Title: A PHASE II, DOUBLE-BLIND, RANDOMIZED, CONTROLLED TRIAL TO ASSESS THE SAFETY AND IMMUNOGENICITY OF A TETRAVALENT DENGUE VACCINE WITH TWO DIFFERENT SEROTYPE 2 POTENCIES IN AN ADULT POPULATION IN SINGAPORE.

NCT Number: NCT02425098

SAP Approve Date: 14 December 2017

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TAKEDA VACCINES, INC.

STATISTICAL ANALYSIS PLAN

STUDY NUMBER: DEN-205

A PHASE II, DOUBLE-BLIND, RANDOMIZED, CONTROLLED TRIAL TO ASSESS THE SAFETY AND IMMUNOGENICITY OF A TETRAVALENT DENGUE VACCINE WITH TWO DIFFERENT SEROTYPE 2 POTENCIES IN AN ADULT POPULATION IN SINGAPORE.

Safety and Immunogenicity with Two Different Serotype 2 Potencies of TDV in Adults

PHASE 2

Version: Final 2.0

Date: 14 December 2017

Prepared by:

PPD

1.1 Approval Signatures

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

2.0 TABLE OF CONTENTS

LIST OF IN-TEXT TABLES

LIST OF IN-TEXT FIGURES

LIST OF APPENDICES

3.0 LIST OF ABBREVIATIONS

AE	Adverse Event
BMI	Body Mass Index
CMI	Cell-mediated Immunity
DEN	Dengue Virus
DENV	Dengue Virus (wild type)
DHF	Dengue Hemorrhagic Fever
DSS	Dengue Shock Syndrome
E	Envelope
ELISA	Enzyme-linked immunosorbent assay
eCRF	electronic Case Report Form
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMT	Geometric Mean Titer
HD-TDV	High dose TDV formulation
HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus
IB	Investigator Brochure
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
Ig	Immunoglobulin
Inc	Incorporated
IRB	Institutional Review Board
IUD	Intrauterine device
IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
MNT	Microneutralization test
CCI	[redacted]
PBMC(s)	Peripheral Blood Mononuclear Cells

PFU	Plaque Forming Units
PPS	Per-Protocol Set
prM	pre-membrane
PT	Preferred term
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SC	Subcutaneous
SOC	System Organ Class
SOP	Standard operating procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TDV	Tetravalent Dengue Vaccine Candidate
US	United States
WHO	World Health Organization

4.0 OBJECTIVES

4.1 Primary Objective

- To assess the post-vaccination neutralizing antibody response against each dengue serotype by vaccine group.

4.2 Secondary Objectives

Safety:

- To assess the safety of HD-TDV and TDV by vaccine group and baseline dengue seropositivity (MNT).

Immunogenicity:

- To assess the post-vaccination neutralizing antibody response against each dengue serotype by vaccine group and baseline dengue seropositivity (MNT).

Vaccine viremia:

- To assess vaccine viremia post-vaccination by vaccine group and baseline dengue seropositivity (MNT).

4.3 Exploratory Objectives

- CCI [redacted]
- To characterize humoral immune response.
- To characterize cell-mediated immunity in the CMI subset.

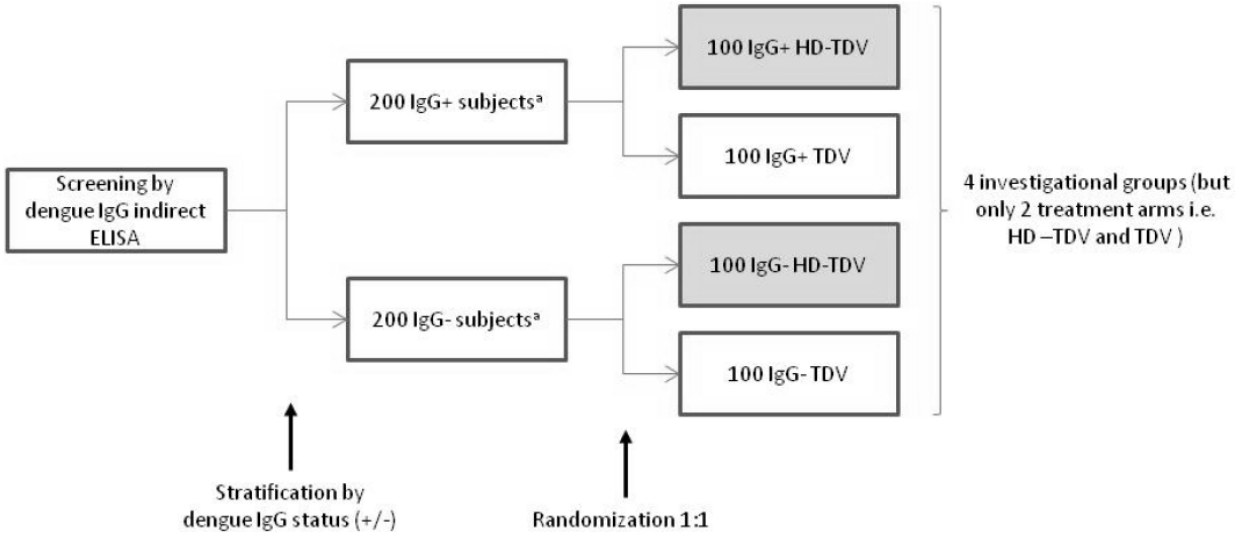
4.4 Trial Design

This is a descriptive, Phase II, double-blind, randomized, and controlled trial in 400 subjects aged 21 to 45 years living in Singapore. Subjects will be randomized (1:1) into two vaccine groups:

- Arm 1 will receive one subcutaneous (SC) dose of HD-TDV;
- Arm 2 will receive one SC dose of TDV.

