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

Abbreviated title: Study of the DENV-4 LVHC Virus Strain in DHIM

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INVESTIGATOR'S AGREEMENT

Study 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

The signature below constitutes the approval of this protocol and the attachments and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Kirsten E. Lyke, MD Principal Investigator Center for Vaccine Development and Global Health University of Maryland School of Medicine	Date

2. SYNOPSIS

Name of Sponsor/Company: The University of Maryland, Center for Vaccine Development and Global Health and/or Institute of Human Virology	
Co-development Partners: U.S. Army Medical Materiel Development Activity (USAMMDA), Ft. Detrick, MD	
Name of Investigational Product: Dengue-4-Virus-Live Virus Human Challenge (DENV-4-LVHC)	
Name of Active Ingredients: Dengue virus (DENV) type 4 strain H-241	
Title of Study: Phase One, Open Label, Assessment of a Dengue-4-Virus-Live Virus Human Challenge - (DENV-4-LVHC) Virus Strain in Healthy U.S. Adults	
Study Centers: Center for Vaccine Development and Global Health (CVD), University of Maryland Baltimore, Maryland	
Principal Investigators (PIs)**:** Kirsten E. Lyke	
Study Period: **Main Study -** Estimated date first volunteer enrolled: 1st quarter 2022 Estimated date last volunteer completed:4th quarter 2022 **Homologous Rechallenge Substudy –** Estimated date first volunteer enrolled: 3rd quarter 2023 Estimated date last volunteer completed: 1st quarter 2024	Phase of Development: 1

Objectives:

Primary:

- To assess the safety and dose 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

Secondary

- To characterize the clinical and virologic responses following increasing dose exposure to the newly derived DENV-4-LVHC viral strain as a DHIM
- To assess the performance of the DENV-4-LVHC viral strain to elicit 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

Exploratory

- To explore the immune response and host-virus interactions following exposure to 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.

Methodology:

Design (Main Study): 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. 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

Substudy Design (Homologous Rechallenge): Willing volunteers previously challenged with DENV-4-LVHC (e.g., Main Study participants) will be eligible to enroll in this substudy. This homologous rechallenge will be conducted at least 12 months from original DENV-4 LVHC challenge. Volunteers of this sub-study will be inoculated with the optimized DENV-4-LVHC subcutaneously using the same dose of the virus used in their previous DENV-4-LVHC challenge. Volunteers will be closely monitored for symptoms, viremia, and safety parameters until Day 28-post inoculation. Sequestration and inpatient hospital admission criteria will follow the same parameters used in the Main Study with additional immunologic timepoints.

Note: Due to the COVID-19 pandemic, additions have been added to the protocol in the event that local transmission of the SARS-CoV-2 virus continues concomitant to study operations.

Estimated Number of Volunteers to be Screened:

Approximately 100 volunteers will be screened

Minimum and Maximum Number of Volunteers Planned for Enrollment: (Main Study)

Minimum: 3 (low dose does not meet safety parameters)

Maximum: 30 volunteers (each dose may require up to ten volunteers)*

*Note: Will not proceed to higher doses if DHIM optimized.

Volunteer Population for Inclusion/Exclusion:

Healthy men and healthy, non-pregnant, non-breastfeeding women between the ages of 18 and 50

Investigational Product Dosage, Schedule, and Mode of Administration:

The investigational product, DENV-4-LVHC is reconstituted with 0.7 mL of water for injection and diluted with formulated Eagle's minimum essential medium (EMEM with human serum albumin, L-glutamine, and lactose monohydrate). Product dilution is listed below but will be adjusted based on latest stability data.

- Low dose: ~0.95 x 10^2 PFU
- Medium dose: ~0.95 x 10^3 PFU
- High dose: ~0.95 x 10^4 PFU

All investigational products will be administered in a single 0.5-1.0 mL inoculation subcutaneously (SC) in the triceps area of the arm. Dose escalation will not occur if parameters are optimized at a lower dose.

Duration of Study:

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

Homologous Rechallenge Substudy: Approximately 6 months after rechallenge inoculation per volunteer.

Criteria for Inclusion/Exclusion:

Inclusion:

- Male or non-pregnant, non-breastfeeding female between 18 and 50 years of age (inclusive) at the time of consent.
- Volunteers must be able to provide written informed consent.
- Volunteers must be healthy as established by medical history and clinical examination at study entry.
- Volunteers must pass a comprehension test and be able to comply with all study requirements.
- Female volunteers of non-childbearing potential (non-childbearing potential is defined as having had one of the following: a tubal ligation at least 3 months prior to enrollment, a hysterectomy, an ovariectomy, or is post-menopausal).
- Female volunteers of childbearing potential may be enrolled in the study, if all the following apply:
  - Practiced adequate contraception (see Definition of Terms, **section 5.4.2.3.**) for 30 days prior to challenge
  - Has a negative urine pregnancy test on the day of DHIM
  - Agrees to continue adequate contraception until two months after completion of the DHIM

Exclusion:

- History of dengue infection or dengue illness, or history of flavivirus infection or vaccination (e.g., yellow fever, tick-borne-encephalitis virus [TBEV], Japanese encephalitis, and dengue). *Note: for the Homologous Rechallenge Substudy, prior DENV-4-LVHC-related dengue infection is not an exclusion.*
- Volunteers positive for antibodies to flaviviruses (FV) to include dengue virus, West Nile virus, Yellow Fever virus, Zika virus, and Japanese encephalitis virus, during screening period for Main Study. *Note: Volunteers of Homologous Rechallenge Substudy are expected to be positive for antibodies to dengue virus due to prior DENV-4-LVHC challenge.*
- Planned administration of any flavivirus vaccine for the entire study duration.
- Any recent (within 4 weeks) or planned travel to any dengue endemic area while participating in the trial
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required)
- Volunteer seropositive for hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies (anti-HCV), or human immunodeficiency virus antibodies (anti-HIV). *Note: Prior volunteers of HIV vaccine studies may result in a false positive HIV antibody test, as such, in this scenario, volunteer will be eligible if they have a negative HIV RNA PCR at screening.*
- Safety laboratory test results at screening that are deemed clinically significant or more than Grade 1 deviation from normal with the exception of PT/PTT, fibrinogen decrease, ALT/AST increase (acceptable to 1.1 ULN), platelet decrease which will be exclusionary at Grade 1 or higher
- Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by physical examination or laboratory screening tests

- History of bipolar disorder, schizophrenia, hospitalization in the past year for a mental health disorder, or any other psychiatric condition, which in the opinion of the investigator prevents the volunteer from participating in the study
- Significant screening physical examination abnormalities at the discretion of the investigator, including a BMI > 35 kg/m^2
- Planned administration or administration of a vaccine/product not planned in the study protocol during the period starting 30 days prior to the DHIM until 56 days after the study completion (routine influenza or COVID-19 vaccination will be allowed if it is not administered within 14 days preceding DHIM)
- Use of any investigational or non-registered product (drug or vaccine) other than the study DHIM during the period starting 30 days preceding the DHIM and/or planned use during the study period
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting 90 days prior to the DHIM (for corticosteroids, this will mean prednisone ≥ 5mg/day or equivalent; inhaled, intranasal and topical steroids are allowed)
- Concurrently participating in another clinical study, at any time during the study period, in which the volunteer has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device)
- Autoimmune disease or history of autoimmune disease
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the study product or related to a study procedure
- Major congenital defects or serious chronic illness
- History of any neurological disorders or seizures
- Acute disease and/or fever (≥37.5°C/99.5°F oral body temperature) at the time of enrollment: note that a volunteer with a minor illness such as mild diarrhea, mild upper respiratory infection, etc., without fever, may be enrolled at the discretion of the investigator
- Administration of immunoglobulins and/or any blood products during the period starting 90 days preceding the DHIM or planned administration during the study period
- Recent history of chronic alcohol consumption (more than 2 drinks per day and/or drug abuse) based on volunteer reported history
- Pregnant or breastfeeding female or female currently planning to become pregnant or planning to discontinue adequate contraception
- Men who intend to father a child during the study period (approximately 2 months)
- Any religious or personal beliefs that bar the administration of blood products, transfusions or serum albumin
- Planned or current administration of an HMG-CoA reductase inhibitor (i.e., lovastatin, simvastatin, atorvastatin, etc.)
- Currently regularly taking anti-coagulant medication, aspirin, or non-steroidal anti-inflammatory drugs (NSAIDs)

Any other condition which, in the opinion of the investigator, prevents the volunteer from participating in the study

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 and maximum (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.

Statistical Methods for Primary Endpoints:

The enrolled population consists of all volunteers who receive the virus inoculation or re-inoculation.

Primary Endpoints:

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

- Occurrence, intensity, 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 hospitalization, whichever is later
- Occurrence, intensity, and duration of solicited systemic symptoms until 28 days post virus inoculation or 7 days post hospitalization, whichever is later
- Occurrence, intensity, and duration of unsolicited systemic symptoms until 28 days post virus inoculation or 7 days post hospitalization, 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 defined Dengue Illness Index)
- 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

Statistical Methods for Secondary Endpoints:

Secondary:

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

Analysis of the secondary endpoints will be applied on 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)

Statistical Methods for Exploratory Endpoints:

Exploratory endpoints will be analyzed using the population of volunteers enrolled in the trial who receive one 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
- 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

Statistical Methods of Dengue Illness Index (DII) of DHIM-4:

The impact of the challenge virus up to 28 days after virus inoculation will be assessed descriptively using the following disease severity index (DSI) parameters:

- Number and percentage of volunteers in each group who develop signs and symptoms of dengue fever as described below
- Time to onset of clinical signs and symptoms of dengue fever as described below

- Magnitude of viremia, time to onset of viremia, duration of viremia, number and percentage of volunteers with measurable and sustainable viremia and area under the curve viremia analysis
- Number and percentage of volunteers in each dosing group with fever greater than or equal to 38°C (100.4°F) measured at least 2 times at least four hours apart in 24 hours
- 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**:
  - Fever greater than or equal to 38°C (100.4°F)
  - 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

  **Note: Post-hoc analysis can recategorize disease severity based upon WHO parameters*

Analysis of the secondary endpoints will be applied on per protocol population. Only those volunteers who receive inoculations will be included in the analysis. Descriptive analysis will compare measurements from vaccinated and unvaccinated study volunteers.

Power and Sample Size:

As this study has no statistical hypothesis test, 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%.

The proposed sample size leads to a reasonable amount of confidence about the capability of the challenge DENV-4-LVHC virus to produce an uncomplicated dengue-like illness that meets the desired Performance Parameters. With 9 of 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%.

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4. LIST OF ABBREVIATIONS

AE	Adverse event, adverse experience
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
β-hCG	Beta human chorionic gonadotropin
BS	Blood sample
BSE	Bovine spongiform encephalopathy
C	Celsius
CBER	Center for Biologics Evaluation and Research (United States Food and Drug Administration)
CDSC	Consortium Data Safety Committee
CFR	Code of Federal Regulation
CI	Confidence interval
CLIA	Clinical laboratory improvement amendments (42 CFR 493)
CMI	Cell-mediated immunity
CMP	Comprehensive metabolic panel
CSSD	Clinical Services Support Division
CTC	Clinical Trials Center
CVD	Center for Vaccine Development and Global Health (Maryland)
D+24h	Within 24 hours after specified event (e.g., D+24h of viremia denotes “within 24 hours after viremia detected”)
DENV	Dengue virus
DENV-1, -2, -3, -4	Dengue virus serotype 1, 2, 3 or 4
DENV-4-LVHC	Dengue-4 Virus – Live Virus Human Challenge
DHF	Dengue hemorrhagic fever
DHIM	Dengue Human Infection Model
DoD	Department of Defense
DPIV	Dengue virus purified inactivated vaccine
eCRF	Electronic case report form
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunospot assay
F	Fahrenheit
FDA	US Food and Drug Administration
GBS	Guillain-Barré syndrome
GCP	Good clinical practice
GCRC	General Clinical Research Center
GLP	Good laboratory practice
GMT	Geometric mean titer
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIPAA	Health Insurance Portability Accountability Act

HIV	Human immunodeficiency virus
HSPO	Human Volunteers Protection Office
IB	Investigator brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
ICS	Intracellular cytokine staining
IDS	Investigational drug services (pharmacy)
IFN	Interferon
IHV	Institute of Human Virology
IND	Investigational new drug
IRB	Institutional review board
mAb	Monoclonal antibody
MD	Medical doctor
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram
mL	Milliliter
mm	Millimeter
NIAID	National Institute of Allergy and Infectious Diseases
NCS	Not clinically significant
NSAID	Non-steroidal anti-inflammatory drug
ORP	Office of Research Protections
PBF	Pilot Bioproduction Facility
PBMC	Peripheral blood mononuclear cells
PDMP	Protocol Deviation Management Plan
PI	Principal investigator
PFU	Plaque forming units
PPAS	Per-protocol analysis set
PSSB	Product Safety Surveillance Branch
PVG	PharmacoVigilance Physician
RBC	Red blood cell
RDE	Remote data entry
RN	Registered nurse
RNA	Ribonucleic acid
RT-PCR	Reverse transcription – polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SMC	Safety Monitoring Committee
SOP	Standard operating procedure
SUNY-UMU	State University of New York, Upstate Medical University
ULN	Upper limit of normal

UMB	University of Maryland, Baltimore
UMB HRPP	University of Maryland, Baltimore Human Research Protection Program
UMMC	University of Maryland Medical System
US	United States
USAMMDA	United States Army Medical Materiel Development Activity
USAMRMC	United States Army Medical Research and Materiel Command
WRAIR	Walter Reed Army Institute of Research
WBC	White blood cell
WFI	Water for injection
WHO	World Health Organization

5. INTRODUCTION

Dengue, a mosquito-borne disease caused by serologically related but antigenically distinct viruses grouped into 4 types (DENV-1 to DENV-4), occurs in more than 128 countries in the tropical and subtropical regions of Asia-Pacific, the Americas, the Middle East, and Africa (estimated 2.5 billion people at-risk). Dengue is the most common arboviral (mosquito-borne) infection of humans and a major cause of acute febrile illness affecting an estimated 100 million people worldwide annually, with 500,000 cases of dengue hemorrhagic fever (DHF) and 22,000 deaths annually.[1] Dengue is associated with explosive urban epidemics and has become a major public health problem, with significant economic, political, and social impact. Dengue is a significant cause of febrile illness in returning travelers, especially those from the Caribbean and Southeast Asia.[2] A recent GeoSentinel analysis of 82,825 ill Western travelers identified 1,910 with acute dengue infection of which 0.9% developed hemorrhagic fever, resulting in one death.[3] The annual proportionate morbidity due to dengue in Southeast Asia during an epidemic is estimated to be 159 cases per 1,000 travelers.[4] The risk is likely higher for deployed military personnel due to longer exposure periods.

Dengue ranks third on the US DoD infectious disease threat list, after malaria and infectious diarrhea. US military operations over the past century have experienced numerous disruptions due to dengue. It has particularly affected US troops in the Pacific Theater, with over 91,000 cases observed in WWII. Dengue infection accounted for approximately 30% of febrile viral illnesses in Vietnam, affecting 40/1000 troops. More recently it was the cause of 289 hospitalizations in Somalia and 406 hospital admissions among US troops in Haiti. Soldiers who are hospitalized due to dengue take 14-21 days to recover to the point where they are fully mission capable. As the United States shifts its foreign policy and military focus to the Pacific region, the number of military and civilian personnel traveling to dengue endemic regions is also expected to rise significantly.[5] Once infected with dengue virus (DENV), the incubation period typically lasts 4 to 7 days, ranging from 3 to 14 days.[6] Infection with dengue virus may cause an undifferentiated febrile illness with a maculopapular rash. The majority of infections, especially in children under age 15, are asymptomatic or minimally symptomatic.[7] Older children and adults sustain either a mild febrile syndrome or the classical, self-limited, but incapacitating illness dengue fever consisting of fever (often 102°F-105°F), myalgias, frontal headache, retro-orbital pain, nausea, vomiting, anorexia, altered taste and olfactory perception, and malaise. The fever typically lasts 2-7 days and viremia lasts an average of 5 days.[8] Following the painful "breakbone" and febrile phase most patients slowly improve. Dengue virus disappears from the bloodstream at approximately the same time that the fever dissipates. Leukopenia and thrombocytopenia are common findings in mild dengue infection.

