Statistical Analysis Plan

NCT Number: NCT06060067

Title: A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial to Investigate the Safety and Immunogenicity of a Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) Administered Subcutaneously to Healthy Subjects Aged 4 to 60 Years in India

Study Number: DEN-302

Document Version and Date: Version 3.0, 09 January 2025

Certain information within this document has been redacted (ie, specific content is masked irreversibly from view) to protect either personally identifiable information or company confidential information.

STATISTICAL ANALYSIS PLAN

Study Number: DEN-302

Study Title: A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial to Investigate the Safety and Immunogenicity of a Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) Administered Subcutaneously to Healthy Subjects Aged 4 to 60 Years in India

Phase: 3

Version: 3.0

Date: 09-Jan-2025

Prepared by: [redacted], MSc

[redacted], Vaccine Statistics

Statistical and Quantitative Science

Based on:

Protocol Version: Amendment 1.0

Protocol Date: 15-June-2023

REVISION HISTORY

Version	Finalization Date	Primary Rationale for Revision
Original version	30-May-2022	Not Applicable
Amendment 1	14-Sept-2023	The main rationale for this amended SAP was the amendment to the trial protocol removing the Day 30 blood sample draw to comply with a request made by Indian Central Drugs Standard Control Organization (CDSCO) following a meeting of the Subject Expert Committee on 16 May 2023.
Amendment 2	09-Jan-2025	[redacted]

TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF ABBREVIATIONS

AE	adverse event
ATC	Anatomical Therapeutic Chemical
CDSCO	Central Drugs Standard Control Organization
CI	confidence interval
COVID-19	coronavirus disease 2019
CRF	case report form
CRO	contract research organization
CSR	clinical study report
DAMP	Data access management plan
DENV	wild type dengue virus
DENV-1, -2, -3, -4	wild type dengue virus serotypes -1, -2, -3, -4
ECG	electrocardiogram
FAS	full analysis set
GM	geometric mean
GMT	geometric mean titer
GMR	geometric mean ratio
GSD	geometric standard deviation
ICH	International Conference on Harmonisation
LLOD	lower limit of detection
LLOQ	lower limit of quantification
M	month
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
SC	subcutaneous
SD	standard deviation
SOC	System Organ Class
TDV	Dengue Tetravalent Vaccine (Live, Attenuated), the Takeda dengue vaccine candidate also known as TAK-003, is referred to as TDV
TLF	tables, listings, and figures
WHODrug	World Health Organization Drug Dictionary

1.0 OBJECTIVES, ENDPOINTS AND ESTIMANDS

1.1 Objectives

1.1.1 Primary Objective(s)

Safety

- *To assess the safety profile of TDV administered as 2 doses given 3 months apart in healthy adults, adolescents, and children.*

Immunogenicity

- *To evaluate the immunogenicity of TDV administered as 2 doses given 3 months apart in healthy adults, adolescents, and children at 1 month post second dose.*

1.1.2 Secondary Objective(s)

Immunogenicity

- *To describe immunogenicity of TDV at baseline and 6 months post second TDV dose when administered as 2 doses given 3 months apart.*
- *To describe seropositivity (% of subjects with reciprocal neutralizing titer ≥10) at baseline, 1 month post second TDV dose, and 6 months post second TDV dose.*

1.2 Endpoints

1.2.1 Primary Endpoint(s)

Safety

- *Frequency and severity of solicited local (injection site) adverse events (AEs) for 7 days (day of vaccination + 6 subsequent days) and solicited systemic AEs for 14 days (day of vaccination + 13 subsequent days) postvaccination at Day 1 (Month [M]0) and Day 90 (M3).*
- *Percentage of subjects with any unsolicited AEs for 28 days (day of vaccination + 27 subsequent days) postvaccination at Day 1 (M0) and Day 90 (M3).*
- *Percentage of subjects with an AE leading to subject withdrawal from the trial from Day 1 (M0) postvaccination through the end of the trial.*
- *Percentage of subjects with an AE leading to TDV or placebo discontinuation from Day 1 (M0) postvaccination through the end of the trial.*
- *Percentage of subjects with a medically-attended AE (MAAE) from Day 1 (M0) postvaccination through the end of the trial.*
- *Percentage of subjects with a serious adverse event (SAE) from Day 1 (M0) postvaccination through the end of the trial.*

Immunogenicity

- *Geometric mean titers (GMTs) of neutralizing antibodies (by microneutralization test [MNT_{50}]) against each of the 4 dengue virus serotypes at Day 120 (M4).*

1.2.2 Secondary Endpoint(s)

Immunogenicity

- *GMTs by MNT_{50} against each of the 4 dengue virus serotypes at Day 1 (M0) and Day 270 (M9).*
- *Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against each of the 4 dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).*
- *Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against multiple (2, 3, or 4) dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).*

1.2.3 [redacted]

[redacted]

1.3 Estimand(s)

Not applicable.

