Statistical Analysis Plan (SAP)

Protocol Title:	Phase One, Open Label, Assessment of a Dengue-4-Virus-Live Virus Human Challenge - (DENV-4-LVHC) Virus Strain in Healthy U.S. Adults
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Revision History

Version	Date	Revisions
0.1	24-Jun-22	Initial draft
1.0	08-Aug-23	Revised draft SAP incorporating changes in protocol v5.0: In section 3 study objectives, Primary objective add “To assess the safety, reactogenicity, and immunogenicity of homologous rechallenge with DENV-4-LVHC”. Secondary objectives add “To characterize the clinical and virologic responses following homologous rechallenge with DENV-4-LVHC”. Exploratory objective add “To explore the immune response and host-virus interactions following homologous rechallenge with DENV-4-LVHC”. In section 4.3 exploratory endpoints add “Comparison analysis of primary DHIM-4 results with homologous rechallenge DHIM-4 results”. In section 5.1 design overview add “Volunteers previously challenged with DENV-4-LVHC (i.e., Main Study participants) will be eligible and invited to enroll into the homologous rechallenge sub-study.” In section 5.5 study duration add “Homologous Rechallenge Sub-study: Approximately 6 months after the rechallenge inoculation per volunteer”. In section 9.2 Physical Examination add “Abnormal physical findings at baseline will be recorded.”.
1.1	10-Jun-24	Removed the “Occurrence, intensity, and duration of unsolicited injection site symptoms until 28 days post virus inoculation or 7 days post inpatient evaluation, whichever is later”; “Occurrence, intensity, and duration of unsolicited systemic symptoms until 28 days post virus inoculation or 7 days post inpatient evaluation, 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 (See appendix: defined Dengue Illness Index)” from the primary endpoints. Changed “If the FAS and PPAS include the same volunteers, only one set of analyses will be produced, in addition to those on the SafAS” to “Following the data review meeting, the FAS and PPAS include the same volunteers, therefore, only one set of analyses

		will be produced, in addition to those on the SafAS" in section 6.3. Added "DII will be reported separately whereby" in section 8. Removed "Occurrence, intensity, and duration of unsolicited injection site symptoms until 28 days post virus inoculation or 7 days post inpatient evaluation, 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" and "Number, intensity, and duration of abnormal laboratory measurements until 28 days post virus inoculation or 7 days post hospitalization, whichever is later" in section 8. Removed "and SARS-CoV-2 RT-PCR/Antigen test (optional) will be summarized by day period." in section 17.2. Added "The calculation of AUC uses linear trapezoidal rule applied to plots of viral RNA levels over time. The AUC calculated from the day of first viral RNA detection and conclude on the day after (Day + 1) of the final viral RNA detection. The x-values represent the timepoints (in days) at which viral RNA measurements were taken. The y-values represent the corresponding viral RNA load (Log10 copies/mL) at each time point." in section 17.2. Removed "at 28 days following inoculation" and "will be summarized at 0, 1, 3, and 6 months" and "CMI data will be analysed separately" from section 17.3. Removed the whole section 17.4.
2.0	12-Jun-24	Final version

Table of Contents

1. List of Abbreviations

Abbreviation	Explanation
AE	Adverse Event
CDISC	Clinical Data Interchange Standards Consortium
CDSC	Consortium Data Safety Committee
CSR	Clinical study report
DENV-4	Dengue virus serotype 4
DENV-4-LVHC	Dengue -4 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
Pes	Physical examinations
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

2. Introduction

This study is a phase 1, open label clinical trial in healthy US adults. This study will examine the safety and effectiveness of the Dengue 4 Live Virus Human Challenge (DENV-4-LVHC) product 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.

The preparation of this SAP has been based on International Conference on Harmonisation (ICH) E9 and Good Clinical Practice (GCP) guidelines.