A small proportion of individuals will progress to a more severe form of dengue infection. Manifestations of severe dengue (DHF or dengue shock syndrome [DSS]) include severe hemorrhage leading to shock through blood loss, sudden increased vascular permeability leading to shock with or without hemorrhage, and severe encephalopathy with hepatitis. There is no pathognomonic sign or symptom for DHF during the acute stage, but as fever remits, characteristic manifestations of plasma leakage appear, making accurate clinical diagnosis possible in many cases. An individual who has been infected with a specific DENV serotype, develops serotype specific antibodies and is considered to be protected from future infection from the same serotype. However, they are not protected against infection from a heterologous serotype, and approximately

10% are at increased risk for progressing to DHF or DSS during the second infection.[9] This is theorized to be due to antibody-enhanced entry of dengue virus into cells.[10] No specific treatment is currently available but symptomatic and supportive measures (e.g., intravenous [IV] fluid management) significantly reduce the morbidity and mortality rates. Currently, vector control is the only method with a demonstrated although limited efficacy, but it is costly. Mosquito control does not offer satisfactory control of dengue and additional prevention methods are required. There are no antivirals registered, but several candidates are under evaluation at preclinical stages. Several vaccine candidates are at various stages of development.

5.1. Rationale for the Study Design

To evaluate the effectiveness of candidate dengue vaccine formulations, it is prudent to develop an appropriate challenge model. To this end, this first-in-human 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.

5.2. Rationale for the Homologous Rechallenge Substudy

Understanding the correlate of protection against DENV infection remains elusive but is crucial to advance dengue vaccine development. The new tool of dengue human challenge models allows for a controlled exposure to weakened dengue virus. An optimized DENV-4 LVHC (as a result of this protocol) is an important tool in interrogating vaccine and biologic therapies against dengue. Additionally, sequential human challenge studies such as in the case of a homologous rechallenge would allow us to compare immune profiles of participants prior to and after a primary and secondary DENV infection, facilitating identification of important immune biomarkers that are associated with protection against DENV-4. While heterologous exposure to a second or subsequent dengue infection carries the risk of antibody dependent enhancement, homologous rechallenge does not carry this risk and has been safely done in the remote past. Post-war studies by Sabin et al. published findings of his seminal human challenge studies that demonstrated that full protection against symptomatic dengue even after 18 months following primary challenge with same (homologous) serotype.[11] Although his findings were groundbreaking, published data from this study is scant with regards to methodology, clinical findings and immunologic characterization.

A large cohort of Nicaraguan children (N >2,800) found four cases of secondary homologous reinfection, implying that immunity against homologous serotype may not always be sterilizing. Further studies are needed to identify causes of partial protection.[12] Nevertheless, it remains widely accepted that natural immunity following DENV infection results in protection against the same serotype based on the lack of epidemiologic evidence for homologous reinfection.[13]

This substudy is designed to evaluate the safety and tolerability of homologous rechallenge as well as to compare clinical and immunologic profiles following primary DENV-4-LVHC challenge and after homologous rechallenge, with the hopes to identify correlates of protection against DENV-4. We hypothesize that volunteers previously challenged with DENV-4-LVHC will be protected against a rechallenge of the same virus even years after primary challenge.

5.3. Name and Description of the Investigational Product

The product used in this study, the Walter Reed Army Institute of Research (WRAIR) challenge lot 1892, DENV-4-LVHC, is a lyophilized powder for injection consisting of: DENV-4 strain H-241 virus, Eagle's minimal essential medium (EMEM, modified) culture medium, human plasma albumin-USP, L-glutamine, streptomycin, neomycin, and lactose. The freeze-dried DENV-4-LVHC is rehydrated with 0.7 mL of water for injection (WFI). The route of administration is subcutaneous (SC) injection for the proposed indication of infection of volunteers to produce an uncomplicated dengue-like illness in order to support development of vaccines and drugs.

5.4. Summary of Pre-clinical and Clinical Trials

5.4.1. Pre-clinical Studies

Refer to the investigator's brochure (IB).

5.4.2. Clinical Studies

This protocol is aligned with a previous protocol (S-14-09) optimizing the DENV-1-LVHC (IND 016332) and performed by the State University of New York, Upstate Medical University (SUNY-UMU).

Refer to the IB for additional safety and immunogenicity data.

5.4.2.1. Early Human Challenge Studies with Dengue Virus

Early studies included transmission studies as early as 1926, performed by service members within the United States Army. Sixty-four volunteers were challenged in transmission experiments including inoculations from virus-infected blood, and mosquito bite challenges from *Aedes aegypti* (47 positive) and *Culex quinquefasciatus* (all negative). Among the sixty four volunteers challenged, dengue was produced in fifty two (81%).[14]

5.4.2.2. Human Challenge Studies with DENV-4 H241

In 2001, study A-9211, "Clinical and Immunological Evaluations of Four Dengue Viruses as Challenge Strains in Immune and Susceptible Volunteers," was completed under BB-IND-8796. The clinical study report (CSR) was submitted on 14 June 2002; serial number (SN) 0018 and a second iteration (for site number 2) was submitted on 02 January 2003 (SN 0020). This was an exploratory study to evaluate possible dengue challenge viruses that will reproducibly cause uncomplicated dengue fever lasting 3-7 days.[15]

Out of 15 volunteers total; two volunteers received DENV-1, three volunteers received DENV-2, three volunteers received DENV-3, four volunteers received DENV-4, and three volunteers received placebo. Of the 12 volunteers who received challenge strains, five (two DENV-1 and three DENV-3 recipients) developed dengue fever (DF) defined as > 2 typical symptoms, > 48h or sustained fever (>100.4 F) and concurrent viremia. DENV-4 H-241 produced symptoms and multiple days of viremia but did not meet the case definition of DF.

Of the 12 volunteers in this study who were challenged with one of 7 different attenuated strains of dengue virus, 7 volunteers developed asymptomatic fluid collections around the liver, spleen, lungs, or heart.[16]

As part of IND#19250 (current protocol), ten volunteers at UMB underwent LVHC with DENV-4 utilizing the low dose aliquot of 95 PFU. All ten volunteers developed mild dengue fever with viremia beginning as early as Day 4 with a mean RNA-emia duration of 8.7 days (range 7-11 days). Compared to DENV-1, the onset of viremia different by ~ 4 days (4.2 vs. 8 days, $P > 0.0001$ and was nearly two days less in duration of viremia (8.7 vs. 10.5 days, $P = 0.04$). Transient leukopenia and mild transaminase elevations were noted and self-resolved by Day 28 in all cases. All volunteers developed mild symptoms, fulfilling our endpoint analyses goals of optimizing a DHIM-4 that reliably induced viremia with mild-moderate symptoms in a safe manner. Of note, a unique feature of this strain (DENV-4) was a transient total body rash (8 of 10 volunteers – Grade 3; 2 of 10 Grade 2). The study was conducted with 3 volunteers followed by 7 additional volunteers (**Section 7.2**) and dose escalation was not required to define the dosing as 'optimized'. Scientific discussion held with sponsors (U.S. Army and MTEC) and the DHIM Consortium members targeted studies assessing homologous rechallenge as high priority and re-allocation of funds to examine this important scientific question were approved, to allow for protocol re-design.

5.4.2.3. Phase 1 Selection of Attenuated Dengue 4 Viruses by Serial Passage in Primary Kidney Cells (WRAIR)

In 1984, a study evaluated a candidate DENV-4 vaccine in human volunteers. Five yellow fever-immune volunteers received inoculation of reconstituted DENV-4 (H-241) at 1.6×10^4 PFU (n = 2) and 2.8×10^4 PFU (n = 3). Two of the five volunteers developed viremia on days 5 (range 5-9) and 6 (range 6-10). Both volunteers had received 2.8×10^4 PFU of the DENV-4 strain. Both volunteers developed symptoms compatible with DF including rash, elevated temperature and transaminitis. Both viremic volunteers developed H1 and N antibody titers to DENV-4. All volunteers recovered without sequelae.[17]

5.4.2.4. Recent Human Studies with Dengue 1 Virus (45AZ5): Phase One, Open Label Assessment of DENV-1-LVHC (IND 16332) (NCT02372175)

In 2016, SUNY Upstate in collaboration with the U.S. Army initiated a study characterizing the safety of the attenuated DENV-1-LVHC (45AZ5) viral strain for use in a Dengue Human Infection Model (DHIM). In addition, clinical, immunological and virologic responses following exposure to the virus were assessed. Six flavivirus naïve volunteers were consented and after meeting all eligibility requirements, were inoculated subcutaneously with 0.5 ml of 6.5×10^3 pfu/mL of the virus. Volunteers were evaluated every day for the first 4 days and then every other day until Day 28 post-inoculation, or until they entered the hospital phase of the study if they met hospitalization criteria. One volunteer did not develop viremia nor had any antibody response. The inoculum for this volunteer was tested and contained viable virus. The remaining five volunteers developed viremia and produced IgM and IgG antibody responses. The viremias began as early as Day 6 and continued as late as Day 14 (undetectable at Day 16 but untested on Day 15). One volunteer developed febrile dengue illness, met hospitalization criteria, was hospitalized, and recovered. The remaining four viremic volunteers had classic afebrile dengue infection with various combinations of grade 1 and 2 symptoms including abdominal pain, eye pain, headache, nausea, muscle pain, joint pain, and rash. One volunteer had a grade 3 rash that was considered not clinically significant.

A recent study (ADVP005 – January 2021) conducted at UMB, in collaboration with the U.S. Army, examined the outcome of a DENV-1-LVHC (45AZ5) in volunteers who had previously been primed with Tetravalent Dengue Virus (TDEN) Purified Inactivated Vaccine (PIV) with Alum adjuvant prime and boosted with Tetravalent Dengue Virus Live Attenuated Vaccine Formulation 17 post transfection (LAV) at 90 or 180 days. Vaccinated volunteers (n = 6) with detectable pre-DHIM TDEN antibody titers and flavivirus-naïve control volunteers (n = 4) were challenged with DENV-1 strain 45AZ5. Volunteers were followed daily from Days 4-16 with quantitative PCR used for detection, and DENV-1 solicited adverse events assessed. A novel Illness Index, incorporating dengue signs and symptoms as well as laboratory abnormalities was employed to score illness. Nine of 10 volunteers developed detectable viremia (5/6 vaccinees and all 4 controls). The mean viremia onset in vaccinees was day 5 (range 5-6) versus day 8 (range 7-10) for controls, P = 0.007. The viremia duration was 8.2 days (range 7-10 days) in vaccinees vs. 9.75 (range 9-11) in controls, P = 0.088. There was no difference in viremia area under the curve (vaccinees 3.35 x 10^7 vs. controls 2.66 x10^7, P = 0.895). Despite earlier onset and shorter duration, the vaccinee Illness Index scored at 17.5 vs controls' 15.1, P= 0.55. We concluded that TDEN-PIV primed, TDEN-LAV boosted volunteers were unprotected against DENV-1 with a trend towards more severe symptoms than flavivirus-naïve controls. These findings highlighted the importance of utilizing DHIM before advanced Phase II/III clinical trials of dengue vaccine candidates.

5.4.2.5. Past Human Studies with Any Dengue Serotypes

Other previous clinical studies, prior to DHIM Consortium studies, with any dengue serotype are listed in **Table 1**.

Table 1: Additional Past Clinical Studies Including Challenge with Any Dengue Serotype

Investigator(s)	Publication Year	Volunteers Inoculated	Dengue Serotype	Comment
Graham[18]	1902	6	Unknown	Infection by mosquito inoculation
Ashburn and Craig[19]	1907	22	Unknown	Injection of blood and mosquito inoculation
Cleland[20]	1919	21	Unknown	Injection of human and animal blood; mosquito inoculation
Siler[14]	1926	48	DENV-4	Infection by mosquito inoculation
Simmons	1931	81	DENV-1	infection by mosquito inoculation
Misao[21]	1944	120	DENV-1	Injection of blood and mosquito inoculation
Hotta[22]	1952	>10	Unknown	Injection of blood
Sabin and Schlesinger[23]	1945	16	DENV-1, DENV-2	Injection of mouse-adapted dengue
Sabin and Schlesinger[23] [a]	1944-6	>125, >75	DENV-1, DENV-2	Injection of mouse-adapted dengue
Taniguchi[24]	1951	35	Unknown	Injection of blood; mouse-, rabbit-, and chick embryo-adapted dengue
Schlesinger[25]	1956	17	DENV-1, DENV-2	Injection of mouse-adapted dengue vs. sterile saline; then unmodified dengue challenge
Wisseman[26]	1966	15	DENV-1	Injection of unmodified virus
Eckels[17]	1984	2	DENV-4	Vaccine trial: injection of virus from cell culture
McKee[27]	1987	2	DENV-1	Vaccine trial: injection of virus from cell culture
Innis[28]	1988	2	DENV-3	Vaccine trial: Injection of virus from cell culture
Edelman[29]	1994	29	DENV-1	Vaccine trial: SC injection of under-attenuated DENV-1 45AZ5
Sun[30]	2013	14	DENV-1, DENV-3	Injection of virus from cell culture
Mammen[15]	2014	15	DENV-1, DENV-2, DENV-3, DENV-4	Injection of virus from cell culture

[a] Telephone communication between Dr. Schlesinger and COL Bruce Innis

5.5. Known and Potential Risks and Benefits to Human Volunteers

5.5.1. Dengue Classification

According to the World Health Organization (WHO) classification, dengue severity is divided into the following: dengue with or without warning signs, and severe dengue 2009.[31]

Dengue without warning signs is defined as fever and two (2) of the following:

- Nausea
- Rash

- Aches and pains
- Leukopenia
- Positive tourniquet test

Dengue with warning signs is dengue defined above with any of the following:

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation (ascites, pleural effusion)
- Mucosal bleeding
- Lethargy, restlessness
- Liver enlargement > 2 cm Laboratory increase in HCT concurrent with rapid decrease in platelet count

Dengue with warning signs requires strict observation and medical intervention. Severe dengue is defined by severe plasma leakage, severe hemorrhage, and severe organ impairment. Severe dengue also requires strict observation and medical intervention. We do not anticipate dengue with warning signs, based upon prior experience with this attenuated DHIM-4 strain, however, we will categorize volunteers into this WHO categorization on a post-hoc basis if symptoms dictate.

5.5.2. Risks/Discomfort to Volunteers and Precautions to Minimize Risk

Outlined below are anticipated and unexpected/unanticipated adverse reactions, and a brief description of procedures to ameliorate risks and symptoms. All known risks and precautions described here are explained in detail in the informed consent. Of note, the challenge strain is an attenuated strain of dengue virus.

5.5.2.1. Local Reactions

Local reactions may include pain, redness, and swelling at the injection site.

5.5.2.2. Systemic Reactions

The symptoms of dengue infection range from asymptomatic infection to a mild febrile syndrome, or the classical, self-limited, but incapacitating illness dengue fever consisting of fever (often 102°F-105°F), joint pain, myalgias, frontal headache, retro-orbital pain, nausea, vomiting, anorexia, rash, increase in liver enzymes, leukopenia, thrombocytopenia, and mild bleeding manifestation (e.g., nose or gum bleed), petechiae, or easy bruising. Any or all of these clinical manifestations may occur. While the incubation period for natural occurring dengue is typically 7 days (range 4 – 10 days) with manifestations of complicated dengue likely to appear by Day 10 (range 3-7 days post symptom onset), the incubation and signs of complicated dengue infection may be different for the study product.

In-patient will receive symptomatic and supportive care as needed. Most will only require oral hydration and acetaminophen for fever and myalgias. Volunteers with more severe symptoms may require IV fluid hydration. The risks for this are pain at the IV site, thrombophlebitis, fluid

extravasation, or volume overload. Local infection or systemic infection (bacteremia) are extremely uncommon risks related to IV insertion.

Severe dengue infection (DHF) with evidence of plasma leakage and hemorrhage is extremely rare in first time infections. Only dengue naïve volunteers will be eligible to participate in this study. No volunteers who have received live attenuated virus strains in prior vaccination studies have developed severe dengue symptoms.[32]

5.5.2.3. Pregnancy

Risks to unborn babies are unknown at this time. Pregnancy is an exclusion criterion for enrollment in this study and contraception should be used by female and male volunteers for the duration of the study. Female volunteers who become pregnant following inoculation will not be discontinued from the trial and will be followed for safety assessment for the duration of the pregnancy. Female partners of male volunteers who father a child during the study will be asked to be followed for the duration of the pregnancy.

A sexually active man is defined as one whose partner is a woman of childbearing potential (see definition below) and has not had a vasectomy performed > 1 year prior to screening. They must agree not to father a child until 2 months after the inoculation. These volunteers must agree to use a barrier method of birth control (e.g., either condom with spermicidal foam/gel/film/cream or partner usage of occlusive cap [diaphragm or cervical/vault caps] with spermicidal foam/gel/film/cream/suppository).

Women of childbearing potential are defined as those who have not been sterilized via tubal ligation, bilateral oophorectomy, bilateral salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with history of documented radiological confirmation test at least 90 days after the procedure (or with use of another birth control method if history of confirmation test not confirmed), still menstruating or < 1 year of the last menses if menopausal. For this study, an effective contraceptive method is defined as one that results in a failure rate of less than 1% per year when it is used consistently and correctly. Adequate contraceptive precautions include- intrauterine contraceptive device, oral contraceptives, diaphragm, or condom in combination with contraceptive jelly, cream, or foam; Norplant® or Depo-Provera®, through 56 days after the dengue challenge injection to minimize any potential risk.