Randomization will be stratified based on the dengue IgG status (positive/negative) at screening, resulting in four investigational groups (Figure 4.a).

Figure 4.a Schematic Showing the Stratified Randomization Process

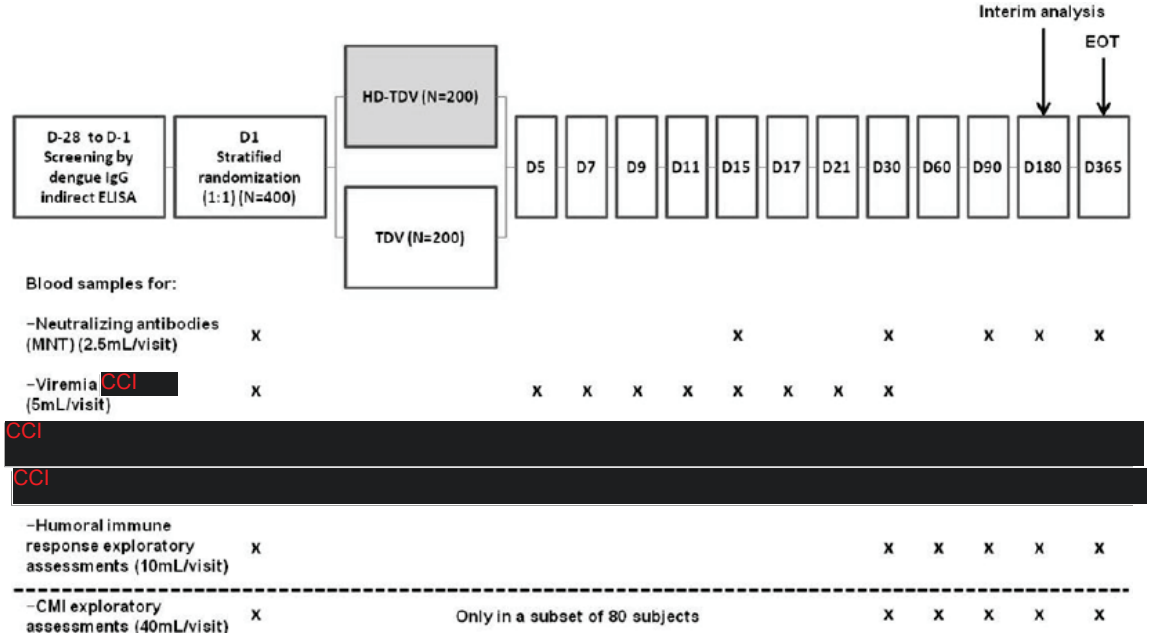


(a) Approximately 40 subjects in each of the IgG positive and negative groups will form the CMI subset.

Subjects will be followed for one year, with 13 scheduled visits, for the assessment of reactogenicity, safety and/or immunogenicity (Figure 4.b). Cell-mediated immunity will be assessed in a subset of approximately 80 subjects (approximately 40 subjects in each of the IgG positive and negative groups). This CMI subset will consist of volunteers identified at the time of randomization.

The duration of the study for each subject will be approximately 365 days (12 months) following vaccination on Day 1 (Month 0). An interim analysis is planned on cleaned data up to 6 months post-vaccination to provide data to support the planning and execution of other studies in the development plan of TDV.

Figure 4.b Schematic Showing Subject Flow through the Trial



Immunogenicity evaluation:

Neutralizing antibodies (MNT) will be measured on blood samples collected pre-vaccination on Day 1 (Month 0) and post-vaccination on Days 15, 30 (Month 1), 90 (Month 3), 180 (Month 6) and 365 (Month 12).

Assessment of viral replication:

Vaccine viremia will be assessed on blood samples collected pre-vaccination on Day 1 (Month 0) and post-vaccination on Days 5, 7, 9, 11, 15, 17, 21 and 30 (Month 1).

Safety evaluation:

Subjects will be provided with a diary card for the recording of:

- Solicited local AEs for 7 days following vaccination (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 vaccination (day of vaccination + 13 days). These will include: fever, headache, asthenia, malaise, and myalgia.
- Unsolicited AEs for 28 days following vaccination (day of vaccination + 27 subsequent days).
- Serious adverse events throughout the trial.

Exploratory assessments:

CCI

[redacted]

Neutralizing and non-neutralizing antibodies will be characterized on blood samples collected pre-vaccination on Day 1 (Month 0), and post-vaccination on Days 30 (Month 1), 60 (Month 2), 90 (Month 3), 180 (Month 6) and 365 (Month 12) by different exploratory assays.

CMI will be assessed in a subset of subjects on blood samples collected pre-vaccination on Day 1 (Month 0), and post-vaccination on Days 30 (Month 1), 60 (Month 2), 90 (Month 3), 180 (Month 6) and 365 (Month 12).

