STATISTICAL ANALYSIS PLAN

A Phase 1, Open-Label Clinical Trial with Dengue-1-Virus Live Virus Human Challenge (DENV-1-LVHC) Assessment of Healthy U.S. Adults Previously Primed with Tetravalent Dengue Virus Purified Inactivated Vaccine (TDEN-PIV) and Boosted with Tetravalent Dengue Virus Live Attenuated Vaccine Formulation (TDEN LAV)

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APPROVAL SIGNATURES

Study Title:	**Study of the ADVP004 vaccinated volunteers with DENV-1-LVHC**
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List of Abbreviations

Abbreviation	Explanation
AE	Adverse event, adverse experience
CDISC	Clinical Data Interchange Standards Consortium
CSR	Clinical study report
DENV-1	Dengue virus serotype 1
DENV-1-LVHC	Dengue -1 Virus-Live Virus Human Challenge
DHIM	Dengue Human Infection Model
eCRF	Electronic case report form
EDC	Electronic data capture
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot assay
EMEM	Eagle's minimum essential medium
FAS	Full analysis set
FDA	US Food and Drug Administration
FV	Flavivirus
GCP	Good Clinical Practice
GE	Genome Equivalents
GLP	Good Laboratory Practice
GMT	Geometric mean titer
LAV	Live Attenuated Vaccine
HIPAA	Health Insurance Portability and Accountability Act
LLOQ	Lower level of quantification
NS-1 NT	Non-structural protein 1 Neutralization titer
ORP HRPO	Office of Research Protections, Human Research Protection Office
PBMC	Peripheral blood mononuclear cells
PIV	Purified Inactivated Vaccine
PFU	Plaque forming units
PPAS	Per-Protocol Analysis Set
PUD	Peptic ulcer disease
RNA-seq	RNA sequencing
RT-PCR	Reverse transcription - polymerase chain reaction
SAE	Serious adverse event
SafAS	Safety analysis set
SAP	Statistical analysis plan
SAS	Statistical analysis software
SC	Subcutaneous
SDTM	Study Data Tabulation Model
TDEN	Tetravalent Dengue Virus
UMB	University of Maryland, Baltimore

1 Introduction

This study is a phase 1, open-label in health adults. This study will examine the level of protection following Dengue 1 Live Virus Human Challenge (DENV-1-LVHC) product in volunteers previously vaccinated with heterologous prime boost utilizing Tetravalent Dengue Virus (TDEN) Purified Inactivated Vaccine (PIV) and Tetravalent Dengue Virus Live Attenuated Vaccine (LAV) as compared to unvaccinated, healthy control volunteers. Additionally, this study will examine the safety and effectiveness of the DENV-1-LVHC and assess the ability of this virus strain to elicit an uncomplicated dengue like illness.

The purpose of this Statistical Analysis Plan (SAP) is to ensure that the summary tables, figures and listings (TLFs) which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the study objectives. Individual study results, appropriate summary statistics for study conduct (including subject disposition and demographics), and safety assessments will be presented.

2 Changes from protocol in study conduct or statistical analysis

During study design and database production, Day 0 was used as the day of inoculation. However, for CDISC data tabulation and statistical reporting under FDA recommendation, Tables 1 and 3 in the protocol were amended to use Day 1 as day of inoculation and Day 181 as final visit day rather than Day 0 and Day 180 as the protocol describes. Accordingly, specific study days in the SAP, TLFs, and CSR were also amended. Note that at some places absolute study day and relative study day might be used interchangeably. For instance, 28 days post inoculation is equivalent to study day 29.

3 Study Objectives

3.1 Primary Objectives

- To further evaluate the safety and reactogenicity of the DHIM-1 human challenge model
- To determine the level of protection to dengue serotype 1 challenge related symptoms provided by previous vaccination with heterologous prime boost utilizing Tetravalent Dengue Virus Purified Inactivated Vaccine (PIV) and Tetravalent Dengue Virus Live Attenuated Vaccine (LAV)

3.2 Secondary Objectives

- To characterize the immunologic responses following dose exposure to the DENV-1-LVHC viral strain as a DHIM in vaccinated compared to unvaccinated volunteers
- To compare viremia kinetics following dose exposure to DHIM-1 in vaccinated compared to unvaccinated groups

3.3 Exploratory Objectives

- To explore the immune response and host-virus interactions following exposure to DENV-1-LVHC