2.0 STUDY DESIGN

This is a phase 3, randomized, multi-site, double-blind, placebo-controlled trial in 480 healthy subjects aged ≥4 to ≤60 years living in India.

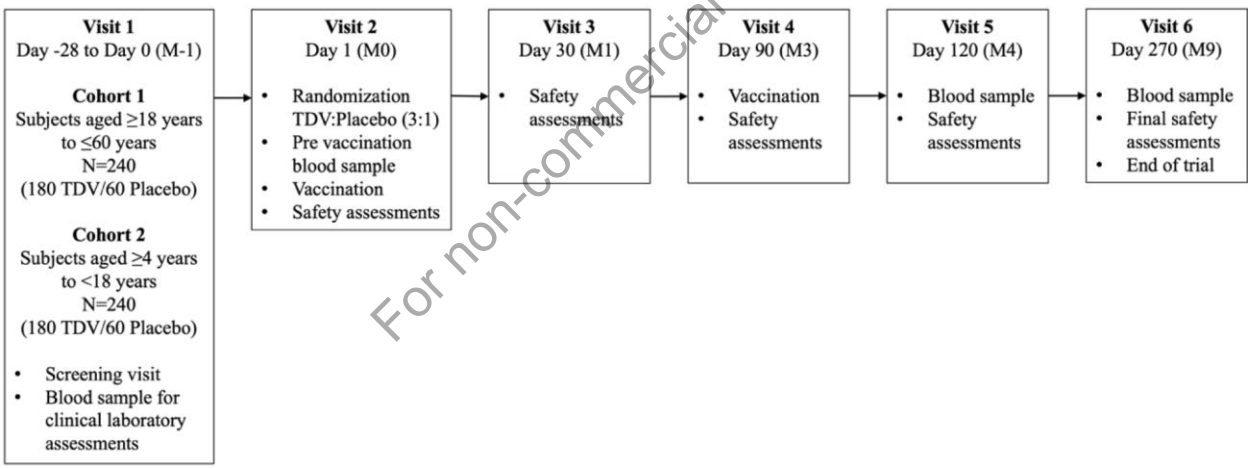
The vaccine will be administered as a 2-dose regimen, 3 months (ie, 90 days) apart, by subcutaneous (SC) injection. Subjects will be randomized into cohorts 1 and 2, in parallel.

Cohort 1: *240 subjects ≥18 to ≤60 years of age will be enrolled and randomly assigned (3:1) to receive either TDV (N=180) or placebo (N=60) at Day 1 (M0) and Day 90 (M3).*

Cohort 2: *240 subjects ≥4 to <18 years of age will be enrolled and randomly assigned (3:1) to receive either TDV (N=180) or placebo (N=60) at 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), or end of trial. *The trial duration will be approximately 270 days (9 months) for each subject. A schematic of the trial design is included in* Figure 2.a.

Figure 2.a Schematic of Trial Design



Abbreviations: M, Month; N, Number of subjects; TDV, Takeda's Dengue Tetravalent Vaccine (Live, attenuated).

Immunogenicity evaluation (all subjects):

Neutralizing antibodies will be measured (by MNT_{50}) using blood samples obtained pre vaccination at Day 1 (M0), 1 month post second vaccination at Day 120 (M4), and at the end of trial (Day 270 [M9] or earlier for early terminations).

Safety evaluation (all subjects):

Diary cards will be distributed at Day 1 (M0) and Day 90 (M3) for the recording of:

- *Solicited local (injection site) AEs for 7 days following administration of TDV or placebo (day of vaccination + 6 days). These will include:*
  - *Injection site pain, injection site erythema, and injection site swelling.*
- *Solicited systemic AEs for 14 days following administration of TDV or placebo (day of vaccination + 13 days). These will include:*
  - *Subjects <6 years of age: fever, irritability/fussiness, drowsiness, and loss of appetite.*
  - *Subjects ≥6 years of age: asthenia, fever, headache, malaise, and myalgia.*

Unsolicited AEs will be collected by interview and recorded for 28 days following administration of TDV or placebo (day of vaccination + 27 days).

All SAEs, MAAEs, AEs leading to subject withdrawal from the trial, and AEs leading to TDV or placebo discontinuation will be collected from Day 1 (M0) postvaccination through the end of the trial. 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.

A blinded interim analysis of safety data will be prepared when all subjects have completed the Day 120 (M4) visit. Details related to the interim analysis are provided in Section 6.7.

Inclusion/exclusion criteria will be checked at trial entry and at first trial dose vaccination (Day 1 [M0]) to confirm subjects are eligible.