5.0 ANALYSIS ENDPOINTS

5.1 Primary Endpoints

- Geometric mean titers (GMTs) of neutralizing antibodies for each of the four dengue serotypes post-vaccination on Days 15, 30 (Month 1), 90 (Month 3), 180 (Month 6), 365 (Month 12).
- Seropositivity rates (% of subjects) for each of the four dengue serotypes post-vaccination on Days 15, 30 (Month 1), 90 (Month 3), 180 (Month 6), 365 (Month 12) where seropositivity is defined as a reciprocal neutralizing titer ≥10.

5.2 Secondary Endpoints

Safety

- Frequency and severity of solicited local (injection site) adverse events (AEs) for 7 days and solicited systemic AEs for 14 days after vaccination.
- Percentage of subjects with any unsolicited AEs for 28 days after vaccination.
- Percentage of subjects with serious adverse events (SAEs) throughout the trial.

Immunogenicity

- Refer to primary endpoints, but will be analyzed by dengue baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes).

Vaccine viremia

- Incidence, duration, and level of vaccine viremia for each of the four dengue serotypes post-vaccination.

5.3 Exploratory Endpoints

- CCI [redacted]
- Additional analyses will be performed to characterize humoral immune response and cell-mediated immune response (CMI subset).

6.0 DETERMINATION OF SAMPLE SIZE

As this study 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 was considered sufficient to meet the endpoints of the study.

7.0 METHODS OF ANALYSIS AND PRESENTATION

7.1 General Considerations

This Statistical Analysis Plan (SAP) was developed based on International Conference on Harmonization E3 [1] and E9 [2] Guidelines, and information provided in Protocol DEN-205, Version 3.0 dated 25 April 2017 [3].

All statistical analyses will be generated using SAS Version 9.2 or higher.

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

A blinded data review will be conducted prior to unblinding of subject's vaccination assignment. This review will assess the accuracy and completeness of the study database, subject evaluability, and appropriateness of the planned statistical methods.

7.1.1 Data Presentation

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

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

All data collected will be presented in listings, sorted by vaccine group, site number, subject number, and date/time of the finding if applicable.

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 or last contact, MedDRA version, WHODrug version, and SAS version used for the analysis will be presented for all screened subjects.

7.1.2 Imputation rules

There will be no imputation for missing data with the exception of missing severity, missing relationship, and missing or partial dates (i.e. start and stop dates for AEs). If the severity of an unsolicited adverse event is missing, "severe" will be imputed. If the relationship to a study drug of a solicited or unsolicited adverse event is missing, "related" will be imputed. Partial dates will

be presented as they are in the listings. The partial dates of AEs will be imputed to determine the relationship between the onset of AE and the study drug vaccination date.

The following methods will be used to impute missing or partial dates of the start date of AEs.

Month/year available and day missing:

If the month and year are the same as those in the vaccination date, the vaccination date is to be used to impute the AE start date.

If the month and year are different from the vaccination date, then the first day of the month will be used for the start date given that this does not result in a date prior to the vaccination date. If it would be prior to the vaccination date, the vaccination date is to be used to impute the start date of the AE.

Year available and month/day missing:

If the year is the same as the year of the vaccination date, the vaccination date is to be used to impute the AE start date. If the year is different from the vaccination date, then the start date is to be set as January 1st as long as that this does not result in a date prior to the vaccination date. If it is prior to the vaccination date, the vaccination date is to be used to impute the start date of the AE.

For imputation of partial end dates for AEs and if the AE is not ongoing by the end of the study the following rules (worst case scenario) will be considered: If only the day is missing but the month and year are known, the last day of the month will be used. If the day and month are missing but the year is known, the last day of the year will be used.

7.1.3 Baseline and Study Day Definitions

Baseline is defined as the last non-missing measurement taken before study drug vaccination. Where the time is available, the time of the collection must be prior to study drug vaccination. Day 1 observations taken after the vaccination are considered post-baseline values.

Study Day 1 is defined as the date of the vaccination, as recorded on the CRF vaccination page. Other study days are defined relative to the Day 1 (e.g. Day -1 is the day prior to Day 1).

7.1.4 Visit Windows

The End of Treatment visit will be remapped to the next scheduled visit if the day of the visit falls within the protocol scheduled window.

7.2 Analysis Sets

Randomized Set: The Randomized Set will consist of all randomized subjects. Subjects will be summarized according to vaccine received.

Safety Set: The Safety Set will consist of all randomized subjects who received the study vaccine (TDV or HD-TDV). Subjects will be summarized according to vaccine received.

Full Analysis Set (FAS): The FAS will consist of all randomized subjects who received the study vaccine (TDV or HD-TDV) and for whom a valid pre-dose and at least one valid post-dose blood sample are taken. Subjects will be summarized according to vaccine received.

Per-Protocol Set (PPS): The PPS will consist of subjects in the FAS who have no major protocol violations as presented in Table 7.a. The major protocol violation criteria will be defined as part of the blinded data review prior to the unblinding of subject's vaccine assignment. The categories of major protocol violations include: (1) not meeting selected entry criteria, (2) receiving wrong study vaccine except for the packaging issue noted below, (3) receiving prohibited therapies, and (4) other major protocol violations that may be identified during blinded data reviews. Any changes to these criteria after approval of the SAP will be documented and approved in a separate document (blinded data review meeting minutes) prior to database lock and unblinding. Subjects will be summarized according to vaccine received.

