Statistical Analysis Plan

NCT Number: NCT03999996

Title: A Phase 3, Follow-Up Trial to Evaluate Long-Term Safety and Antibody Persistence, and the Impact of a Booster Dose of a Tetravalent Dengue Vaccine Candidate in Healthy Adolescents and Adults in Areas Non-Endemic for Dengue.

Study Number: DEN-303

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STATISTICAL ANALYSIS PLAN

STUDY NUMBER: DEN-303

A Phase 3, Follow-Up Trial to Evaluate Long-Term Safety and Antibody Persistence, and the Impact of a Booster Dose of a Tetravalent Dengue Vaccine Candidate in Healthy Adolescents and Adults in Areas Non-Endemic for Dengue

Long-Term Safety and Antibody Persistence of TDV and the Impact of a Booster Dose

PHASE 3

Version: Final, 3.0

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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
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CI	Confidence Interval
CMI	Cell-Mediated Immunity
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CSR	Clinical Study Report
DENV	Wild Type Dengue Virus
DENV-1, -2, -3, -4	Wild Type Dengue Virus Serotypes 1, 2, 3, And 4
EC_{50}	Half Maximal Effective Concentration
eCRF	Electronic Case Report Form
ECG	Electrocardiogram
FAS	Full Analysis Set
FAS-B	Full Analysis Set – after TDV Booster Administration
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IFN-γ ELISPOT	Interferon-Gamma Enzyme-Linked Immunospot
IND	Investigational New Drug
IRT	Interactive Review Board
LAR	Legally Acceptable Representative
LLOD	Lower Limit of Detection
LLOQ	Lower Limit of Quantification
LOD	Limit of Detection
M0, 6, 12, 15, 16, 18, 21, 42, 43, 48	Month 0, 6, 12, 15, 16, 18, 21, 42, 43, 48
MAAE	Medically Attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
MNT_{50}	Microneutralization Test 50%
NC	Negative Control
NS1	Non-Structural protein 1
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal Investigator
PPS	Per-Protocol Analysis Set
PPS-B	Per-Protocol Analysis Set – after TDV booster administration
PT	Preferred Term
SAE	Serious Adverse Event
SAF	Safety Set

SAF-B	Safety Set – after TDV booster administration
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SC	Subcutaneous
SFC	Spot Forming Cells
SOC	System Organ Class
TDV	Tetravalent Dengue Vaccine Candidate
TDV-1	TDV-1 Dengue Serotypes 2/1 Recombinant Chimeric Strain
TDV-2	TDV-2 Molecularly Characterized, Attenuated Dengue Serotype 2 Strain
TDV-3	TDV-3 Dengue Serotypes 2/3 Recombinant Chimeric Strain
TDV-4	TDV-4 Dengue Serotypes 2/4 Recombinant Chimeric Strain
WHODrug	World Health Organization Drug Dictionary

4.0 OBJECTIVES

4.1 Primary Objectives

- To describe antibody persistence for each of the 4 dengue serotypes for up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).
- To describe the impact of a Tetravalent Dengue Vaccine Candidate (TDV) booster dose vs placebo on antibody response for each of the 4 dengue serotypes at 1 month and 6 months post administration of the TDV booster or placebo.

4.2 Secondary Objectives

Immunogenicity:

Antibody Persistence

- To describe the overall trend in antibody decay for all 4 dengue serotypes from values obtained after the primary vaccination series in the parent trials through 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and through 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).

Impact of a TDV Booster Dose

- To describe the impact of a TDV booster on antibody response for each of the 4 dengue serotypes for up to 69 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and for up to 42 months following the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States).

Safety:

- To describe the long-term safety of Takeda's TDV for up to 63 months in previously vaccinated subjects from parent trial DEN-315 (Mexico) and for up to 36 months in previously vaccinated subjects from parent trial DEN-304 (United States).
- To assess safety for 6 months following administration of the TDV booster or placebo in Groups 1 and 2, respectively.

4.3 Exploratory Objectives

Applicable to subjects from parent trial DEN-315 (Mexico only):

- To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 63 months after the first vaccination in the primary vaccination series in the parent trial (DEN-315); this is inclusive of, but not restricted to, an assessment of the anti-dengue Non-Structural protein 1 (NS1) antibody response.

Applicable to subjects from parent trial DEN-304 (United States only):

- To evaluate aspects of the long-term humoral immune response to Takeda's TDV in all subjects at 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304), and in the cell-mediated immunity (CMI) subset at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, an assessment of the anti-dengue NS1 antibody response.
- To evaluate aspects of the long-term cell-mediated immune response to Takeda's TDV up to 36 months after the first vaccination in the primary vaccination series in the parent trial (DEN-304) and at 1 month and 6 months post booster in the current trial; this is inclusive of, but not restricted to, the magnitude (Interferon-gamma Enzyme-Linked Immunospot [IFN-γ ELISpot]) of the long-term T cell-mediated immune response to TDV (CMI subset only).

4.4 Study Design

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

This is a phase 3 follow-up trial that will evaluate the long-term antibody persistence and safety of Takeda's TDV in healthy adolescents and adults in areas non-endemic for dengue, in addition to assessing the impact of a booster dose in this population. Subjects who previously received TDV in two parent trials, DEN-304 and DEN-315, will be invited to participate in this follow-up trial. DEN-303 will include up to 600 healthy subjects aged ≥13 to ≤63 years at trial entry. To enable the assessment of a booster dose, the trial will be double-blinded, randomized, and placebo-controlled from Visit 3 onwards.

Antibody persistence and safety will be assessed from Visit 1 through Visit 3; up to 63 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-315 (Mexico) and up to 36 months after the first vaccination in the primary vaccination series for subjects from parent trial DEN-304 (United States). Further characterization of the long-term humoral and cell-mediated immune responses to Takeda's TDV will be undertaken in a subset of approximately 50 volunteers identified at enrollment (CMI subset: participation is on a voluntary basis from DEN-304 only) up to Visit 5. A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) between site visits and to remind the subject/the subject's LAR of any upcoming site visits. At Visit 2 the site will discuss any information that is pertinent to the booster phase of the trial with the subject. A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18).

At Visit 3, following all scheduled blood draws, all subjects will be screened for 'booster eligibility' to determine if they are eligible to go on to receive the TDV booster in the booster phase. Any subject who fails to meet the criteria for 'booster eligibility' will end the trial at Visit 3. All eligible subjects will be randomized using an interactive response technology (IRT) at Visit 3 to 1 of 2 trial groups (Group 1 and Group 2) in a 1:1 ratio stratified by parent trial and serostatus at baseline in the parent trials. Subjects allocated to Group 1 will receive the TDV booster (single dose) and subjects allocated to Group 2 will receive placebo. The impact of the TDV booster on neutralizing antibody titers and seropositivity rates will be assessed at 1 month and 6 months after administration of the TDV booster or placebo. Safety assessments will continue for 6 months following TDV booster or placebo administration for subjects in Groups 1 and 2.