3.0 STATISTICAL HYPOTHESES AND DECISION RULES

No inferential analyses will be performed for this trial, ie, all analyses described in this statistical analysis plan (SAP) will be descriptive only. Therefore, no statistical hypotheses, statistical decision rules or multiplicity adjustments are required.

4.0 SAMPLE-SIZE DETERMINATION

The analysis of this trial is descriptive and is not based on testing formal null hypotheses. [redacted] *The number of subjects receiving placebo will be 60 in each cohort (N=120 in total), as per the random assignment of 3:1 (TDV:placebo). Therefore, the total trial sample size is 480 subjects.*

5.0 ANALYSIS SETS

5.1 All Screened

The All Screened Set will consist of all subjects who signed the informed consent, regardless of whether they were screen failures.

5.2 Randomized Set

The Randomized Set will include all randomized subjects, regardless of whether a dose of TDV or placebo was received.

5.3 Safety Analysis Set

The Safety Set will consist of *all subjects who received at least 1 dose of TDV or placebo.*

5.4 Full Analysis Set

The Full Analysis Set (FAS) will consist of *all randomized subjects who received at least 1 dose of TDV or placebo, and for whom a valid pre dose* measurement*, and at least 1 valid post dose* measurement *are received for immunogenicity assessments.*

5.5 Per-Protocol Analysis Set

The Per-Protocol Analysis Set (PPS) will consist of *all subjects in the FAS who have no major protocol violations.*

The provisional criteria for major protocol violations, as described in Table 5.a, will be used to identify the subjects who will be excluded from the PPS. These subjects will be identified during the blinded data review (ie, prior to the unblinding of the subject's assignment to TDV or placebo). Other major protocol violations may be identified during the blinded data review and/or during the periodic reviews of the deviation logs conducted 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.

Table 5.a Criteria for Exclusion from the PPS

Criteria for Exclusion	Probable Method of Identification
Not receiving at least one dose of the trial vaccine [(a)]	Identified programmatically using dosing data
Not having a valid pre-dose (baseline) and at least 1 valid post-dose measurement [(b)]	Identified programmatically using immunogenicity data
Not having a valid immunogenicity assessment at 1 month post vaccination 2 (ie, Day 120 [M4])	Identified programmatically using immunogenicity data
Not receiving both doses of the trial vaccine	Identified programmatically using dosing data
Receiving the second trial vaccination inadmissibly outside of the scheduled visit window (ie, outside Day 90 [-15/+25 days])	Identified programmatically using dosing data
Receiving the incorrect trial vaccine(s) for Vaccination 1 and/or Vaccination 2	Identified after unblinding
Product preparation error	Identified through protocol deviation review/medical review
Subject meets any of the following exclusion criteria: 1, 2a, 2c, 2d, 5, 6, 7, 13, 14	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

Abbreviations: CRF, case report form; M, month; PPS, per-protocol set.

(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.

Reasons for exclusion of subjects from analysis sets will be summarized based on the Randomized Set following the description provided in Section 6.1 for continuous and categorical variables.

6.0 STATISTICAL ANALYSIS

6.1 General Considerations

This SAP was developed based on the information provided in Protocol DEN-302, Version 2.0, dated 15 June 2023 [1] and on International Conference on Harmonisation (ICH) E3 [2] and E9 [3] Guidelines.

Summary tables for categorical variables will display both frequencies (n) and percentages (%). For those categorical variables with defined categories in the case report form (CRF), all possible categories will be displayed, even if the subject count is zero. For any other categorical variables recorded (eg, category of AE or medication/vaccination), only categories with at least 1 subject count will be displayed. Unless otherwise specified, percentages will be presented with 1 decimal place (eg, 80.3%), percentages that round to <0.1 will be reported as "<0.1" and percentages for counts of zero (n=0) will be left blank.

Summary tables for continuous variables will display the number (n) of subjects with non-missing values, means or geometric means (GM), medians, SD or geometric standard deviations (GSD), and minimum and maximum values. Minimum and maximum values will be presented with the same number of decimal places as the recorded data. Means, geometric means, and medians will be presented with 1 more decimal place than the recorded data. SD will be presented with 2 more decimal places than the recorded data. Decision on decimal precision for derived data (ex. log-transformed) may be addressed during review of blinded data/table review.

Summaries for selected immunogenicity and safety variables may also include the CI around parameter estimates (means or percentages). The CI will be presented with the same number of decimal places as the parameter estimate itself.

All collected data will be displayed in the listings sorted by cohort, age group if where applicable, trial group, site number, subject number, and by date/time of the recorded event if where applicable (eg, date/time of vaccination, date/time of blood draw, date/time of AE). If applicable, screen failure subjects will be grouped and listed separately, at the end.