5.5.2.4. Lactation

Risks to nursing infants are unknown at this time. Females who are breastfeeding a child will be excluded from enrollment into the study. Females will be instructed not to become pregnant for the duration of the study.

5.5.2.5. Venipuncture

Blood sampling carries a minimal risk of minor discomfort and the possibility of minor bruising at the site of the needle puncture and, rarely, the possibility of infection at the needle puncture site and/or nerve damage.

5.5.2.6. Allergic Reaction

As with any investigational new drug (IND) product administration and no matter what precautions are taken, there is always the risk of a serious, or even life-threatening, allergic reaction. Medical emergency equipment is located at the study site. This is available to handle emergencies, such as anaphylaxis, angioedema, bronchospasm, and laryngospasm.

5.5.2.7. Guillain-Barré Syndrome (GBS)

A serious but rare, neurologic disorder called Guillain-Barré syndrome can occur after certain infections, due to immune activation which damages the body's peripheral nerves, resulting in muscle weakness and some degree of paralysis. GBS can last for weeks to months. Approximately 85% of people eventually recover completely or nearly completely, but some people have permanent nerve damage, and between 3% and 4% of people who develop GBS die. To decrease this risk, individuals with a history of GBS or other significant reaction to vaccines will be excluded from participating in the study. Thus far, GBS does not seem to be associated with dengue virus infection.

5.5.2.8. Observations From Natural Dengue Infection

Patients who have antibody from an earlier heterotypic dengue virus infection and who are subsequently infected by a dengue virus strain of another serotype, have been shown to be at higher risk (approximately 100-fold) for DHF and/or dengue shock syndrome. The virus to be used in this study is designed to induce Dengue-4 virus sero-responsiveness. There is the possibility that a volunteer exposed in the future to serotypes 1, 2, or 3 may develop a more severe case of dengue infection (i.e. DHF) than if they had been dengue naïve. To date, there is no evidence that attenuated dengue virus vaccines (monovalent and tetravalent) place adults at increased risk for severe dengue.[33] However, a chimeric dengue vaccine (Dengvaxia®) produced by Sanofi, has demonstrated a safety signal suggesting increased risk in seronegative pediatric patients less than nine years of age.[34]

5.5.2.9. Theoretical Risks

Theoretical risks based on symptoms/outcomes typically associated with wild-type dengue infection, but not seen in prior DHIM studies may include:

- Volunteer(s) may develop dengue fever with one or more of the following events
  - Hemorrhage
  - Evidence of end organ involvement defined by clinical or lab findings greater than grade 2 severity
  - Intravenous (IV) fluid requirement for hemodynamic instability (not for comfort or the inability to take oral fluids)
- Volunteer(s) may develop dengue hemorrhagic fever (DHF) with symptoms similar to dengue fever plus, any one of the following
  - Severe and continuous pain in abdomen
  - Ascites and or/pleural effusions

  - Bleeding from the nose, mouth and gums or skin bruising
  - Frequent vomiting with or without blood
  - Black stools, like coal tar
  - Excessive thirst (dry mouth)
  - Pale, cold skin
  - Restlessness, or sleepiness
- Volunteer(s) may develop dengue shock syndrome (DSS), which can be defined as DHF plus the following symptoms
  - Weak rapid pulse
  - Hypotension
  - Respiratory distress
  - Altered level of consciousness
  - Narrow pulse pressure (less than 20 mm Hg)
  - Cold, clammy skin
  - Restlessness
- A volunteer who receives this viral challenge could be primed for more severe disease, if exposed to another dengue virus following this study.

There is a very low risk of acquiring dengue in the greater Baltimore-Washington area, and although the mosquito vector exists in the southern United States and along the mid-Atlantic coastal regions, transmission from one individual with imported dengue to another individual is still very rare in the continental US. However, if a volunteer traveled to a part of the world where dengue is present, they have the potential to contract a second infection. There are four distinct, but closely related, serotypes of the virus that cause dengue (DEN-1, DEN-2, DEN-3, and DEN-4). Recovery from infection by one provides lifelong immunity against that particular serotype. However, cross-immunity to the other serotypes after recovery is only partial and temporary. Subsequent infections by other serotypes increase the risk of developing severe dengue. In primary wild type dengue infection, there is a less than 1% risk of developing DHF or DSS. With a secondary infection, this risk increases to about 5-10%.[9]

Globally, there are an estimated 50 to 100 million cases of dengue fever (DF) and several hundred thousand cases of dengue hemorrhagic fever (DHF) per year. There are approximately 30,000 deaths per year related to severe dengue. There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates below 1%. (CDC-2012). The patients who succumb to dengue fever are typically very young, very old, or have underlying immune issues present.

- Mosquitos may transmit the DENV-4-LVHC challenge strain to people in the community surrounding the clinical site.

- Female volunteers with excessive menstrual bleeding and hypermenorrhea have been associated with both severe and uncomplicated dengue.[35]

5.5.2.10. Unknown Risks

As with all research there is the remote possibility of risks that are unknown or that cannot be foreseen based on current information. This would include late effects that have been seen with some vaccines.

5.5.2.11. SARS-CoV-2 Precautions

On December 31, 2019, a novel coronavirus termed SARS-CoV-2, was described. Since that time, the virus has spread and has been termed a global pandemic by the World Health Organization. As such, special attention must be paid to volunteers participating in human clinical trials. The Center for Vaccine Development and Global Health has been on the leading edge of implementing safety guidelines for the safe conduct of clinical studies during this outbreak (while conducting Covid-19 vaccine studies). Policies are continually updated based upon the circulating risk of SARS-CoV-2. The center follows a policy of strict pre-screening of all volunteers admitted to the University campus. This includes mandatory mask use on campus, the completion of a pre-screening questionnaire assessing for the presence of COVID-19 symptoms and a temperature check. All clinical staff will don appropriate personal protective equipment during study operations. Additionally, all rooms and equipment are wiped down between each volunteer and strict social distancing practices conducted. Upper respiratory, either nasopharyngeal (NP) or nasal, swab for the presence of the SARS-CoV-2 virus by PCR, may be performed prior to dengue inoculation and if a temperature develops following this inoculation, if the prevalence of SARS-CoV-2 remains high in the community. Results may not be available prior to initiation of challenge. Volunteers will be asked to maintain self-quarantine conditions or sequester within a local hotel (e.g., The Hotel Monaco) from the day of challenge inoculation until completion of daily follow-up.

Multiple vaccines have been issued EUAs for prevention of COVID-19. We will provide vaccine exception for volunteers who have the opportunity to receive the vaccine or booster, to receive one as early as 21 days following the DHIM, out of an abundance of caution and safety during the pandemic (defined in the Exclusion Criteria).

5.5.3. Alternatives to This IND Product or Study

An alternative is to not participate in this study.

5.5.4. Intended Benefit for Volunteers

There is no direct medical benefit for volunteers participating in this study.

5.5.5. Risks to the Study Personnel and the Environment

The principal risk in the clinical setting is handling needles that may be contaminated with the challenge strain or human pathogens. Adherence to universal precautions will reduce the risk of exposure.

There are no known risks to the environment other than those associated with the generation of biohazardous waste attendant to inoculation of humans. All biohazardous waste will be disposed of as stipulated by local, state, and federal regulations and in accordance with study site SOPs.

5.6. Route of Administration, Dosage Regimen, and Experimental Vaccination

The investigational product, DENV-4-LVHC will be administered in a single inoculation subcutaneously (SC) over the triceps muscle of the arm.

The volume of a single dose is 0.5-1.0 mL for each challenge. See **Section 7.3.7.** for details regarding the challenge strain.

5.7. Compliance Statement

The study will be conducted according to the protocol and in compliance with International Conference on Harmonization (ICH) Good Clinical Practice (GCP), Belmont Principles, and other applicable regulatory and Department of Defense (DoD) requirements. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with the ICH GCP guidelines and clinic site SOPs. A list of roles and responsibilities is included in the sponsor's information sheet.

5.8. Study Population

This study will enroll healthy male and non-pregnant, non-breastfeeding female volunteers between the ages of 18 and 50 (inclusive).

Enrollment is planned for a total of 30 volunteers (based on outcomes as indicated in section)

Refer to **Section 11.4** for a statistical justification of the sample size.

5.9. Study Sites

This study will be conducted at the Center for Vaccine Development and Global Health (CVD) and/or the Institute for Human Virology (IHV) at the University of Maryland, Baltimore (UMB), Maryland. The UMB CVD is a clinic-based research center affiliated with the UMB School of Medicine (SOM) and is located on the 3rd and 4th floors of the Health Sciences Facility (HSF) Building. The IHV is located across the street from the CVD with outpatient facilities available. The outpatient phase will occur at the UMB CVD or IHV. A sequestration phase, precipitated upon detection of asymptomatic dengue viremia, will occur at local hotel which is within a few blocks of the CVD. The inpatient phase will occur at one of two sites: Pharmaron is a biopark facility located across the street from the CVD and IHV and within two blocks of the University of Maryland Medical Center (UMMC). The General Clinical Research Center is an alternative site located on the 10th floor of University Hospital itself. Only one inpatient site will be used per challenge period and the site will be chosen based upon inpatient site schedule availability and volunteer numbers. The CVD is staffed with Infectious Disease physicians, Internists and Pediatricians, with robust nursing and laboratory support. It is physically attached to UMMC, an 800-bed hospital with access to its tertiary care inpatient facilities, including radiology, cardiology and an Intensive Care Unit (ICU).

6. TRIAL OBJECTIVES

6.1. Primary Objectives

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

6.2. 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

6.3. Exploratory Objectives

- 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.

7. TRIAL DESIGN

7.1. Study Endpoints

7.1.1. 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 unsolicited injection site symptoms until 28 days post virus inoculation or 7 days post inpatient evaluation, whichever is later.
- Occurrence, intensity, and duration of solicited systemic 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 defined **Dengue Illness Index**)
- 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

7.1.2. 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

7.1.3. 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)

7.2. Overall Study Design

7.2.1. Main Study

The goal of the study will be to produce uncomplicated dengue-like illness in volunteers at the lowest dose of challenge virus capable of meeting the safety endpoint and performance parameters.

The performance parameters (the level of uncomplicated dengue sought) are defined as:

1. Measurable viremia by RT-PCR and duration in days
2. Viremia by quantitative RT-PCR (pfu/mL) and calculated AUC
3. Number, percentage, and severity score of volunteers with each of the following clinical or laboratory symptoms as measured by the **Dengue Illness Index**:
    - Fever greater than or equal to 38°C (100.4°F)
    - 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

**Note: Disease severity can be re-classified into the WHO categorizations (Section 5.4.1) in the unlikely event that complicated symptoms develop*

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 in a staggered start design. A 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 $\geq$ 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.

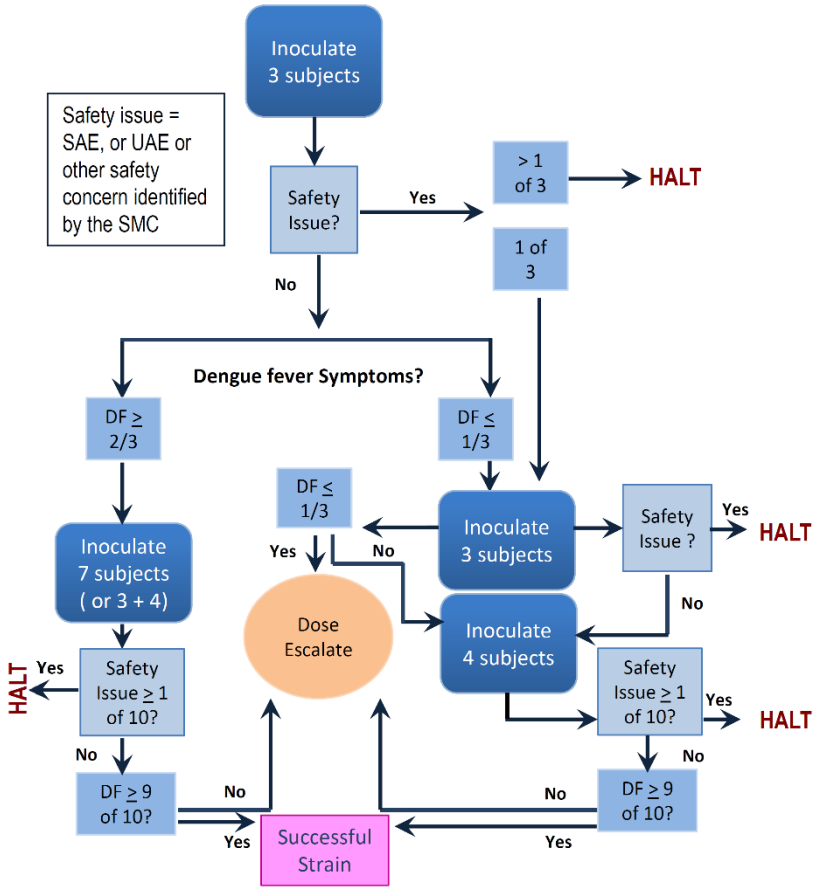
b) After 28 days, if $\leq$ 1 of 3 volunteers have met performance parameters and no safety issues have occurred, an additional 3 volunteers will be inoculated in the low dose cohort. If the performance parameters remain at $\leq$ 1 of 3 volunteers, without safety concerns, dose escalation to the next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

Figure 1: Algorithm for Dengue strain selection

Figure 1: Algorithm for Dengue strain selection

7.2.2. Homologous Rechallenge Substudy

Volunteers previously challenged with DENV-4-LVHC (i.e., Main Study participants) will be eligible and invited to enroll in this sub-study. This homologous rechallenge 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).[36,37]

7.3. Investigational Product

A summary description of the investigational product is presented in **Table 2**. The DENV-4-LVHC investigational challenge material consists of the dengue-4 virus strain H-241 as a powder, lyophilized for reconstitution. The product is reconstituted with 0.7 mL of water for injection (WFI, manufactured by APP Pharmaceuticals) for injection at a concentration of 1.9 x 10^4 PFU/mL. Stability data is conducted on an interval basis and may result in adjusted concentrations and PFU. This may alter the quantity of injection (currently estimated as ~0.5 mL). For the medium and low doses, the reconstituted DENV-4 LVHC will be diluted in EMEM (BPR No.: BPR-1169-00 Lot 1898, 4.5 mL), which is provided as a sterile, vialed product.

Table 2: Investigational Product

Product Name	Dengue 4 Live Virus Human Challenge (DENV-4-LVHC)
Diluent Name	Diluent Eagle's minimal essential medium (EMEM, modified)
Dosage Form	Liquid injectable
Unit Dose*	Low dose: 0.5 mL of 1.9 x 10^2 PFU/mL = 0.95 x 10^2 PFU Medium dose: 0.5 mL of 1.9 x 10^3 PFU/mL = 0.95 x 10^3 PFU High dose: 0.5 mL of 1.9 x 10^4 PFU/mL = 0.95 x 10^4 PFU
Route of Administration	Subcutaneous in triceps region of arm
Physical Description	White to yellow dense cake (clear to pink solution when reconstituted with WFI)
Manufacturer	WRAIR Pilot Bioproduction Facility
Lot Number	1892

***** Written formulation above is based on initial stability data. Stability data is obtained on an interval basis and may result in varying concentration of measured virus. Formulation will be adjusted to ensure injection of 0.95 x 10^2 PFU for the low dose, 0.95 x 10^3 PFU for the medium dose and 0.95 x 10^4 PFU for the high dose.

7.3.1. Investigational Product Packaging and Labeling

Sample investigational product labels are as follows:

Dengue Virus Type 4 (H-241) (Live Virus)Vial # XXXX
for Human Challenge
BPR No.: **BPR-1164-00** Lot No.: 1892
Contents: 0.7 ml (Freeze Dried) Storage: -15 to -30 °C
Caution: New Drug – Limited by Federal (or United States) law to investigational use.
Date of Mfg.: 28 Oct 2014
Manufactured By: WRAIR, Silver Spring, MD 20910

EMEM Diluent Vial # XXXX
BPR No.: BPR-1169-00 Lot No. 1898
Contents: 4.5 mL Storage 2 – 8ºC
Caution: New Drug – Limited by Federal (or United States) law to investigational use.
Date of Mfg: 14 Nov 2014
Manufactured By: WRAIR, Silver Spring, MD 20910

7.3.2. Investigational Product Shipment

DENV-4-LVHC and the EMEM diluent will be shipped under controlled temperature conditions; -15°C to -30°C for the DENV-4-LVHC and 2ºC and 8ºC for the EMEM. DENV-4-LVHC and the EMEM diluent will be quarantined upon receipt by the study site, and temperature records will be downloaded and submitted to the designated representative for review. Upon verification that the cold chain was maintained during transit, WRAIR will provide assurance of the product's integrity and authorization for use. Any temperature excursion, (i.e., temperature outside the defined range of

storage) must be reported to the sponsor's representative as soon as detected. After exposure to a temperature deviation, the product will not be used until written approval has been given by the sponsor's representative.