4 Study Endpoints

4.1 Primary Endpoints

Following are the primary endpoints for this study:

- Occurrence, grade, and duration of solicited injection site symptoms until 7 days post virus inoculation
- Occurrence, intensity, and duration of unsolicited injection site symptoms until 28 days post virus inoculation or 7 days post inpatient, whichever is later
- Occurrence, intensity, and duration of solicited systemic symptoms until 28 days post virus inoculation or 7 days post inpatient, whichever is later
- Number, intensity, and duration of abnormal laboratory measurements until 28 days post virus inoculation or 7 days post inpatient, whichever is later
- Occurrence, intensity and duration of dengue like symptoms/adverse events until 28 days post virus inoculation or 7 days post inpatient whichever is later
- Occurrence, intensity, and duration of unsolicited systemic symptoms until 28 days post virus inoculation or 7 days post inpatient, whichever is later
- Number of SAEs until 28 days post virus inoculation or 7 days post hospitalization, whichever is later
- Number of SAEs until 6 months post virus inoculation
- The occurrence of fever defined as greater than or equal to 38°C (100.4° F) measured at least 2 times at least 4 hours apart

4.2 Secondary Endpoints

The secondary endpoints align with the viremia and immunogenicity objectives. Dengue viremia will be examined both as a binary endpoint (present or not present), peak, and as a function of area under the curve (AUC)

- Viremia by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) up to 28 days post virus inoculation
- MN50 antibody GMT titers at 28 days following inoculation in vaccinated and unvaccinated volunteers

4.3 Exploratory Endpoints

Exploratory endpoints will be analyzed using the population of volunteers enrolled in the trial who receive dengue virus inoculation. Analysis will be done using the previously mentioned assays and appropriate statistical tests will be performed. The immune response to the challenge virus at each dose will be characterized descriptively by:

- Geometric mean titer (GMT) and geometric mean titer rates (GMTRs) of neutralizing antibodies (measured by dengue neutralization titer (NT) at 0, 1, 3, and 6 months after virus inoculation (> 10 defined as response)
- Cell mediated immunity (CMI)
- Proteomics - microarray
- Transcriptomics (to include single-cell analyses)

  Evolutionary analysis of DHIM-1 strain whole genome sequence (consensus and quasi-species)

4.4 Derived Endpoints

4.4.1 Adverse Events

4.4.1.1. Duration

For solicited local AEs over 7 days post virus inoculation, duration will be displayed by period as following:

- 1-3 days
- 4-7 days
- ≥ 8 days
- Missing

For solicited systemic AEs, unsolicited AEs, and laboratory test abnormalities 28 days post virus inoculation or 7 days post hospitalization, whichever is later, duration will be displayed by period as following:

- 1-3 days
- 4-7 days
- 8-16 days
- ⩾ 17 days
- Missing

4.4.1.2. Maximum intensity

The maximum intensity of laboratory test abnormalities, solicited AEs and unsolicited AEs will be the highest severity of if the occurrences occurs more than once. If an AE or laboratory test abnormality is not continuous (i.e., occurring over two separate periods of time intervened by at least one day) then the maximum intensity is the maximum intensity of the two periods of time.

4.4.2 Viral RNA Detection

Viral RNA is expressed in Ct value (xx.xx). A test for RNA will be considered positive if the assay value is detectable and negative if the assay value is below the level of detection.

Genome Equivalent/microliter of serum (yyyyyy.yy) is from the quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) assay. Final reported data will be recorded as GE/microliter x 1000 (GE/mL). A positive result is defined as two detectable replicates, reported as a single value.

4.4.2.1 Duration

The duration of the viremia will be computed as: Duration = last date of positive viral RNA - start date of positive viral RNA + 1.

4.4.2.2 Maximum intensity

The maximum intensity of viral RNA is calculated as the maximum GE/microliter (x 1000) value among all days with positive viral RNA.

4.4.2.3. Area under the Curve

Dengue viremia will be examined both as a binary endpoint (present or not present), peak, and as a function of area under the curve (AUC). The AUC will be calculated from the day of first viral RNA detection and conclude on the day after (Day + 1) of the final viral RNA detection.