In all outputs, trial groups will be labeled as:

- TDV
- Placebo

Similarly, in all outputs the cohort will be presented and labeled as follows (depending on the cohort):

- Cohort 1: ≥18 to ≤60 years of age.
- Cohort 2: ≥4 to <18 years of age.

And if applicable (eg, if requested by the local health authority), by the following age subgroups:

- ≥12 to <18 years of age.
- ≥4 to <12 years of age.
- ≥6 years of age.

Solicited local (injection site) AEs and solicited systemic AE outputs will be presented as follows:

- ≥4 to <6 years of age.
- ≥6 years of age.

In general, summaries will be presented by cohorts combined (≥4-≤60 years of age) and trial group (TDV; Placebo), followed by each individual cohort and trial group, unless otherwise specified. If requested by the local health authority, subgroup analyses will also be presented by age group and trial group.

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) measurements taken after the first trial vaccination are considered post-baseline values.

A windowing convention for immunogenicity and safety (laboratory tests and vital signs) data will be used to determine the analysis value of a variable for a given trial visit. Details of this are described in Section 9.2.3.

6.1.1 Handling of Treatment Misallocations

Summary tables generated for the Randomized Set will present trial groups "as randomized", ie, according to the vaccination a subject was assigned to receive, which may be different from the vaccination the subject actually received. For example, a subject randomized to TDV but vaccinated with placebo will be analyzed in the TDV group.

All summaries generated for the Safety Set will present trial groups "as treated", ie, according to the trial vaccine the subject actually received rather than the trial vaccine to which the subject was randomized. For example, a subject assigned randomly to TDV but vaccinated at both vaccination visits with placebo will be analyzed in Placebo.

Subjects who received different vaccinations at the first and second vaccinations (if any, eg, subject vaccinated with TDV at the first vaccination and with placebo at the second vaccination or vice versa) will be considered in a separate group. Data for this group will be labelled as "Unplanned vaccine sequence" and will be displayed in selected summaries, all listings, and all subject mappings generated for the Safety Set.

Summary tables generated for the FAS and PPS will present trial groups "as randomized".

6.2 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 and the date of last subject's last procedure for collection of data for the primary endpoint. In addition, the Medical Dictionary for Regulatory Activities (MedDRA) version, the World Health Organization Drug Dictionary (WHODrug) version, and the SAS version used for the analyses will also be presented.

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

Disposition for all randomized subjects will be summarized and will include the following disposition categories:

- Number of subjects randomized by trial site.
- Number of subjects randomized, but not vaccinated (including primary reason for being randomized, but not vaccinated).
- Number of subjects in the Randomized Set, Safety Set, FAS and PPS (including reason for exclusion, based on the Randomized Set).
- Number of subjects who completed the vaccination regimen/trial.
- Number of subjects who prematurely discontinued the vaccination regimen/trial (including primary reason for premature discontinuation).
- Number of significant protocol deviations based on the Randomized Set.

An additional listing and summary table may be provided including all protocol deviations (significant and non-significant) related to any exceptional circumstances (eg, the Coronavirus Disease 2019 [COVID-19] pandemic), if applicable.

Analyses based on the Safety Set (except for AEs), FAS and PPS will include measurements obtained following the analysis visit windows defined in Table 9.b only.

6.3 Demographic and Other Baseline Characteristics

6.3.1 Demographics

Demographic characteristics including age, sex, race, ethnicity, weight, height and BMI will be summarized descriptively based on the Randomized Set, Safety Set, FAS and PPS. Age groups (≥18-≤60/≥4-<6/≥4-<12/≥4-≤16/≥4-<18/≥6/≥6-<12/≥12-≤16/≥12-<18), may also be

summarized, as required. The statistics presented are described in Section 6.1 for continuous and categorical variables.

6.3.2 Medical History and Concurrent Medical Conditions

Medical history and concurrent medical conditions will be coded using the MedDRA coding system. The version used of the coding dictionary will be identified on the applicable listings and/or tables.

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

Summary tables will be provided by System Organ Class (SOC) and Preferred Term (PT) based on the Safety Set.

In case the "End Date" field is missing on the medical history/concurrent medical conditions form of the CRF then the "End Date Relative to Signing informed Consent" field will be used to determine a medical history or concurrent medical condition. In case the "End Date" field is a partial date and it can't be concluded that the event is clearly a medical history, then the event will be considered as a concurrent medical condition.

6.3.3 Baseline Characteristics

The summaries of demographic characteristics will include the following baseline characteristics: overall dengue serostatus (seropositive [reciprocal neutralizing titer ≥10 for at least 1 dengue serotype] or seronegative [reciprocal neutralizing titer <10 for all dengue serotypes]), dengue serostatus for each serotype and for multiple serotypes. The statistics presented will be as described in Section 6.1.