7.3.3. Investigational Product Storage

At the study site, the investigator or his or her designee (e.g., Investigational Drug Pharmacy) will be responsible for product management. The lyophilized DENV-4-LVHC to be administered to the volunteers must be stored at -15°C to -30°C in a safe and locked place with restricted, controlled access. The temperature of the storage unit will be monitored daily with a validated temperature monitoring device(s) and documented. The WFI used for reconstitution will be stored at 20°C to 25°C. The EMEM used for diluting the investigational product will be stored under controlled refrigerator temperature at 2°C to 8°C. Any temperature excursion (i.e., temperature outside the defined range of storage) must be reported to the sponsor's representative within 24 hours of knowledge of the excursion. After exposure to a temperature excursion, the product will not be used until written approval has been given by the sponsor's representative.

7.3.4. Investigational Product Accountability

The sponsor's representative is responsible for receipt of the investigational product at the study site. The sponsor's representative has delegated drug accountability responsibility for this product to PI; however, the sponsor's representative has ultimate responsibility for product accountability. After the investigational product is distributed, the PI is responsible for and will maintain logs of investigational product receipt, storage, reconstitution, accountability by volunteer, and investigational product remaining before final disposition. The PI may delegate, in writing, this responsibility to another individual, but the PI is ultimately responsible for the investigational product and its proper storage upon receipt at the study site until it is transferred back to the sponsor's representative or designee or is destroyed, as directed by the sponsor's representative.

All vials (unused, partially used, and spent) will be retained by the study staff for accountability. No vials should be disposed of or destroyed without sponsor's representative's approval. The disposition records will account for all remaining investigational products.

7.3.5. Investigational Product Preparation

Detailed instructions for handling and diluting DENV-4-LVHC are provided in the Pharmacy guidelines and a summary is provided below.

The reagents for preparing the investigational product reconstitution and dilutions are:

- Investigational Product: Dengue Virus Type 4 (H-241) (Live Virus) for Human Challenge, BPR No. 1164-00, Lot No. 1892, and volume: 0.7 mL upon rehydration with WFI
- WFI: Sterile Water for Injection USP, Preservative Free, APP Pharmaceuticals, LLC, 5 mL
- Diluent: EMEM diluent BPR No.: BPR-1169-00 Lot 1898, 4.5 mL

Prior to preparation, all study products must be inspected visually for cracks, broken seals, correct label content, and extraneous particulate matter and/or discoloration, whenever solution and

container permit. If any of these conditions exist, the product will not be used. A replacement dose will be used, and the event will be reported to the sponsor's representative.

The investigational products are removed from the freezer and refrigerator and prepared at room temperature. All drug product reconstitutions and dilutions will be conducted in a certified cleaned biological safety cabinet by trained personnel wearing appropriate personal protective equipment (PPE).

The DENV-4-LVHC will be reconstituted with WFI prior to injection. The reconstituted DENV-4-LVHC will be further diluted in EMEM. Per Pharmacy guideline, the diluted DENV-4-LVHC will be drawn into a syringe, labeled and stored at 2°C to 8°C. Once the product is reconstituted it is stable at 2°C to 8°C for up to 6 hours.

7.3.6. Investigational Product Administration

Prior to administration, study product must be inspected visually for correct label content, and extraneous particulate matter and/or discoloration, whenever solution and container permit. If any of these conditions exist, the product will not be administered. A replacement dose will be used, and the event will be reported to the sponsor's representative.

Volunteer must be inoculated within 30 minutes of the investigational product being removed from 2°C-8°C. DENV-4-LVHC will be administered SC in the triceps of the upper arm in a volume of ~0.5-1.0 mL based on foundational concentration of product determined at interval stability analysis.

Volunteers will be kept under observation for at least 30 minutes after each inoculation to ensure their safety, and any reactions during this period will be documented. Appropriate medical equipment and emergency medications, including epinephrine (1:1000), will be available on site in the event of an anaphylactic or other immediate allergic reaction.

If a vial or syringe is accidentally broken and the DENV-4-LVHC spills out, appropriate disinfection procedures must be used.

The pharmacy label from the vaccine syringe will be placed in the source document.

7.3.7. Investigational Product Dose Selection

There are three possible doses of DENV-4-LVHC to be administered as a single injection (fill volume 0.7 mL). The product was originally vialed at 1.9×10^4 PFU/mL but concentration is measured on an interval basis and the team will adjust formulation to meet the targeted PFU listed below:

- Low dose: ~0.95×10^2 PFU*
- Medium dose: ~0.95×10^3 PFU
- High dose: ~0.95×10^4 PFU

This study is intended to be a proof-of-concept study to demonstrate that the challenge product can safely create uncomplicated dengue illness and to determine how well the challenge product performs in this regard. This study is an exploratory study and is not designed to deliver a dose

response relationship. Based on the data from previous clinical studies using DENV-4 H-241, we have chosen a starting dose (0.95 x 10^2 PFU). If the newly re-derived strain meets performance parameters but is too reactogenic and elicits safety concerns at this dose, the dose can be reduced by an additional 1 log to 0.95 x 10^1 PFU and administered to a new cohort, provided the PI, sponsor, and CDSC agree to this plan. In a previous study, a 4.35 x 10^3 PFU/mL dose of strain H-241 caused symptoms and viremia in one volunteer.[15] If the medium dose (0.95 x 10^3 PFU) does not achieve the performance parameters among volunteers, we have built in a higher dose (0.95 x 10^4 PFU) as well. If a dose proves safe but does not meet performance parameters, and an increase to the next level meets performance parameters, but proves to be too reactogenic, the PI, sponsor, and CDSC will discuss the possibility of administering a one-half step incremental dose decrease to a new group beginning with a 3-volunteer cohort. *If the dose is optimized at a lower dilution, it will not increase to the next log dilution.

7.3.8. Investigational Product Replacement Doses

There is no blinding or randomization code for this study, so if a dose needs to be replaced, another vial of product from the site's supply can be used.

7.4. Identification of Data to be Recorded on the Case Report Forms

The electronic case report form (eCRF) data will be transcribed from source documentation. No source data will be recorded directly in the eCRF (i.e., without prior written or electronic record of data). The transcribed data will be consistent with the source documents, or the discrepancies will be explained. For more information on data handling, refer to **Section 15.0.**

8. SELECTION AND WITHDRAWAL OF VOLUNTEERS

8.1. Recruitment of Volunteers

Volunteers will be recruited from the greater Baltimore-Washington area. Volunteers will be recruited via IRB approved flyers, email, advertisements, word of mouth, site database and social media.

Refer to **Section 5.7.** for a detailed description of the study population.

8.2. Eligibility Screening

Each volunteer must meet all inclusion and no exclusion criteria. The PI or designee will make the final decision of the eligibility. Only eligible volunteers will be given the investigational product.

8.2.1. Volunteer Inclusion Criteria

Volunteers must meet all of the following criteria to be included in the study:

1. Male or non-pregnant, non-breastfeeding female between 18 and 50 years of age (inclusive) at the time of consent
2. Volunteers must be able to provide written informed consent.
3. Volunteers must be healthy as established by medical history and clinical examination at study entry
4. Volunteers must pass a comprehension test and be able to comply with all study requirements.
5. Female volunteers of non-childbearing potential (non-childbearing potential is defined as having had one of the following: a tubal ligation at least 3 months prior to enrollment, a hysterectomy, an ovariectomy, or is post-menopausal).
6. Female volunteers of childbearing potential may be enrolled in the study, if all the following apply:
    a. Practiced adequate contraception (see Definition of Terms, **section 5.4.2.3.**) for 30 days prior to challenge.
    b. Has a negative urine pregnancy test on the day of DHIM.
    c. Agrees to continue adequate contraception until two months after completion of the DHIM.

8.2.2. Volunteer Exclusion Criteria

Volunteers meeting any of the following criteria will be excluded from the study:

1. History of dengue infection or dengue illness, or history of flavivirus infection or vaccination (e.g., yellow fever, tick-borne-encephalitis virus [TBEV], Japanese encephalitis, and dengue). ***Note:*** *For the Homologous Rechallenge Sub-study, prior DENV-4-LVHC-related dengue infection is not an exclusion.*

2. Volunteers positive for antibodies to flaviviruses (FV) to include dengue virus, West Nile virus, Yellow Fever virus, Zika virus, and Japanese encephalitis virus**, as part of screening for the Main Study.** ***Note:*** *Volunteers of Homologous Rechallenge Sub-study are expected to be positive for antibodies to dengue virus due to prior DENV-4-LVHC challenge.*
3. Planned administration of any flavivirus vaccine for the entire study duration.
4. Any recent (within 4 weeks) or planned travel to any dengue endemic area while participating in the trial.
5. Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
6. Volunteer seropositive for hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies (anti-HCV), or human immunodeficiency virus antibodies (anti-HIV). ***Note:*** *Prior volunteers of HIV vaccine studies may result in a false positive HIV antibody test, as such, in this scenario, volunteer will be eligible if they have a negative HIV RNA PCR at screening*.
7. Safety laboratory test results at screening that are deemed clinically significant or more than Grade 1 deviation from normal with the exception of PT/PTT, fibrinogen decrease, ALT/AST increase (acceptable to 1.1 ULN), platelet decrease which will be exclusionary at Grade 1 or higher.
8. Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by physical examination or laboratory screening tests
9. History of bipolar disorder, schizophrenia, hospitalization in the past year for a mental health disorder, or any other psychiatric condition, which in the opinion of the investigator prevents the volunteer from participating in the study
10. Significant screening physical examination abnormalities at the discretion of the investigator, including a BMI > 35 kg/m^2
11. Planned administration or administration of a vaccine/product not planned in the study protocol during the period starting 30 days prior to the DHIM until 56 days after the study completion (routine influenza or COVID-19 vaccination will be allowed if it is not administered within 14 days preceding DHIM)
12. Use of any investigational or non-registered product (drug or vaccine) other than the study DHIM during the period starting 30 days preceding the DHIM and/or planned use during the study period
13. Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs during the period starting 90 days prior to the DHIM (for corticosteroids, this will mean prednisone 5 mg/day or equivalent; inhaled, intranasal and topical steroids are allowed)
14. Concurrently participating in another clinical study, at any time during the study period, in which the volunteer has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device)

15. Autoimmune disease or history of autoimmune disease
16. History of any reaction or hypersensitivity likely to be exacerbated by any component of the study product or related to a study procedure
17. Major congenital defects or serious chronic illness
18. History of any neurological disorders or seizures
19. Acute disease and/or fever (≥37.5°C/99.5°F oral body temperature) at the time of enrollment: note that a volunteer with a minor illness such as mild diarrhea, mild upper respiratory infection, etc., without fever, may be enrolled at the discretion of the investigator
20. Administration of immunoglobulins and/or any blood products during the period starting 90 days preceding the DHIM or planned administration during the study period
21. Recent history of chronic alcohol consumption (more than 2 drinks per day and/or drug abuse) based on volunteer reported history
22. Pregnant or breastfeeding female or female currently planning to become pregnant or planning to discontinue adequate contraception
23. Men who intend to father a child during the study period (approximately 2 months)
24. Any religious or personal beliefs that bar the administration of blood products, transfusions, or serum albumin
25. Planned or current administration of an HMG-CoA reductase inhibitor (i.e., lovastatin, simvastatin, atorvastatin, etc.)
26. Currently regularly taking anti-coagulant medication, aspirin, or non-steroidal anti-inflammatory drugs (NSAIDs)
27. Any other condition which, in the opinion of the investigator, prevents the volunteer from participating in the study

8.3. Temporary Exclusion Criteria

Volunteers meeting any of the following criteria may be temporarily excluded from the study until the condition for exclusion resolves or is no longer applicable:

1. Acute disease and/or fever (≥ 38°C/100.4°F oral body temperature) at the time or within 6 hours of challenge inoculation: note that a volunteer with a minor illness such as mild upper respiratory infection, etc., without fever, and with a negative SARS-CoV-2 upper respiratory (NP or nasal) swab PCR on the day of inoculation, may be enrolled at the discretion of the investigator.
2. Recent blood donation (within prior 56 days).
3. Recent or scheduled receipt of any live vaccine 30 days and/or inactivated or sub-unit vaccine 14 days prior to inoculation.
4. Safety labs may be repeated once. If outside the 90-day screening window, the volunteer may be rescreened except for flavivirus, hepatitis, and HIV viral screens.

8.4. Volunteer Withdrawal Criteria

Each volunteer may withdraw consent at any time during the study without penalty. Counseling about the volunteer's health will be provided if he/she decides to discontinue participation in the study. Medical advice regarding what is in the best interest of the volunteer will be provided.

The PI may discontinue the volunteer's activity without the volunteer's consent if any of these criteria is met:

- A volunteer fails to comply with study procedures.
- A volunteer's safety or health may be compromised by further participation.

8.4.1. When and How to Withdraw Volunteers

If a volunteer withdraws, the PI will make a reasonable effort to determine the reason for the withdrawal from the study and to complete termination procedures as described in **Section 8.4.3**. and **Section 8.4.5.** For volunteers leaving the study, a targeted examination may be performed, if medically indicated and if permitted by the volunteer.

If a volunteer meets withdrawal conditions for a concomitant medication violation or noncompliance, this should clearly be stated in the source document and the study termination electronic CRF.

8.4.2. Data Collected for Withdrawn Volunteers

All data collected up to the time of withdrawal will be reported. The study termination eCRF will be completed, with the reason for withdrawal specified.

8.4.3. Lost to Follow-up Procedure

In the case of volunteers who fail to return for a follow-up examination, documented reasonable effort (i.e., telephone calls and certified mail) should be undertaken to locate or recall them, or at least to determine their health status while fully respecting their rights. These efforts should be documented in the source documents.

8.4.4. Replacement of Volunteers

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. Homologous rechallenge volunteers will be comprised of willing volunteers who participated in the main study. If funds allow, 2-3 unique, dengue-naïve participants may be recruited as a comparison arm to the homologous rechallenge cohort.

8.4.5. Follow-up for Withdrawn Volunteers

If a volunteer withdraws from the study, the study team will attempt to schedule at least one follow up visit before or on Day 56. If a volunteer drops out in the first 30 days before symptoms manifest, the risks as discussed during the consent process will be discussed with the volunteer. Volunteers will be asked to return for a close out visit at which time safety and immunologic samples will be drawn depending on the volunteer's preference. For volunteers where the reason for early termination was lost to follow-up or if the volunteer withdrew informed consent and specified that they do not want to be contacted again and it is documented in the source document, the site will

not attempt to obtain further safety information. No follow up for withdrawn volunteers will occur once the study termination eCRF is completed.

9. TREATMENT OF VOLUNTEERS

The schedule of study procedures is provided in **Table 3** and **Table 4**. More detailed descriptions of the study procedures are provided in the following sections. The procedures in **Table 3** will be followed until a volunteer meets the criteria for sequestration or inpatient admission (**Section 9.4**). At that point, the procedures in **Table 4** will be followed. Protocol deviations will not occur for visits that do not commence (i.e., if a volunteer is hospitalized and discharged on Day 4, there is no protocol deviation for assessments not performed on Day 5 of hospitalization). This will also be true for procedures not done on the non-hospitalized schedule while the volunteer is hospitalized. The blood volumes collected at each visit and for specific assessments for non-hospitalized and hospitalized volunteers are provided in **Table 3** and **Table 4**, respectively.

Table 3: DHIM-4 Study Schedule – Represents each study sequence following 3+7 algorithm

Table 3: DHIM-4 Study Schedule – Represents each study sequence following 3+7 algorithm

Study Day	D-90 to D-1	-28 to -1	0	2	4	5	6	7	8	9	10	11	12	13	14	15	16	19	22	25	28	56	90	180
Visit Number	S1	S2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Window (+/-)			0	1	0	0	0	0	0	0	0	0	0	0	0	0	0[g]	1	1	1	2	3	7	7
Eligibility Criteria	X	X	X																					
Medical History	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam[a]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DHIM			X																					
Illness index card/ Performance Criteria[c]					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Adverse Events				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Labs[d]	11		11		11		11		11		11		11		11		11				11			
HIV, HbSAg, anti-HCV	4																							
Flavivirus Screen[e]		14																						
Urine β-HCG	X		X																		X			
Type and Screen					7																			
RT-qPCR[f]				2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
Immunoassays																								
Serum-Microarray/ELISA[h]		10			7				7[h]						7						7		7	7
Core Cellular Assays		100			25		10		10		10		10		40						50		50	50
Transcriptome		3		3	3		3		3		3		3		3						3			
Daily Volume (mL)	15	127	11	5	55	2	26	2	33	2	26	2	26	2	63	2	13	2	2	2	73		57	57
Cumulative Volume (mL)	15	142	153	158	213	215	241	243	276	278	304	306	332	334	397	399	412	414	416	418	491		548	605

[a] Physical exam to be targeted after screen

[b] Vital signs performed q 8h as inpatient (BP, HR, respiratory rate)

[c] Performance Parameters as defined for DF criteria. Index card to be collected at D28 or D7 post-discharge, whichever is later

[d] Serum chemistry (Cr, glucose, AST/ALT), CBC with differential, fibrinogen, albumin, PT/PTT, will be obtained at screen and qod during daily fu

[e] Flavivirus- DENV, W. Nile, Yellow Fever, Jap B encephalitis, zika neutralizing abs

[f] Real-time PCR to be sent to be processed daily; if negative at Day 16, continue q2-3 days until D28

[g] Daily Visits last until RT-PCR is negative x 2 or until Day 16 (whichever is last)

[h] Sera to be drawn upon awareness of viremia (D+24 or Day 8 if aviremic) and after cessation of viremia.