5 Trial Design

5.1 Design Overview

Volunteers will be 18-50 years, inclusive, at enrollment. This study is a Phase 1 open-label, study with 3 groups of 5 volunteers each. This will include 5 unvaccinated dengue naïve volunteers, 5 volunteers vaccinated with PIV followed by LAV at 90 day, and 5 volunteers vaccinated with PIV followed by LAV at 180 days. If 5 volunteers are not determined to be eligible, the remainder will be filled by eligible vaccinated volunteers. If funds allow and volunteers express interest, a fourth group of 5 previously vaccinated volunteers may be recruited. If an inadequate number of volunteers vaccinated on these schedules are available volunteers vaccinated with PIV followed by LAV 28 days later may also be included.

Both vaccinated and unvaccinated volunteers will be challenged with the DHIM-1 challenge strain at University of Maryland's CVD in at least two separate challenge events. Volunteers who meet the criteria for inpatient analysis (i.e., develop fever, detectable viremia, and/or symptoms, signs or laboratory criteria concerning for development of severe dengue) will be hospitalized at a designated inpatient unit.

After inoculation, volunteers will be seen and evaluated (including blood draw) closely (qd or qod per study schedule) until Day 28-post inoculation. If a volunteer develops viremia, symptoms or laboratory findings that meet sequestration (viremia) or hospital admission criteria (viremia plus symptoms and/or laboratory findings) he or she will be admitted. During hospitalization they will receive additional clinical and laboratory evaluations if determined necessary by treating physicians. Volunteers will be eligible for discharge when they have fever resolution, improvement in symptoms and absence of virus detection by polymerase chain reaction (PCR).

5.2 Study Population and Eligibility Criteria

This study will enroll up to 20 healthy, male and female subjects who are 18-50 years old at the time of consent. Each subject must meet all inclusion and no exclusion criteria, as provided in the protocol. The PI or designee will make the final decision of the eligibility. Control volunteers must have a negative flavivirus screen and vaccinated ADVP003 and 004 volunteers must have positive DENV flavivirus screen. Only eligible subjects will be given the investigational product.

5.3 Replacement of Subjects Withdrawn from Study

At the investigator's discretion, subjects may be replaced during the study if they are unable to complete the study procedures required during the screening period and before dengue human infection inoculation (DHIM) if they are withdrawn for reasons not related to the study.

5.4 Randomization and Allocation Procedures

This is an open label study.

5.5 Study Duration

Within 90 days of screening visit 1 and within 28 days of screening visit 2, subjects that are enrolled will receive 1 inoculation with the challenge strain and will be followed for 6 months/180 days.

5.6 Sample size and Power

As this study has no statistical hypothesis test, there is no formal power calculation. The sample of 10 total vaccinated volunteers with 5 volunteers from each of the two most immunogenic dengue prime boost vaccination schedules has been determined primarily on the basis of available funding for a study of this nature along with the available pool of previously vaccinated volunteers who remain available and potentially willing to participate in this study. The control arm of previously unvaccinated and flavivirus naïve volunteers has been set at 5 based upon standards set using previous challenge models which will allow a reasonable comparison group for those symptoms and criteria that are expected to occur in >90% of subjects inoculated which would include viremia and at least one dengue like symptoms based on previous studies with this challenge virus strain.

Due to the sample size, only adverse events with high incidence rates will be detected. With 10 volunteers, the probability of observing at least 1 AE is approximately 95% if the true incidence rate is 26%. At least 7/10 volunteers must seroconvert to establish a true seroconversion rate of no less than 30% with 95% confidence.

With 10 consecutive successes of meeting the desired performance parameters, it can be concluded with 95% confidence that the future success rate of the DENV-1-LVHC virus challenge is expected to be greater than 74%.

6 Analysis Populations and Disposition

Three analysis sets will be used: The Per-Protocol Analysis Set (PPAS), the Full Analysis Set (FAS), and the Safety Analysis Set (SafAS).

6.1 Safety Analysis Set

The SafAS is defined as those volunteers, who meet the eligibility criteria, received the virus inoculation, and for whom safety data are available

6.2 Full Analysis Set

The FAS is defined as those volunteers, who meet the eligibility criteria, received the virus inoculation, and for whom performance data are available.