6.4 Medication History and Concomitant Medications

Medication history, vaccine history, concomitant medications, and concomitant vaccines will be coded using the WHO Drug coding system. The version used of the coding dictionary will be identified on the applicable listings and/or tables.

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

Summary tables for medication history and concomitant medications will be provided by Anatomical Therapeutic Chemical (ATC) 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.

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 as 'prior' only if the partial end date indicates that it was stopped before the first trial vaccination. A vaccine will be considered as 'prior' only if the partial vaccination date indicates that it was given before the first trial vaccination. In all other cases the medication(s) or vaccine(s) will be considered concomitant.

6.5 Immunogenicity Analysis

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 ≥LLOD and below the lower limit of quantification (LLOQ, which differs between serotypes), then 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 (the LLOD) and <18 (the LLOQ) will be imputed with a value of 14 for this serotype.

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

6.5.1 Primary Endpoint(s) Analysis

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

The number (n) of subjects with non-missing assessments, GM with 95% CI, GSD, 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 supplementary analysis will be provided using the FAS instead.

Potential sensitivity analyses may be performed to assess the effect of an exceptional circumstance (eg, the COVID-19 pandemic), if applicable.

6.5.2 Secondary Endpoint(s) Analysis

The secondary immunogenicity endpoints are:

- *GMTs by MNT_{50} against each of the 4 dengue virus serotypes at Day 1 (M0) and Day 270 (M9).*
- *Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against each of the 4 dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).*
- *Seropositivity rates (% of subjects with reciprocal neutralizing titer ≥10) against multiple (2, 3, or 4) dengue virus serotypes at Day 1 (M0), Day 120 (M4), and Day 270 (M9).*

GMTs of dengue neutralizing antibodies (derived from dengue MNT_{50} results) on Day 1 (M0), and Day 270 (M9) will be derived and analyzed descriptively, in analogy to the primary immunogenicity endpoint.

In addition, GMTs (including 95% CIs) over time (all visits) and reverse cumulative distribution curves (for all visits except baseline) will be plotted (using line plots) by dengue serotype and visit based on the PPS.

For the seropositivity rates for each dengue serotype, the percentage of seropositive subjects and the exact 2-sided 95% CIs will be presented by visit. The exact 2-sided 95% CI of seropositivity rate will be calculated based on the 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).

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.

6.5.3 [redacted]

[redacted]

6.5.4 Subgroup Analyses

Subgroup analyses will be performed, for any of the above primary or secondary endpoints, by age group and trial group (TDV; Placebo).

For the clinical study report (CSR), the age groups will be as follows:

- ≥18 to ≤60 years of age.
- ≥4 to <18 years of age.
- ≥4 to <6 years of age.
- ≥6 to <12 years of age.
- ≥12 to <18 years of age.

The results of the above age group analyses will be discussed in the CSR.

[redacted]

6.6 Safety Analysis

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

6.6.1 Adverse Events

The primary safety endpoints are listed in Section 1.2.1.

Unless otherwise specified, AEs will be summarized after the first trial vaccination, after the second trial vaccination, and after any trial vaccination. In general, summaries will be presented by age group and trial group (TDV; Placebo).

For the clinical study report (CSR), the age groups will be as follows:

- ≥18 to ≤60 years of age.
- ≥4 to <18 years of age (except for solicited local [injection site] AEs and solicited systemic AEs).
- ≥4 to <6 years of age.
- ≥6 to <12 years of age.
- ≥12 to <18 years of age.

The results of the above age group and trial group presentations will be discussed in the CSR.

To answer potential requests from local health authorities, additional presentations by age group and trial group will be presented for:

- ≥4 to ≤60 years of age (except for solicited local [injection site] AEs and solicited systemic AEs).
- ≥4 to <12 years of age (except for solicited local [injection site] AEs and solicited systemic AEs).
- ≥6 years of age.

[redacted]

Reactogenicity (Solicited AEs)

Solicited AEs will be collected for at least 30 minutes after each vaccination at the site (in-clinic assessment). In addition, subjects will be 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 will also be provided with a diary card for the recording of solicited systemic AEs (<6 years of age: fever, irritability/fussiness, drowsiness, and loss of appetite; ≥6 years of age: fever, asthenia, malaise, headache, 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. 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 the severity of solicited safety parameters are given in Section 9.2.4.

Missing data for solicited AEs will not be imputed unless otherwise specified. For each solicited AE, the denominator for the percentage will exclude subjects with completely missing data (ie, a subject that 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 postvaccination:

- 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 to 7 (daily, solicited local [injection site] AEs).
- Days 1 to 14 (daily, solicited systemic AEs).
- Days 1 to 3 and Days 4 to 7 (solicited local [injection site] AEs).
- Days 1 to 7 and Days 8 to 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 trial vaccine (TDV or Placebo) (assessed by the Investigator) for the following intervals:

- 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 trial vaccine.