Cell-Mediated Immunity Subset (CMI): Cell-mediated immunity will be assessed in a subset of approximately 80 subjects (approximately 40 subjects in each of the IgG positive and negative groups). The CMI subset is a subset of the PPS and will consist of subjects identified at the time of randomization. Subjects will be summarized according to vaccine received.

Background for summarizing data based on the vaccine received instead of randomized vaccine:

This study uses two Dengue vaccine lots (DB0021213 for HD-TDV and DB0040714 for TDV). During the first two packaging campaigns, incorrect vaccine lots were assigned to the two vaccine groups, i.e. DB0021213 as TDV and DB0040714 as HD-TDV. As the labels are blinded, medication identification numbers are used on the vials for identification. Due to this packaging error the medication identification numbers associated with TDV were used on the vials containing HD-TDV and vice versa. Two hundred and fifty subjects had been vaccinated with either TDV or HD-TDV from these two campaigns, and hence these subjects had their vaccine transposed. In this study, subjects are vaccinated only once. The error was identified before the third packaging campaign started, and therefore the only impacted vaccine stock is that from the first and second packaging campaigns.

Due to this packaging issue, the primary analyses of this trial will be based on a PPS that will be adapted in order to exclude all subjects with major protocol violations (as stated above) with the exception that it will include the 250 subjects affected by this packaging issue (provided that there is no other major protocol violation for the subjects). This PPS will analyze the subjects according to the vaccine received. A sensitivity analysis will be performed on the FAS that will also be adapted in order to summarize subjects according to the vaccine received.

This packaging issue is considered to have no impact on the overall distribution of the number of subjects across vaccine groups due to the 1:1 randomization, and for all 250 subjects the vaccine was misallocated 1:1. Furthermore, the study was still ongoing and blinded at the time of these adaptations.

All 250 subjects will be treated as randomization errors within the interactive web response system (IWRS). The deviation will be documented and filed in the Trial Master File. A programming update of the IWRS will be implemented which will flag all 250 subjects with

transposed vaccine groups. This will be visible on the data transfer to ensure correct data in the clinical database/analyses.

7.2.1 Protocol Deviations

The criteria described in Table 7.a will be used to identify subjects who will be excluded from the PPS and will be identified prior to database lock and unblinding. These criteria are considered to have a potentially significant impact on the immunogenicity, and also vaccine viremia results of the subject. Subjects excluded from PPS due to receiving incorrect vaccine will be identified after unblinding. Reasons for exclusion of subjects from the analysis sets will be summarized based on the Randomized Set.

Table 7.a Criteria for Exclusion from the PPS

Criteria for Exclusion	Probable Method of Identification
Not receiving the study vaccine administration [(a)]	Identified programmatically using "End of Study Drug" page question "Did the subject complete the study vaccine?" and/or dosing data
Not having a valid pre-dose and at least 1 valid post-dose measurement for dengue neutralizing antibodies [(b)]	Identified programmatically using immunogenicity data
Not receiving the assigned vaccine, except for the 250 subjects whose vaccines were transposed because two packaging campaigns assigned incorrect vaccine lots, which was identified on 16Oct2015.	Identified after unblinding (e.g. subject who was randomized to TDV but received HD-TDV, subject who was randomized to HD-TDV but received TDV)
Product preparation error	Identified through protocol deviation log
Subject meets inclusion criterion 5 or any of exclusion criteria 1, 2a, 2c, 2d, 3, 4, 5 or 6 (subject to blinded medical review)	Subjects identified programmatically using CRF-recorded data. Subjects will be identified before unblinding, and a blinded review list sent for clinical science review to determine evaluability status for each identified subject. Note that exclusion criteria 2d and 3 identify subjects' use of prohibited medications prior to enrollment.
Use of prohibited medications at study start (subject to blinded medical review)	Potential prohibited medications (Appendix C) to be identified by sending a blinded review list of CRF-recorded medication data for medical review to determine evaluability status for each identified subject.

(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.

Measurements for dengue neutralizing antibodies and viremia collected outside the windows as specified below will be excluded from the respective visits in the Per-Protocol analysis:

- Dengue neutralizing antibodies: D15 ± 5 days, D30 ± 5 days, D90 ± 15 days, D180 ± 15 days, D365 ± 15 days
- Viremia: D5 ± 1 day, D7 ± 1 day, D9 ± 1 day, D11 ± 1 day, D15 ± 1 day, D17 ± 1 day, D21 ± 2 days, D30 ± 5 days

Other significant deviations may be identified based on blinded data reviews of deviation logs throughout the study, subject to medical review.

In addition, all significant protocol deviations identified based on the deviation logs will be summarized based on the safety set.

7.3 Disposition of Subjects

The reason for screen failure will be summarized based on all screened subjects.

Disposition of all subjects from the safety set will be summarized by vaccine received, overall, and additionally by baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes). A sample is considered seropositive if the reciprocal neutralizing titer ≥10. Disposition categories will include:

- Completed study
- Did not complete study
- Primary reason for discontinuation from study

A subject is assumed to be ongoing unless he/she completed the end of study CRF, indicating either completion or early termination. The primary reason for study discontinuation will be summarized.