3. Study Objectives

3.1 Primary objective

- To assess the safety of a newly derived DENV-4-LVHC viral strain intended for use in a Dengue Human Infection Model (DHIM) as defined by clinical and laboratory parameters.
- To assess the safety, reactogenicity, and immunogenicity of homologous rechallenge with DENV-4-LVHC

3.2 Secondary objectives

- To characterize the clinical and virologic responses following inoculation with a DENV-4-LVHC viral strain as a DHIM
- To assess the performance of the DENV-4-LVHC viral strain in eliciting an uncomplicated dengue-like illness based upon clinical and laboratory parameters
- To characterize the clinical and virologic responses following homologous rechallenge with DENV-4-LVHC

3.3 Exploratory objective

- To explore the immune response and host-virus interactions following exposure to manufactured, attenuated DENV-4-LVHC
- To explore the immune response and host-virus interactions following homologous rechallenge with DENV-4-LVHC
- To explore immunologic correlates of protection to DENV-4.

4. Study Endpoints

4.1 Primary endpoints

The primary endpoints to address safety are as follows:

- Number, intensity, and duration of abnormal laboratory measurements until 28 days post virus inoculation or 7 days post inpatient evaluation, whichever is later
- Occurrence, intensity, and duration of solicited injection site symptoms until 7 days post virus inoculation
- Occurrence, intensity, and duration of solicited systemic symptoms until 28 days post virus inoculation or 7 days post inpatient evaluation, whichever is later
- Number of serious adverse events (SAE) until 28 days post virus inoculation or 7 days post inpatient evaluation, 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) and as a function of area under the curve (AUC):

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

4.3 Exploratory endpoints

The endpoints for the characterization of response to the challenge virus may include the following:

- 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)
- Proteomics – microarray
- Cell mediated immunity (CMI)
- Transcriptomics
- Evolutionary analysis of DHIM-4 strain whole genome sequence (consensus and quasi-species)
- Comparison analysis of primary DHIM-4 results with homologous rechallenge DHIM-4 results

5. Trial Design

5.1 Design Overview

Volunteers will be 18-50 years, inclusive, at enrollment. This is a study of the safety, tolerability, immunogenicity and efficacy of vialed DENV-4 LVHC strain(s) with dose escalation in a 3+7 design.

A dose escalation design will be followed to consider both the safety and performance profile (Figure 1). Each dose cohort will enroll up to 10 volunteers. The sentinel cohort of 3 volunteers in each dose group (starting with the low dose) will be inoculated and followed through Day 28 prior to additional volunteers being inoculated.

Depending upon safety outcome and achievement of performance parameters, the algorithm proceeds as follows:

a) After 28 days, if ≥2 of 3 volunteers have met performance parameters and no safety issues have occurred, the remaining volunteers [up to seven more volunteers (or a 3+4 scheme)], in the low dose cohort will be inoculated for a total of 10 volunteers.

b) After 28 days, if ≤1 of 3 volunteers have met performance parameters and no safety issues have occurred, an additional 3 volunteers will be inoculated in the low dose cohort. If the performance parameters remain at ≤1 of 3 volunteers, without safety concerns, dose escalation to the next dose, without additional inoculation will occur. However, if performance parameters are noted in ≥ 2 of 3 volunteers, an additional 4 volunteers will be inoculated.

c) After 28 days, if a safety issue is noted in 1 of the 3 volunteers, consideration will be made as to whether to halt or to proceed with caution to inoculate an additional 3 volunteers. The Consortium Data Safety Committee (CDSC) serving as the safety monitoring committee (SMC) for the study will approve inoculation of further volunteers. If no safety issue is identified in the next 3 volunteers, an additional 4 volunteers will be inoculated.