A summary of the day of first onset of each event and the number of days subjects reported each event will be presented postvaccination. 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 postvaccination 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 trial vaccine).
- 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 recorded 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 of the unsolicited AE summaries or listings.

Unsolicited AEs

Unsolicited AEs will be assessed for 28 days following administration of each trial vaccine dose (day of vaccination + 27 subsequent days). MAAEs, SAEs and AEs leading to trial vaccine withdrawal or trial discontinuation will be recorded from first trial vaccine dose (Day 1 [M0]) until early termination date or the end of the trial (Day 270 [M9]). Unsolicited AEs, MAAEs, SAEs, and AEs leading to trial vaccine 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 only be counted 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 vaccine dose.

Unsolicited AEs up to 28 days postvaccination will be summarized as follows:

- By SOC and PT.
- By SOC and PT including events with frequency greater than 5% (or a lesser value, such as 2% or 3% as dictated by the data).
- By SOC and PT for trial vaccine related events.
- By SOC and PT for trial vaccine related events with frequency greater than 5% (or a lesser value, such as 2% or 3% as dictated by the data).
- By SOC, PT, and severity (mild, moderate, severe).

MAAEs (postvaccination through the end of the trial) will be summarized as follows:

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

SAEs (postvaccination through the end of the trial) will be summarized as follows:

- By SOC and PT (including a separate group of subjects who received different trial vaccines at first and second vaccination [if any]).
- By SOC and PT for trial vaccine related events.

AEs leading to trial vaccine withdrawal or trial discontinuation (postvaccination through the end of the trial) will be summarized as follows:

- By SOC and PT (including a separate group of subjects, [if any], who received different IPs trial vaccines at first and second vaccination).
- By SOC and PT for trial vaccine related events.

In addition, overview tables will be generated for unsolicited AEs (recorded up to 28 days postvaccination), SAEs, MAAEs, and AEs leading to trial vaccine withdrawal or trial discontinuation and will include the variables as outlined in Table 6.a.

Table 6.a Overview of Unsolicited Adverse Events

	All AEs (28 Days Postvaccination)	SAEs	MAAEs	AEs Leading to Vaccination Withdrawal and/or Discontinuation from Trial
Relationship to trial vaccine	✓	✓	✓	✓
Relationship to trial procedure	✓	✓	✓	✓
Severity	✓	✓	✓	✓
AEs leading to vaccination withdrawal and/or discontinuation from trial	✓	✓	✓	
AEs leading to vaccination withdrawal	✓	✓	✓	✓
AEs leading to discontinuation from trial	✓	✓	✓	✓
MAAEs	✓			✓
SAEs and Non-serious AEs	✓			✓
Deaths	✓	✓		✓

Abbreviations: AE, adverse event; MAAE, medically-attended adverse event; SAE, serious adverse event.

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 trial vaccine withdrawal or discontinuation from trial.

Based on clinicaltrials.gov results posting requirements, another summary table by SOC and PT for AEs postvaccination will be provided and will include all non-serious events (ie, all non-serious unsolicited AEs up to 28 days postvaccination, all MAAEs postvaccination through the end of the trial, and all non-serious AEs leading to trial discontinuation postvaccination through the end of the trial) with frequency greater than 5% (or a lesser value, such as 2% or 3% as dictated by the data) in any trial group.

Missing information regarding 'relationship to trial vaccine' (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 to (ie, Vaccination 1 or Vaccination 2). An AE should be temporally allocated to the correct dose using the following rules:

- If the AE start and end dates are both completely missing, then the AE will be allocated to the first trial vaccination.
- If at least month and/or the year of the AE start date is/are available, then the AE will be allocated to 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, then 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, then the AE will be allocated to the first trial vaccination.

6.6.2 Other Safety Analysis

Vital signs will be measured at Day -28 (M -1), Day 1 (M0), Day 30 (M1), Day 90 (M3), Day 120 (M4), and Day 270 (M9) for each subject. 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).

Clinical laboratory assessments will be performed at screening (Day -28 [M -1]) for each subject. This data will be presented in a listing.

6.6.3 Extent of Exposure and Compliance

The Investigator records all injections of the trial vaccine given to the subject on the CRF.

Investigational product compliance will be summarized by trial group (including a separate group of subjects who received a different trial vaccine at first and second vaccination [if any]) 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 trial vaccine (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 to 30 days, 31 to 90 days, 91 to 120 days, 121 to 270 days, >270 days. Additionally, the duration of follow-up (days) after the second dose of trial vaccine (calculated as end of trial date – second vaccination date + 1 day) will be summarized in a similar way as a continuous variable and as a categorical variable for the following intervals: 1 to 30 days, 31 to 90 days, 91 to 180 days, >180 days.