Number of subjects randomized but not vaccinated, in the safety set, full analysis set, per-protocol set, and cell-mediated immunity subset will also be presented. Additionally, the number of subjects will be presented by site and dengue screening IgG status for the safety set.

7.4 Demographic and Baseline Characteristics

Demographic and baseline characteristics will include age, gender, ethnicity, race, weight, height, BMI (calculated based on weight and height measurements at baseline), baseline seropositivity status, and baseline seropositivity status for each dengue serotype. These demographic and baseline characteristics will be summarized descriptively by vaccine received based on the safety set and PPS.

In addition, the demographic and baseline characteristics will also be summarized by baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes).

Demographic characteristics of screen failure subjects will be summarized.

Summary statistics (number of subjects [n], mean, median, SD, minimum, and maximum) will be generated for continuous variables (age, height, weight, and BMI), and the number and percentage of subjects within each category will be presented for categorical variables (gender, ethnicity, race, and baseline seropositivity).

7.5 Medical History and Concurrent Medical Conditions

Medical history and concurrent medical conditions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, Version 16.1, or higher) coding system. Concurrent medical conditions are conditions that are recorded as ongoing at screening.

Medical history and concurrent medical conditions will be summarized by SOC, PT, and vaccine received based on the safety set.

7.6 Medication History, Vaccination History, Concomitant Medications, and Concomitant Vaccinations

Medication history, vaccination history, concomitant medications, and concomitant vaccinations will be coded using the World Health Organization DRUG dictionary (WHO Drug Version March 2013 or higher). Medication history (prior medications), vaccination history, concomitant medications, and concomitant vaccinations will be summarized separately by Anatomical Therapeutic Chemical class level 2, preferred medication name, and vaccine received based on the safety set.

A prior medication or vaccine is any medication or vaccine taken before study vaccination (i.e. intake was stopped before study vaccination). A concomitant medication or vaccine is any medication or vaccine ongoing at the time of study vaccination or taken on or after study vaccination.

7.7 Study Vaccination and Follow-Up

The duration of follow-up after study vaccination will be summarized as a continuous variable, and also in categories using the following intervals: 1–30 days, 31–180 days, 181–365 days, and >365 days, by vaccine received based on the safety set. The duration of follow-up is defined as the number of days since vaccination to end of the study.

7.8 Efficacy Analyses

Not applicable.

7.9 Pharmacokinetic/Pharmacodynamic Analysis

Not applicable.

7.10 Other Outcomes

7.10.1 Primary Immunogenicity Analyses

The primary immunogenicity endpoints of the study are GMTs of neutralizing antibodies for each of the four dengue serotypes at post-vaccination visits and seropositivity rates (% of subjects) for each of the four dengue serotypes at post-vaccination visits, where seropositivity is defined as a reciprocal neutralizing titer ≥10.

Both endpoints are derived from dengue microneutralization test (MNT) results. All subjects will undergo blood sampling for neutralizing antibodies measurement at Days 1, 15, 30 (Month 1), 90 (Month 3), 180 (Month 6), and 365 (Month 12).

The number of subjects with non-missing assessment, geometric mean, median, associated 2-sided 95% confidence interval (CI) and associated geometric SD, minimum and maximum will be presented for neutralizing antibody titers for each dengue serotype by vaccine received and visit. Geometric mean titers will be calculated, for each relevant time point as anti-logarithm of ∑(log transformed titer/n), where n is the number of subjects with titer information. The 95% CI for GMT will be calculated as the anti-log transformation of upper and lower limits for a two-sided CI of the mean of the log-transformed titers (based on student's t-distribution).

A value of 5 (midpoint between 0 and the lower limit of detection) will be used for Dengue neutralizing antibody titers which are below the lower limit of detection (10). If a reported value is between the lower limit of detection and the lower limit of quantification (differs between serotypes) this value will be replaced with the mid-point between the two.

Geometric mean titers will be plotted over visit, by vaccine received and serotype. In addition, reverse cumulative distribution curves will be plotted by vaccine received, serotype, and visit. These plots will be provided overall and by baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes) based on the PPS.

Seropositivity rates along with exact 2-sided 95% CI, will be presented for each dengue serotype by vaccine received and visit for each dengue serotype. The exact 2-sided 95% CI of seropositivity rate will be calculated based on Clopper-Pearson method [4]. Seropositivity rates by vaccine received and visit will also be graphically presented by dengue serotype, for at least trivalent, and for tetravalent seropositivity using bar graphs including the percentage of subjects seropositive and corresponding 95% CIs.

Primary immunogenicity analyses will be summarized by vaccine received based on the PPS. A supportive analysis will be provided using the FAS.

7.10.2 Secondary Immunogenicity Analyses

The primary immunogenicity analyses will be repeated by baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes) for the PPS. In addition, the percent of subjects with monovalent (seropositive for only one of the four dengue serotypes), bivalent (seropositive for any two of the four dengue serotypes), trivalent (seropositive for any three of the four dengue serotypes), and tetravalent (seropositive for all four

dengue serotypes) seropositivity, as well as at least bivalent (seropositive for ≥2 dengue serotypes) and at least trivalent (seropositive for ≥3 dengue serotypes) seropositivity will be summarized by vaccine received and by baseline seropositivity status at each visit for the PPS.

