Title: An Open-Label, Phase 3 Trial to Investigate the Immunogenicity and Safety of Tetravalent Dengue Vaccine Candidate (TDV) at the End of Shelf Life in Healthy Adults in Non-Endemic Country(ies) for Dengue

NCT Number: NCT03771963

SAP Approve Date: 15 March 2019

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STATISTICAL ANALYSIS PLAN

STUDY NUMBER: DEN-307

An Open-label, Phase 3 Trial to Investigate the Immunogenicity and Safety of Tetravalent Dengue Vaccine Candidate (TDV) at the End of Shelf Life in Healthy Adults in Non-Endemic Country(ies) for Dengue

Immunogenicity and Safety of TDV at the End of Shelf Life in Healthy Adults

PHASE 3

Version: Final, 1.0

Date: 15 March 2019

Prepared by:

PPD

Based on:

Protocol Version: 1.0

Protocol Date: 21 June 2018

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
BMI	Body Mass Index
CRF	Case Report Form
FAS	Full Analysis Set
GMT	Geometric Mean Titer
ICH	International Conference on Harmonization
IP	Investigational Product
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
MNT_{50}	Microneutralization Test 50%
PPS	Per-Protocol Set
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SC	Subcutaneous
SOC	System Organ Class
TDV	Tetravalent Dengue Vaccine candidate
WHO Drug	World Health Organization Drug Dictionary

4.0 OBJECTIVES

4.1 Primary Objective

To describe the neutralizing antibody response against each dengue serotype of a naturally aged (>12 months stored at 2°C to 8°C) lot of a Tetravalent Dengue Vaccine candidate (TDV) at 1 month post second dose.

4.2 Secondary Objectives

Immunogenicity

- To describe the seropositivity rates for all dengue serotypes at 1 month and at 6 months post second TDV dose, where seropositivity is defined as a reciprocal neutralizing titer ≥10.
- To describe the persistence of the immune response at 6 months post second dose of TDV.

Safety

- To assess the safety profile following each vaccination.

4.3 Additional Objectives

Not applicable.

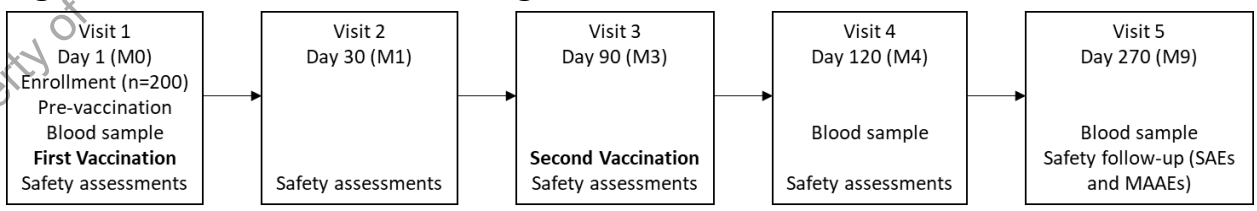
4.4 Study Design

This is an open-label phase 3 trial in country(ies) non-endemic for dengue to investigate the immunogenicity and safety of a lot of TDV with potency values representative of those at the end of shelf life, administered as a 2 dose regimen 3 months apart via subcutaneous (SC) injection. One single trial group of 200 healthy subjects aged 18 to 60 years, inclusive, will receive a naturally aged (>12 months stored at 2°C to 8°C) lot of TDV on Day 1 (M0) and Day 90 (M3).

All subjects will be followed-up for 6 months post second vaccination on Day 90 (M3) through Day 270 (M9), so the trial duration will be approximately 270 days (9 months) for each subject.

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

Figure 4.a Schematic of Trial Design



M=Month; MAAEs=Medically Attended Adverse Events; SAEs= Serious Adverse Events.

Immunogenicity evaluation:

Neutralizing antibodies will be measured (by microneutralization test 50% [MNT_{50}]) using blood samples collected pre first vaccination (Day 1 [M0]) and post second vaccination (Day 120 [M4] and Day 270 [M9]).