6.7 Interim Analyses

In anticipation of interest by the local health authority, a blinded interim analysis (IA) *of safety data will be prepared when all subjects have completed the Day 120 (M4) visit. An interim CSR will not be prepared. If requested, the unblinded* IA *will be tabulated and submitted to the local health authority for review.* Trial personnel in this scenario, will remain blinded to the individual subject data (including treatment assignments) and only aggregate data, at trial group-level, will be available. No subject-level listings are anticipated to be submitted to the local health authority for review. Separate unblinded teams will be set up at both Takeda and the contract research organization (CRO) to perform oversight, in terms of checking the data and outputs prior to the submission to the local health authority. Further details on this process and the blinded/unblinded teams will be described in the data access management plan (DAMP).

The IA will include descriptive summary tables for demographic and baseline characteristics, as well as safety data. The methodology for these tables will generally be as described in the respective sections of the SAP, with the exception that only data through the time of the interim data cut will be included. The IA will not be used to alter the trial conduct but just to provide the necessary data to the local health authority for review.

Trial results will be reported in the final CSR. All tables, listing and figures (TLFs) will be re-run including additional and/or final data. Any major changes from the IA will be described in the full CSR. Trial personnel will remain blinded to the individual subject data until unblinding after trial completion (database lock for data through the Month 9 visit).

7.0 REFERENCES

1. A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial to Investigate the Safety and Immunogenicity of a Dengue Tetravalent Vaccine (Live, Attenuated) (TDV) Administered Subcutaneously to Healthy Subjects Aged 4 to 60 Years in India. Takeda Vaccines, Inc. Protocol No. DEN-302, Version 2.0, dated 15 June 2023.
2. ICH Harmonised Tripartite Guideline – Clinical Trial Reports: Structure and Content, E3 (https://database.ich.org/sites/default/files/E3_Guideline.pdf).
3. ICH Harmonised Tripartite Guideline – Statistical Principles for Clinical Trials, E9 (https://database.ich.org/sites/default/files/E9_Guideline.pdf).
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.

8.0 CHANGES TO PROTOCOL PLANNED ANALYSES

Italicized font was used in the SAP to indicate text that was copied from the study protocol. Places where clarifications or changes were made in the copied text is unitalicized.

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

The protocol states that age, sex, race, and other baseline characteristics will be summarized descriptively for all randomized subjects. However, these data will be summarized based on the Safety Set, FAS and PPS. Age groups will also be included in the summaries, as required.

Solicited local (injection site) reactions and systemic events are labeled in the SAP as solicited local (injection site) AEs and solicited systemic AEs to keep consistency between SAPs and TLFs across the dengue program level. The same is true for AEs leading to subject discontinuation which are labeled here as AEs leading to trial discontinuation (ie, subject discontinued from the trial because of the AE).

9.0 APPENDIX

9.1 Changes from the Previous Version of the SAP

Changes made from the previous version of the SAP that have a material impact to the planned statistical analysis methods are described below. In addition, there were textual changes purely to improve the flow, organization and clarity. As these represent cosmetic changes with no impact to the planned statistical analyses, they are not included in the table below.

SAP Section	Change	Rationale for Change
1.1.2	Removal of the Day 30 immunogenicity assessments from the secondary objectives	To comply with the removal of the Day 30 blood sample draw per protocol amendment 1
1.2.2	Removal of the Day 30 immunogenicity assessments from the secondary endpoints	To comply with the removal of the Day 30 blood sample draw per protocol amendment 1
2.0	Removal of the Day 30 blood draw from the trial design schematic and the immunogenicity evaluation sub-section	To comply with the removal of the Day 30 blood sample draw per protocol amendment 1
6.5.2	Removal of the Day 30 immunogenicity assessments from the secondary endpoints analysis.	To comply with the removal of the Day 30 blood sample draw per protocol amendment 1
1.1.3	[redacted]	[redacted]
1.2.3	[redacted]	[redacted]
6.5.3	[redacted]	[redacted]

9.2 Data Handling Conventions

9.2.1 General Data Reporting Conventions

Data will be presented in the listings as reported. For the summaries and analysis, the conventions described in the respective sections of the SAP, will be used.

9.2.2 Implausible Values

Data that are not within the plausible ranges as defined in Table 9.a will be excluded from respective analyses but presented as recorded in data listings including a flag that highlights implausible values.