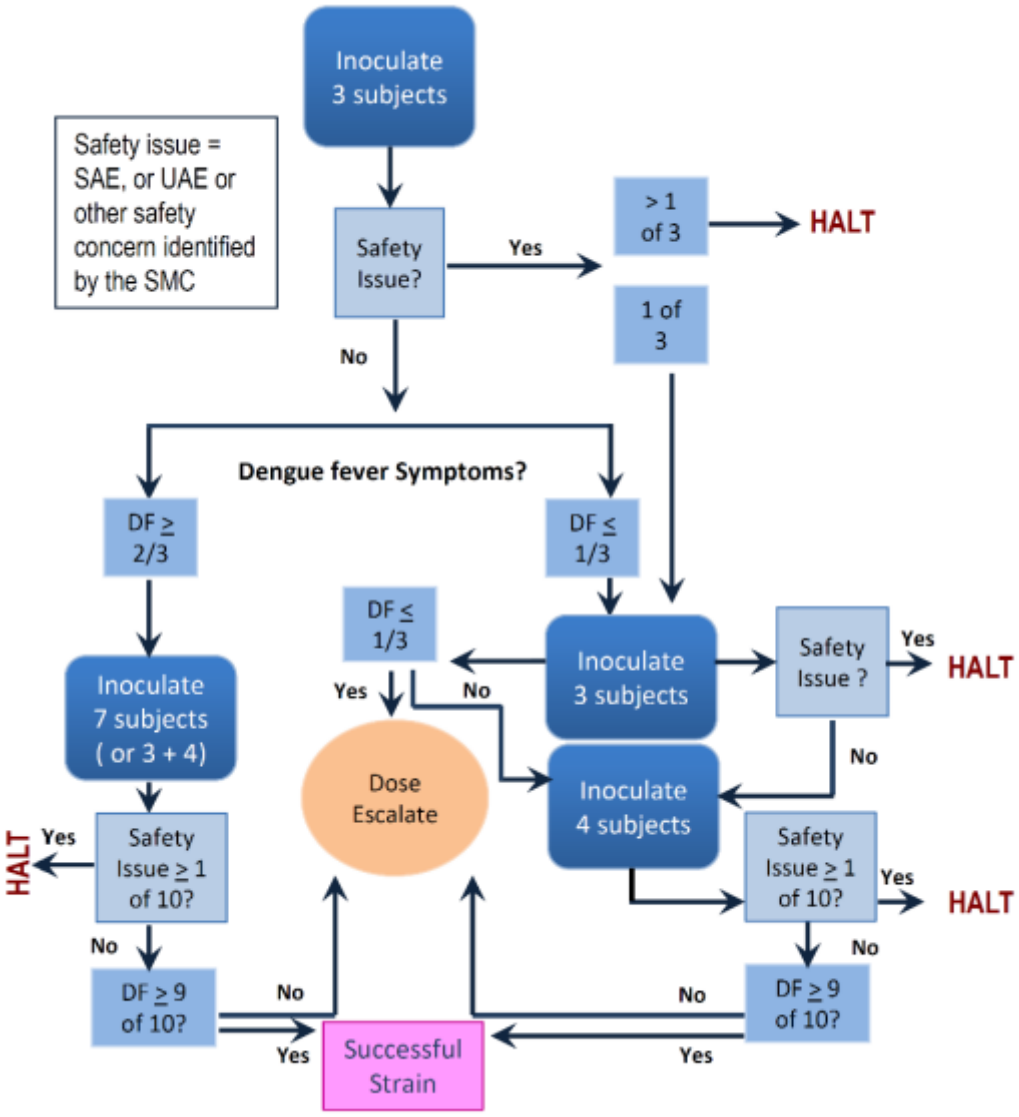
Day 28 data from the inoculated volunteers will be compiled and presented to a CDSC to determine if a dose escalation is warranted or if the current dose represents a successful strain. A dose escalation will follow the same schedule as the low dose, staggering the first three volunteers.

As with the low dose, after day 28 safety and performance parameters will be assessed, and the algorithm will be followed as outlined in Figure 1. The above escalation scheme will be followed for the middle dose and data will go to the CDSC to determine if the criteria meets that of a successful strain or if it warrants going to the next higher dose.