Immunogenicity evaluation:

- Neutralizing antibodies (by Microneutralization Test 50% [MNT50]) will be measured using blood samples collected from all subjects at Visit 1, Visit 2, and Visit 3, and at Visit 4 and Visit 5 for subjects who are randomized to Groups 1 and 2.
- At Visit 3, a larger volume of blood will be collected from all subjects to assess exploratory markers of the long-term humoral immune response to Takeda's TDV.
- Applicable to subjects from parent trial DEN-304 (United States only): Additional blood samples will be collected from subjects in the CMI subset to allow further characterization of the long-term humoral and cell-mediated immune response to Takeda's TDV at Visit 1, Visit 2, and Visit 3, and in subjects randomized to Groups 1 and 2 at Visit 4 and Visit 5.

Safety evaluation:

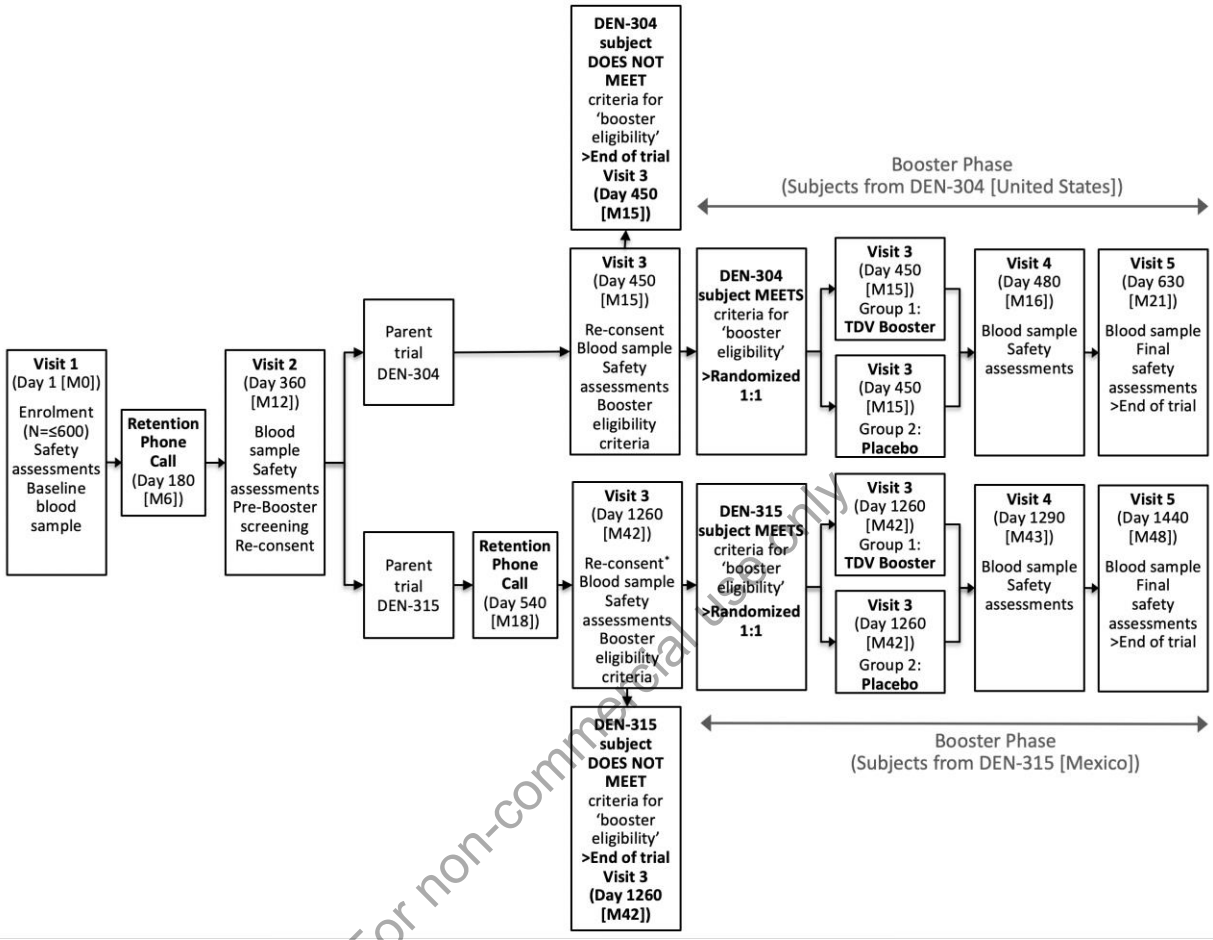
- Diary cards will be distributed to Groups 1 and 2 at Visit 3 for the recording of:
  - Solicited local (injection site) reactions for 7 days (day of vaccination + 6 days) following administration of the TDV booster or placebo. These include: injection site pain, injection site erythema, and injection site swelling.
  - Solicited systemic adverse events (AEs) for 14 days (day of vaccination + 13 days) following administration of the TDV booster or placebo. These include: fever, headache, asthenia, malaise, and myalgia.
- Unsolicited AEs will be collected by interview and recorded for 28 days (day of vaccination + 27 days) following administration of the TDV booster or placebo.
- All serious adverse events (SAEs) and any AEs leading to subject discontinuation and withdrawal will be collected for the current trial duration for all subjects.
- Medically attended adverse events (MAAEs) will be collected, for Groups 1 and 2, following administration of the TDV booster or placebo from Visit 3 through Visit 5. 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.

- For subjects in Groups 1 and 2 the final safety assessments (vital signs, MAAEs, and record SAEs and any AEs leading to subject discontinuation or withdrawal) will be performed approximately 6 months after administration of the TDV booster or placebo at Visit 5.

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

For subjects from parent trial DEN-315 (Mexico), the duration of the current trial will be 42 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 (inclusive of 3 site visits, 3 blood draws) and 48 months for all other subjects (inclusive of 5 site visits, 5 blood draws). For subjects from parent trial DEN-304 (United States), the trial duration will be 15 months for subjects who fail to meet the criteria for 'booster eligibility' at Visit 3 (inclusive of 3 site visits, 3 blood draws) and 21 months for all other subjects (inclusive of 5 site visits, 5 blood draws). A schematic of the trial design is included in Figure 4.a. A schedule of trial procedures is provided in Appendix A.

Figure 4.a Schematic of Trial Design



For all subjects, Visit 1 and Visit 2 correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3, Visit 4, and Visit 5 in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3, Visit 4, and Visit 5 in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series, respectively.

* Due to changes in the trial design in Mexico (protocol amendment 3, dated 22 August 2022), all subjects from parent trial DEN-315 will be asked to re-consent using an updated informed consent form (ICF) or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the eCRF.

5.0 ANALYSIS ENDPOINTS

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

5.1 Primary Endpoints

- Geometric mean titers (GMTs) of neutralizing antibodies (by MNT50) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 1, Visit 2, Visit 3 (prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) summarized for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials.
- GMTs of neutralizing antibodies (by MNT50) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 4 and Visit 5 for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trial.