Table 4: DHIM-4 Clinical Observation – Inpatient Management Phase Algorithm

Table 4: DHIM-4 Clinical Observation– Inpatient Management Phase Algorithm

Study Day	5	6	7	8	9	10	11	12	13	14	15	16	D1 Post	D3 Post	D7 Post	28[j]
Visit Number	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Window (+/-)	0	0	0	0	0	0	0	0	0	0	0	0[g]	1	1	2	2
Physical Exam[a]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Vital Signs and temp[b]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Fluid Intake/Output	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Performance Criteria[c]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Illness Index Card Review[e]	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Safety Labs[d]		11		11		11		11		11		11				11
RT-qPCR[f]	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Telephone Consult													X			
Immunoassays																
Serum-Microarray/ELISA				7[h]						7						7
Core Cellular Assays[h]		10		10		10		10		40						50
Transcriptome[h]		3		3		3		3		3						3

[a] Physical exam to be targeted after screen

[b] Vital signs performed q 8h as inpatient (BP, HR, respiratory rate) but not sequestration

[c] Performance Parameters as defined for DF criteria based on illness index card and labs

[d] Serum chemistry (Cr, glucose, AST/ALT), CBC with differential, fibrinogen, albumin, PT/PTT, will be obtained qod during daily fu

[e] Day Illness index card will continue through in-patient/sequestration to Day 28 or D7 post-discharge, whichever is later.

[f] Real-time PCR to be sent to be processed daily; if negative at Day 16, continue q2-3 days until D28

[g] Daily Visits last until RT-PCR is negative x 2 or until Day 16. Day of viremia detection and discharge will vary between study volunteers

[h] Sera to be drawn upon awareness of viremia (D+24 or Day 8 if aviremic)

[i] Day 28 visit links to Table 3 and continues with Day 56, 90 and 180 FU

Table 5: DHIM-4 Homologous Rechallenge Sub-study Schedule of Events

Table 5: DHIM-4 Homologous Rechallenge Substudy

Study Day	Day-90 to Day-1	0	2	4	5	6	7	8	9	10	11	12	13	14	15	16	19	22	25	28	56	90	180
Visit Number	S3	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Window (+/-)		0	1	0	0	0	0	0	0	0	0	0	0	0	0	0[f]	1	1	1	2	3	7	7
Eligibility Criteria	X	X																					
Medical History	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Exam[a]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[b]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
DHIM		X																					
Illness index card/ Performance Criteria[c]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Meds	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Labs[d]	11	11		11		11		11		11		11		11		11				11			
HIV, HbSAg, anti-HCV	4																						
Urine b-HCG	X	X																		X			
Type and Screen				7																			
RT-qPCR [e]			2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2			
Immunoassays																							
Serum- Microarray/ELISA [g]	10			7				7[g]						7[g]						7		7	7
Core Cellular Assays	100			25		10		10		10		10		40						50		50	50
Transcriptome	3		3	3		3		3		3		3		3						3			
Daily Volume (mL)	128	11	5	55	2	26	2	33	2	26	2	26	2	63	2	13	2	2	2	73		57	57
Cumulative Volume (mL)	128	139	144	199	201	227	229	262	264	290	292	318	320	383	385	398	400	402	404	477	477	534	591

[a] Physical exam to be targeted after screen
[b] Vital signs performed q8h if inpatient (BP, HR, RR)
[c] Performance Parameters as defined for DF criteria. Index card to be collected at D28 or D7 post-discharge, whichever is later
[d] Serum chemistry (Cr, glucose, AST, ALT), CBC with differential, fibrinogen, albumin, PT/PTT, will be obtained at screen and qod during daily visits.
[e] Real-time PCR to be sent to be processed daily; if negative at Day 16, continue q2-3 days until D28.
[f] Daily visits last until Day 16 or if viremic, until RT-PCR are negative x 2.
[g] Sera to be drawn upon awareness of viremia (D+24 or Day 8 if aviremic) and after cessation of viremia (or Day 14 if aviremic).
[h] PBMC and transcriptomes will be drawn on Day 14 or Day after cessation of viremia (whichever comes first)

9.1. Informed Consent Process

A written informed consent document will be signed by the volunteer before any study-related procedures are initiated for that volunteer. Prior to agreeing to participate in the study, volunteers will participate in an information session about the study. The investigator or designee will explain the study, outline participation requirements, review the consent form in detail with volunteers, and then answer any questions. Volunteers will then be provided ample time to read the informed consent form and ask questions. If a volunteer decides to participate, he/she will sign and date the informed consent form (ICF). A signed copy of the ICF will be provided to the volunteer before any study procedure is performed.

Volunteers will be given a test on their understanding of the information from this informed consent, and they must score at least 75% correct in order to continue with the screening process. In case they do not pass the test on the first try with a score of 75%, they will be given 2 additional opportunities to pass the test (3 total). All questions and answers will be reviewed for all candidate volunteers.

Volunteers who wish to participate in the **Homologous Rechallenge Sub-study** will have an addendum written informed consent document signed to participate in this sub-study.

9.2. Screening (Main Study)

9.2.1. Screening Visit 1 (Day -90 to 0)

After signing and dating the ICF, volunteers will be sequentially assigned a unique volunteer identifier number starting with DHIM-4-100. The screening process is a two-step process occurring over a minimum of two visits. The procedures and assessments listed in **Table 3**, Day -90 and Day -30, respectively, will be carried out for each volunteer to determine their eligibility for participation in the study.

- The informed consent process will be performed
- Inclusion and exclusion criteria will be assessed
- Demographic information will be collected
- A complete medical history will be collected
- Concomitant medications will be recorded
- A complete physical examination will be performed including the collection of vital signs (Heart rate and blood pressure) and temperature
- Urine will be collected for pregnancy test, females only
- Blood will be drawn for screening serology tests (valid for 90 days prior to enrollment after which tests will be repeated)
    - Blood will be drawn for safety labs which consist of complete blood count and differential (WBC, hemoglobin, platelets, neutrophil and lymphocyte counts), liver function tests (AST/ALT), serum chemistry (Cr, glucose), prothrombin time/partial prothrombin time, albumin, and fibrinogen

- Screening serologies for antibodies to HIV-1, hepatitis C, and hepatitis B surface antigen.

9.2.2. Screening Visit 2

After the initial screening visit, available information will be reviewed to assess eligibility. If the volunteer is eligible to continue, a second screening visit will be completed:

- Inclusion and exclusion criteria will be re-assessed
- Medical history will be updated
- Concomitant medications will be reviewed and updated
- Vital signs (Heart rate and blood pressure) and temperature will be performed
- Blood will be drawn for screening serology test to include neutralizing antibodies to dengue, West Nile, Yellow Fever, Japanese B encephalitis and Zika.
- Safety labs that were exclusionary grade 1 in abnormality may be repeated if the investigator believes that there is a laboratory error or reasonable transient explanation for the abnormality (e.g., elevated AST after vigorous exercise), or is the result of a normal variant of a healthy state.
- Blood will be drawn for research assays which consist of serum for microarray and ELISAs, PBMC for core cellular assays and transcriptome analysis

After all screening information has been reviewed, inclusion and exclusion criteria will be assessed.

All screening related procedures and assessments should be completed no more than 90 days prior to inoculation. If volunteer is not inoculated within 90 days of screening, they will be re-screened before inoculation with the possible exception of FV, HIV, and hepatitis serologies (if < 6 months). Laboratory evaluation for FVs will be considered evaluable for inclusion/exclusion for up to 12 months if the volunteer has not traveled to a dengue endemic area in that time.

If a volunteer tests positive for Zika antibodies, the results will be reported to local public health authorities according to state requirements. Although most antibody positive cases are asymptomatic, there is a chance for sexual transmission. We will counsel the volunteer to abstain from sexual activity, reduce the exposure to mosquito bites and see their doctor immediately.

When a volunteer has completed the screening visits and is eligible and willing to continue participation, he/she will be scheduled for inoculation (Day 0). Information on volunteers that fail the screening visit(s) will not be recorded in the data management system.

9.3. Inoculation and Follow-up Visits (Main Study)

Volunteers will follow the procedures listed in **Table 3**. This schedule will be followed until the volunteer meets the criteria for sequestration or inpatient admission (**Section 9.5**). Upon admission to the hospital, the procedures for hospitalized volunteers (**Table 4**) will be followed.

9.3.1. Day 0 Inoculation

To confirm a volunteer is still eligible the following procedures will be completed prior to inoculation:

- Inclusion and exclusion criteria will be reviewed
- Medical history will be reviewed
- A targeted physical examination, including vital signs (HR and BP) and temperature, will be conducted
- Concomitant medications will be reviewed
- Screening safety lab results will be re-reviewed
- In the event of ongoing local transmission of the COVID-19 virus, an upper respiratory (e.g., NP or nasal) swab sample will be collected for same day testing for the SARS-CoV-2 virus. Volunteers with positive results will be referred to their personal physician or local public health department for definitive testing and examination. Results will not be available prior to the challenge inoculation. A positive test in an asymptomatic individual would result in quarantining but will not result in study removal following challenge if DHIM had occurred.
- Urine will be collected; pregnancy test (females only) will be performed, and results will be reviewed
- Blood will be drawn for CBC with differential (WBC, hemoglobin, platelets, neutrophil and lymphocyte counts), chemistry (glucose and Cr), AST/ALT, albumin, fibrinogen, PT/PTT (henceforth termed 'safety labs').
- If the volunteer is still eligible, DENV-4-LVHC will be administered as a single SC inoculation in the triceps area of the arm. **Note**: *Dengue-naïve volunteers that may potentially be enrolled in the homologous rechallenge sub-study would receive low dose SC inoculation.*
- Volunteers will be kept under observation for at least 30 minutes after inoculation to ensure their safety, and any reactions during this period will be documented in the source document and the eCRF. The injection site will be evaluated, and AEs and SAEs will be assessed.

Volunteers will be provided with a 28-day illness index card, thermometer and instructions for use. The thermometers will be checked prior to distribution to ensure they are functional. Volunteers will be reminded before they leave to complete the illness index card as instructed and to bring the completed illness index card to all clinic visits. Volunteers will also be told to notify the site of any SAE. Volunteers will also be provided with a wallet card to carry with them during the study that provides 24-hour contact information for the study team with language to carry with them in the future to alert potential treating physician of their involvement in this study, should they become ill while traveling in or after travelling to dengue endemic areas.

9.3.2. Day 2 (+/- 1)

The following procedures will be performed at the Day 2 visit:

- A symptom-driven physical examination will be performed, vital signs will be obtained, and temperature will be measured

- Medical history will be reviewed
- Concomitant medications will be reviewed
- The injection site will be evaluated
- Solicited and unsolicited AEs and SAEs will be assessed
- Memory cards will be reviewed and Illness Indices updated
- Blood will be drawn for RT-qPCR
- Blood will be drawn for research transcriptome labs
- The volunteer will be reminded to notify the site in the event of an SAE

9.3.3. Days 4 to 16 Daily Follow-up

Beginning on Day 4, volunteers will be followed daily to evaluate for symptoms and viremia. Optimization studies for DHIM-1 revealed that 0/6 volunteers developed viremia before Day 6 (however Day 5 was not tested) and symptoms were minor. More recent results documented 2 of 10 volunteers developed viremia on Day 4 with mild symptoms.

- A symptom-driven physical examination will be performed, vital signs will be obtained, and the volunteer's temperature will be measured
- Concomitant medication will be reviewed
- The injection site will be evaluated through Day 7
- Solicited and unsolicited AEs and SAEs will be assessed
- Memory cards will be reviewed
- Blood will be drawn for RT-qPCR
- Blood will be drawn for research labs on Days 4, (sera within 24 hours of the time of viremia (D+24h) and within 24 hours of the cessation of viremia), Days, 6, 8, 10 12, and 14.
- Blood will be drawn for safety labs on Days 4, 6, 8, 10, 12, 14, and 16.
- For volunteers who become viremic, daily RT-PCR and every other day safety labs will be done until he/she is aviremic for two successive days.

9.3.4. Days 19, 22, and 25(+/- 1), and 28 (+/- 2) Post-Daily Follow-up

Following the period of daily follow-up when viremia is expected to occur (note: 0/6 volunteers in the DHIM-1 optimization experienced viremia after Day 16), volunteers who are viremia negative will complete a visit every 3 days until Day 28. The following procedures will be performed at each visit:

- A symptom-driven physical examination will be performed, vital signs will be obtained, and the volunteer's temperature will be measured
- Concomitant medication will be reviewed
- Solicited and unsolicited AEs and SAEs will be assessed
- Memory cards will be reviewed

- Blood will be drawn for research labs
- Blood will be drawn for safety labs (Day 28)
- Blood will be drawn for RT-qPCR
- The volunteer will be reminded to notify the site in the event of an SAE
- Memory card collected (Day 28 only)

9.3.5. Days 56 (± 3 days), 90 and 180 (± 7 days) Safety Follow-ups

The following procedures will be performed:

- A symptom-driven physical examination will be performed, vital signs will be obtained, and temperature will be measured
- Concomitant medication will be reviewed
- AEs and SAEs will be assessed
- The volunteer will be reminded to notify the site in case of an SAE
- Blood will be drawn for research assays on Days 90 and 180
- AEs and SAEs will be assessed

9.4. Homologous Rechallenge Sub-study Procedures

The homologous rechallenge sub-study will follow the schedule of activities as detailed and summarized in Table 5.

9.4.1. Screening (S3): Day -90 to Day -1

The following screening assessments must be done to determine subject eligibility. Written consent will be obtained before conducting any study procedures. Potential naïve controls will be follow **Section 9.3, Main Study**, activities.

- Screen for inclusion/exclusion criteria (see Section 8.2)
- Medical history
- Physical examination, including weight, height, and vital signs (blood pressure, respiratory rate, heart rate, and temperature)
- Concomitant medications.
- Safety labs includes CBC with differential, fibrinogen, albumin, PT/PTT, and serum chemistry (creatinine, glucose, ALT, and AST). Safety labs that were exclusionary grade 1 in abnormality may be repeated if the investigator believes that there is a laboratory error or reasonable transient explanation for the abnormality (e.g., elevated AST after vigorous exercise), or is the result of a normal variant of a healthy state.
- Re-screen for HIV antibody, HBsAg, anti-HCV antibody.
- Urine β-HCG (pregnancy) test for women of childbearing potential.
- Blood will be drawn for serum, core cellular assays, and transcriptomes.

9.4.2. Day 0: DENV-4-LVHC Rechallenge

Screening tests must be available before the subject is eligible for participation. Once screening is completed and volunteer meets all eligibility criteria, the subject will obtain baseline evaluations. Study product administration may occur after completion of all baseline procedures.

Before DENV-4-LVHC rechallenge:

- Review eligibility criteria and confirm eligibility of volunteer.
- Review of interim medical history.
- Targeted physical examination if needed.
- Review of concomitant medications.
- Baseline (pre-challenge) vital signs including HR, RR, BP, and temperature.
- Urine pregnancy test for women of childbearing potential.
- In the event of ongoing local transmission of the COVID-19 virus, an upper respiratory swab sample will be collected and tested following procedure as in Main Study (see Section 9.3.1).
- Blood will be draw for safety labs.

DENV-4-LVHC rechallenge and post-inoculation procedures:

- Eligible volunteers will be administered a single SC inoculation of DENV-4-LVHC in the triceps area of the arm. The DENV-4-LVHC dose that will be used will be the same dose that the volunteer received during his/her prior DENV-4-LVHC challenge (e.g., dose given during Main Study participation).
- Volunteers will be kept under observation for at least 30 minutes after inoculation to ensure their safety and any reactions during this period will be documented in the source document and the eCRF. Injection site will be evaluated, and AEs and SAEs will be assessed.

Volunteers will be provided with a 28-day illness index card, thermometer, and instructions for use. The thermometers will be checked prior to distribution to ensure they are functional. Volunteers will be reminded before they leave to complete the illness index card as instructed and to bring the completed illness index card to all clinic visits. Volunteers will also be told to notify the site of any SAE. Volunteers will also be provided with a wallet card to carry with them during the study that provides 24-hour contact information for the study team with language to carry with them in the future to alert potential treating physician of their involvement in this study, should they become ill while traveling in or after travelling to dengue endemic areas.