6.3 Per-Protocol Analysis Set

The PPAS will include all volunteers who meet the definition of the SafAS and had none of the following protocol deviations:

1. Administration of inoculation was not done as per protocol (site and route of administration)
2. Volunteer received a dose other than the one that he/she was expected to receive
3. Volunteer received a protocol-restricted medication

4. Volunteer did not complete the study due to being lost to follow up (during the 28 days after inoculation or through 7 days post hospital discharge, whichever is later), but not due to withdrawn consent

The numbers and percentages of enrolled subjects and safety set subjects will be presented for each treatment group. A summary of subject disposition will summarize, overall and by treatment group, the numbers and percentages of subjects who are enrolled, withdrawal by subject or physician decision, lost to follow-up, and who complete the study. Subjects prematurely withdrawn from the study will be summarized overall and for each of the reasons defined in the protocol and Discontinuation of Treatment CRF (DS2). The percentages will be based on the number of enrolled subjects.

7 Subject Characteristics

Characteristics of subjects will be summarized or listed as described below.

7.1 Demographics:

The following will be summarized for the all enrolled subjects and Safety Analysis population:

- Age
- Sex
- Ethnicity
- Race

Age will be calculated as the number of years elapsed between birth date and the date of informed consent, adjusted for whether the birthday has passed as of the day of signing. (This corresponds to the typical calculation of age a person would use in conversation, namely, Age= floor ((Date of informed consent - date of birth)/365.25))

Race categories will be summarized.

A listing of demographics will also be presented.

7.2 Baseline Medical History

Medical history is collected at the screening visit. A listing for subjects medical history will be presented for the Safety Analysis population.

8 Safety Assessment

8.1 Adverse Events

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

8.2 Solicited Adverse Events

A solicited AE is a predetermined event. The solicited AEs for this study include:

- Fever ≥ 38 ◦ C (100.4 ◦ F)
- Rash
- Headache/retroorbital pain
- Muscle pain (Myalgia)
- Joint pain or bone pain
- Fatigue/malaise
- Pain at injection site
- Swelling at injection site
- Erythema at injection site
- GI symptoms (Abdominal pain, nausea, vomiting)

Solicited AEs will be captured during all clinical visits to Day 28. The solicited AEs will be summarized by categories defined in Section 4.4.1.1.

8.3 Unsolicited Adverse Events

Treatment-emergent AEs (TEAEs) will be summarized and tabulated using MedDRA, by System Organ Class (SOC) and Preferred Term (PT). A TEAE is defined as an AE that first occurs or worsens in severity on or after the administration of study vaccine.

The relationship of each AE with (DENV-1-LVHC) will be classified as related (Unlikely-Definite) or Not related.

8.4 Laboratory Test Abnormalities

Clinical laboratory evaluations (blood draw for safety labs) consist of serum chemistry and hematology assessments as specified in the protocol. If the lab abnormality results in the diagnosis of a new medical condition, that condition should be captured as an AE.

8.5 Severity Assessment

All AEs will be assessed for severity by the investigator. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, potentially life-threatening, or fatal using the criteria in the FDA's Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Trials (2014) summarized in Appendix A in the protocol. Any AE not included in the scale will be graded according to Table 8 in the protocol. For laboratory results, any event identified as abnormal according to the local laboratory normal ranges will also be graded according to the FDA's Toxicity Grading Scale in Appendix A in the protocol.

8.6 Physical Examination

Physical examination will be listed for enrolled subjects only, presenting date of evaluation if exam was done. Abnormal findings before the inoculation will be listed as medical history and abnormal findings after the inoculation will be listed as adverse events.

8.7 Vital Signs

Blood pressure (systolic and diastolic), heart rate, and oral temperature recorded on ADVP005 CRF VS2 will be listed for each of the planned assessment times during the study.

8.8 Concomitant Medications

Prescriptions and over-the-counter medications (including vitamins and supplements) will be recorded on the ADVP005 CRF CM1 during each of the planned review visits. The trade name and/or generic name of the medication, medical indication, the start and end dates of treatment were to be recorded on the CRF. Concomitant medications (CMs) will be coded using the WHO Drug coding. The listing of CMs will display entries from CRF CONCOMITANT MEDICATIONS, ordered within subject by the "Start Date." The listing will display the recorded term from the CRF CONCOMITANT MEDICATIONS and, adjacent to that, the WHO Drug preferred term and medication name and therapeutic indication.

9 General Statistical Methodology

The analysis will be performed under the responsibility of ICON.

9.1 Data Sources

CRF data are extracted from the clinical data base. Data will be tabulated and reported following the CDISC standards.