All volunteers will receive a single SC inoculation of DENV-4-LVHC (the inoculation). After the inoculation, volunteer will be seen and evaluated in the clinic beginning on Day 4 and then every day until Day 16 in the absence of viremia, upon which volunteers will be seen every other day until Day 28 post virus inoculation. If

a volunteer develops asymptomatic viremia (during May-October), they will enter the sequestration phase of the study. If a volunteer meets inpatient criteria listed in protocol section 9.4.1, they will be admitted to the inpatient facility (GCRC or Pharmaron) and will follow the inpatient phase schedule. Data will be collected and compiled during the 28 days after inoculation or through 7 days post inpatient discharge, whichever is later.

Figure1: Algorithm for Dengue Strain Selection



Volunteers will be enrolled in staggered-start, dose-ranging cohorts, with the lowest dose being introduced first. Volunteers (up to 30 per DENV) will be followed by close observation of safety parameters, viremia and symptoms meeting pre-defined performance parameters designed to optimize DHIM for safe use in humans. 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/or absence of virus detection by polymerase chain reaction (PCR).

After inoculation of up to 3 volunteers at a given dose level, the safety and performance data collected for the 28 days will be presented to the Consortium Data Safety Committee (CDSC) which will serve as the study safety monitoring committee (SMC) and that will provide a recommendation as per the following options:

1. Discontinue further inoculation –pursued if CDSC or clinical team/PI determines that there are unacceptable safety concerns
2. Repeat same dose on three or four new volunteers – pursued if CDSC determines potential but acceptable safety issue
3. Proceed onto the dose verification cohort with seven new volunteers – pursued if CDSC determines acceptable safety profile
4. Escalate to higher dose in three new volunteers – pursued if CDSC determines acceptable safety profile in the six to ten volunteers inoculated at same dose, but performance parameters are not met in 90% of volunteers

Volunteers previously challenged with DENV-4-LVHC (i.e., Main Study participants) will be eligible and invited to enroll into the homologous rechallenge sub-study. This sub-study will be conducted at least 12 months from the original DENV-4 LVHC challenge (final DHIM – June 9, 2022). Volunteers of this sub-study will be inoculated with the DENV-4-LVHC subcutaneously using the optimized dose of the challenge virus used in their previous DENV-4-LVHC challenge. If funds allow, 2-3 naïve volunteers will also be recruited as a comparison group both clinically and immunologically, given the adjusted immunologic parameters. Volunteers will be closely monitored for symptoms, viremia, and endpoint parameters until Day 28-post inoculation. Sequestration and inpatient hospital admission criteria will remain unchanged. Long term follow-up will be up to Day 181. The immunologic parameters will be adjusted slightly to allow for an in-depth analysis of the response to homologous rechallenge and based on RNAseq data acquired from both the DHIM-1 and DHIM-3 results (personal communication).

5.2 Study Population and Eligibility Criteria

This study will enroll up to 30 healthy men and healthy, non-pregnant, non-breastfeeding women between the ages of 18 and 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 eligibility decision.

5.3 Replacement of Subjects Withdrawn from Study

Volunteers may be replaced during the study if they are unable to complete the study procedures required during the first 28 days of the study, or at the investigator's discretion if they are withdrawn for reasons not related to the study.

5.4 Randomization and Allocation Procedures

This is an open label study, there is no blinding or randomization code for this study.

5.5 Study Duration

Main Study: Approximately 6 months after inoculation (Day 0-180) per volunteer.

Homologous Rechallenge Sub-study: Approximately 6 months after the rechallenge inoculation per volunteer

5.6 Sample size and Power

As this study has no statistical hypothesis tests, there is no formal power calculation.

Since this is a modified 3+7 first-in-human study with focus on safety, the number of volunteers exposed to test product needs to be limited. Due to the sample size, only adverse events with high incidence rates will be detected. With 10 volunteers per cohort, 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-4-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. Volunteers will be analyzed according to the inoculation they received. The safety analysis set (SafAS) will be used for the analysis of safety data in this study.