7.10.3 Vaccine Viremia Analysis

Vaccine Viremia is defined as a positive result from the Dengue Vaccine Screening Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

The vaccine viremia endpoints of the study are the vaccine RNA level, incidence, and duration, of vaccine viremia for each of the four TDV strains (TDV-1, TDV-2, TDV-3, TDV-4) post-vaccination.

Vaccine viremia will be assessed on blood samples collected pre-vaccination on Day 1 (Month 0) and post-vaccination on Days 5, 7, 9, 11, 15, 17, 21 and 30 (Month 1).

Vaccine viremia summaries will be presented by vaccine received and visit, overall and by baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes) based on the Safety Set.

The number of subjects with non-missing assessment, mean, associated 2-sided 95% confidence interval (CI) and associated SD, median, minimum, and maximum will be presented for vaccine RNA (vRNA) levels (expressed as $\log_{10}$ [genome equivalents per mL]) measured by RT-PCR for each TDV strain.

Incidence rates of vaccine viremia along with exact 2-sided 95% CI, will be presented for each TDV strain. The exact 2-sided 95% CI of incidence rate will be calculated based on the Clopper-Pearson method.

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

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

7.10.4 CCI

CCI

[redacted]

CCI

[redacted]

7.10.5 Characterization of Humoral Immune Response

Data on characterization of the humoral immune response will be summarized descriptively by vaccine received based on the PPS.

7.10.6 Cell-Mediated Immunity Response

Subjects in the CMI subset of the PPS will have their CMI response summarized descriptively by vaccine received.

7.11 Safety Analysis

All summaries of safety data will be performed using the Safety Set. The safety data will be summarized by vaccine received.

7.11.1 Adverse Events

Reactogenicity (Solicited adverse events [AEs])

Subjects are provided with a diary card for the recording of solicited local AEs, including injection site pain, injection site erythema, and injection site swelling, for 7 days following vaccination (day of vaccination + 6 days). Subjects are also provided with a diary card for the recording of solicited systemic AEs (fever, headache, asthenia, malaise, and myalgia) for 14 days following vaccination (day of vaccination + 13 days). Any solicited local or systemic AE observed as continuing on study Day 7 (local reactions) or Day 14 (systemic reactions) will be recorded as an unsolicited AE on the AE eCRF. Any solicited local or systemic AE that resolved before that time but which recurs at a later time will be recorded as an unsolicited AE on the AE eCRF. The details of solicited local and systemic AEs, severity of solicited safety parameters are given in Appendix B.

For each solicited AE, the number of subjects and the percentage of subjects will be summarized by event severity:

- 30 minutes after study vaccination
- for each day (Days 1 to 7 after vaccination for local AEs, and Days 1 to 14 after vaccination for systemic AEs) and overall (within 7 days after vaccination for local AEs, within 14 days after vaccination for systemic AEs)
- for Day 1 to Day 3 and Day 4 to Day 7 for local AEs, Day 1 to Day 7 and Day 8 to Day 14 for 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:

- 30 minutes after study vaccination
- Within 14 days after vaccination

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 AEs, the subject will be counted under the related category.

The number of subjects who had their first onset of each event will be provided for each day. The total number of days for each AE term will also be summarized.

Additional subgroup analyses of AE severity will be performed by baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes) for summaries of solicited local AEs 30 minutes after study vaccination, solicited systemic AEs 30 minutes after study vaccination, solicited local AEs within 7 days after study vaccination, and solicited systemic AEs within 14 days after study vaccination. Solicited local AEs within 7 days after study vaccination and solicited systemic AEs within 14 days after study vaccination will also be repeated by occurrence of viremia (subjects with viremia /subjects without viremia).

Additional subgroup analyses of AE relationship to study vaccine will be performed by baseline seropositivity status for summaries of solicited systemic AEs 30 minutes after study vaccination and solicited systemic AEs within 14 days after study vaccination. A summary of solicited systemic AEs within 14 days after study vaccination by relationship will also be performed by subgroup of occurrence of viremia.

For solicited AEs, missing data will be handled as follows. For each vaccine group and solicited AE, the denominator for the percentage will exclude subjects with completely missing data (i.e. subject does not have at least one recorded result of none, mild, moderate, or severe) for the solicited AE in the period being summarized.

Prolonged solicited AEs that continue beyond Day 7 (for local AEs) or Day 14 (for systemic AEs) will be identified during the blinded data review based on the question "Did the symptom continue beyond Day 7/14?" and start/end dates of the corresponding unsolicited AEs. These prolonged solicited AEs will be presented in a separate listing.

Unsolicited AEs and Serious Adverse Events (SAEs)

Any AE identified as a prolonged solicited AE will not be included in any unsolicited AE summary or listing.

Unsolicited AEs will be assessed for 28 days following vaccination (day of vaccination + 27 subsequent days). Collection of SAEs will commence from the time that the subject is vaccinated (Day 1 [Month 0]). Routine collection of SAEs and AEs leading to discontinuation from study will continue until the end of the study (Day 365 [Month 12]). Unsolicited AEs and SAEs will be coded according to 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 adverse events/ subjects with any adverse events) 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. Unsolicited AEs will be summarized as follows: by SOC; by SOC and PT; by PT; including only non-serious events with frequency greater than 2% (>2% of subjects in any vaccine group in order to provide this information for Clinicaltrials.gov); by SOC, PT, and relationship to the study vaccine; by SOC, PT, and severity; and by SOC, PT, and outcome. Study vaccine-related AEs will be summarized by SOC, PT; by SOC, PT, and severity; and by SOC, PT, and outcome.