9.2 Missing Data

Missing data will be handled according to Table 1. Unless stated otherwise in the sections below, missing data will not be replaced with imputed values.

Table 1: Methods for Handling Missing Data and Outliers

Data	Handling Method
Safety	Missing data will not be replaced with imputed values. Data not uploaded from the source documents can be included.
Causality	Non-serious unsolicited AEs and SAEs with missing causality will be considered as related to inoculation.
Measurements	Missing measurement (for temperature) will not be replaced. Nevertheless, the following rule will be applied: If temperature is partially missing after decimal point, the data will be analyzed replacing "MD" by zero (whatever the group). By example, a "39.MD" daily temperature (MD means missing data) will be considered as "39.0°C" at the time of analysis.
Intensity	Missing intensity will not be imputed.
Start and Stop Dates	Missing or partially missing stop dates after Day 28 for injection site or Day 28 for systemic reactions will not be recorded.
Action Taken	Missing action taken will not be imputed.
Assessment of Outcome	Assessment of outcome will not be imputed.

Seriousness (for SAE)	Missing seriousness will not be imputed. Missing seriousness will be indicated as such in the data listings.

9.3 Multiple Study Centre

This study will be conducted at one center (UMB).

9.4 Baseline Assessments

Results of baseline assessments will be summarized with other data of the relevant type. Hypothesis tests will not be used for comparison of pretreatment data among cohorts. Clinical judgment of the importance of any differences among treatment groups will be addressed in the study report.

9.5 Definition of Baseline and Change from Baseline

When analysis requires identification of a baseline value, the last value prior to administration of the DENV-1-LVHC vaccine will be used.

9.6 Covariates and Subgroups

No adjustment will be made for effects of covariates and subgroups.

9.7 Sample Size Reassessment

No sample size reassessment was planned for this study.

9.8 Interim/Preliminary Analysis

Raw data directly extracted from database system will be shared for CDSC review at each decision time point. No formal statistical analysis will be performed.

9.9 Test Size

Hypotheses will not be tested as part of the analysis of study data, and so test sizes are not relevant to this study.

9.10 Multiple Testing

Hypotheses will not be tested as part of the analysis of study data, and so control of the effect of multiple comparisons is not relevant to this study.

9.11 Analysis Population

Three analysis sets will be used: The Per-Protocol Analysis Set (PPAS), the Full Analysis Set (FAS), and the SafAS.

8.11.1 **Safety Analysis Set**

The SafAS is defined as those subjects, who meet the eligibility criteria, received the virus inoculation, and for whom safety data are available.

8.11.2 **Full Analysis Set**

The FAS is defined as those subjects, who meet the eligibility criteria, received the virus inoculation and for whom performance data are available.

8.11.3 **Per Protocol Analysis Set**
The PPAS will include all subjects who meet the definition of the FAS and are protocol compliant, with the absence of the following major protocol deviations:

1. Administration of inoculation was not done as per protocol (site and route of administration)
2. Subject received a dose other than the one that he/she was expected to receive
3. Subjects did not complete the study due to being lost to follow up (during the 28 days after inoculation or through 7 days post hospital discharge, whichever is later), but not due to withdrawn consent.

9.12 Data Display Characteristics

Data displays produced for this study will include two types—summary tables and data listings. Unless stated otherwise, data listings will be produced for all recorded data. Summary tables will be produced as specified in the following sections. Additional data listings will be produced for outcome measures that involve extensive procedures to derive the analyzed outcomes.

Data listings will simply list the data recorded in the clinical data base or derived for each subject. They will be ordered by cohort (Vaccinated ADVP003 or -004 subjects or controls), subject number, and date/time of assessment. Data listings will also display Study Day (day of study relative to the day of the study DHIM). For example, inoculation day is displayed as Day 0 and Day before Day 0 is displayed as Day -1 and Day after Day 0 is Day 1. When expedient, additional levels of ordering hierarchy may reflect subsets of assessments within subject. Data listings will not display subject initials or any unique identifiers that violate HIPAA.

Summary tables will display summary statistics calculated for each of the cohorts (if applicable), unless described otherwise in following sections.