Safety evaluation:

- Diary cards will be distributed for the recording of solicited adverse events (AE):
  - Solicited local (injection site) reactions for 7 days following each TDV dose on Day 1 (M0) and Day 90 (M3) (day of vaccination + 6 days). These will include: injection site pain, injection site erythema, and injection site swelling.
  - Solicited systemic events for 14 days following each TDV dose on Day 1 (M0) and Day 90 (M3) (day of vaccination + 13 days). These will include: fever, headache, asthenia, malaise, and myalgia.
- Unsolicited AEs for 28 days following each TDV dose on Day 1 (M0) and Day 90 (M3) (day of vaccination + 27 days) will be collected by interview and recorded for all subjects.
- Serious adverse events (SAE), medically attended adverse events (MAAE) and AEs leading to subject discontinuation and withdrawal will be collected for the entire trial duration. MAAEs are defined as AEs leading to an unscheduled visit to or by a healthcare professional including visits to an emergency department, but not fulfilling seriousness criteria.

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

5.0 ANALYSIS ENDPOINTS

5.1 Primary Endpoint

- Geometric Mean Titers (GMT) of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes at Day 120 (M4).

5.2 Secondary Endpoints

Immunogenicity

- Seropositivity rates (% of seropositive subjects) for each of the 4 dengue serotypes at Day 120 (M4) and Day 270 (M9).
- Seropositivity rates (% of seropositive subjects) for each of the multiple (2, 3, or 4) dengue serotypes at Day 120 (M4) and Day 270 (M9).

Note: Seropositivity is defined as a reciprocal neutralizing titer $\geq$10.

- GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes at Day 270 (M9).

Safety

- Frequency and severity of solicited local (injection site) reactions for 7 days (day of vaccination + 6 days) and solicited systemic events for 14 days (day of vaccination + 13 days) after each TDV dose on Day 1 (M0) and Day 90 (M3).
- Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 days) after each TDV dose on Day 1 (M0) and Day 90 (M3).
- Percentage of subjects with SAEs throughout the trial.
- Percentage of subjects with MAAEs throughout the trial.

6.0 DETERMINATION OF SAMPLE SIZE

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

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-307, Version 1.0 dated 21 June 2018 [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 9.2 or higher.

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 exploratory only.

A data review will be conducted prior to database lock. This review will assess the accuracy and completeness of the trial database, subject evaluability, and appropriateness of the planned statistical methods.

7.1.1 Data Presentation

Unless specified otherwise, number of subjects with non-missing observations, mean or geometric mean, SD or geometric SD, median, minimum, and maximum will be presented for continuous data. Frequency and percent will be presented for categorical data. In summary tables for categorical data for which categories are defined on 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/vaccinations), 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, with possible exceptions made for derived 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 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 (vital signs) data 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 relative to the days when each trial dose was administered (Day 1 [M0] and Day 90 [M3]).

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. Both scheduled and unscheduled visits will be considered equally for the visit mapping for both immunogenicity and safety (vital signs) data.

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)	Scheduled Dose	Analysis Visit Windows		
			Safety Set	Full Analysis Set	Per-Protocol Set
V1	Day 1 (M0)	Dose 1	Prior [≤1 day] [(a)] to Dose 1	Prior [≤1 day] [(a)] to Dose 1	Prior [≤1 day] [(a)] to Dose 1
V2	Day 30 (M1)		2 – 60 [(b)] days after Dose 1	Not applicable (no blood sample is taken at V2)	Not applicable (no blood sample is taken at V2)
V3	Day 90 (M3)	Dose 2	61 – 115 [(b)] days after Dose 1 and Prior [≤1 day] [(a)] to Dose 2	Not applicable (no blood sample is taken at V3)	Not applicable (no blood sample is taken at V3)
V4	Day 120 (M4)		2 – 105 [(b)] days after Dose 2 or 116 – 195 [(b)] days after Dose 1 [(c)]	2 – 105 [(b)] days after Dose 2 or 2 – 195 [(b)] days after Dose 1 [(c)]	29 – 37 [(b)] days after Dose 2
V5	Day 270 (M9)		≥106 [(b)] days after Dose 2 or ≥196 [(b)] days after Dose 1 [(c)]	≥106 [(b)] days after Dose 2 or ≥196 [(b)] days after Dose 1 [(c)]	173 – 194 [(b)] days 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).
(c) Applies to subjects who missed the second dose at V3.

7.1.3 Handling of Missing Data

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 between the LLOD and 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 between 10 and 18 will be imputed with 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) for solicited systemic and unsolicited AEs 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 solicited systemic and unsolicited AEs with missing relationship will be considered as 'related'.