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. Volunteers will be analyzed according to the inoculation they received. The full analysis set (FAS) will be used for the analysis of the efficacy data in this study.

Performance data includes viremia and any of the following solicited symptoms:

- Fever ≥ 38°C (100.4°F)
- Rash
- Headache
- Retro-orbital (eye) pain

- Muscle pain (Myalgia)
- Joint pain and/or bone pain
- Fatigue and/or malaise (weakness)
- Pain at injection site
- Swelling at injection site
- Erythema at injection site
- GI symptoms (abdominal pain, nausea and/or vomiting)

Volunteers will be analyzed according to the inoculation they received.

6.3 Per-Protocol Analysis Set

The PPAS will include all volunteers who meet the definition of the FAS 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 performance analyses will be performed on the FAS and PPAS. Volunteers will be analyzed according to the inoculation they received.

Following the data review meeting, the FAS and PPAS include the same volunteers, therefore, only one set of analyses will be produced, in addition to those on the SafAS.

7. Subject Characteristics

7.1 Demographics:

The following subject characteristics will be collected

- Age
- Sex at birth
- Ethnicity
- Race
- Weight (kg)
- Height (cm)
- BMI (kg/m^2)

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)).

7.2 Concomitant Medications

Prescriptions and over-the-counter medications (including vitamins and supplements) will be recorded on the CRF 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.

7.3 Baseline Medical History

A complete medical history will be collected.

7.4 Flavivirus screen

Lab specimen collection date and time and test results will be collected.

7.5 ABO blood group

The ABO blood group will be collected.

8. Endpoint Assessments

Solicited Adverse Events

A solicited AE is a predetermined event, identified in the investigator's brochure (IB), which may reflect safety concerns related to the investigational product. The solicited AEs for this study include:

- Fever ≥ 38°C (100.4°F)
- Rash
- Headache
- Retro-orbital (eye) pain
- Muscle pain (Myalgia)
- Joint pain and/or bone pain
- Fatigue and/or malaise (weakness)
- Pain at injection site
- Swelling at injection site
- Erythema at injection site
- GI symptoms (abdominal pain, nausea and/or vomiting)

Solicited AEs will be captured during all clinical visits. Solicited events will be the basis of categorizing the presence of dengue fever, along with the presence of detectable viremia.

Solicited injection site symptoms of interest occur until 7-days post virus inoculation. Solicited systemic symptoms of interest occur through to 28-days post virus inoculation or 7 days post inpatient evaluation (whichever is later).

Unsolicited Adverse Events

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

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the investigator brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the investigator brochure listed only cerebral vascular accidents. “Unexpected,” as used in this definition, also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

Unsolicited AEs of interest occur through to 28-days post virus inoculation or 7 days post inpatient evaluation (whichever is later) and include

- Abnormal laboratory measurements
- Injection site symptoms excluding solicited injection site symptoms
- Systemic symptoms excluding solicited systemic symptoms

Dengue Illness Index (DII) of DHIM-4

DII will be reported separately whereby a Dengue Illness Index will be calculated for each volunteer. The number, percentage and severity score of volunteers in each group with each of the following clinical or laboratory symptoms as measured by the Dengue Illness Index will be determined based upon the following:

- Fever greater than or equal to 38°C (100.4°F) measured at least twice at least 4 hours apart
- Headache/retro-orbital pain
- Rash
- Fatigue and/or malaise
- Myalgia
- Arthralgia and/or bone pain
- GI symptoms (nausea, vomiting, abdominal pain)
- Liver function tests (ALT, AST)
- Leukopenia
- Thrombocytopenia

Occurrence, intensity, and duration of solicited injection site symptoms until 7 days post virus inoculation will be used a scoring system (0 = none, 1 = mild, 2 = moderate, and 3 = severe) on a daily basis and a total will be calculated representing the mean of the summation of total number of days of symptoms, the number of symptoms per day and the maximum severity score (grade 0-3).