Unless stated otherwise in relevant sections to follow, continuous data will be summarized with the number of non-missing values, mean, and standard deviation, minimum, median, and maximum. Unless stated otherwise, categorical data will be summarized with the number of non-missing values and the numbers of values equal to each of the possible categories. Unless stated otherwise, percentages of subjects with each of the possible values will be calculated using the number of subjects with non-missing data for endpoints where results are expected to be obtained including lab tests and diary-recorded data. For AEs, since these are spontaneous reports collected by date of onset, the number of subjects in the corresponding analysis population will be used as the denominator. Means, standard deviations, and medians will be displayed with one more decimal digit than the data that they summarize. Unless otherwise specified percentages will be displayed to 1 place after the decimal point.

9.13 Data Grouping for Analysis

Unless otherwise stated, there will be no additional grouping of the two cohorts for any summaries.

10 Statistical Methods

Analysis of the data from this study will be descriptive in nature. Confidence intervals and p-values will not be generated as part of the final summaries due to the small sample size of this study. Mean, standard deviation, minimum, maximum, and possibly median and quartiles will be used for continuous data and number and percentage will be used for categorical data, unless specified otherwise in the section below.

There will be a final statistical analysis conducted following the end of all study visits. No formal statistical analysis will be performed for CDSC reviews.

10.1 Statistical Analysis for Primary Objectives

There are no hypotheses. All of the main analyses will be descriptive by cohort (vaccinated and controls) and overall. The safety analysis set (SafAS) will be used for the analysis of safety data in this study.

9.1.1 Laboratory Test Abnormalities

All clinical safety laboratory parameters will be listed by subject and date/time and study day of sample, sorted within treatment group. There will be separate listings for hematology and chemistry. The listings will also include the normal range for the parameter. Laboratory data will be summarized descriptively for each of the planned assessment. In addition, a summary of subjects with shifts from baseline (relative to normal range) in laboratory test results will be prepared.

Abnormal laboratory measurements that occur following DHIM over the 28-day follow-up period will be summarized overall, by group, by duration and by toxicity grade for each component of the trial. A summary of subjects with shifts from baseline (relative to normal range) in laboratory test results will be provided.

9.1.2 Solicited AEs

Individual solicited local AEs over the 7-day follow-up period and solicited systemic AEs over the 28-day follow-up period will be analyzed.

The incidence of individual solicited AEs to the vaccine will be calculated overall, by group, type of reactions, by duration and maximum intensity. Presentations will include the number and percentage of subjects with at least one solicited symptom (local or systemic), at least one local symptom, and at least one general (systemic) symptom, as well as the incidence of each symptom individually.

9.1.3 Unsolicited AEs

The number of subjects with at least one report of an unsolicited treatment emergent adverse event (TEAE)reported up to 28 days after inoculation will be summarized overall and by immunization dose group. The intensity and temporal relationship of the unsolicited symptoms to immunization will also be assessed. Presentations will also summarize unsolicited AEs by preferred term, organ body system, grade, and relatedness to virus inoculation.

For the tabulation of the TEAEs by preferred term, organ body system, a subject will be counted only once in a given body system. For example, a subject reporting nausea and diarrhea will be reported as one subject, but the symptoms will be listed as two separate TEAEs within the class. Therefore, the total number of TEAEs reported within a body system may exceed the number of subjects within the body system reporting TEAEs.

For summaries by severity, if a subject has more than one event within the same preferred term, the most severe event episode will be counted.

For summaries of vaccine-associated (related) events, if a subject has more than one event within the same preferred term, and if one event is considered "not associated" and the other "associated", the subject will be counted as "associated" for that term.

Data Listings will present the verbatim-reported event along with the Preferred Term (PT) and System Organ Class (SOC), onset and stop dates, severity, relatedness (dengue associated or not), SAE status, action taken, and outcome.

These summaries will be repeated for TEAEs with onset on or after the day of the inoculation through 181 days following the inoculation. Any events in the database with onset not within any of the periods described above will not be summarized but will be included in subject AE listing.

Serious AEs will be listed, and depending upon the number of serious TEAEs, may be summarized. A list of AEs for which any action was taken will be provided.

10.2 Statistical Analysis for Secondary Objectives

The performance of the challenge virus at a certain dose up to 28 days after virus inoculation will be assessed descriptively by group using the following parameters:

- number and percentage of subjects with quantitative RNA by RT-PCR up to 28 days post virus inoculation by cohort (vaccination and controls) and overall.
- number and percentage of subjects with detectable RNA ≥ 4 days duration by cohort (vaccination and controls) and overall.
- number and percentage of subjects with maximum detectable RNA ≥ 10^6 Genome Equivalents (GE)/ml by cohort (vaccination and controls) and overall.
- Number and percentage of subjects with fever greater than or equal to 38 ◦ C (100.4 ◦ F) by cohort (vaccination and controls) and overall, measured at least 2 times at least four hours apart in 24 hours but not lasting more than 72 hours duration
- Number and percentage of subjects with each of the following clinical or laboratory symptoms: by cohort (vaccination and controls) and overall.
  - Headache/retro-orbital pain ≤ grade 2
  - Rash ≤ grade 2
  - Malaise/fatigue ≤ grade 2
  - Myalgia ≤ grade 2
  - Arthralgia ≤ grade 2
  - GI Symptoms (anorexia, nausea, vomiting, diarrhea or abdominal pain) ≤ grade 2

- Liver function tests (ALT, AST) ≤ grade 2
- Leukopenia ≤ grade 2
- Thrombocytopenia ≤ grade 2

- Total number and percentage of subjects of the following clinical or laboratory values by cohort (vaccination and controls).
  - Headache/retro-orbital pain > grade 2
  - Rash > grade 2
  - Malaise/fatigue > grade 2
  - Myalgia > grade 2
  - Arthralgia > grade 2
  - GI Symptoms (anorexia, nausea, vomiting, diarrhea or abdominal pain) > grade 2
  - Liver function tests (ALT, AST) > grade 2
  - Leukopenia > grade 1
  - Thrombocytopenia > grade 1

- Number and percentage of subjects with detectable RNA ≥ 4 days duration, maximum detectable RNA≥ 10^6 GE/ml, and two or more of the following clinical or laboratory symptoms:
  - Headache/retro-orbital pain ≤ grade 2
  - Rash ≤ grade 2
  - Malaise/fatigue ≤ grade 2
  - Myalgia ≤ grade 2
  - Arthralgia ≤ grade 2
  - GI Symptoms (anorexia, nausea, vomiting, diarrhea or abdominal pain) ≤ grade 2
  - Liver function tests (ALT, AST) ≤ grade 2
  - Leukopenia ≤ grade 2
  - Thrombocytopenia ≤ grade 2

- Time to onset of clinical signs and symptoms as described above, which will be displayed by period as 'Day 0–4', 'Day 5–16', and 'After Day 16'.

Analyses of the secondary endpoints will be applied on the full analysis set and per protocol population. Analyses of the secondary endpoints are focused on the AEs with grade ≤ 2 and other safety analyses for the mentioned symptoms by grade are discussed in Section 9.1.

10.3 Statistical Analysis for Exploratory Objectives

Exploratory analysis will be performed with data measuring Serum antibody levels in geometric mean titers to dengue, Neutralizing antibody in geometric meant titer rates (GMTRs) levels, measured by dengue neutralizing titer (NT) at 0, 1, 3, and 6 months after virus inoculation (> 10 defined as response), Proteomics, Cell mediated immunity (CMI), Transcriptomics, Cytokine changes, immunophenotyping, The analysis will be mainly descriptive in nature to characterize the

response to the challenge virus. In general, the continuous variable will be summarized by the number of non-missing values, mean (standard deviation), median, minimum and maximum; and the categorical variable will be summarized by the frequency and proportion, by dose cohorts and time points if measurements at multiple time points are available.

11 Statistical Analysis for CDSC Review

There are no statistical criteria for study termination in this DHIM clinical trial. Raw data on safety and performance for 28 days post inoculation will be extracted from EDC database system and presented to the study CDSC for recommendation of appropriate follow-up options.

12 References

1. Phase One, Open Label Assessment of a Dengue-3-Virus-Live Virus Human Challenge - (DENV-3-LVHC) Virus Strain. IND 019231; Sponsor Protocol 2019-01-UMU; Assessment of DENV-3-LVHC. Version 3. 07 Jul 2020.
2. Food and Drug Administration Center for Biologics Evaluation and Research (September 2007). Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. FDA Maryland.
3. U.S. Department of Health and Human Services, National Institutes of Health, National Cancer Institute (2009). Common Terminology Criteria for Adverse Events (CTCAE) Version 4.03. 2, http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06

13 Summary Tables, Listings, and Figures

The list and specifications of the table, listings and figures are included in a separate document.