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 dose date of the most appropriate vaccination that the AE should be allocated 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 to the vaccination prior to the AE end date. 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. 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	Plausible range
Demographic/ Physical examination	Height	110 – 210 cm
	Weight	20 – 200 kg
Solicited AEs	Erythema	≤ 500 mm
	Swelling	≤ 500 mm
	Body Temperature [(a)]	32 – 43ºC
Vital Signs	Heart Rate	40 – 200 beats/min
	Systolic Blood Pressure	70 – 180 mmHg
	Diastolic Blood Pressure	30 – 120 mmHg
	Respiratory Rate	5 – 80 breaths/min

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

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: 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 pre-dose (Baseline) measurement and at least 1 valid post-dose measurement are available for immunogenicity assessments.

Per-Protocol Set (PPS): All subjects from the FAS who have no major protocol violations, excluding subjects who are seropositive for any serotype of dengue virus at Baseline (Seropositivity is defined as a reciprocal neutralizing titer ≥10).

The criteria (ie, the major protocol violations) as described in Table 7.c will be used to identify subjects who will be excluded from the PPS and will be identified prior to database lock. These criteria are considered to have a potentially significant impact on the immunogenicity results of the subject.

Other major protocol violations may be identified during 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 database lock.

Reasons for exclusion of subjects from analysis sets will be summarized based on all enrolled subjects.

Table 7.c Criteria for Exclusion from the PPS

Criteria for Exclusion	Probable Method of Identification
Not receiving at least one dose of the 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
Subjects seropositive to any serotype of dengue neutralizing titers at Baseline (Day 1 [M0])	Identified programmatically using immunogenicity data
Not receiving both doses of the IP	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 log
Subject meets any of the following exclusion criteria: 6, 7, 9, 10, 11, 12, 16, 20, 21, 22, 23	Identified programmatically using CRF-recorded data; Identified through protocol deviation review/medical review
Use of prohibited medications/vaccines	Identified through medical review based on CRF-recorded data

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

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 last subject's last visit, 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 age (in days) of the TDV lot at the time of first subject's first vaccination, the age (in days) of the TDV lot at the time of last subject's second vaccination, the Medical Dictionary for Regulatory Activities (MedDRA) version, the World Health Organization Drug Dictionary (WHO Drug) version, and the SAS version used for analysis.

The age of the TDV lot (per subject per dose) will be calculated as the date of injection (ie, Vaccination 1 or Vaccination 2) – the date of transfer of TDV lot to 2-8°C (ie, 17Jan2018) + 1 day.

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 for all enrolled subjects will be summarized including the following disposition categories:

- Number of subjects enrolled by trial site.
- Number of subjects enrolled, but not vaccinated (including primary reason for being enrolled, but not vaccinated).
- 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.
- Primary reason for premature discontinuation of the IP regimen/trial.

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

7.4 Demographic and Other Baseline Characteristics

Age, gender, race, and other Baseline characteristics will be summarized descriptively based on the Safety Set, FAS and PPS. These summaries will include overall dengue seropositivity status (seropositive [reciprocal neutralizing titer ≥10 for at least 1 dengue serotype] or seronegative [reciprocal neutralizing titer <10 for all dengue serotypes]), dengue seropositivity status for each serotype and for multiple serotypes.

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 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, vaccine history, concomitant medications, and concomitant vaccines will be coded using the current version of WHO Drug.

A prior medication/vaccine (history) is any medication/vaccine which intake was stopped before 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. Vaccine history and concomitant vaccines will be summarized by vaccine type and name as recorded on 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 on the CRF.

Investigational product compliance will be summarized for the Safety Set presenting the number and percentage of subjects receiving:

- Both vaccinations;
- First vaccination only.

The duration of follow-up [days] after the first dose of IP (calculated as end of trial date – first vaccination date + 1 day) will be summarized for the Safety Set as a continuous variable (number of subjects [n], mean, SD, median, minimum, and maximum), and also as categorical variable (frequency and percentage of subjects) for the following intervals: 1 – 30 days, 31 – 90 days, 91 – 120 days, 121 – 270 days, >270 days. Additionally, the duration of follow-up [days] after the second dose of IP (calculated as end of trial date – second vaccination date + 1 day) 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, >180 days.

For each subject and each dose the age of the TDV lot will be calculated (as defined in Section 7.3) and listed together with the dosing data.

7.8 Efficacy Analysis

Not applicable.

7.9 Pharmacokinetic/Pharmacodynamic Analysis

Not applicable.