Unless otherwise specified, unsolicited AEs will be summarized for the following 3 time periods:

1. Overall up to 28 days post-vaccination (onset between 1 and 28 days post-vaccination)
2. With onset between 1 and 14 days post-vaccination
3. With onset between 15 and 28 days post-vaccination

SAEs will be summarized as follows: by SOC and PT; by SOC, PT, and relationship to the study vaccine; and by SOC, PT, and outcome.

AEs leading to study discontinuation will be summarized by SOC, PT.

Additional subgroup analyses will be performed by baseline seropositivity status (seropositive for at least 1 dengue serotype/seronegative for all dengue serotypes) as well as for occurrence of viremia for overviews and summaries of unsolicited AEs, serious AEs, and AEs leading to study discontinuation; summaries of study vaccine-related AEs; relationship to the study vaccine of serious AEs.

7.11.2 Clinical Laboratory Evaluations

Not applicable.

7.11.3 Vital Signs

The vital signs collected in the study include systolic and diastolic blood pressure (mmHg), heart rate (bpm), body temperature (°C), weight (kg) and height (cm). Measurements are taken at screening, Day 9, Day 17, and Day 30 (Month 1). Note that height and weight will only be measured at screening. BMI will be calculated based on the height and weight at screening (weight (kg)/height^2 (m^2)). Vital signs will be summarized descriptively by visit. The measurement parameters will be sorted or grouped in a clinically meaningful order.

Descriptive statistics (n, mean, median, SD, minimum and maximum) of vital sign parameters (observed and change from baseline) except height, weight, and BMI will be summarized by vaccine received at each visit. Only vital sign measurements at the scheduled visits will be included in the summaries.

7.12 Interim Analysis

No interim analysis will be conducted for this trial.

7.13 Changes in the Statistical Analysis Plan

Descriptive summarizations for all randomized subjects are now based on the safety set due to reasons described in Section 7.2.

7.13.1 Amendment History

Date	Amendment Number
07 Sep 2016	Initial Analysis Plan
14 Dec 2017	1

7.13.2 Summary of Changes

This section describes major changes to the SAP Version 1.0, dated 07 September 2016.

Final Version	Section	Description of Change.
2.0	General	The main rationale for this amended SAP was the Protocol Version 3.0, dated 25 April 2017 where the planned interim analysis including data up to Day 180 (Month 6) was removed. Other sections were updated for clarification and harmonization across the Dengue program.
	1.1	Administrative updates and use of eSignatures.
	5.3	Deletion of "(all subjects)" from the exploratory endpoint regarding humoral immune response for consistency to other endpoints.
	7.1.2	Imputation rules for missing severity and missing relationship to study drug were added for AEs for consistency across the Dengue program.
	7.2	The Randomized Set was introduced.
	7.2.1	Table 7.b was updated. Removal of the exclusion of single measurements as only subjects can be excluded from the Per-Protocol Set. Measurements outside of windows will not be included in the respective Per-Protocol analysis. Removal of criterion "Inadvertent unblinding of subject's vaccine assignment to relevant site personnel and study team" as such a criterion is more relevant for pharma studies. As we have blood sample assessments for vaccine studies there is no bias here for the immunogenicity analysis results.

7.6	Clarification was added for definition of prior/concomitant medications/vaccinations.
7.10.1	Plots for seropositivity rates were added.
7.10.3	Details were added for summary of nucleotides at attenuation loci's.
7.10.5	Details added for analysis of humoral immune response data.
7.10.6	Details added for analysis of CMI data.
7.11.1	Handling of prolonged solicited AEs was added.
7.12	Update of Interim Analysis Section as no interim analysis will be conducted for this trial.
8.0	Reference Section was updated to capture all references mentioned in the SAP.

8.0 REFERENCES

1. 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).
2. 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).
3. A Phase II, Double-Blind, Randomized, Controlled Study to Assess the Safety and Immunogenicity of a Tetravalent Dengue Vaccine with two Different Serotype 2 Potencies in an Adult Population in Singapore. Takeda Vaccines, Inc. Protocol No. DEN-205, Version 3.0, dated 25 April 2017.
4. Clopper CJ and Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika. 1934;26: 404-13.
5. Marcy SM, Kohl KS, Dagan R, Nalin D, Blum M, Jones MC, et al. Fever as an adverse event following immunization: case definition and guidelines of data collection, analysis and presentation. Vaccine. 2004;22(5-6):551-6.