Table 9.a Plausible Data Ranges

	Parameter	Applicable Age Ranges	Plausible Range
Solicited adverse events	Swelling	<6 years	≤250 mm
		≥6 years	≤500 mm
	Erythema	<6 years	≤250 mm
		≥6 years	≤500 mm
	Body Temperature [(a)]	All ages	32°C-43°C
Demographics	Height	4-5 years	60-140 cm
		6-11 years	90-180 cm
		12-17 years	100-210 cm
		18-60 years	110-210 cm
	Weight	4-5 years	5-50 kg
		6-11 years	10-120 kg
		12-17 years	20-200 kg
		18-60 years	20-200 kg
Vital Signs	Heart Rate	All ages	40-200 beats/min
	Systolic Blood Pressure	All ages	70-180 mmHg
	Diastolic Blood Pressure	All ages	30-120 mmHg

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

9.2.3 Definition of Visit Windows

Following the schedule of trial procedures, analysis visit windows will be calculated relative to the day on which each trial vaccine 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, then 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 (laboratory tests and vital signs) data.

The analysis visit windows for each trial visit are displayed in Table 9.b.

Table 9.b Analysis Visit Windows

Visit (V)	Scheduled Visit Day (Month [M])	Scheduled Dose	Analysis Visit Windows		
			Safety Set	**Full Analysis Set**	**Per-Protocol Set**
V1	Day -28 (M-1)		Up to 28 days prior [-28 to -1 day] to Dose 1	Up to 28 days prior [-28 to -1 day] to Dose 1	Up to 28 days prior [-28 to -1 day] to Dose 1
V2	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
V3	Day 30 (M1)		2-60 [(b)] days after Dose 1	2-60 [(b)] days after Dose 1	29-37 [(b)] days after Dose 1
V4	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 V4)	Not applicable (no blood sample is taken at V4)
V5	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
V6	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 assessments 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 V3 number of days after V2 is calculated as [Date of V3] – [Date of V2] + 1 (day).

(c) Applies to subjects who missed the second dose at V4.

9.2.4 Definitions of Severity of Solicited Safety Parameters

The definitions of severity of solicited safety parameters are displayed for infant/toddler/child (<6 years) and child/adolescent/adult (≥6 years) in Table 9.c and Table 9.d, respectively.

Table 9.c Severity of Solicited Safety Parameters for Infant/Toddler/Child (<6 Years)

Adverse Event	Severity Grade	Severity
Pain at injection site	0	None
	1	Mild: Minor reaction to touch
	2	Moderate: Cries/protests on touch
	3	Severe: Cries when limb is moved/spontaneously painful
Erythema at injection site [(a)]	0	<10 mm
	1	Mild: ≥10-≤20 mm
	2	Moderate: >20-≤40 mm
	3	Severe: >40 mm
Swelling at injection site [(a)]	0	<10 mm
	1	Mild: ≥10-≤20 mm
	2	Moderate: >20-≤40 mm
	3	Severe: >40 mm
Drowsiness	0	Behavior as usual
	1	Mild: Drowsiness easily tolerated
	2	Moderate: Drowsiness that interferes with normal activity
	3	Severe: Drowsiness that prevents normal activity
Irritability/fussiness	0	Behavior as usual
	1	Mild: Crying more than usual/no effect on normal activity
	2	Moderate: Crying more than usual/interferes with normal activity
	3	Severe: Crying that cannot be comforted/prevents normal activity
Loss of appetite	0	Appetite as usual
	1	Mild: Eating less than usual/no effect on normal activity
	2	Moderate: Eating less than usual/interferes with normal activity
	3	Severe: Not eating at all
Fever [(b)]	NA	None
	NA	38.0°C-<38.5°C
	NA	38.5°C-<39.0°C
	NA	39.0°C-<39.5°C
	NA	39.5°C-<40.0°C
	NA	40.0°C-<40.5°C
	NA	40.5°C-<41.0°C
	NA	≥41.0°C

(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 for measurement [5].

Table 9.d Severity of Solicited Safety Parameters for Child/Adolescent/Adult (≥6 Years Old)

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 activity with or without treatment
Asthenia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Malaise	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Myalgia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Fever [(b)]	NA	None
	NA	38.0°C-<38.5°C
	NA	38.5°C-<39.0°C
	NA	39.0°C-<39.5°C
	NA	39.5°C-<40.0°C
	NA	40.0°C-<40.5°C
	NA	40.5°C-<41.0°C
	NA	≥41.0°C

(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 for measurement [5].

9.3 Analysis Software

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

Signature Page for DEN-302 Statistical Analysis Plan Version 3.0, 09 January 202
Title: DEN-302 Statistical Analysis Plan

Approval Task	[redacted] Statistics 14-Feb-2025 22:11:05 GMT+0000

Document Number: TDN-000002500 v4.0