7.10 Other Outcomes

7.10.1 Primary Immunogenicity Analyses

The primary immunogenicity endpoint is the GMTs of dengue neutralizing antibodies (derived from dengue MNT_{50} results) for each of the 4 dengue serotypes (DENV-1, DENV-2, DENV-3, DENV-4) at Day 120 (M4).

The number of subjects with non-missing assessments, geometric mean with 95% CI, geometric SD, median, minimum and maximum will be presented for neutralizing antibody titers for each dengue serotype. 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 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 titers (based on student's t-distribution).

The primary immunogenicity endpoint will be summarized based on the PPS. A supportive analysis will be provided using the FAS.

7.10.2 Secondary Immunogenicity Analyses

The secondary immunogenicity endpoints are:

- Seropositivity rates (% of subjects seropositive) for each of the 4 dengue serotypes at Day 120 (M4) and Day 270 (M9).
- Seropositivity rates (% of subjects seropositive) for multiple (2, 3, or 4) dengue serotypes at Day 120 (M4) and Day 270 (M9).
- GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes at Day 270 (M9).

For the seropositivity rates for each dengue serotype, the percentage of subjects seropositive along with exact 2-sided 95% CIs will be presented by visit. The exact 2-sided 95% CI of seropositivity rate will be calculated based on Clopper-Pearson method [4]. 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).

GMTs of dengue neutralizing antibodies (derived from dengue MNT_{50} results) at Day 270 (M9) will be analyzed in analogy to the primary immunogenicity endpoint.

The secondary immunogenicity endpoints will be summarized by visit based on the PPS. Supportive analyses will be provided based on the FAS.

In addition, GMTs (including 95% CIs) over time (all visits) and reverse cumulative distribution curves (all visits except Baseline) will be plotted (line plots) by dengue serotype and visit based on the PPS. Seropositivity rates will be graphically presented by dengue serotype, for at least trivalent, and for tetravalent seropositivity and by visit (except Baseline) using bar graphs including the percentage of subjects seropositive and corresponding 95% CIs.

7.11 Safety Analysis

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

7.11.1 Adverse Events

Unless otherwise specified, AEs will be summarized after first trial vaccination, second trial vaccination, and any trial vaccination.

Reactogenicity (Solicited AEs)

Solicited AEs are collected for at least 30 min after each vaccination at the site (in-clinic assessment). In addition, subjects are provided with a diary card for the recording of solicited local (injection site) AEs, including injection site pain, injection site erythema, and injection site swelling, for 7 days following vaccination (day of vaccination + 6 days). Subjects are also provided with a diary card for the recording of solicited systemic AEs (fever, headache, asthenia, malaise, and myalgia) for 14 days following vaccination (day of vaccination + 13 days). For the local (injection site) AEs erythema and swelling, the subject/the subject's representative will record the length of the longest diameter in mm. However, for the analysis these data will be displayed in cm. For the systemic AE fever, the subject/the subject's representative will record the body temperature in either °F or °C. For the analysis, all data will be displayed in °C. Severity grades for erythema and swelling will be derived from the recorded diameters, and fever will be presented using the proposed temperature increments published by the Brighton Collaboration Fever Working Group [5]. Details of solicited local (injection site) and systemic AEs, and severity of solicited safety parameters are given in Appendix B.

Missing data for solicited AEs will not be imputed unless otherwise specified in Section 7.1.3. For each solicited AE, the denominator for the percentage will exclude subjects with completely missing data (ie, subject does not have at least 1 recorded result [ie, none, mild, moderate, or severe]) for the solicited AE in the period being summarized.

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

- 30 minutes (in-clinic assessment of solicited local [injection site] and systemic AEs – analyzed separately from diary-recorded solicited AEs);
- Within 7 days (solicited local [injection site] AEs);
- Within 14 days (solicited systemic AEs);
- Days 1 – 7 (daily, solicited local [injection site] AEs);
- Days 1 – 14 (daily, solicited systemic AEs);
- Days 1 – 3 and Days 4 – 7 (solicited local [injection site] AEs);
- Days 1 – 7 and Days 8 – 14 (solicited systemic AEs).

For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

For solicited systemic AEs, the number and percentage of subjects will also be summarized by relationship to IP (assessed by the Investigator) for the following intervals post-vaccination:

- 30 minutes;
- Within 14 days.