Appendix A Schedule of Trial Procedures

Visits		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13 EOT [a]**
Day (Month)	Screening	D1 (Month 0)	D5	D7	D9	D11	D15	D17	D21	D30 (Month 1)	D60 (Month 2)	D90 (Month 3)	D180 (Month 6)	D365 (Month 12)
Time window	-28 to -1 days		±0 days	±1 day	±1 day	±1 day	±1 day	±1 day	±2 days	±4 days	±5 days	±5 days	±10 days	±15 days
Signed Informed Consent [b]	X													
Assessment of Eligibility Criteria [c]	X	X												
Demographics	X													
Medical History	X													
Concomitant medication [d]	X	X												X
Complete physical examination [e]	X													
Targeted physical examination [f]		X												X
Symptom-directed physical examination [g]			X	X	X	X	X	X	X	X	X	X	X	
Vital signs [h]	X				X			X		X				
Pregnancy test [i]	X	X												
Screening results		X												
Randomization		X												
Vaccine administration		X												
Injection Site Evaluation [j]		X		X										
Diary card distribution [k]		X												
Diary card collection and review				X			X			X				
Serious Adverse Events (SAEs) [l]		X	X	X	X	X	X	X	X	X	X	X	X	X

Visits		1	2	3	4	5	6	7	8	9	10	11	12	13 EOT [a]
Blood Collection [m]														
Blood sample for screening dengue IgG indirect ELISA (2.5 mL)	X													
Blood sample for dengue MNT (2.5 mL)		X					X			X		X	X	X
CCI CCI CCI														
Blood sample for vaccine viremia (2.5 mL)		X	X	X	X	X	X	X	X	X				
Blood sample for humoral immune response (10 mL)		X								X	X	X	X	X
Blood sample for cell-mediated immunity (40 mL) [o]		X								X	X	X	X	X

Notes: ELISA=enzyme-linked immunosorbent assay; EOT=End Of Study; Ig=immunoglobulin; MNT=microneutralization test; CCI 1

(a) End of the Trial (EOT) is Day 365 (Month 12), which corresponds to 365 days following vaccination. If the subject terminates early, Day 365 procedures should be performed.
(b) The signed informed consent of the subject needs to be obtained before performing any other study procedure.
(c) Eligibility by review of inclusion/exclusion criteria will be documented at screening and may be re-assessed before randomization on Day 1 (Month 0).
(d) Concomitant therapy (all medications) and vaccine history from 4 weeks prior to TDV vaccination will be collected on Day 1 (Month 0) and Day 365 (Month 12).
(e) Physical examination including measurement of weight and height; body mass index (BMI) will be calculated automatically.
(f) Including (but not limited to) the measurement of vital signs.
(g) Symptom-directed examinations should assess clinically significant changes from the baseline examination.
(h) These will include (but are not limited to) systolic blood pressure/diastolic blood pressure, heart rate and temperature.
(i) In women of childbearing potential, urine pregnancy tests will be performed after informed consent is obtained at screening and before vaccination on Day 1 (Month 0) (within one day prior).
(j) Injection site evaluation (pain, erythema and swelling) at 30 minutes after vaccination on Day 1 (Month 0) and on Day 7.
(k) Diary cards will be distributed for the collection of:

- Solicited local AEs until Day 7 (day of vaccination + 6 subsequent days),
- Solicited systemic AEs until Day 14 (day of vaccination + 13 subsequent days), and
- Unsolicited AEs until Day 28 (day of vaccination and + 27 subsequent days).

(l) SAEs will be reported to the Sponsor within 24 hours of the Investigator becoming aware of the event.
(m) Blood samples on Day 1 (Month 0) need to be collected before vaccination.
(n) Blood collection at screening is used to determine dengue baseline immune status (i.e., dengue naïve and pre-exposed subjects).
(o) Cell-mediated immunity (CMI) will be assessed in a subset of subjects, referred to as the CMI subset.

Appendix B Solicited Local and Systemic Adverse Events and Severity

Table 8.a Solicited Local and Systemic AEs

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

(a) Fever is defined as greater than or equal to 38°C or 100.4°F regardless of method used.

Table 8.b Severity of Solicited Safety Parameters

Adverse Event	Intensity grade	Severity/Intensity
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	0	< 25 mm
	1	Mild: 25 - ≤ 50 mm
	2	Moderate: > 50 - ≤ 100 mm
	3	Severe: > 100 mm
Swelling at injection site	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 [(a)]	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

(a) Fever is defined as greater than or equal to 38°C or 100.4°F regardless of method taken.
NA = not applicable

Appendix C Potential Prohibited Therapies

1. Chronic use of oral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0) (use of inhaled, intranasal, or topical corticosteroids is allowed).
2. Receipt of parenteral steroids (equivalent to 20 mg/day prednisone ≥12 weeks/≥ 2 mg/kg body weight/day prednisone ≥2 weeks) within 60 days prior to Day 1 (Month 0).
3. Administration of immunoglobulins and/or any blood products within the 3 months prior to Day 1 (Month 0) or planned administration during the study.
4. Receipt of immunostimulants within 60 days prior to Day 1 (Month 0).
5. Immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within 6 months prior to Day 1 (Month 0).
6. Receipt of any other vaccine within 14 days (for inactivated vaccines) or 28 days (for live vaccines) prior to Day 1 (Month 0) or planning to receive any vaccine within 28 days after Day 1 (Month 0).
7. Participation in any clinical study with another investigational product 30 days prior to Day 1 (Month 0) or intent to participate in another clinical study at any time during the conduct of this study.
8. Previous participation in any clinical study of a dengue candidate vaccine, or previous receipt of a dengue vaccine.

Signature Page for DEN-205 Statistical Analysis Plan, Version 2.0, 14 Dec 2017

Title:

Approval	PPD
Approval	PPD
Approval	PPD

Document Number: TAK-003-01091 v2.0