9.4.3. Day 2 (±1)

Day 2 visit procedures will follow same procedures as in Main Study (see Section 9.3.2).

9.4.3. Days 4 to 16 Daily Follow-up

Beginning Day 4, volunteers will be followed daily to evaluate for symptoms and viremia. Daily follows will continue until Day 16 for those who remain aviremic or until two negative DENV

RT-PCR tests for those who develop viremia. Daily follow-up procedures are summarized in **Table 5**, and listed as follows:

- A symptom-driven physical examination will be performed, vital signs obtained, and volunteer's temperature measured.
- Review concomitant medications.
- Evaluate injection site through Day 7.
- Assess solicited and unsolicited AEs and SAEs
- Review memory cards
- Blood collection for DENV RT-qPCR on Day 2 and Days 4-16 until two negative DENV RT-qPCRs.
- Blood collection for safety labs every other day starting Day 4 until Day 16 (if aviremic) or until two negative DENV RT-qPCR (if viremic).
- Blood collection for PBMC and PAXgene RNA on Days 4, 6, 8, 10, 12; and Day 14 or after cessation of viremia (whichever comes first).
- Blood collection for serum (microarray and ELISA) on Day 4, Day 8 (or upon awareness or viremia), and Day 14 (or after cessation of viremia).

9.4.3. Days 19, 22, and 25(+/- 1), and 28 (+/- 2) Post-Daily Follow-up

Post-daily follow-up visits (Day 19, 22, 25, and 28) procedures for the Homologous Rechallenge Sub-study will follow same procedures as in the Main Study (see Section 9.3.4) with the following changes:

- Blood will be drawn for PBMC (core cellular assays) on Day 28.

9.4.3. Days 56 (± 3 days), 90 and 180 (± 7 days) Safety Follow-ups

Long term safety follow-up visits (Day 56, Day 90, and Day 180) procedures for the Homologous Rechallenge Sub-study will follow same procedures as in the Main Study (see Section 9.3.5).

9.5. Inpatient Admission

9.5.1. Criteria for Admission to Inpatient Service

Due to the possibility of signs and symptoms associated with dengue fever, and the presence of mosquitoes theoretically competent for dengue virus, volunteers will be admitted to an inpatient service for observation if defined criteria are met. Viremia is expected, on average, to occur between days 5-16 after challenge (D0). *Aedes aegypti* and *Aedes albopictus***,** mosquito dengue vectors found in the United States, are present in the mid-Atlantic region of Maryland, and have the potential to support Zika, dengue, chikungunya, and other viruses.[38] Infected mosquitoes are not present in this area but their presence requires vigilance by sequestration to ensure that viremic volunteers are not able to infect local mosquito vectors. The CDC website notes 12 imported cases,

and no locally transmitted cases of dengue were detected in the state of Maryland and reported through ArboNET in 2019 (as of October 2, 2019).[39] Volunteers will be sequestered within a local hotel if asymptomatic save for viremia or admitted to the inpatient facility (Pharmaron or the General Clinical Research Center) for closer observation if any of the following additional criteria are met (or arise during the sequestration phase). If volunteers remain viremic for periods beyond Day 16, they will remain sequestered or an inpatient until documented clearance. If a DHIM occurs in late autumn/winter months in which there are no circulating *Aedes spp*., viremia will not result in sequestration (sustained temperatures above 59° F required for *Aedes albopictus).*[40] This would conservatively range from November to April. *Note: If local pandemic conditions continue, volunteers will be encouraged to sequester at the local hotel from the time of challenge onwards. If local, they will be asked to self-quarantine at home and apply social distancing standards to minimize any inadvertent exposure to the SARS-CoV-2 virus. Temperatures and vital signs will be monitored daily and an upper respiratory (e.g., NP or nasal) swab obtained for SARS-CoV-2 PCR at the first temperature (≥100.4℉) to distinguish the fever from that related to COVID-19 (if circulating SARS-CoV-2 remains sufficiently high as to warrant).*

Sequester Criteria:

- Viremia (D+24h) as detected by RT-qPCR

Inpatient Criteria:

- ***Viremia (D+24h) as detected by RT-qPCR <u>and two or more of the following symptoms</u>:***

Symptoms/lab parameters:

- Fever (> 100.4°F) at 2 time points at least 4 hours apart in the absence of antipyretic medication or any two or more of the following (per FDA Guidance for Industry). *Note: If local pandemic conditions continue, a repeat upper respiratory (e.g., NP or nasal) swab for SARS-CoV-2 detection via PCR will be performed on the day of the first temperature (defined as > 100.4℉).*
- Headache ≥ grade 2
- Eye pain ≥ grade 2
- Bone pain ≥ grade 2
- Joint pain ≥ grade 2
- Abdominal pain ≥ grade 2
- Muscle pain ≥ grade 2
- Nausea and/or Vomiting ≥ grade 2
- Liver function tests (ALT, AST) ≥ grade 2
- Leukopenia ≥ grade 2
- Thrombocytopenia ≥ grade 2
- Weakness/malaise ≥ grade 2

- Any symptoms determined by the PI to warrant hospital admission

The volunteers will remain sequestered/inpatient for observation until the criteria for their admission (i.e., viremia and/or ≥ grade 2 symptoms) resolve and meet the discharge criteria (**Section 9.5.3.**).

Volunteers will not be admitted if, in the opinion of the PI, an unrelated medical condition is responsible for the volunteer meeting hospitalization criteria. If required, the volunteer will be referred to their primary care physician for evaluation. Of special concern is the underlying risk of exposure to SARS-CoV-2. Close attention will be paid to symptoms consistent with COVID-19 illness to include upper respiratory symptoms (new or increased nasal discharge, sore throat, shortness of breath, wheezing, or the onset of loss of taste or smell). If necessary, additional testing or referrals for nasopharyngeal swabs for COVID-19 PCR may be done.

9.5.2. Procedures During Inpatient Admission

Once a volunteer is admitted to the hospital, the Dengue Human Infection Model Clinical Observation and Inpatient Management Phase Algorithm, will be followed. The procedures are listed in **Table 4** and summarized below.

During inpatient admission and sequestration, the following procedures will be performed daily: A symptom-driven physical exam, AE/SAE assessment, concomitant medication review, and illness index card review if applicable. The presence of viremia (RT-qPCR) will be checked daily from Days 4-16 (or extended if viremia continues past Day 16). Research sample collection will be performed according to **Table 3** and **Table 4**. Safety labs will be performed every other day (**Table 4**) and consist of complete blood count, liver function tests, Cr, glucose, prothrombin time/partial prothrombin time, and fibrinogen. In addition to the previously described procedures, the following daily additional procedures will be followed for the inpatient phase of the study. The volunteer's temperature and other vital signs will be measured at least 3 times per day (q 8 hours +/- 1 hour), or more if needed for volunteer care, and fluid intake and output measurement will be recorded on the volunteer's medical record. The standard of care is at the discretion of the PI and may include medication for management of pain, antipyretics (acetaminophen), the management of fluid loss through oral or IV hydration, monitoring and periodic clinical assessment, and judicious fluid replacement.

Although past clinical trials with the DENV-1, -3, or -4 strains have not produced any cases with severe symptoms, dengue with warning signs or severe symptoms may occur. Experienced clinicians will be expected to treat patients in accordance with the protocol and using the CDC and WHO guidelines as reference.

9.5.3. Criteria for Discharge/Release from Sequestration or Inpatient Admission

The actual discharge day will vary by volunteer. For volunteers to be discharged from observation, the following criteria must be met:

Sequestration:

- Two negative qualitative RT-PCR tests for dengue run on samples obtained via 2 different blood samples obtained at least 8 hours apart

Inpatient admission:

- Amelioration of clinical symptoms to ≤ grade 2 admission criterion* ***and:***
- No fever (< 100.4°F)
- Laboratory parameters resolving and are ≤ grade 2, at clinician discretion

  *Note: Will continue daily follow-up until two negative qualitative RT-PCR tests for dengue obtained on 2 difference blood samples at least 8 hours apart, as per protocol.

All volunteers discharged from sequestration or inpatient admission will be contacted via telephone the day after discharge and will return for follow up 3 days post discharge and 7 days post discharge. Volunteers will be seen at 28 days post inoculation, only if discharge occurs prior to Day 28. All volunteers will be seen on Days 56, 90, and 180 (2, 3, and 6 months post inoculation) according to **Table 3** and **Table 4**.

9.6. Biological Samples

9.6.1. Biological Sample Handling

Samples will be coded with information including, but not limited to, volunteer study number, study time-point, sample type and date/time. Samples will not be labeled with information that directly identifies the volunteer. Samples will be held in storage until exhausted through use in research assays or until directed to be destroyed by overseeing IRB or other oversight authority (e.g., FDA, USAMRDC etc.). Samples sent to WRAIR will follow these same guidelines and will be dispositioned and tracked by the WRAIR VDB quality management unit and held in WRAIR VDB cold storage facilities.

9.6.2. Future Use of Human Samples

Samples collected under this protocol will be used for protocol mandated and future research, research related to the development, maintenance, quality assurance, and improvement of the laboratory tests described in this protocol, the development of new test methods, and making sure that new tests work reliably and are comparable to previous methods.

It may be desirable to use the samples acquired in this study for future research, not described in this protocol. Therefore, all volunteers will be asked to give a specific consent to allow the sponsor's representative to use the samples for future research.

Volunteers not authorizing future use of samples will be tracked, and their samples will be destroyed upon completion of the study. Samples stored for future use will be stored indefinitely, unless local rules, regulations or guidelines require different timeframes or different procedures.

A volunteer may decide at any point during the study to withdraw consent for the future use of his/her samples. Should a volunteer withdraw consent for the future use of his/her samples,

remaining samples will be destroyed after the conclusion of testing for this clinical study. Testing sites will provide verification of destruction in writing to the clinical site.

9.7. Concomitant Medications

Volunteers are not being asked to discontinue current medications. In the event that medical conditions not related to the study arise after inoculation and dictate use of medications, volunteers are encouraged to obtain appropriate care, comply with the course of therapy as prescribed by their physician, and inform the investigators as soon as practicable. Details of all medications taken during this study must be recorded on the volunteer's record. Some medications will be exclusionary (e.g., HMG-CoA reductase inhibitors which have been shown to ameliorate dengue viremia).

Volunteers should not use any aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs), including Advil, Motrin, Nuprin, Aleve, Naproxen, and Celebrex, during the 28 days after inoculation or through 7 days post hospital discharge, whichever is later. Aspirin and NSAIDs can interfere with the ability of the blood to clot. It is not anticipated that any volunteers will have severe dengue infection with bleeding problems; however, it is still recommended to avoid these drugs. The investigators may recommend Tylenol or Paracetamol, which can be used safely for fevers and body aches.

9.8. Research Assessments

A summary of research specimen assays is summarized below in **Table 6**:

Table 6: Assessment Summary

Assessment	Method
Serum antibodies	ELISA or binding antibody
Antibody quantification	MN50 (microneutralization)
Viremia (RNAemia)	Quantitative RT-PCR
Viremia (RNAemia) for discharge	Qualitative RT-PCR
Serologic Microarray	Protein microarray
PBMC cellular immune response	ELISPOT/Flow cytometry
Transcriptomics	PAXgene - To be determined

9.9. Serum Antibodies

Blood samples will be obtained for the measurement of antibodies against the four dengue serotypes, DENV-1-4 and quantification of neutralizing antibodies via the MN50 microneutralization assay to be performed at WRAIR. Anti-dengue IgG, IgA and IgM may be measured using research lab (WRAIR) or commercial ELISA kits. Other functional assays, such as in vitro antibody-dependent enhancement (ADE) assays or antibody depletion assays may also be used to characterize serum antibodies. Screening flavivirus neutralization assays will be performed by the WRAIR Pilot Bioproduction facility using their microneutralization assay.

9.10. Polymerase Chain Reaction (PCR) Detection of Viremia

Blood samples will be obtained for measurement of levels of viremia using qualitative and/or quantitative (qPCR). Samples will be analyzed in real time (RT) in house using nucleic acid-based methods for the detection and quantification of dengue virus. WRAIR laboratories may be involved in the performance of this testing if needed.

9.11. Immune Responses

Cryopreserved peripheral blood mononuclear cells (PBMCs) will be tested for innate, B cell profiling and/or cellular immune responses, antigen-specific cytokine responses, chemokines and cell surface markers in order to examine the acquisition of T cell memory response following challenge. Conventional flow cytometry (CFCM) and CyTOF® and Helios® mass cytometers may be utilized for novel examinations of the memory response. Panels of 30-38 parameters are designed to identify innate mechanisms of immune response, effector/memory and peripheral T follicular helper cell subsets, pro-inflammatory cytokine/chemokine production, T cell homing patterns, B cell profiling, and the activation status of various T cell subsets. The use of a human challenge model will allow for investigation of predominant anti-DENV immune responses and the kinetics to examine early responses, and identify immune signatures, which differ in susceptible and resistant volunteers that may elucidate important correlates of protection. If funds allow, studies to investigate the T cell responses against whole cell DENV homogenate will be performed.

9.12. Peptide Microarray

Serum will be preserved and archived for microarray examination. Aliquots will be sent to Dr. Phil Felgner of UC Irvine for probing against a 1,2,3,4-dengue protein/flavivirus microarray constructed with 15 antigens per virus type (60 total). Additionally, Zika antibodies will also be printed to assess cross-reactivity. Microarray allows mapping antibody signatures against a pathogen's protein antigens that result after infection. The goal for this array is to identify antibody biomarkers associated to dengue disease onset and susceptibility to DF as well as assess ADE and cross-reactivity to Zika Virus.

9.13. Transcriptomics

A global approach to transcriptional analyses will be performed for gene expression analysis during primary infection using the HTA 2.0 array and/or total ribonucleic acid sequencing (RNA-Seq). This approach allows for unbiased analysis of genes regulated in response to dengue infection and has the potential to identify key pathways modulated. Initial analysis will focus on activation of innate immune pathways, as this type of analysis has not been possible in the natural infection setting. Given that the innate immune system is key to generating an effective acquired immune response, identification of key innate pathways regulated during infection may provide rational targets for enhancing a protective immune response. Subsequent confirmation of select targets will be done by quantitative PCR to confirm upregulation. Blood will be collected directly into PaxGene sample preparation tubes optimized for stabilizing RNA for long-term storage prior to transcriptional profiling. Genetic testing may be performed on these samples only after specific informed consent is acquired for such testing and IRB approval is obtained. WRAIR laboratories will be involved in the performance of some of this testing.

10.0. SAFETY ASSESSMENT

10.1. Consortium Data Safety Committee

The core Consortium Data Safety Committee (CDSC) includes, at a minimum, each site Principal Investigator (or designate), the protocol Independent Safety Monitor/Research Monitor (ISM/RM), and the IND sponsor Medical Officer. Representatives of the U.S. Army (i.e., WRAIR, USAMMDA, MRDC) will be asked to participate on the CDSC. Participants in the CDSC reviews may also include site Assistant/Associate Investigators, or designated team representatives including but not limited to study coordinators, study clinicians, or protocol specialists. The CDSC performs the safety reviews for the DHIM's conducted as part of the Dengue Consortium operations, and will perform safety reviews and review the summary of study safety data reports on a weekly or bimonthly basis through 4 weeks after the last volunteer receives the last study injection in order to ensure that the DHIM has an acceptable safety profile. Otherwise, if there have been no DHIM, in the prior 4 weeks, the CDSC will monitor safety data on a monthly basis through completion of the last study visit. At each site, an ISM/RM (advanced practitioner or research physician) will participate in study safety meetings and conduct a safety review of all UPIRTSOs and SAEs as well as submit a short summary of the event. The CDSC will evaluate and respond to safety concerns in a timely manner.

10.2. Clinical Laboratory Assessment

Safety laboratory assays will be performed at the study site-designated Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. All safety-related clinical laboratory values will be reviewed, and all abnormal values will be assessed by the investigators as clinically significant or not, with respect to safety. See **Table 7** for further details.

Urinalysis testing for pregnancy (urine β-hCG) will be assessed at screening, on the day of challenge and at Day 28 following challenge. Volunteers with a positive pregnancy test result will be discontinued from the study treatment but will be followed as explained in **Section 10.9.1.**

The laboratory safety assessments at specified study time points are summarized in **Tables 3** and **Table 7**.

Table 7: Clinical Laboratory Evaluations

Volunteers	**Assessments**	**Laboratory**
UMB	WBC count Hemoglobin (hematocrit will be reviewed) Platelet count Total neutrophils and lymphocytes within manual differential Biochemistry (creatinine, glucose (for screen only)) Liver Panel (AST, ALT) Fibrinogen, Albumin, PT/PTT	Garcia Clinical Laboratories 2195 Spring Arbor Road Jackson, MI 49203-2797 and/or University of Maryland Medical Systems Laboratory of Pathology 22 South Greene Room, N2W49 Baltimore, Maryland 21201

10.3. IND Safety Reporting Definitions

The following terms, as defined by 21 CFR 312.32, apply to IND safety reporting.