The scoring system will also be used in the following tables:

- Occurrence, intensity, and duration of solicited systemic symptoms until 28 days post virus inoculation or 7 days post inpatient evaluation, whichever is later

9. Safety Assessments

9.1 Laboratory Test Abnormalities

Clinical laboratory evaluations 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, the condition should be captured as an AE.

9.2 Physical Examination

Physical examinations (PEs) including weight and height will be conducted at baseline. Abnormal physical findings at baseline will be recorded. A targeted exam may be conducted at all subsequent visits.

Physical examination will be presented by treatment group using frequency counts and percentages.

9.3 Vital Signs

Vital signs (Heart rate, respiratory rate and blood pressure) and temperature will be conducted at baseline and all subsequent visits.

10. General Statistical Methodology

General Statistical Methodology

10.1 Data Sources

CRF data will be extracted from the clinical data base, PCR qualification and microneutralization antibody data will be provided by a vendor. Data will be tabulated and reported following CDISC standards.

10.2 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.

10.3 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.

10.4 Definition of Baseline and Change from Baseline

When analysis requires identification of a baseline value, the last value prior to administration of the DENV-4-LVHC vaccine will be used.

11. Statistical Methods

11.1 Variates and Subgroups

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

11.2 Sample Size Reassessment

No sample size reassessment was planned for this study.

11.3 Interim/Preliminary Analysis

No formal interim analysis will be performed.

11.4 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.

11.5 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.

11.6 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, 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 descriptive statistics calculated for each of the treatment groups (if applicable), unless described otherwise in following sections. Treatment groups will be categorized into first exposure and second exposure(rechallenge). First exposure includes low dose main study, low dose sub-study and combined (low dose main study and low dose sub-study).

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, median, Q1 and Q3 values will be displayed with one more decimal digit than what the original value. Min and max will keep the same number of decimal places as the original value. Standard deviations and standard errors will be displayed two more decimal places than the original value. For categorical summary results, percentages will be displayed one decimal place unless the value is 100. Zero counts will not be accompanied by a percentage.

11.7 Data Grouping for Analysis

Unless otherwise stated, there will be no additional grouping for any summaries. One exception will be the cohort of homologous rechallenge participants (n = 6) who will be summarized as a separate standing group.

Analysis of the data from this study will be descriptive in nature. For hematology and serum chemistry tests, any clinically significant changes from baseline value will be identified. 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.

12. Subject Disposition

Subject Disposition will be summarized by treatment group. The numbers and percentage of subjects screened, enrolled (treated and not treated), safety analysis set, full analysis set, per-protocol analysis set, completed study, and the primary reasons for discontinuation from study will be tabulated. Subject disposition will be listed. A listing of each subject excluded from an analysis population will be listed as well as the reason why they were excluded from the population.

13. Demographic and Baseline Characteristics

Demographics will be summarized descriptively [number of subjects, (n), mean, SD, median, minimum and maximum] for Full analysis set.

Medical history is collected at the screening visit and will be summarized using WHO version xxx for the Safety Analysis Set.

14. Treatments

15. Concomitant Medications

Concomitant medications will be listed, ordered within subject by the “Start Date.” The listing will display the recorded term and, adjacent, the WHO Drug preferred term and medication name and therapeutic indication.

16. Important Protocol Deviations

Important deviations will be summarised using counts and percentage by dose group for the Full Analysis Set

17. Endpoint Analyses

17.1 Statistical Analysis for Primary Endpoints

The primary endpoints will be analysed as follows:

- Number, intensity, and duration of abnormal laboratory measurements until 28 days post virus inoculation or 7 days post inpatient evaluation, whichever is later

- Occurrence, intensity, and duration of solicited injection site symptoms until 7 days post virus inoculation
- Occurrence, intensity, and duration of solicited systemic symptoms until 28 days post virus inoculation or 7 days post inpatient evaluation, whichever is later
- Number of serious adverse events (SAE) until 28 days post virus inoculation or 7 days post inpatient evaluation, 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

17.2 Statistical Analysis for Secondary Endpoints

Analysis of the secondary endpoints will be applied to the per protocol population. Only those volunteers who receive inoculations will be included in the analysis. A scoring system will be used to evaluate symptoms daily and sum a total calculated symptom value based upon the assignation of scores (0 = none, 1 = mild, 2 = moderate, and 3 = severe).

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

- Viremia by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) up to 28 days post virus inoculation.
- Area Under the Curve (AUC) for Viremia by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) up to 28 days post virus inoculation will be summarized descriptively by treatment group. Data will only be imputed for those with detectable dengue viremia. The calculation of AUC uses linear trapezoidal rule applied to plots of viral RNA levels over time. The AUC calculated from the day of first viral RNA detection and conclude on the day after (Day + 1) of the final viral RNA detection. The x-values represent the timepoints (in days) at which viral RNA measurements were taken. The y-values represent the corresponding viral RNA load (Log_{10} copies/mL) at each time point.
- Number and percentage of subjects with quantitative RNA by RT-PCR up to 28 days post virus inoculation will be summarized by day period.

17.3 Statistical Analysis for Exploratory Endpoints

There are no hypotheses. All the main analyses will be descriptive. The immune response to the challenge virus at a certain dose will be characterized descriptively by:

- GMT and geometric mean titer rates (GMTRs) of MN50 antibody (measured by dengue neutralizing titer) will be summarized by treatment groups at 0, 1, 3, and 6 months
- Number and percentage of volunteers with a titer ≥10
- Genetic (RNA) data will be analysed separately

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.

Analysis of the exploratory endpoints will be applied on the full analysis set and per protocol population. Only those volunteers who receive inoculations will be included in the analysis.

18. Safety Analyses

18.1 Adverse Events

AEs will be presented by body system, preferred term, severity grade, and relatedness to virus inoculation. For the tabulation of the AEs by body system, a volunteer will be counted only once in a given body system. For example, a volunteer reporting nausea and diarrhea will be reported as one volunteer, but the symptoms will be listed as two separate AEs within the class. Therefore, the total number of AEs reported within a body system may exceed the number of volunteers reporting AEs within the body system.

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.

18.2 Deaths and Serious Adverse Events

Serious AEs and death will be listed and summarized.

18.3 Laboratory Data

All clinical safety laboratory parameters will be listed by subject and date/time and study day of sample, sorted within treatment group and grouped into those with viremia versus those without viremia. 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 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.

18.4 Vital Signs

Visit values and changes from baseline for vital sign measurements (blood pressure, oral temperature, heart rate and respiratory rate) will be summarized by treatment group at each visit using descriptive statistics. Visit values will be calculated as the mean of all available measurements per parameter.

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

18.5 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.

19. Statistical Analysis for CDSC Review

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

20. 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

21. Summary Tables, Listings, and Figures

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

22. Appendix

Table: Dengue Illness Index Card

Symptoms	Day after DHIM																
Clinical	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Duration of Sx
Fever (Temp ≥ 100.4°F; 38°C)																	
Headache and/or retro-orbital pain																	
Rash																	
Malaise and/or fatigue																	
Myalgia (muscle pain)																	
Arthralgia and/or bone pain																	
GI Symptoms*																	
Laboratory																	
Elevated ALT or AST																	
WBC (Leukopenia)																	
Platelets (Thrombocytopenia)																	
																	Total =
Number of Sx each day																	Total =
Max Severity Score (1-3)**																	Total =
DII - Dengue Illnesss Index =		$\frac{[A+B+C]}{3}$	A = Duration of Symptoms			B = Number of Symptoms per Day				C - Max Severity Score							#VALUE!

* GI symptoms include either anorexia, nausea, vomiting, diarrhea and/or abdominal pain

** Severity score corresponds to Mild (1), Moderate (2) and Severe (3). Laboratory values would coincide with protocol specific FDA-CIBR guidelines.