be described in the full CSR.

7.13 Changes in the Statistical Analysis Plan

Section 5.2 defines the secondary endpoints frequency and magnitude of IFN-γ ELISPOT responses to TDV and the phenotype characterization of cellular immune responses to TDV by region (Asia/Latin America). However, for the analysis of the secondary endpoints "by region" was changed to "by country" as this trial includes two countries only (Philippines and Panama) ie, 1 country per region.

The SAP 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
29 Apr 2019	Initial Analysis Plan
14 May 2020	1

7.13.2 Summary of Changes

This section describes major changes to the SAP Version 1.0, dated 29 April 2019.

Final Version	Section	Description of Change.
2.0	General	The main rationale for this amended SAP was to add clarity on the analyses related to cellular immune response measured by IFN-γ ELISPOT.
	3.0	Abbreviations were updated.
	7.1.3	Clarification was added for imputation of MNT values = 10.
	7.1.4	Respiratory rate as deleted from the Table 7.b as it's not collected for this trial.
	7.2	Definition of ICS Subset was added. Clarification was added that 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 only.

Final Version	Section	Description of Change.
	7.3	An additional listing and summary table were added for protocol deviations related to the COVID-19 pandemic for the final analyis.
	7.4	Demographics and baseline characteristics will also be provided for the ICS subset. Summaries will be repeated by age group and combination of subgroups. Clarification added that baseline characteristics also include Baseline seropositivity status and sentence referring to additional summaries deleted to avoid confusion.
	7.10.2	Details were added for the analyses related to cellular immune response measured by IFN-γ ELISPOT and by ICS and for analyses related to dengue neutralizing antibodies. • A second definition for cellular immune response was added for comparability with other dengue studies: IFN-γ ELISPOT response that is ≥4 times higher compared to a negative control, and ≥50 SFC/10^6 PBMCs. • Details for peptide pools (Table 7.a) were added. • Clarification was added for analysis of magnitude of IFN-γ ELISPOT responses and for ICS. • Clarification was added for graphs to be provided for GMTs/reverse cumulative distribution curves/seropositivity rates of dengue neutralizing antibodies (ie, for all subjects and by Baseline seropositivity status). • Addition of additional analyses/summaries that may be provided for the final analysis to assess the impact of the COVID-19 pandemic.
	7.12	Clarification was added for the handling of the interim and final/full CSR.
	7.13	Additional analyses/summaries added that may be provided to assess the impact of the COVID-19 pandemic.

8.0 REFERENCES

1. An open label, Phase 2 Study to Investigate Cell-mediated Immunity and Safety of a Tetravalent Dengue Vaccine Candidate (TDV) Administered Subcutaneously in Healthy Children Aged 4 to 16 Years. Takeda Vaccines, Inc. Protocol No. DEN-313, Version 1.0, dated 01 July 2016.
2. ICH Harmonized Tripartite Guideline – Clinical Trial Reports: Structure and Content, E3 (http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/structure-and-content-of-clinical-study-reports.html).
3. ICH Harmonized Tripartite Guideline – Statistical Principles for Clinical Trials, E9 (http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/statistical-principles-for-clinical-trials.html).
4. Clopper CJ and Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika. 1934;26: 404-13.

Appendix A Schedule of Trial Procedures

Table 8.a Schedule of Trial Procedures

	Screening [a]	Day 1 (Month 0)	Day 14 [b]	Day 30 (Month 1) [c]	Day 90 (Month 3)	Day 120 (Month 4) [c]	Day 270 (Month 9)	Annually for 3 years post second vaccination	Follow-up Visit [d]
Visit window	Up to 28 days prior to Day 1 (Month 0)		± 2 days	-1/+ 7 days	± 15 days	-1/+ 7 days	± 21 days	365 days from last vaccination ± 45 days	
Visits	X	X	X	X	X	X	X	X	
Signed informed consent/assent	X								
Assessment of eligibility criteria [e]	X	X							
Check contraindications to vaccination					X				
Check criteria for delay of vaccination					X				
Demographics	X								
Medical history	X	X			X				X
Concomitant medications/ vaccinations history [f]	X	X		X	X	X	X	X	X
Complete physical examination [g]	X	X			X				
Targeted physical examination [h]			X	X		X	X	X	X
Pregnancy test [i]	X	X			X				
Vaccine administration [j]		X			X				
Blood sample for screening by dengue IgG indirect ELISA (3 mL) [k]	X								
Blood sample [l]		X	X	X	X	X	X	X	
Surveillance for dengue fever [m]		X							
Febrile illness blood sample [n]		X							
Documentation of unsolicited adverse events (AEs) and medically attended AEs [o]		X	X	X	X	X	X		
Serious AEs and AEs leading to subject discontinuation or withdrawal [p]		X							

(a) Screening and the Day 1 (M0) visit may be performed on the same day depending on the availability and requirement of the dengue immunoglobulin G indirect enzyme-linked immunosorbent assay (dengue IgG indirect ELISA) result before first vaccination. All marked under either Screening or Day 1 (M0) is to be performed only once if screening is performed at Day 1 (M0).
(b) Visit only for subjects >10 years of age. See also footnote (l).
(c) Visits at Day 30 (M1) and Day 120 (M4) should occur at least 28 days after the day of first and second vaccination at Day 1 (M0) and Day 90 (M3), respectively. The window period (ie, -1/ + 7 days) for these visits will be calculated from the 30th day after each vaccination (ie, day of vaccination + 29 days).
(d) Follow-up visit is only applicable if the subject terminates early. The follow-up visit should be performed as soon as possible and preferably at least 28 days after the last trial vaccination.
(e) Eligibility by review of inclusion/exclusion criteria will be documented before entry into the trial.
(f) History of vaccination against Japanese Encephalitis or Yellow Fever irrespective of time of administration and including the vaccine type as well as any supportive documentation for these vaccinations, all concomitant medications and vaccine history from 1 month (minimum 28 days) prior to administration of each dose of TDV 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).
(g) Physical examination including measurement of weight and height; body mass index will be calculated automatically. Measurement of height is only required at Screening and at Day 1 (M0).
(h) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, body temperature, height and weight. Measurement of height is only required at Day 270 (M9), and annually for 3 years post second vaccination at Day 90 (M3).
(i) Pregnancy testing (serum or urine) for females of childbearing potential. Results must be confirmed and documented as negative prior to each IP administration. Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator. Female of childbearing potential who are sexually active will be reminded during trial visits to adhere to acceptable contraceptive methods up to 6 weeks post second vaccination.
(j) Subjects will be observed for at least 30 minutes after administration of each vaccine dose.
(k) A blood sample (approximately 3 mL) will be collected from all subjects to perform a dengue IgG indirect ELISA during the screening period to ensure that approximately 40% or more subjects of either dengue Baseline seropositivity status are enrolled at each trial center.
(l) Blood samples for assessment of dengue neutralizing antibodies and cellular immune responses will be collected from all subjects at pre-first vaccination (Day 1 [M0]), 1 month post first vaccination (Day 30 [M1]), pre-second vaccination (Day 90 [M3]), 1 month and 6 months post second vaccination (Day 120 [M4] and Day 270 [M9], respectively), and annually for 3 years post second vaccination. One additional blood sample will be collected at Day 14 from subjects >10 years of age. Blood volumes for assessment of dengue neutralizing antibodies will be 2.5 mL at Day 30 (M1) and 5 mL at each of the other specified visits. The blood volume for assessment of cellular immune responses will be 20 mL at each specified visit. In subjects ≤10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 3 mL/kg or 50 mL in total (whichever is lower) within 8 weeks. In subjects >10 years of age, volumes of blood samples for assessment of cellular immune responses will be adjusted so as not to exceed 5 mL/kg within 8 weeks.
(m) The subjects or the subject's parent(s)/LAR, as applicable, will be contacted at least weekly (eg, phone calls, home visits, or school-based surveillance) to ensure robust identification of febrile illness by reminding subjects or the subject's parent(s)/LAR, as applicable, of their obligation to return to the site in case of febrile illness.
(n) Subjects with febrile illness (defined as fever ≥38°C on any 2 of 3 consecutive days) or clinically suspected dengue will have a blood sample (4 mL) taken during the acute phase of the disease (ie, as soon as possible and preferably within 5 days after the onset of fever). Additional blood samples may be taken for diagnosis and clinical management of the subject as per standard medical practice.
(o) Unsolicited AEs during the 28-day period (day of vaccination + 27 subsequent days) after administration of each vaccine dose will be collected by interview and will be recorded for all subjects at Day 30 (M1) and Day 120 (M4). Medically attended AEs occurring from first vaccination at Day 1 (M0) up to 6 months post second vaccination at Day 90 (M3) will be collected by interview and will be recorded at Day 30 (M1), Day 90 (M3), Day 120 (M4) and Day 270 (M9).
(p) All serious AEs and AEs leading to subject discontinuation or withdrawal from first vaccination until the end of the trial.

Signature Page for DEN-313 Statistical Analysis Plan, Version 2.0, 14 May 2020

Title: DEN-313 Statistical Analysis Plan

Approval	PPD	

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