Subjects will only be counted once if the subject has more than 1 episode of the same event. In the case where the subject has both related and unrelated solicited systemic AEs, the subject will be counted under the related category. All solicited local (injection site) AEs are considered as related to IP.

A summary of the day of first onset of each event and the number of days subjects reported each event will be presented post-vaccination. The number of days a subject reported each event is calculated as the total of all days the subject reported the event, regardless of whether the event was reported on consecutive days.

An overview table for solicited AEs post-vaccination will be provided including:

- 30 minutes in-clinic assessment (solicited local [injection site] and systemic AEs combined);
- Solicited AEs (solicited local [injection site] and systemic AEs combined);
- Solicited local [injection site] AEs;
- Solicited systemic AEs (overall and by relationship to IP);
- Prolonged solicited AEs (overall and for solicited local [injection site] and systemic AEs separately).

Prolonged solicited AEs that continue beyond Day 7 (for local [injection site] AEs) or Day 14 (for systemic AEs) will be captured on the AE CRF with appropriate indication ("continued solicited AE"). These prolonged solicited AEs will be presented in separate listings and will not be included in any unsolicited AE summary or listing.

Unsolicited AEs

Unsolicited AEs will be assessed for 28 days following administration of each trial dose (day of vaccination + 27 subsequent days). MAAEs, SAEs and AEs leading to IP withdrawal or trial discontinuation will be collected from first trial dose (Day 1 [M0]) until the end of the trial (Day 270 [M9]). 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. Percentages will be 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;
- By SOC and PT including events with frequency greater than 2%;
- By SOC and PT including non-serious events with frequency greater than 2%;
- By SOC and PT for IP related events;
- By SOC and PT for IP related events with frequency greater than 2%;
- By SOC, PT, and severity (mild, moderate, severe).

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

- By SOC and PT;
- By SOC and PT for IP related events;
- By SOC, PT, and severity (mild, moderate, severe).

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

- By SOC and PT;
- By SOC and PT for IP related events.

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

- By SOC and PT;
- By SOC and PT for IP related events.

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

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.

Table 7.d Overview of Unsolicited Adverse Events

	All AEs (28 days post-vaccination)	SAEs	MAAEs	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	✓	✓		✓

7.11.2 Clinical Laboratory Evaluations

Not applicable.

7.11.3 Vital Signs

Vital signs will be measured on Day 1 (M0), Day 30 (M1), Day 90 (M3), Day 120 (M4), and Day 270 (M9). Summary statistics (number of subjects [n], mean, SD, median, minimum, and maximum) will be calculated and presented by visit (observed data and changes from Baseline).

7.11.4 12-Lead ECGs

Not applicable.

7.11.5 Other Observations Related to Safety

Not applicable.

7.12 Interim Analysis

No interim analysis is planned for this trial.

7.13 Changes in the Statistical Analysis Plan

Definitions for "All Screened" and "All Enrolled" subjects were added and the other analysis sets were updated to make them more clear (no changes to the definitions itself).

The protocol states that age, gender, race, and other Baseline characteristics will be summarized descriptively for all enrolled subjects. However, these data will be summarized based on the Safety Set, FAS and PPS.

Solicited local (injection site) reactions and systemic events are labeled in the SAP as solicited local [injection site] AE and solicited systemic AEs to keep consistency between SAPs and tables, listings and graphs. The same is true for AEs leading to subject discontinuation which are labeled here as AEs leading to trial discontinuation (i.e. subject discontinued from the trial due to the AE).

8.0 REFERENCES

1. An Open-Label, Phase 3 Trial to Investigate the Immunogenicity and Safety of Tetravalent Dengue Vaccine Candidate (TDV) at the End of Shelf Life in Healthy Adults in Non Endemic Country(ies) for Dengue. Takeda Vaccines, Inc. Protocol No. DEN-307, Version 1.0, dated 21 June 2018.
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.
5. Marcy SM, Kohl KS, Dagan R, Nalin D, Blum M, Jones MC, et al. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis and presentation. Vaccine. 2004;22(5-6):551-6.