10.3.1. Adverse Event/Suspected Adverse Reaction/Unexpected Adverse Reaction

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.

See **Section 10.6** for Adverse Event Reporting

10.3.2. Solicited Adverse Event

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.

Refer to **Section 10.9.1** for adverse event reporting.

10.3.3. Serious Adverse Event/Serious Suspected Adverse Reaction

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the PI or Sponsor's Representative, it results in any of the following outcomes:

- Death
- Life-threatening adverse event
- Unanticipated hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the PI or Sponsor's Representative, its occurrence places the patient or volunteer at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or volunteer and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

For the purposes of this study, planned inpatient admission for dengue symptoms without serious deterioration in health, will not be considered an SAE as it is an expected outcome. Inpatient admission (defined as admission resulting in an overnight stay, > 24 hours) not related to dengue symptoms will be considered an SAE.

See **Section 10.9.2** for SAE reporting.

10.3.4. Unanticipated Problems Involving Risks To Volunteers Or Others (UPIRTSOs)

Federal regulations require that unanticipated problems involving risks to volunteers or others be promptly reported to the IRB. These events encompass a broader category of events than SAEs and may include issues such as problems with loss of control of volunteer data or the

investigational product, adverse psychological reactions, or breach of confidentiality. Risks to others (e.g., program personnel) must also be reported.

Unanticipated problems involving risks to volunteers or others (UPIRTSOs) are any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the procedures that are described in the protocol, investigators brochure or informed consent document; and (b) the characteristics of the volunteer population;
- Related or possibly related to a volunteer's participation in the study; and
- Suggests that the study places volunteers or others at a greater risk of harm than was previously known or recognized.

The IRB will evaluate the PI's and safety monitor's reports to determine whether a given incident, experience or outcome constitutes an unanticipated problem involving risk to volunteers or others and, in coordination with the Sponsor's Representative, ensure upward reporting of the unanticipated problems involving risk to volunteers or others to the appropriate regulatory offices.

See **Section 10.9.2.** for UPIRTSO reporting.

10.3.5. Pregnancy

Volunteers who become pregnant after Day 0 will be followed to term, and the following information will be gathered for outcome, date of delivery, health status of the mother and child including the child's gender, height, and weight. Complications and or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale.

See **Section 10.10.1.** for pregnancy reporting.

10.4. Relationship to Investigational Product

The PI must assign a relationship of each AE to the receipt of the investigational product. The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. The guidelines shown in **Table 8** should be used by investigators to assess the relationship of an AE to study product administration. Only a physician can make this determination.

Table 8: Adverse Event Relationship to Investigational Product Categories

Category	Description
Not related	No relationship to investigational product. Applies to those events for which evidence exists that there is an alternate etiology.
Unlikely	Likely unrelated to the investigational product. Likely to be related to factors other than investigational product but cannot be ruled out with certainty.
Possible	An association between the event and the administration of investigational product cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the volunteer's clinical status or underlying factors including other therapy.
Probable	There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the volunteer's clinical state or factors including other therapy.
Definite	An association exists between the receipt of investigational product and the event. An association to other factors has been ruled out

To align consortium protocols, AE relationship uses 5 categories of relatedness (defined above), which are to be used for this clinical trial. The category of "Not Related" maps to the dual category of "Not Related," while the categories of "Unlikely," "Possible," "Probable," and "Definite" map to the dual category of "Related."

10.5. 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 1 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**. Any AE not included in the scale will be graded according to **Table 9**. 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**.

FDA guidelines for toxicity will be followed; however, if a volunteer is evaluated in an emergency room for nonlife threatening illness or symptoms (i.e., visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed and severity of the adverse event will be assessed according to the volunteer's clinical signs and symptoms.

As defined by the ICH guideline for GCP, the term "severe" is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious", which is based on volunteer/event outcome or action criteria usually associated with events that pose a threat to a volunteer's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Table 9: Adverse Event Severity Categories

Category	Grade	Description
Mild	1	Does not interfere with routine activities Minimal level of discomfort
Moderate	2	Interferes with routine activities Moderate level of discomfort
Severe	3	Unable to perform routine activities Significant level of discomfort

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3.

10.6. Recording Adverse Events

The PI will report all AEs to the UMB CVD IRB in the appropriate safety, annual, and/or final reports. The study site will provide data files for preparation of annual and final reports to the FDA.

10.6.1. Methods/Timing for Assessing, Recording, and Analyzing Safety Endpoints

AEs and SAEs will be assessed at all study visits, documented in the source records, and recorded on the eCRF's using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The PI will assess all AEs for seriousness, relationship to investigational product, severity, and other possible etiologies. When an event has not resolved by the end of the volunteer's participation (6 months), it will be documented on the AE eCRF as "not recovered/not resolved".

The timeframe for the collection of AEs begins at time of inoculation (Day 0) through end of volunteer's participation (6 months). SAEs will also be collected from Day 0 through end of volunteer's study participation (6 months).

The timeframe for the collection of solicited AEs begins at the administration of investigational product through Day 28 (Local injection site solicited AEs will be collected through Day 7). All solicited AEs collected in the first 28 days will be considered 'definitely related' to the investigational product unless they can be attributed to a known cause unrelated to the investigational agent.

10.6.2. Duration of Follow-Up of Volunteers after Serious Adverse Events

Investigators are required to follow SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. The SAE outcomes will be reported to the sponsor's representative using the Serious Adverse Event Report Form.

Investigators are not obligated to actively seek SAEs in former volunteers; however, if a SAE, considered to be related to the investigational product is brought to the attention of the investigator

at any time following completion of the study, the event will be reported to the sponsor's representative's safety office as defined in **Section 10.9.2**, **Table 10**.

10.7. Study Halting Criteria

The PI, study site safety office, or the sponsor's representative will place the study on hold for any of the following criteria:

Further enrollment and study inoculation will be halted for safety review if any of the following are reported:

1. Any volunteer experiences a study product-related SAE from the time of the study product administration through the volunteer's last study visit.
2. Any volunteer experiences laryngospasm, bronchospasm, generalized urticaria or anaphylaxis within 1 day after administration of study product that is considered related to study product.

This trial will also be halted for safety review if, within 7 days after administration of any challenge inoculation, any of the following occurs:

1. Two or more volunteers experience a Grade 3 unsolicited AE in the same MedDRA system organ class after administration of challenge inoculation that is considered related to study product and not resolved or improved to lower grade within 2 days.
2. Two or more volunteers experience a Grade 3 solicited local adverse event that is considered related to study product and not resolved or improved to lower grade within 2 days.
3. One or more volunteers experience a Grade 3 solicited systemic adverse event that is considered related to study product (excluding fever) and not resolved or improved to lower grade within 2 days.
4. Two or more volunteers experience a Grade 3 laboratory adverse event (excluding leukopenia that would be expected with a viral infection) that is considered related to study product.

In addition, the PI, sponsor's representative, UMB IRB or the FDA may place this study on hold at any time.

Grading scales for solicited local (application site) and systemic (subjective and quantitative) AEs are included in **Appendix A**.

Grading scales for clinical safety laboratory adverse events are included in **Appendix A**.

10.8. Termination Rules

If the AE/SAE profile is not acceptable to the PI, sponsor's safety office, sponsor's representative, DHIM Consortium Safety Review or FDA, the trial may be terminated.

If the trial is terminated, a notification (via memo) indicating the reasons for suspending the study will be provided by the sponsor's representative to the PI for submission to the IRB.

10.9. Reporting Requirements

10.9.1. Reporting Adverse Events

Adverse Events (including Solicited Adverse Events and Unsolicited Adverse Events) will be reported in the volunteer source document and in the eCRF.

The PI will report all AEs to the sponsor's representative's safety office and the UMB IRB in the appropriate safety, annual, and/or final reports.

10.9.2. Reporting Serious Adverse Events/Unanticipated Problems Involving Risk to Volunteers or Others

Initial Report: All SAEs, and UPIRTSOs, whether or not the event is considered related to the study product, must be reported promptly (within 24 hours of site awareness of the event) to the sponsor's representative's office and the sponsor's safety office (**Table 10**).

Follow-up report: The site will provide a follow up SAE report at any point additional information related to the event is available to the sponsor's safety office and the safety monitor.

Contact information for reporting SAEs/UPIRTSOs are provided in the table below.

Table 10: Study Contacts for Reporting Serious Adverse Events, Unanticipated Problems Involving Risk to Volunteers or Others

Sponsor's Safety Office	
Research Monitor/Independent Safety Monitor	Justin R. Ortiz, MD, MS, FACP, FCCP Center for Vaccine Development University of Maryland School of Medicine 685 W. Baltimore Street, Room 480 Baltimore, MD 21201 Telephone: 410-706-3502 Fax: 410-706-6205 E-mail: jortiz@som.umaryland.edu Alternate: Wilbur Chen, MD Center for Vaccine Development University of Maryland School of Medicine 685 W. Baltimore Street, Room 480 Baltimore, MD 21201 Telephone: 410-706-1188 Fax: 410-706-6205 E-mail: wchen@som.umaryland.edu
Institutional Review Board	Human Research Protections Office University of Maryland, Baltimore 800 W. Baltimore Street, Suite 100 Telephone: 410-706-5037 Fax: 410-706-4189 E-mail: HRPO@som.umaryland.edu

All notification will be provided to the sponsor's safety office. Further, the investigator should comply with relevant study site SOPs on reporting SAEs. The minimum information that the investigator will provide to the sponsor's safety office is specified in **Table 11**. The sponsor's representative may request additional information for purposes of the study.

Table 11: SAE Information to be reported to the Sponsor's Safety Office

Notification Method	Information to Be Provided
E-mail or Telephone (within 24 hours of site awareness)	IND number, sponsor study number, name of the investigational product, and investigator name and contact number
	Volunteer identification number
	SAE, onset date, date of investigational product administration, severity, relationship, and volunteer's current status
AND	
E-mail or Fax	Cover sheet or letter
	Adverse event case report form
	Serious adverse event report form
	Concomitant medication case report form or a list of concomitant medications
	Medical record progress notes including pertinent laboratory/diagnostic test results
NOTE: When submitting SAE reports via e-mail, the volunteer line of each email notification will read as follows: **SAFETY REPORT – IND # _____, Sponsor Study #_____, Volunteer# _____, Event Term: _____**	

10.9.3. Reporting to the UMB IRB

UPIRTSOs, SAEs related to participation in the study, and all volunteer deaths related to participation in the study should be promptly reported within 24 hours of site awareness by telephone, email, or fax to the UMB IRB (**Table 10**). A complete written report should follow the initial notification.

Investigators are required to forward safety information provided by the sponsor's representative to the IRB.

10.9.4. Sponsor Reporting Requirements to FDA

In order to comply with 21 CFR 312.32 (c), the sponsor must report any suspected adverse reaction that is both serious and unexpected to the FDA as an IND safety report within 15 calendar days. Any unexpected fatal or life-threatening suspected adverse reaction must be reported to the FDA within 7 calendar days.

10.10. Reporting Additional Immediately Reportable Events to the Sponsors Safety Office and HRPO

10.10.1. Pregnancy

Each pregnancy must be described on the Pregnancy Report Form and reported immediately (within 24 hours of site awareness) by email, fax or phone to the sponsor's safety office and the sponsor's office.

Volunteers who become pregnant after Day 0 will be followed until 30 days after delivery to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The following information will be gathered for outcome, date of delivery, health status of the mother and child including the child's gender, height and weight. Complications and

or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion, an elective termination for medical rationale, or the infant has a congenital anomaly/birth defect.

Report the incident to UMB HRPO in accordance with HRPO policy.

10.10.2. AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported immediately (within 72 hours of identification) by email or fax to the sponsor's safety office as per **Table 11**. Report the withdrawal to local IRB in accordance with IRB policy.

10.10.3. Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters, or actions taken by any regulatory agency including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the local IRB and the sponsor's representative.

10.10.4. Final Report

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications" and ICH E3 Guideline "Structure and Content of Clinical Study Reports" and provided to the sponsor's representative for review and approval. The sponsor's representative will use this report to prepare the final clinical study report for submission to the FDA.

The PI will report all AEs to the UMB IRB in the appropriate safety, annual, and/or final reports. After appropriate data cleaning and query resolution between the clinical site, sponsor's clinical monitor, and clinical data manager, SAEs from the clinical database will be reconciled with the sponsor's SAE database. SAEs and AEs for inclusion in annual and final reports to the FDA will be provided from the clinical database by the clinical data manager at the UMB CVD.

The final study report submitted to the IRB, including a copy of any acknowledgement documentation and any supporting documents, will be submitted to the HRPO as soon as all documents become available.

11.0. STATISTICS

Detailed statistical procedures, listings, table shells and figures will be provided in a separate statistical analysis plan (SAP) written shortly after protocol approval but before any volunteer enrollment. The SAP will be finalized before study close-out and database lock. The following key statistical components will be considered, and a detailed description will be documented in the SAP:

- Primary and secondary endpoints and how they will be measured,
- Statistical methods and tests that will be used to analyze the endpoints,
- Strategy that will be used if the statistical test assumptions are not satisfied,
- Specification of potential adjusted analyses and a statement with which covariates or factors will be included,
- Planned exploratory analyses and justification of their importance, and
- Any subgroup effects with biological justification and support from within and outside the study.

11.1. Description of Statistical Methods

Descriptive analysis of safety and reactogenicity outcomes will include all volunteers who meet the eligibility criteria, receive inoculation, and for whom safety data are available. Summary tables will be created in which incidence, severity, and relationship to the use of investigational product of individual solicited local and systemic signs, symptoms, or trending unsolicited events are delineated by study group, severity, gender, and overall. Unsolicited AEs and SAEs will be analyzed in a similar fashion.

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.

11.1.1. Analysis Addressing the Primary Endpoints

There are no hypotheses. All the main analyses will be descriptive.

The safety analysis set (SafAS) will be used for the analysis of safety data in this study. The SafAS population consists of all volunteers who are inoculated.

Individual solicited local AEs over the 7-day follow-up period and solicited systemic AEs over the 28-day follow-up period or 7 days post hospitalization (whichever is later) will be analyzed.

The incidence, intensity, and relationship of individual solicited AEs to the inoculation will be calculated overall and by dose group. Abnormal laboratory measurements that occur following each inoculation will be summarized overall, and by toxicity grade for each component of the trial. Presentations will include the number and percentage of volunteers with at least 1 solicited symptom (local or systemic), at least 1 local symptom, and at least 1 general (systemic) symptom, as well as the incidence of each symptom individually.

The number of volunteers with at least 1 report of an unsolicited adverse event reported up to 28 days after inoculation or 7 days post hospitalization (whichever is later) will also be summarized overall and by dose group. The intensity and temporal relationship of the unsolicited symptoms to inoculation will also be assessed. Presentations will also summarize unsolicited AEs by body system, 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 1 volunteer, but the symptoms will be listed as 2 separate AEs within the class. Therefore, the total number of AEs reported within a body system may exceed the number of volunteers within the body system reporting AEs. SAEs occurring at any point during the trial will be summarized and relatedness to virus inoculation will be assessed. Serious and/or unexpected AEs will also be discussed on a case-by-case basis.

A **Dengue Illness Index** (**Table 12**) will be calculated for each volunteer. A calculated score will be generated on each volunteer which will allow for a statistical analysis of the values tabulated between the vaccinated and unvaccinated controls. The Dengue Illness Index will be compared between vaccinated and unvaccinated volunteers using statistical testing such as Fisher's exact test or T-test to evaluate for statistically significant differences between these two groups. P-values less than 0.05 will be considered significant for analysis performed within this protocol. 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 2 times at least four hours apart in 24 hours]
- 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 or AST graded to the higher value)
- Leukopenia
- Thrombocytopenia

A scoring system will be used to evaluate symptoms (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 (A), the number of symptoms per day (B) and the maximum severity score (grade 0-3) (C) (See **Table 12**). Volunteers may also be categorized based upon the 2009 revised WHO guidelines (Dengue without warning signs, dengue with warning signs and severe dengue).[41] *Note: Tourniquet tests will be performed at investigator discretion.*

Table 12: 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.

11.1.2. Multiple Comparison Endpoint

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.

11.1.3. Analysis Addressing the Secondary Endpoints

There are no hypotheses. All the main analyses will be descriptive.

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

Analysis of the secondary endpoints will be applied on 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).

11.1.4. Analysis Addressing the 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 neutralizing antibody titer levels (measured by dengue neutralizing titer) at 0, 1, 3, and 6 months after inoculation
- Number and percentage of volunteers with a titer ≥10
- CMI data: descriptive statistical summaries will be provided to describe data by treatment group.
- Genetic (RNA) data to include minor variant sequence analysis, whole genome RNA-seq, and/or transcriptomic analysis

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.