5.2 Secondary Endpoints

Immunogenicity:

Antibody Persistence

- Geometric Mean Ratio (GMR) of neutralizing antibodies for each of the 4 dengue serotypes for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials for:
  - Visit 1 vs Visit 2.
  - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 3 in the current trial.
  - Day 270 (Month 9) in the parent trials (9 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 1 and Visit 2 in the current trial.

Impact of a TDV Booster Dose

- GMR of neutralizing antibodies for each of the 4 dengue serotypes for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials for:

- Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 4 in the current trial.
- Visit 3 vs Visit 4.
- Visit 3 vs Visit 5.
- Visit 4 vs Visit 5.
- Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 5 in the current trial.

Safety:

- Frequency and severity of solicited local (injection site) AEs for 7 days (day of vaccination + 6 subsequent days) and solicited systemic AEs for 14 days (day of vaccination + 13 subsequent days) after the TDV booster or placebo dose at Visit 3 by trial group.
- Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 subsequent days) after the TDV booster/placebo dose at Visit 3 by trial group.
- Percentage of subjects with any MAAEs following administration of the TDV booster or placebo from Visit 3 through Visit 5 by trial group.
- Percentage of subjects with any SAEs from Visit 1 through Visit 3 prior to administration of the TDV booster or placebo.
- Percentage of subjects with any SAEs following administration of the TDV booster or placebo from Visit 3through Visit 5 by trial group.

5.3 Exploratory Endpoints

Applicable to subjects from parent trial DEN-315 (Mexico only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3. Additional exploratory techniques may be added as the field evolves.

Applicable to subjects from parent trial DEN-304 (United States only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3, and from subjects in the CMI subset at Visit 4 and Visit 5. Additional exploratory techniques may be added as the field evolves.
- The assessment of the long-term cell mediated response to TDV will include, but it is not restricted to, the frequency (percentage of subjects) and magnitude (number of Spot Forming Cells [SFC]/10^6 PBMCs of IFN-γ ELISpot responses to TDV using blood samples collected

from subjects in the CMI subset at Visit 1, Visit 2, Visit 3, Visit 4, and Visit 5. Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥50 spots per 10^6 PBMC. Additional exploratory techniques may be added as the field evolves (CMI subset only).

6.0 DETERMINATION OF SAMPLE SIZE

As the analysis of this trial is descriptive and is not based on testing formal null hypotheses, the sample size was not determined based on formal statistical power calculations. The sample size is considered sufficient for the evaluation of the objectives of the trial.

7.0 METHODS OF ANALYSIS AND PRESENTATION

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

7.1 General Principles

This statistical analysis plan (SAP) was developed based on the information provided in Protocol DEN-303, Version 5.0 dated 22 August 2022 [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 (Statistical Analysis System)® Version 9.4 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 defined in this SAP will be descriptive only.

Blinded data reviews will be conducted prior to unblinding of subject's trial group assignment. These reviews will assess the accuracy and completeness of the trial database and subject evaluability.

7.1.1 Data Presentation

In general, descriptive summaries will be provided by trial group (Group 1: TDV booster; Group 2: placebo) post-vaccination up to Visit 5. For pre-vaccination, summaries up to Visit 3 will be provided.

Unless specified otherwise, number of subjects with non-missing observations, mean or geometric mean, SD (standard deviation) 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 eCRF (Electronic Case Report Form), 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 (confidence interval) 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 and, if not stated otherwise, will be sorted by trial group and/or parent trial ID.

7.1.2 Definition of Study Days

Study Day 1 is defined as the date on which a subject had their first study visit in the current trial. Other study days are defined relative to the Study Day 1 with Day 1 being Study Day 1. Additionally, a booster vaccination study day is defined relative to the date on which a subject had their booster vaccination. These study days will be presented in the listings, where applicable.

7.1.3 Definition of Study Visit Windows

The visit window conventions, as defined in the protocol, will be used to determine the analysis value of a variable for immunogenicity analyses based on the Per-Protocol Set (PPS) and Per Protocol Set-Booster (PPS-B) at a given trial visit. The only exception is that for Visit 3, for subjects from parent trial DEN-304, the visit window considered for analysis inclusion will be the same as for subjects from parent trial DEN-315, ie, it will be extended to -120/+120 days. For analyses based on the Full Analysis Set (FAS), Full Analysis Set-Booster (FAS-B), Safety set (SAF), and Safety Set-Booster (SAF-B), all measurements from scheduled visits will be considered, even if collected outside the protocol defined visit windows.

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. The End of Study visit will be remapped to the next scheduled visit only if the day of the visit falls within the protocol scheduled window. If a scheduled visit and End of Study visit get mapped to the same scheduled visit, the visit closest to the protocol specified scheduled visit date will be used.

7.1.4 Handling of Missing Data

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

Table 7.a Plausible Data Ranges

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

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

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, ie, <10) will be imputed with a value of 5 (ie, half of the LLOD). If a reported value is greater than or equal to the LLOD (LLOD, ≥10) and less than 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 ≥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.

Humoral response data

No imputation methods will be used for missing humoral response data and all analyses will be based on complete records only. Imputation may be applied to negative responses ie, below a positivity cut-off and/or quantification cut-off.

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 vaccination so that the AE can be allocated to either prior or post trial vaccination. Note: The term 'trial vaccination' in the SAP will henceforth refer to the injection of TDV booster or placebo.

An AE should be temporally allocated to prior or post the trial vaccination based on the following rules:

- If the AE start and end dates are both completely missing, the AE will be allocated to post trial vaccination.
- If at least month and year, or just year, of AE start date are available and these match the month/year of the trial vaccination or indicate AE start date is after the trial vaccination, then the AE will be allocated to post trial vaccination. Otherwise, the AE will be allocated to prior trial vaccination.
- If the AE start date is completely missing, or the available start date information is insufficient to distinguish between prior or post trial vaccination, 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 period (prior or post trial vaccination), during which the AE ends. If partial end date information indicates possible association with both prior and post trial vaccination, the AE will be allocated to post trial vaccination.

Prior/concomitant medication/vaccination data

Missing and partial medication/vaccination dates will be assessed to distinguish if a medication or vaccine was started prior to start of the trial (Visit 1) or prior to trial vaccination (Visit 3, ie, prior to the booster vaccination) or concomitantly. A medication will be considered prior to start of the trial (Visit 1) only if the partial end date indicates that it was stopped before the trial starts ie, prior to the signature of the informed consent form (ICF). A medication will be considered prior to trial vaccination only if the partial end date indicates that it was stopped before the trial vaccination (Visit 3, ie, prior to the booster vaccination).

Similar rules will apply to prior vaccines. A vaccine will be considered prior to start of the trial (Visit 1) only if the partial vaccination date indicates that it was given before the start of the trial (ie, prior to the signature of the ICF). A vaccine will be considered prior to trial vaccination only if the partial vaccination date indicates that it was given before the trial vaccination (Visit 3, ie, prior to the booster vaccination). In all other cases medications or vaccines 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 it cannot be concluded from the partial date that the event is clearly a medical history, the event will be considered a concurrent medical condition. In case there is a partial end date available similar rules as the prior/concomitant medication/vaccination data will apply to medical history/concurrent medical conditions.

7.2 Analysis Sets

All Screened: All subjects who agreed to participate in the current trial.

Safety Set (SAF): All subjects who agreed to participate in the current trial, did not screen fail, and who received at least one dose of Takeda's TDV in the parent trials.

Full Analysis Set (FAS): All subjects who did not screen fail and received at least one dose of Takeda's TDV in the parent trials and for whom there is at least one valid follow-up measurement up to and including Visit 3 for immunogenicity assessments in the current trial.

Per Protocol Set (PPS): All subjects from the FAS who received two doses of Takeda's TDV in the parent trials with no new major protocol violations in this trial prior to administration of the trial vaccination at Visit 3 that could potentially confound the primary endpoints in the current trial. The violations are described in Table 7.b.

All Screened-Booster: All subjects who agreed to participate in the current trial and who were screened for 'booster eligibility' to determine if they are eligible to go on to be randomized to Group 1 or Group 2.

Randomized Set-Booster: All subjects randomized at Visit 3 regardless of whether they received the trial vaccination in the current trial. Subjects in this set will be summarized according to randomized treatment.

Safety Set-Booster (SAF-B): All subjects who received at least one dose of Takeda's TDV in the parent trials and who received the trial vaccination in the current trial. Subjects in this set will be summarized according to actual treatment.

Full Analysis Set-Booster (FAS-B): All subjects who received at least one dose of Takeda's TDV in the parent trial, the trial vaccination in the current trial, and for whom there is at least one valid follow-up measurement after administration of the trial vaccination at Visit 3 for immunogenicity assessments in the current trial. Subjects in this set will be summarized according to randomized treatment.

Per Protocol Set-Booster (PPS-B): All subjects from the FAS-B who received two doses of Takeda's TDV in the parent trials with no new major protocol violations after administration of the trial vaccination at Visit 3 that could potentially confound the primary endpoints in the current trial. Subjects in this set will be summarized according to randomized treatment. The criteria (ie, the major protocol violations) are described in Table 7.b.

Table 7.b Criteria for Exclusion from the PPS/PPS-B

Criteria for Exclusion	Probable Method of Identification	PPS	PPS-B
Not receiving at least one dose of TDV in the parent trials [(a)]	Identified programmatically using dosing data	X	X
Not receiving two doses of TDV in the parent trials	Identified programmatically using dosing data	X	X
Not having at least one valid follow-up measurement up to and including Visit 3 [(b)]	Identified programmatically using immunogenicity data	X	NA
Not having at least one valid follow-up measurement post-booster vaccination up to and including Visit 5 [(c)]	Identified programmatically using immunogenicity data	NA	X
Subjects who do not meet all the entry criteria	Identified programmatically using CRF-recorded data; Identified through protocol deviation review/medical review	X	X
Use of prohibited medications/vaccines, or therapies within the relevant periods (PPS: Prior to Visit 1, PPS-B: Prior to Visit 3)	Identified through medical review based on CRF-recorded data	X	X
Subject who is not eligible to receive the IP (TDV booster or placebo) at Visit 3 based on the booster eligibility criteria	Identified programmatically using CRF-recorded data; Identified through protocol deviation review/medical review	NA	X
Not receiving the IP (TDV booster or placebo) [(d)]	Identified programmatically using dosing data	NA	X
Performing Visit 4 outside the visit window (subjects randomized to Groups 1 and 2 only)	Identified programmatically using dosing data.	NA	X
Receiving the incorrect IP	Identified after unblinding	NA	X
Product preparation error	Identified through protocol deviation review/medical review	NA	X

NA = Not applicable.
(a) Subjects with this protocol violation will be excluded from all analysis sets, apart from All Screened.
(b) Subjects with this protocol violation will be excluded from the FAS, and thus also from the PPS.
(c) Subjects with this protocol violation will be excluded from the FAS-B, and thus also from the PPS-B.
(d) Subjects with this protocol violation will be excluded from the SAF-B/FAS-B, and thus also from the PPS-B.

The major protocol violation criterion will be defined as part of blinded data review(s) prior to unblinding and analysis.

CMI subset: The CMI subset is a subset of the PPS and will consist of volunteers from parent trial DEN-304 only. The subjects will be assigned to the CMI subset at trial entry to permit a more detailed characterization of the long-term humoral and cell-mediated immune response to Takeda's TDV (up to and including Visit 3).

CMI-booster (CMI-B) subset: The CMI-B subset is a subset of the PPS-B and will consist of volunteers from parent trial DEN-304 only. This will permit a further detailed characterization of the long-term humoral and cell-mediated immune response to Takeda's TDV (from Visit 3 to Visit 5). Subjects in this set will be summarized according to randomized treatment.

7.3 Disposition of Subjects

Trial information in the current trial 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 last subject's last MNT blood sample collection, the date of the trial vaccination and the date of the first dose in the parent trial. In addition, the Medical Dictionary for Regulatory Activities (MedDRA) version, the World Health Organization Drug Dictionary (WHODrug) version, and the SAS version used for analysis, will be presented, where applicable.

Disposition of all screened subjects will be summarized overall descriptively, including a summary of the number of screened subjects, the number of screen failures, reasons for screen failure, the number of subjects eligible for randomization and the primary reason for not being eligible for randomization.

Additional disposition categories include:

- Number of subjects screened by trial site.
- Number of subjects who prematurely discontinued the trial (including primary reason for premature discontinuation).

Number of subjects in the SAF, FAS, PPS and CMI subset will also be summarized.

Disposition for the randomized set-booster subjects will be summarized overall and by trial group, as applicable. Disposition categories include:

- Number of subjects randomized by trial site.
- Number of subjects randomized and vaccinated.
- Number of subjects randomized, but not vaccinated (including primary reason for being
- randomized, but not vaccinated).
- Number of subjects who completed the trial.
- Number of subjects who prematurely discontinued the trial (including primary reason for premature discontinuation).

Number of subjects in the SAF-B, FAS-B, PPS-B and CMI-B subset will also be summarized.

Significant protocol deviations will be summarized based on the SAF and SAF-B. An additional listing and summary table will be provided including all protocol deviations (significant and non-significant) related to the Coronavirus Disease 2019 (COVID-19) pandemic, if applicable. The listing will be sorted by trial group and/or parent trial ID and will include the date of the protocol deviation, and description of the protocol deviation.

7.4 Demographic and Other Baseline Characteristics

Age (including age at informed consent in the parent trial), gender, race, height, weight, and body mass index (BMI) will be summarized descriptively for all subjects in the current trial, and also presented by parent trial and parent trial baseline serostatus based on the SAF, SAF-B, FAS,

FAS-B, PPS, PPS-B, CMI subset and CMI-B subset. The characteristics of screen failures will also be summarized descriptively for all screened subjects.

7.5 Medical History and Concurrent Medical Conditions

Medical history and concurrent medical conditions will be coded according to the MedDRA coding system. The version of the dictionary used will be specified in the CSR (Clinical Study Report).

All medical history and concurrent medical condition data will be listed based on the general data presentation (Section 7.1.1) and presented based on the SAF/SAF-B, depending on the time of the trial. The listing will also contain SOC, PT, start and stop dates of the medical history and concurrent medical conditions and the study day/booster vaccination study day when the medical history or concurrent medical condition occurred.

Medical History and Concurrent Medical Conditions at the start of the trial

A medical history at the start of the trial is defined as any significant condition/disease that stopped prior to the start of the trial (ie, prior to the signature of the ICF). A concurrent medical condition is defined as any significant condition/disease that is ongoing at the start of the trial (on/after the ICF signature).

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

Medical History and Concurrent Medical Conditions at the time of trial vaccination

A medical history at the time of trial vaccination is defined as any significant condition/disease that stopped prior to the trial vaccination dose. A concurrent medical condition is defined as any significant condition/disease that is ongoing on the day of or after the trial vaccination is administered.

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

7.6 Medication/Vaccination History and Concomitant Medications/Vaccinations

Medication history, vaccine history, concomitant medications, and concomitant vaccines will be coded according to WHODrug. The version of the dictionary used will be specified in the CSR.

Separate listings for medication and vaccination history and concomitant medications and vaccinations will be produced based on the general data presentation (Section 7.1.1) and based on the SAF/SAF-B, depending on the time of the trial. Medication listings will contain ATC code, preferred medication name, dose, frequency, unit, route, stop date and reason for use.

Medication/Vaccination History and Concomitant Medications/Vaccination at the start of the trial

A prior medication/vaccine (history) at the start of the trial is any medication/vaccine for which administration was stopped before the trial start (ie, prior to the signature of the ICF). A concomitant medication/vaccine is any medication/vaccine taken on or after the start of the trial (ie, on/after the ICF signature).

Summary tables for medication history and concomitant medications will be provided by Anatomical Therapeutic Chemical (ATC) class level 2, preferred medication name and parent trial based on the SAF. Vaccine history and concomitant vaccines will be summarized by vaccine type, vaccine name and parent trial as recorded on the CRF based on the SAF.

Medication/Vaccination History and Concomitant Medications/Vaccination at the time of trial vaccination

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

Summary tables for medication history and concomitant medications will be provided by Anatomical Therapeutic Chemical (ATC) class level 2, preferred medication name and parent trial based on the SAF-B. Vaccine history and concomitant vaccines will be summarized by trial group, vaccine type, vaccine name and parent trial as recorded on the CRF based on the SAF-B.

7.7 Investigational Product Exposure and Compliance

The investigator records all injections of the trial vaccination given to the subject on the CRF.

The duration of follow-up (calculated as end of the trial – date of trial vaccination + 1 day) will be summarized for the SAF-B as a continuous variable (number of subjects [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 – 90 days, 91 – 180 days, >180 days.

7.8 Efficacy Analysis

Not applicable.

7.9 Pharmacokinetic/Pharmacodynamic Analysis

Not applicable.

7.10 Other Outcomes

All analyses described in the next sections will be exploratory only.

7.10.1 Primary Immunogenicity Analyses

The primary immunogenicity endpoints of this trial are:

- GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the 4 dengue serotypes and multiple (2, 3 or 4) at Visit 1, Visit 2, Visit 3 (prior to administration of the TDV booster or placebo for subjects randomized to Groups 1 and 2, respectively) summarized for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials.
- GMTs of neutralizing antibodies (by MNT_{50}) for each of the 4 dengue serotypes and seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) for each of the dengue serotypes and multiple (2, 3 or 4) at Visit 4 and Visit 5 for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trial.

For GMTs, prior to trial vaccination, the number of subjects with non-missing assessments, geometric mean with 95% CI, geometric SD, median, minimum and maximum will be presented for all subjects, all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials. The same descriptive statistics will be provided for GMTs assessed at Visit 3 through 5, presented for all subjects by trial group, for all subjects by trial group and parent trial, and for all subjects by trial group and serostatus at baseline in the parent trials. 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).

Number and percentage of seropositive subjects and corresponding 95% CIs calculated by the exact (Clopper-Pearson) method [4] will be presented similarly to the description of the GMTs above.

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 seropositive for:

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

The primary immunogenicity endpoints will be summarized based on either PPS or PPS-B, based on the visit (prior to trial vaccination [Visits 1 to 3], or visit after trial vaccination [Visit 4 and Visit 5]). Similarly, sensitivity analyses, will be provided based on the FAS/FAS-B, respectively.

In addition, GMTs (including 95% CIs) over time (all visits) and reverse cumulative distribution curves (all visits) will be plotted (line plots) by dengue serotype, trial group (only for Visit 4 and

Visit 5), parent trial, serostatus at baseline in the parent trials and visit based on the PPS/PPS-B. Seropositivity rates will be graphically presented by dengue serotype, for at least trivalent, and for tetravalent seropositivity, and by trial group (only for Visit 4 and Visit 5), parent trial, serostatus at baseline in the parent trials and visit using bar graphs including the percentage of subjects seropositive and corresponding 95% CIs.

Additional analyses and/or summary tables may be performed to assess the impact of the COVID-19 pandemic. Those may include sensitivity analyses ignoring the protocol defined visit windows for the PPS/PPS-B for subjects impacted by COVID-19. In addition, depending on the number of subjects who received concomitant COVID-19 vaccination(s), further sensitivity analyses may be performed comparing the subjects with and without a concomitant COVID-19 vaccination(s).

7.10.2 Secondary Immunogenicity Analyses

The secondary immunogenicity endpoints of this trial are:

Antibody Persistence

- GMR of neutralizing antibodies for each of the 4 dengue serotypes for all subjects, for all subjects by parent trial, and for all subjects by serostatus at baseline in the parent trials for:
    - Visit 1 vs Visit 2.
    - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 3 in the current trial.
    - Day 270 (Month 9) in the parent trials (9 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 1 and Visit 2 in the current trial.

Impact of a TDV Booster Dose

- GMR of neutralizing antibodies for each of the 4 dengue serotypes for subjects randomized to Groups 1 and 2 by trial group, by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials for:
    - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 4 in the current trial.
    - Visit 3 vs Visit 4.
    - Visit 3 vs Visit 5.
    - Visit 4 vs Visit 5.
    - Day 120 (Month 4) in the parent trials (4 months after the first vaccination in the primary vaccination series in the parent trials) vs Visit 5 in the current trial.

GMRs and the visit comparisons for both antibody persistence and impact of a TDV booster will be summarized descriptively, similarly to the GMTs in Section 7.10.1.

7.10.3 Exploratory Analyses

The exploratory endpoints of this trial are:

Applicable to subjects from parent trial DEN-315 (Mexico only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3. Additional exploratory techniques may be added as the field evolves.

Applicable to subjects from parent trial DEN-304 (United States only):

- The assessment of the long-term humoral response to TDV will include, but is not restricted to, the measurement of the anti-dengue NS1 antibody response by enzyme-linked immunosorbent assay (average concentration [relative units/mL] of anti-dengue NS1 antibodies for each of the 4 dengue serotypes) using blood samples collected from all subjects at Visit 3, and from subjects in the CMI subset at Visit 4 and Visit 5. Additional exploratory techniques may be added as the field evolves.
- The assessment of the long-term cell-mediated response to TDV will include, but is not restricted to, the frequency (percentage of subjects) and magnitude (number of SFC/10^6 PBMC) of IFN-γ ELISpot responses to TDV using blood samples collected from subjects in the CMI subset at Visit 1, Visit 2, Visit 3, Visit 4, and Visit 5. Cellular immune response is defined as an IFN-γ ELISpot response that is >3 times higher compared with background (no peptide) and ≥50 spots per 10^6 PBMC. Additional exploratory techniques may be added as the field evolves (CMI subset only).

The CMI data up to and including Visit 3 will be summarized overall and based on the CMI subset. CMI data from Visit 3 to Visit 5 will be summarized by trial group and based on the CMI-B subset.

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)

along with exact 2-sided 95% CIs calculated based on the Clopper-Pearson method [4] will be presented as summary tables as well as graphically (bar charts).

The magnitude of the CMI response is measured as number of SFC/10^6 PBMCs. The magnitude of the CMI response of a serotype matching pool is calculated by adding all magnitude measures of each individual peptide within that peptide pool. The number of subjects evaluated [n], min, Q1, median, Q3 and max will be summarized for all subjects as well for positive CMI responders only.

Before summarizing the magnitude of the CMI 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 CMI response will also be graphically presented including min, Q1, median, Q3 and max.

Data on characterization of the humoral immune response will be summarized for all subjects at Visit 3. Humoral immune response will also be summarized for all subjects in the CMI subset at Visit 1, Visit 2 and Visit 3, and all subjects in the CMI-B subset at Visit 4 and Visit 5.

7.11 Safety Analysis

Unless specified otherwise, the summaries of safety will be based on the subjects in SAF-B.

7.11.1 Adverse Events

Solicited local (injection site) and systemic AEs are collected for at least 30 min after trial vaccination at the site (in-clinic assessment) and then using diary cards that are provided to the subject. Unsolicited AEs are collected by interview. Subjects will be evaluated for solicited local (injection site) AEs for 7 days (day of vaccination + 6 days), solicited systemic AEs for 14 days (day of vaccination + 13 days), and unsolicited AEs for 28 days (day of vaccination + 27 days), following trial vaccination. SAEs and AEs leading to trial discontinuation will be collected from first trial visit (Visit 1) until the end of the trial (Visit 5). MAAEs will be collected for each trial group from Visit 3 through Visit 5.

Reactogenicity (Solicited AEs)

Solicited local (injection site) AEs include injection site pain, injection site erythema, and injection site swelling. For the local (injection site) AEs erythema and swelling, the subject/the subject's LAR will record the length of the longest diameter in mm. However, for the analysis these data will be displayed in cm.

Solicited systemic AEs include fever, headache, asthenia, malaise, and myalgia. For the systemic AE fever, the subject/the subject's LAR 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 7.1.4. For each trial group and solicited AE, the denominator for the percentage will exclude subjects with completely missing or implausible 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 within 7 days of IP vaccination for solicited AEs or within 14 days of IP vaccination for systemic AEs 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 (Yes response to CRF question "Is this event a continuation of a Solicited Adverse Event?"). 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, MAAEs, SAEs, and AEs leading to 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 overall for events occurring post-vaccination (Visit 3 until Visit 5) 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 SAF-B.

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

MAAEs post-vaccination 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 will be summarized as follows:

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

AEs leading to trial discontinuation post-vaccination will be summarized as follows:

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

In addition, SAEs and AEs leading to trial discontinuation collected pre-vaccination (up to Visit 3) will be summarized by SOC and PT similarly to events collected post-vaccination (Visit 3 until Visit 5) based on the SAF.

Overview tables overall for events occurring pre-vaccination (Visit 1 until Visit 3) and by trial group for events occurring post-vaccination (Visit 3 until Visit 5) will be generated as outlined in Table 7.c.

Table 7.c Overview of Unsolicited Adverse Events

	All AEs (up to 28 days post-vaccination)	SAEs (pre-vaccination & post-vaccination)	MAAEs (post-vaccination)	AEs leading to trial discontinuation (pre-vaccination & post-vaccination)
Relationship to IP	✓	✓	✓	✓
Relationship to trial procedure	✓	✓	✓	✓
Severity	✓	✓	✓	✓
AEs leading to trial discontinuation	✓	✓	✓	
MAAEs	✓			✓
SAEs and Non-serious AEs	✓			✓
Deaths	✓	✓		✓

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

Based on clinicaltrials.gov results posting requirements, another summary table by SOC and PT for AEs post-vaccination is needed including all non-serious events (ie, all non-serious unsolicited AEs up to 28 days post-vaccination, all MAAEs post-vaccination throughout the trial duration, and all non-serious AEs leading to trial discontinuation post-vaccination throughout the trial duration) with frequency greater than 2% in any trial group.

Selected AE tables may be repeated by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials.

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 and body temperature. Summary statistics (number of subjects [n], mean, SD, median, minimum, and maximum) will be presented by visit in all subjects, and in subjects randomized to Groups 1 and 2 by trial group and visit (observed data and changes from Study Day 1) based on the SAF/SAF-B depending on the time of the visit (prior or post administration of vaccination).

7.11.4 12-Lead ECGs

Not applicable.

7.11.5 Other Observations Related to Safety

Not applicable.

7.12 Interim Analysis

Due to significant delays (of >2 years) to booster administration for subjects from parent trial DEN-315 (Mexico), an interim analysis (IA) of the safety and immunogenicity data is planned when all subjects from parent trial DEN-304 (United States) have completed their last trial visit (Visit 5 [Day 630 (M21)]). This IA will be performed in an unblinded manner and will include the necessary steps to ensure that no database modifications are made after unblinding for subjects at trial sites in the United States. Unblinding of subjects from parent trial DEN-315 (Mexico) will occur after all subjects at trial sites in Mexico have completed their last trial visit (Visit 5 [Day 1440 (M48)]) and the trial database has been locked. No modifications to the trial are planned based on the results of this IA. An interim CSR of data from parent trial DEN-304 (United States) will not be prepared; all trial results will be reported in the final CSR. More details regarding data access management for the IA will be provided in a Data Access Management Plan.

7.13 Changes in the Statistical Analysis Plan

The SAP describes additional analyses/summaries that may be provided to assess the impact of the COVID-19 pandemic, as compared to the protocol.

The SAP also describes additional analysis sets, as compared to the protocol: CMI subset and CMI-booster subset.

7.13.1 Amendment History

Date	Amendment Number
21 Jul 2020	Initial Analysis Plan
24 Jun 2021	1
13 Sep 2022	2

7.13.2 Summary of Changes

This section describes major changes to previous SAP versions.

Final Version	Section	Description of Change
2.0	General	The main rationale for this amended SAP was the amendment to Protocol Version 3.0, dated 09 Mar 2020 to account for the change in the booster dose administration for subjects from parent trial DEN-315 (Mexico). This will now be administered at 45 months after the first vaccination in the primary vaccination series (with an expanded visit window), instead of 36 months. This has an impact to the visit references in most sections of the SAP, hence all changes are not mentioned in this summary.

Final Version	Section	Description of Change
	7.1	Updated the study protocol version and date.
	7.11.1	The general statement that AE summaries will be summarized by trial group after vaccination was deleted as AEs leading to withdrawal and SAEs can also occur from Visit 1 until pre-booster vaccination at Visit 3/3B. Similarly, the general statement that AEs occurring post-vaccination will be presented for each trial group, was also deleted as this is summarized later in Table 7.c. Text was included to describe overall summary tables for unsolicited AEs from Visit 1 until pre-booster vaccination at Visit 3/3B and post-vaccination (Visit 3/3B until Visit 5).
	7.13	Addition of this new section.
	Appendix A	Updated to add new Table 8.a.
3.0	General	The main rationale for this amended SAP was the amendment to Protocol Version 4.0, dated 22 Feb 2021 to account for the change in the booster dose administration for subjects from parent trial DEN-315 (Mexico). This will now be administered at 63 months after the first vaccination in the primary vaccination series (with an expanded visit window), instead of 45 months. The earlier introduced Visit 3B was changed back to Visit 3. Introduction of an interim analysis for subjects from parent trial DEN-304 (United States) once they have completed their last trial visit (Visit 5). Other minor updates/clarifications were made as well.
	4.0, 5.0, 7.0	Updates made to reflect changes in Protocol Version 5.0, dated 22 Aug 2022 related to change in booster administration and naming of visits (eg, changing Visit 3B back to Visit 3).
	7.1.3	Clarification added to analysis visit windows for the PPS and PPS-B for subjects from parent trial DEN-304 (United States) to align with subjects from parent trial DEN-315 (Mexico) as there is no scientific rational why this should be handled in a different way.
	7.10.3	Clarification added to the CMI analysis.
	7.11.1	Clarification added that for some selected AE tables summaries may be repeated by trial group and parent trial, and by trial group and serostatus at baseline in the parent trials.
	7.12	Interim analysis was added.
	7.13	Previous changes in the SAP that were addressed in the latest protocol amendment were removed.
	8.0	Protocol reference was updated.
	Appendix A	Table 8.a was updated.

8.0 REFERENCES

1. A Phase 3 , Follow-up Trial to Evaluate Long-Term Safety and Antibody Persistence, and the Impact of a Booster Dose of a Tetravalent Dengue Vaccine Candidate in Healthy Adolescents and Adults in Areas Non-Endemic for Dengue Takeda Vaccines, Inc. Protocol No. DEN-303, Version 5.0, dated 22 August 2022.
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. Brighton Collaboration Fever Working Group: Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis and presentation. Vaccine. 2004; 22(5-6): 551-556.

Appendix A Schedule of Trial Procedures

Table 8.a Schedule of Trial Procedures

Note, for all subjects Visit 1 (Day 1 [M0]) and Visit 2 (Day 360 [M12]) correspond to 21 months and 33 months after the first vaccination in the primary vaccination series in the parent trial. For subjects from parent trial DEN-315 (Mexico), Visit 3 (Day 1260 [M42]), Visit 4 (Day 1290 [M43]), and Visit 5 (Day 1440 [M48]) in this trial correspond to 63 months, 64 months, and 69 months after the first vaccination in the primary vaccination series, respectively. For subjects from parent trial DEN-304 (United States), Visit 3 (Day 450 [M15]), Visit 4 (Day 480 [M16]), and Visit 5 (Day 630 [M21]) in this trial correspond to 36 months, 37 months, and 42 months after the first vaccination in the primary vaccination series in the parent trial, respectively.

Visit **By parent trial (DEN-304/DEN-315)**	**1** **(Day 1[M0]/ Day 1[M0])**	**2 [a]** **(Day 360 [M12]/ Day 360 [M12])**	**3 [b]** **(Day 450 [M15]/ Day 1260 [M42])**		**4 [c]** **(Day 480 [M16]/ Day 1290 [M43])**	**5 [c]** **(Day 630 [M21]/ Day 1440 [M48])**
Visit Window (Days)	**-15/+75 (Linked to Parent Day 1)**	**-45/+45 (Linked to Visit 1)**	**-45/+45 (Linked to Visit 1)**	**-120/+120 (Linked to Visit 1)**	**-1/+7 (Linked to Visit 3)**	**-0/+60 (Linked to Visit 3)**
Applicable to subjects from parent trial	**DEN-304 DEN-315**	**DEN-304 DEN-315**	**DEN-304**	**DEN-315**	**DEN-304 DEN-315**	**DEN-304 DEN-315**
Site visit	X	X	X		X	X
Phone contact	X [d]	X [d]				
Signed Informed Consent /Pediatric Assent Form [e]	X		X			
Eligibility criteria	X [f]		X [g]			
Randomization [h]			X			
Demographics	X					
Pregnancy test [i]			X			
Medical history update	X [j]		X [c]			
Prior medication update	X [j]		X [c]			
Concomitant medication update [k]		X	X [c]		X	
Concomitant vaccination update [l]	X	X	X		X	X
Complete physical examination [m]	X		X[c]			
Targeted physical examination [n]					X	X
Vital signs [o]	X	X	X		X	X
Review of systems			X [c]			X
Pregnancy avoidance guidance [p]		X	X [c]		X	

Visit **By parent trial (DEN-304/DEN-315)**	**1** **(Day 1[M0]/ Day 1[M0])**	**2** [a] **(Day 360 [M12]/ Day 360 [M12])**	**3** [b] **(Day 450 [M15]/ Day 1260 [M42])**		**4** [c] **(Day 480 [M16]/ Day 1290 [M43])**	**5** [c] **(Day 630 [M21]/ Day 1440 [M48])**
Visit Window (Days)	**-15/+75 (Linked to Parent Day 1)**	**-45/+45 (Linked to Visit 1)**	**-45/+45 (Linked to Visit 1)**	**-120/+120 (Linked to Visit 1)**	**-1/+7 (Linked to Visit 3)**	**-0/+60 (Linked to Visit 3)**
Applicable to subjects from parent trial	**DEN-304 DEN-315**	**DEN-304 DEN-315**	**DEN-304**	**DEN-315**	**DEN-304 DEN-315**	**DEN-304 DEN-315**
Investigational product [q] administered by SC injection			X [c]			
Injection site evaluation [r]			X [c]			
Diary card [s] — Distribution			X [c]			
Diary card [s] — Review/collection of solicited local reactions and systemic AEs			X [c]		X	
Unsolicited AEs [t]			X [c]		X	
MAAEs [u]			X [c]		X	X
SAEs and AEs leading to subject discontinuation or withdrawal [v]	X	X	X		X	X
Criteria for delay of blood sampling	X	X	X		X	X
Blood draw (10 mL) [w]	X	X	X		X	X
Blood draw (40 mL) [w]			X			
Additional blood draw (10 mL CMI subset only, n=50) [x]					X	X
Additional blood draw (40 mL CMI subset only, n=50) [x]	X	X	X		X	X

AE: Adverse event; CMI: Cell mediated immunity; M: Month; MAAE: Medically attended adverse event; SAE: Serious adverse event; SC: Subcutaneous.

(a) At Visit 2, subjects will be reminded of any issues relating to administration of/or eligibility for the upcoming booster dose at Visit 3. This includes, but is not limited to, the collection of any SAEs and any AEs leading to subject discontinuation or withdrawal, a review of prohibited therapies, pregnancy avoidance guidance and information on acceptable methods of contraception (for female subjects of childbearing age).
(b) Visit 3 will be delayed until Day 1260 (M42) for subjects from parent trial DEN-315 (Mexico).
(c) Applicable only to subjects randomized to Group 1 or Group 2 at Visit 3.
(d) A retention phone call will be made between Visits 1 and 2 on Day 180 (M6) to maintain contact with the subject/the subject's legally acceptable representative (LAR) and to remind the subject/the subject's LAR about the upcoming site visit. A second retention phone call will be made to subjects from parent trial DEN-315 (Mexico) between Visits 2 and 3 on Day 540 (M18).
(e) 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. Adolescents from parent trial DEN-315 (Mexico) who become 18 years of age during the course of the trial will be asked to return to the investigational site for an additional site visit to provide the appropriate written informed consent. This should be done as soon as possible after their 18th birthday. Due to changes in the trial design in Mexico (protocol amendment 3, dated 22 Aug 2022), all subjects from parent trial DEN-315 (Mexico) will now be asked to re-consent using an updated ICF or an updated informed consent and pediatric assent form, as applicable, at Visit 3 before any further protocol-directed procedures are performed. An oral summary of any major changes that have been made to the study will be provided to the subject and the subject's LAR where applicable in addition to the ICF/Assent Form; this will be documented in the medical chart as part of the re-consent process. Re-consent date should also be documented in the electronic Case Report Form (eCRF).
(f) After informed consent has been obtained, eligibility of the subject will be assessed by review of inclusion/exclusion criteria for trial entry at Visit 1.
(g) A review of the 'booster eligibility' will be performed prior to randomization at Visit 3.
(h) Stratified by parent trial and serostatus at baseline in the parent trials.
(i) For female subjects of childbearing potential, pregnancy testing (urine) will be performed at Visit 3. Results must be confirmed and documented as negative prior to administration of the TDV booster or placebo at Visit 3. Additional pregnancy tests may be performed during the trial if deemed necessary by the investigator; where the results of a urine pregnancy test are in doubt, a serum pregnancy test will be performed to verify the result.
(j) Any relevant information collected during the parent trials will be accessed via the database and updated as necessary throughout the trial conduct.
(k) All medications from 1 month (minimum 28 days) prior to administration of the TDV booster or placebo at Visit 3 and up to 1 month (minimum 28 days) thereafter; steroids and immunostimulants within 60 days prior to Visit 3; immunoglobulins and blood products within 3 months prior to Visit 3; and immunosuppressive therapy within 6 months prior to Visit 3.
(l) Any inactivated vaccines administered ≤14 days and any live attenuated vaccines administered ≤28 days prior to blood sample collection or TDV booster/placebo administration at Visit 3.
(m) Physical examination including measurement of weight and height – body mass index will be calculated automatically.
(n) Subjects may undergo a targeted symptom-directed physical examination. Clinically significant changes from the baseline examination (Visit 1) should be recorded in the subject's source documents and eCRF.
(o) Vital signs including (but not limited to) the measurement of systolic blood pressure/diastolic blood pressure, heart rate, and body temperature.
(p) 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 at Visit 1 stating that they understand the requirements for avoidance of pregnancy and donation of ova. Further guidance with respect to the avoidance of pregnancy will be provided to all female subjects of childbearing potential at Visit 2 and to all female subjects of childbearing potential who are randomized to Group 1 or 2 at Visit 3, and at Visit 4. Females of childbearing potential, who are randomized to Group 1 or 2 and are sexually active, will also be reminded to adhere to acceptable contraceptive methods for up to 6 weeks after TDV booster or placebo administration.
(q) Subjects who meet the criteria for 'booster eligibility' and are subsequently randomized to Group 1 or Group 2 at Visit 3 will receive the TDV booster or placebo, respectively.
(r) Injection site pain, erythema, and swelling assessed by trial staff for 30 minutes post-vaccination.

(s) Diary cards (paper or electronic) will be distributed to subjects in Groups 1 and 2 at Visit 3 for the collection of 1) solicited local (injection site) reactions for 7 days (day of vaccination + 6 subsequent days) following TDV booster or placebo administration, and 2) solicited systemic AEs for 14 days (day of vaccination + 13 subsequent days) following TDV booster or placebo administration. The investigator will assess causality of solicited systemic AEs to vaccine administration (related or not related).

(t) Unsolicited AEs will be collected by interview and recorded for Groups 1 and 2 for 28 days (day of vaccination + 27 subsequent days) following TDV booster or placebo administration. The investigator will categorize each event by severity (mild, moderate or severe) and will assess causality to trial vaccine administration (related or not related). If solicited local (injection site) reactions and systemic AEs continue on Day 8 and Day 15 (after administration of the TDV booster or placebo), respectively, record the full duration of the event on the "Adverse Event" eCRF.

(u) MAAEs will be collected for Groups 1 and 2 from Visit 3 through Visit 5.

(v) Any SAEs and any AEs leading to subject discontinuation or withdrawal will be collected for all subjects for the trial duration.

(w) At Visit 3, a 10 mL blood sample and 40 mL blood sample will be taken from all subjects. For those subjects who are randomized to Groups 1 and 2 at Visit 3, all blood samples should be taken prior to administration of the TDV booster dose or placebo. The blood samples taken at Visit 4 should be taken at least 29 days (-1, +7 days) after administration of the TDV booster or placebo at Visit 3.

(x) One additional 40 mL blood sample will be collected from a subset of 50 subjects (CMI subset; from DEN-304 only) for exploratory immunogenicity analyses from Visit 1 through Visit 5. An additional 10 mL blood sample will also be collected from subjects in the CMI subset at Visit 4 and Visit 5 for further exploratory immunogenicity analyses.

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

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

Solicited local (injection site) AEs:	Pain
	Erythema
	Swelling
Solicited systemic AEs:	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 of Solicited Safety Parameters

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

NA = not applicable
(a) Subjects are to record greatest surface diameter in mm on the diary card.
(b) Fever is defined as a body temperature ≥38°C (100.4°F) regardless of the method used [5].

Signature Page for DEN-303 Statistical Analysis Plan, Version 2.0, 24 June 2021

Title:

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