Appendix A Schedule of Trial Procedures

Table 8.a Schedule of Trial Procedures

Visit	**1**	**2 [a]**	**3**	**4 [a]**	**5**
Day	**Day 1 M0**	**Day 30 M1**	**Day 90 M3**	**Day 120 M4**	**Day 270 M9 (ET)[b]**
Visit window (days)	**1±NA**	**30 days (-1/+7) after Visit 1**	**90 days (-4/+7) after Visit 1**	**30 days (-1/+7) after Visit 3**	**180 days (-7/+14) after Visit 3**
Informed consent [c]	X				
Assessment of eligibility criteria [d]	X				
Demographics	X				
Medical history	X				
Prior and Concomitant medications/vaccinations [e]	X	X	X	X	X
Check contraindications to TDV administration			X		
Check criteria for delay of TDV administration			X		
Complete physical examination [f]	X		X		
Targeted physical examination [g]		X		X	X
Vital signs [h]	X	X	X	X	X
Pregnancy test [i]	X		X		
Pregnancy avoidance guidance [j]	X	X	X	X	
Blood sample for dengue neutralizing antibodies (5 mL) [k]	X			X	X
TDV administration by SC injection	X		X		
Injection site evaluation [l]	X		X		
Diary card[m] – Distribution	X		X		
Diary card[m] – Review/collection of solicited local (injection site) reactions and systemic events		X		X	
Unsolicited AEs [n]	X	X	X	X	
SAEs, AEs leading to subject discontinuation or vaccine withdrawal, MAAEs [o]	X	X	X	X	X

AEs=Adverse Events, D=Day, ET=Early Termination, M=Month, MAAEs=Medically Attended Adverse Events, NA=Not Applicable, SAEs=Serious Adverse Events; SC= Subcutaneous; TDV=Tetravalent Dengue Vaccine Candidate.

(a) Visit 2 and Visit 4 should occur 30 days (at least 29 days) after the 1st and 2nd trial vaccination, respectively.

(b) The final visit will be made on Day 270 (Month [M]9). If the subject terminates early, Day 270 (M9) procedures should be performed at their last visit, if possible.
(c) Prior to the subject entering into the trial, and before any protocol-directed procedures are performed. Up to 28 days prior to the day of enrollment.
(d) Review of inclusion/exclusion criteria will be performed prior to Tetravalent Dengue Vaccine Candidate (TDV) administration on Day 1 (M0). After written informed consent has been obtained and eligibility is assessed, subjects will be enrolled to receive 2 doses of TDV by subcutaneous injection.
(e) All medications and vaccine history from 1 month (minimum 28 days) prior to administration of each trial 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). Concomitant medication/vaccination will be collected throughout the trial conduct.
(f) Physical examination including measurement of weight and height; Body Mass Index (BMI) will be calculated. Measurement of height is only required at Day 1 (M0).
(g) Subjects may undergo a brief symptom-directed physical examination. Clinically significant changes from the baseline examination should be recorded in the subject's source documents and electronic "Adverse Event" Case Report Form (CRF).
(h) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature.
(i) 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.
(j) Females 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 trial vaccination at Day 90 (M3). 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.
(k) The blood sample on Day 1 (M0) should be taken prior to administration of Dose 1. The blood sample on Day 120 (M4) should be taken at least 29 days (-1, 7 days) after administration of Dose 2. At Day 270 (M9) a final blood sample will be taken.
(l) Injection site pain, erythema, and swelling assessed by trial staff for 30 minutes post-vaccination.
(m) Diary cards (paper or electronic) will be distributed for the collection of 1) solicited local (injection site) reactions for 7 days (day of vaccination + 6 subsequent days) following each trial vaccination, and 2) solicited systemic events for 14 days (day of vaccination + 13 subsequent days) following each trial vaccination. The investigator will categorize events by severity (mild, moderate or severe) and will assess causality of solicited systemic events to vaccine administration (related or not related).
(n) Unsolicited Adverse Events (AEs) for 28 days (day of vaccination + 27 subsequent days) following each trial vaccination will be collected by interview and recorded for all subjects at Day 30 (M1) and Day 120 (M4). The investigator will categorize events by severity (mild, moderate or severe) and will assess causality to trial vaccine administration (related or not related). If solicited local reactions or systemic events continue on Day 8 or Day 15, respectively, following each trial vaccination, record the end date of the event on the "Adverse Event" CRF.
(o) Serious AEs, AEs leading to subject discontinuation or vaccine withdrawal and Medically Attended AEs and will be collected for the trial duration.

Appendix B Solicited Local (Injection Site) Reactions, Systemic Events and Severity Scales

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 [5].

Table 8.c Severity Scales for 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 [5].

Signature Page for DEN-307 Statistical Analysis Plan, Version 1.0, 15 March 2019

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