11.2. Planned Enrollment and Reason for Sample Size

As this study has no statistical hypothesis test, there is no formal power calculation. As we have not progressed to the medium or high dose arms, we remain within our recruitment estimates.

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-4-LVHC virus challenge is expected to be greater than 74%.

11.3. Procedures for Reporting Deviations from the Original Statistical Plan

Any deviation(s) from the original statistical plan as indicated in the protocol will be described in an amendment to the protocol and in the SAP.

11.4. Selection of Volunteers to be Included in Analyses

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

11.4.1. 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

11.4.2. 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.

11.4.3. 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

11.4.4. Populations Used in Analyses

The safety analyses will be performed on the SafAS. Volunteers will be analyzed according to the inoculation they received.

The performance analyses will be performed on the FAS and PPAS. Volunteers will be analyzed according to the inoculation they received.

NOTE: If the FAS and PPAS include the same volunteers, only 1 set of analyses will be produced. They will generally be identified as summarizing or listing data from the PPAS.

If exploratory endpoint data become available, exploratory analysis set will be defined in separate documents defining the analyses planned for those populations and data.

11.5. Handling of Missing Data and Outliers

Missing data will be handled according to **Table 13.**

Table 13: Methods for Handling Missing Data and Outliers

Data	Handling Method
Safety	Missing data will not be replaced with imputed values.
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.

12.0. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Volunteers will be identified on eCRFs by a unique volunteer identification number and on source documents by name and date of birth. No personal identifier will be used in any publication or communication used to support this research study. The volunteer identification number will be used if it becomes necessary to identify data specific to a single volunteer. Representatives of the sponsor's representative, the UMB IRB, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human volunteers in clinical research. Personal identifiers will be removed from photocopied medical and research records.

13.0. QUALITY CONTROL AND QUALITY ASSURANCE

13.1. Study Monitoring

Study monitoring will be the responsibility of the external contractor (e.g., ICON). Upon successful approval of the protocol and establishment of the regulatory file, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records. As needed, the clinical monitor may witness the

informed consent process or other applicable study procedures to assure the safety of volunteers and the investigators' compliance with the protocol and GCPs.

Monitoring visits by a sponsor's representative-designated clinical monitor will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last volunteer has completed the study. A report of monitoring observations will be provided to the PI and the sponsor's office, at a minimum.

13.2. Audits and Inspections

Auditing of the clinical trial may be conducted at any time during the study to ensure continued compliance with regulations, policies, and procedures. Authorized representatives of the sponsor, the FDA, the independent ethics committee, or IRB may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guideline of the ICH, and any applicable regulatory requirements. Audit findings will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

The investigator should contact the sponsor's office immediately if contacted by a regulatory agency about an inspection.

14.0. ETHICS

14.1. Ethical Conduct of the Study

The study will be conducted in accordance with all applicable regulatory requirements including ICH Guideline for GCP, all applicable volunteer privacy requirements, and the study will be conducted in accordance with applicable regulations and policies including the Declaration of Helsinki, ICH Guidelines, US 32 CFR 219 (Protection of Human Volunteers), US 21 CFR Part 50 [Protection of Human Volunteers (Informed Consent)] and Part 56 (IRBs) and Part 312 (Investigational New Drug Application), AR 40-7, and AR 70-25, and the principles of respect for persons, beneficence, and justice described in the Belmont Report.

The procedures set out in this study are designed to ensure that the sponsor's representative and all study personnel abide by the principles of the ICH GCP Guideline and other applicable regulatory requirements. The investigator confirms this by signing approval for this study protocol and FDA Form 1572.

14.1.1. Confidentiality

The Health Insurance Portability and Accountability Act (HIPAA) requires that researchers obtain the volunteer's permission (HIPAA Authorization) to use and disclose health information about the volunteer that is either created by or used in connection with this research. The information includes the entire research record and supporting information from the volunteer's medical records, results of laboratory tests, and both clinical and research observations made during the individual's participation in the research.

In this research, the volunteer's health information will be collected and used to conduct the study; to monitor the volunteer's health status; to measure effects of the investigational product; to determine research results, and possibly to develop new tests, procedures, and commercial products. Health information is used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. After the study ends, each volunteer has the right to see and receive a copy of his/her information.

The sponsor's representative, the IRB, and the FDA are eligible to photocopy, and review records related to this protocol as a part of their responsibility to protect the participants of this protocol. In addition, these representatives are eligible to witness the applicable study procedures to assure the safety of volunteers.

No personal identifier will be used in any publication or communication used to support this research study. The volunteer's identification number will be used in the event it becomes necessary to identify data specific to a single volunteer.

14.1.2. Compensation

Volunteers will be compensated for time and inconvenience in accordance with the standards for compensation at the site. Compensation may vary depending upon the number of days that may be required for the daily evaluations in the period after a DHIM as those who become viremic early will require fewer days of clinic visits than those who have delayed or no viremia. Details are provided in the ICF.

14.1.3. Written Informed Consent

The site will prepare a model ICF which will embody the ICH GCP as well as sponsor-required elements. Freely given and written informed consent must be obtained from each volunteer prior to participation in the study. Homologous rechallenge participants will have an revised or addendum consent to review.

The informed consent process and document will be reviewed and approved by the IRB and sponsor's representative prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and any alternate vaccination/preventative options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. The consent document indicates that by signature, the volunteer permits access to relevant study records by the sponsor's representative and by representatives of the FDA. The sponsor's representative will submit a copy of the initial IRB- and sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRB.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, and the Belmont Principles, will be signed by the volunteer before any study-related procedures are initiated for that volunteer. This consent document must be retained by the investigator as part of the study records. The investigators or their designees will present the protocol in lay terms to individual volunteers. Questions on the purpose of the protocol, protocol procedures, and risks to the volunteers will then be solicited. Any question that cannot be answered will be referred to the principal investigator. No volunteer should grant consent until he or she has had ample time to

read the informed consent document and questions have been answered to his/her satisfaction. The volunteer should understand that the study product is an investigational drug and is not licensed by the FDA for commercial use but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the volunteer be informed about the principal potential risks and benefits. This information will allow the volunteer to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary
- Withdrawal from participation can occur at any time
- Refusal to participate involves no penalty
- Questions to understand the nature of the protocol can be asked
- A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law.

Should the protocol be modified, the volunteer ICF must be reviewed and revised, if applicable, to reflect the changes made to the protocol. If a previously enrolled volunteer is directly affected by the change, the volunteer will receive a copy of the revised informed consent document to read. If the volunteer agrees to continue participating in the study, the approved revision will be signed and dated by the volunteer.

14.1.4. Medical Care for Research-Related Injury

All non-exempt research involving human volunteers shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6).

If a volunteer suffers an injury directly related to participation in this project, UMB and/or one of its affiliated institutions or health care groups will help obtain medical treatment for the specific injury and provide referrals to other health care facilities, as appropriate. UMB and/or its affiliated institutions or health care groups will not provide financial compensation or reimbursement for the cost of care provided to treat a research-related injury or for other expenses arising from a research-related injury. The institution or group providing medical treatment will charge the volunteer's insurance carrier, the volunteer, or any other party responsible for treatment costs. If the volunteer incurs uninsured medical costs, they are the responsibility of the volunteer.

14.2. Ethics Review

The study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed by the UMB IRB; the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the volunteers will be respected; the physicians conducting the study will ensure that the risks do not outweigh the potential benefits; the results to be reported will be accurate; volunteers will give their informed consent and will be competent to do so and not under duress; and all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

14.2.1. Review/Approval of Study Protocol

Before the clinical study is initiated, the study protocol and other required documents will be submitted to the University of Maryland Human Research Protection Program IRB for review and/or approval, with the final review by the FDA.

Enrollment and screening will not begin until IRB approvals have been obtained and the formal authorization letter is received by the PI. Initial IRB approval and all materials approved by the IRB for this protocol, including the patient consent form and recruitment materials, must be maintained by the protocol physician (PI) and made available for inspection.

Volunteer informed consent will be obtained prior to the initiation of any study procedure. The PI will be responsible for preparing and submitting continuing review reports per institution and IRB requirements.

14.2.2. Institutional Review Board

The IRB of record will serve as the responsible IRB and will review the protocol, informed consent, and progress reports on a continuing basis in accordance with all applicable regulations, including Title 21, Code of Federal Regulations (CFR), Parts 50 and 56.

The PI must obtain IRB approval for the study. Initial IRB approval and all materials approved by the IRB for this protocol, including the patient consent form and recruitment materials, must be maintained by the protocol physician (PI) and made available for inspection.

The PI will be responsible for preparing and submitting continuing review reports per institution and IRB requirements.

14.2.3. Protocol Modifications

All modifications to the protocol and supporting documents (i.e., informed consent, study-specific procedures, SOPs, recruitment materials, etc.) must be reviewed and approved by the sponsor and by the IRB prior to implementation. Any protocol amendment will be agreed upon and approved by the sponsor's representative prior to submission to the local IRB and prior to implementation of said change or modification. The ICF must be revised to concur with any amendment as appropriate and must be reviewed and approved with the amendment. Any volunteer already enrolled in the study will be informed about the revision and asked to sign the revised informed consent document if the modification directly affects the individual's participation in the study. A copy of the revised, signed, and dated informed consent document will be given to the volunteer. All original versions of the informed consent document will be retained in the protocol regulatory file, and a copy will be retained in the volunteer study chart.

Any modification that could potentially increase risk to volunteers must be submitted to the IRBs for approval prior to implementation.

15.0. DATA HANDLING AND RECORD KEEPING

The primary source document for this study will be the volunteer study case report form (CRF). If separate research records are maintained by the investigator(s), the medical record and the

research records will be considered the source documents for the purposes of auditing the study. The source documents will be retained at the site.

For this study, an electronic data capture (EDC) database system will be used for the collection of the study data in an electronic format. The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the EDC database system. The investigator is ultimately responsible for the accuracy of the data collected on the eCRF. Data monitoring and management will be performed in the EDC database system by the study clinical monitor and the designated Data Management group.

All individuals who will be expected to use the EDC will be given the training necessary to perform their assigned tasks as described in (21 CRF 11.10(i)). Training will be conducted by qualified individuals initially and on a continuing, as needed basis. The training documentation will be maintained at the trial site. The sponsor will also keep a record of the training files.

A detailed data management plan will be written and approved by the study team and the PI prior to study start, with approval by the sponsor's data manager. All updates to the data management plan must be approved before study close-out and database lock.

15.1. Inspection of Records

The sponsor's representative or designee will be allowed to conduct site visits at the study site for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the storage area, investigational product stocks, accountability records, volunteer charts, study source documents, and other records relative to study conduct.

Volunteers' study chart information will be used to report results of research to the sponsor's representative and Federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. The consent document indicates that by signature, the volunteer permits access to relevant study chart information by the sponsor's representative and by representatives of the FDA.

15.2. Retention of Records

Following closure of the study, the investigator must maintain all site study records in a safe and secure location. The records must be maintained to allow easy and timely retrieval, when needed (e.g., audit or inspection), and, whenever feasible, to allow any subsequent review of data in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g., microfiche, scanned, electronic for studies with an eCRF); however, caution needs to be exercised before such action is taken. The investigator must assure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure there is an acceptable back-up of these reproductions and that an acceptable quality control process exists for making these reproductions.

The investigator must maintain all documentation relating to the study. The CVD will retain records on site for the duration of the active IRB protocol. If it becomes necessary for the sponsor's representative or designee or the FDA to review any documentation relating to the study, the investigator must permit access to such records. An archival at an off-site facility, Iron Mountain, will hold records in perpetuity and the records are easily retrievable.

The PI will be responsible for retaining sufficient information about each volunteer, i.e., name, address, telephone number, Social Security number, and volunteer identifier in the study, so that the sponsor's representative, the local IRB, the FDA, employees of USAMRMC, or other regulatory authorities may have access to this information should the need arise.

16.0. PUBLICATION POLICY

16.1. IND Annual Reports and Final Clinical Study Reports

After appropriate data cleaning and query resolution between the clinical site, sponsor's clinical monitor, and clinical data manager, SAEs from the clinical database will be reconciled with the sponsor's SAE database. SAEs and AEs for inclusion in annual and final reports to the FDA will be provided from the clinical database by the clinical data manager.

16.1.1. IND Annual Report to the FDA

The PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the sponsor's representative. Each annual report will summarize IND activity for one year. The sponsor's representative will notify the PI of the due date with sufficient time for the PI to assemble the required information.

16.1.2. Study Results and Clinical Trial Registries

Following completion of the study, the investigator may publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine (NLM). Other biomedical journals are considering adopting similar policies. It is the responsibility of DMID to register this trial in an acceptable registry. In compliance with Public Law 110-85, the Food and Drug Administration Amendments Act of 2007 (FDAAA), DMID will also post the results of the trial in accordance to the legal requirements.

The ICMJE defines a clinical trial as any research project that prospectively assigns human participants to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (*e.g.*, Phase 1 trials), would be exempt from this policy. As a result, this study can but is not required to be registered in the NLM registry, ClinicalTrials.gov.

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APPENDIX A. TOXICITY GRADING SCALES FOR LABORATORY (CHEMISTRY AND HEMATOLOGY), LOCAL REACTOGENICITY, VITAL SIGNS AND SYSTEMIC ADVERSE EVENTS

Laboratory Toxicity Grading Scale for Chemistries

Table 14: Toxicity Grading Scale for Laboratory Abnormalities

Serum[a,d]	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**[b]
Sodium – Hyponatremia (mEq/L)	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia (mEq/L)	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia (mEq/L)	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia (mEq/L)	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia (mg/dL)	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting (mg/dL) Random (mg/dL)	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN (mg/dL)	23-26	27 – 31	> 31	Requires dialysis
Creatinine (mg/dL)	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia (mg/dL)	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia (mg/dL)	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia (mg/dL)	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia (mg/dL)	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK (mg/dL)	1.25 – 1.5 x ULN[c]	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia (g/dL)	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia (g/dL)	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

a The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

a The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a Grade 3 parameter (125-129 mEq/L) should be recorded as a Grade 4 hyponatremia event if the volunteer had a new seizure associated with the low sodium value.

c ULN is the upper limit of the normal range.

d Laboratory values that fall in the institutional normal reference range do not receive a toxicity grade

Table 15: Safety Laboratory Toxicity Grading Scale for Hematology Adverse Events

Hematology Parameter[a,d]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin for Females (gm/dL)[b]	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin for females change from baseline value (gm/dL)	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin for Males (gm/dL)	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin for Males change from baseline value (gm/dL)	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase (cell/mm^3)	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease (cell/mm^3)	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease (cell/mm^3)	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease (cell/mm^3)	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils (cell/mm^3)	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased (cell/mm^3)	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase (mg/dL)	400 – 500	501 – 600	> 600	--
Fibrinogen decrease (mg/dL)	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

a The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

b In cases where ABNORMAL laboratory values fall below grade 1 the lab abnormality will be graded as GRADE 1.

c In cases where ABNORMAL laboratory values fall between two FDA grades, the ABNORMAL value will be graded as the highest grade of the two.

d Laboratory values that fall in the institutional normal reference range do not receive a toxicity grade

Table 16: Toxicity Grading Scale for Local Reactions

Local Reaction	Normal (Grade 0)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Redness/Erythema[a]	<25 mm	25-50 mm	51-100 mm	>100 mm	Necrosis or exfoliative dermatitis
Swelling/ Induration[a, b]	<25 mm	25-50 mm and does not interfere with activity	51-100 mm or interferes with activity	>100 mm or prevents daily activity	Necrosis
Pain	None	Does not interfere with activity	Interferes with activity or repeated use of non-narcotic pain reliever	Prevents daily activity or repeated use of narcotic pain reliever	ER visit or hospitalization

[a] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[a, a] Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Table 17: Toxicity Grading Scale for Vital Signs

Vital Signs[a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) (°F) [b]	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute [c]	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock

[a] Volunteer should be at rest for all vital sign measurements.
[a] Oral temperature, no recent hot or cold beverages or smoking.
[a] When resting heart rate is between 60 to 100 beats per minute. Physician investigators will use clinical judgment. when characterizing bradycardia among some healthy volunteer populations, for example, conditioned athletes when it comes to inclusion into the study during screening.

Table 18: Toxicity Grading Scale for Systemic Adverse Events

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue/malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Nausea/vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Rash	Localized skin eruption	Diffuse skin eruption up to 50% of the body surface area (BSA)	Generalized skin eruption involving > 50% BSA, or – Rash with bullae, vesicles, mucous, membrane ulceration, target lesions, purpura, or with epidermal detachment	Stevens Johnson Syndrome / Toxic epidermal syndrome
Other Systemic Symptoms: Illness or clinical AE (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization