

A PHASE 1, RANDOMIZED, OPEN-LABEL, SINGLE-CENTER, COMPARISON OF HETEROLOGOUS PRIME-BOOST VACCINATION SCHEDULES OF TETRAVALENT DENGUE VIRUS PURIFIED INACTIVATED VACCINE (PIV) AND TETRAVALENT DENGUE VIRUS LIVE ATTENUATED VACCINE (LAV) IN HEALTHY ADULTS IN A NONENDEMIC REGION

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Investigator's Agreement

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"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Conference on Harmonisation Good Clinical Practice Guideline and FDA, DoD, and United States Army Regulations."

Simon Pollett, MBBS
Principal Investigator

Date

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1. SYNOPSIS

Name of Sponsor: The Surgeon General, Department of the Army	
Name of Investigational Product: 1. Tetravalent Dengue Virus Purified Inactivated Vaccine (PIV) with Alum adjuvant 2. Tetravalent Dengue Virus Live Attenuated Vaccine (LAV) Formulation 17 post transfection	
Name of Active Ingredient: 1. Inactivated dengue virus types 1-4 2. Live attenuated dengue virus types 1-4	
Title of Study: A PHASE 1, RANDOMIZED, OPEN-LABEL, SINGLE-CENTER, COMPARISON OF HETEROLOGOUS PRIME-BOOST VACCINATION SCHEDULES OF TETRAVALENT DENGUE VIRUS PURIFIED INACTIVATED VACCINE (PIV) AND TETRAVALENT DENGUE VIRUS LIVE ATTENUATED VACCINE (LAV) IN HEALTHY ADULTS IN A NONENDEMIC REGION	
Study Center(s): Clinical Trials Center, Walter Reed Army Institute of Research (CTC, WRAIR)	
Principal Investigator: Simon Pollett, MBBS Subinvestigators: LCDR Marvin Sklar; COL Paul Keiser, MD; Simon Pollett, MBBS; LTC James Moon, MD; Kristin Mills, DO; MAJ Nathaniel Copeland, MD; MAJ Melinda Hamer, MD; Christine Lee, MD; CPT Jack Hutter, MD; MAJ Justin Curley, MD;	
Study Period (years): Estimated date first subject enrolled: July 2017 Estimated date last subject will completed: January 2019 Estimated time all study related testing and data analysis will be complete: January 2021	Phase of development: 1
Objectives: Primary: <ul style="list-style-type: none"> To further evaluate the safety and reactogenicity of 2 tetravalent dengue vaccine (TDENV) candidates administered in dengue naïve subjects in a heterologous prime boost fashion with a PIV followed by LAV 180 days later. To evaluate the safety and reactogenicity of a previously untested vaccination schedule in dengue naïve subjects consisting of PIV followed by LAV 90 days later Secondary: <ul style="list-style-type: none"> To further evaluate the humoral and cell-mediated immunogenicity of the PIV/LAV Day 0,180 administration schedule and compare it to the humoral and cell-mediated immunogenicity of the PIV/LAV Day 0,90 schedule Exploratory: <ul style="list-style-type: none"> To evaluate the rate of decay and durability of humoral immunity in the Day 0,90 PIV/LAV prime-boost vaccination schedule and the Day 0,180 PIV/LAV prime-boost vaccination schedule To characterize the cell-mediated immune response of the Day 0,90 PIV/LAV prime-boost vaccination schedule compared to the 0, 180 day PIV/LAV prime-boost vaccination schedule 	
Methodology: This study is a Phase 1, randomized, open-label, study with 2 treatment groups (N=40): Group 1 (n=20): TDENV-PIV 4 µg + Alum adjuvant (Day 0), TDENV-LAV F17 (Day 180) Group 2 (n=20): TDENV-PIV 4 µg + Alum adjuvant (Day 0), TDENV-LAV F17 (Day 90)	

Estimated Number of Subjects to Screen: 80-120
Maximum Number of Subjects to Enroll: 40
Main Criteria for Inclusion/Exclusion: Healthy male or female dengue naïve subjects between 18 and 42 years of age (inclusive) at the time of consent.
Investigational Product Dosage, Schedule, and Mode of Administration: TDENV-PIV Dosage: 0.5 mL of DENV serotypes 1-4 (4 µg / serotype) in alum adjuvant Mode of administration: intramuscular (IM) into the subject's upper arm, deltoid area of the subject's arm; vaccination will be given in the non-dominant arm whenever possible TDENV-LAV F17 Dosage: 0.5 mL of the post-transfection LAV F17 vaccine Mode of administration: subcutaneously into the upper-outer triceps/deltoid area of the subject's arm; vaccination will be given in the non-dominant arm whenever possible Schedule Varied, 2 vaccinations per volunteer in a heterologous prime-boost strategy. First dose on Day 0 (PIV) AND a second dose (LAV) on Day 180 for Group 1. First dose on Day 0 (PIV) AND a second dose (LAV) on Day 90 for group 2.
Duration of Subject Participation: Approximately 18 Months
Criteria for Evaluation: Safety: <ul style="list-style-type: none"> Solicited adverse events (AEs) during the 28 days follow up after prime vaccination and 28 days following boost vaccination. Unsolicited adverse events during the 28 days following each vaccination. Hematological and biochemical abnormalities on vaccination day, Day 7, and Day 28 after each vaccination. Occurrence of serious adverse events (SAEs) during the entire study. Occurrence of potential immune-mediated diseases (pIMDs) during the entire study. Occurrence of medically attended adverse events (AEs) during the entire study. Vaccine induced viral replication measured by DENV serotype specific reverse transcription-polymerase chain reaction (RT-PCR) or equivalent assay at Days 6 thru 14 after LAV vaccination (5 consecutive days of testing per subject within this window excluding weekends) Immunogenicity: <ul style="list-style-type: none"> Microneutralizing (MN) and flow cytometry based neutralizing dengue antibody titers before first (prime) vaccination, 28 days after, immediately prior to the second (boost) vaccination (90 or 180 days), then 28 days, 90 days, 180 days, 270 days, and 360 days following the second(boost) vaccination. Geometric mean titers (GMTs) of neutralizing antibody against each DENV serotype. Seropositivity rates for each DENV type 1-4. Trivalent and tetravalent rates of seroconversion. CMI assays

Statistical Methods:

Demography:

Demographic characteristics (age at first visit in years, sex, race (geographic ancestry), and ethnicity of each study cohort will be tabulated. The distribution of subjects enrolled will be tabulated as a whole and per study arm. For continuous variables (ie, age), mean or medians will be presented with range and standard deviations or interquartile range depending on the normality of the data. For categorical variables, proportions will be presented. Proportions, means and/or medians will be compared between the 2 arms using chi-squared, t-test, or Mann-Whitney U respectively.

Safety and reactogenicity:

The following results will be tabulated by group on the total vaccinated cohort:

- The number and proportion of subjects with at least one local solicited adverse event (AE), at least one general solicited AE and any solicited AE during the 7 day and 28-day follow-up periods for prime and boost vaccination will be tabulated with 95% confidence interval (CI) after each vaccine dose and overall.
- The number and proportion of subjects reporting each individual solicited local (any, grade 3) and general AE (any, grade 3, any related and grade 3 related) during the 7-day and 28-day follow-up periods after each vaccination will be tabulated with 95% CIs. Occurrence of fever will be reported in 0.5°C cumulative increments. Duration of fever will be recorded as the number of consecutive days of fever.
- The number and proportion of subjects reporting at least one of each type of unsolicited AE during the 28-day (Day 0 to Day 27) follow-up period after each vaccination will be tabulated with 95% CIs. The verbatim reports of unsolicited AEs will be reviewed by a physician and the signs and symptoms will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA). Every verbatim term will be matched with the appropriate Preferred Term and categorized by system organ class. Similar tabulations will be done for grade 3 unsolicited AEs and, or unsolicited AEs related to vaccination.
- The proportion of subjects with at least one report of any SAE classified by the MedDRA and reported up to 28 days (Day 0-Day 27) after each vaccination will be tabulated with 95% CIs.
- SAEs, withdrawals due to AEs, pIMDs, pregnancies, and all related AEs will be described in detail.
- The number and proportion of subjects who receive at least one concomitant medication/vaccination during the 28 days follow-up period (Day 0 to Day 27) after each vaccination will be tabulated with 95% CIs. Additionally, the number and percentage of subjects receiving non-steroidal anti-inflammatory drugs (NSAIDs) or any prophylactic antipyretics will be calculated by group for the first 7 days after each dose.
- The proportion of subjects with abnormal hematological or biochemical laboratory values will be presented with 95% CIs at baseline and at each specified time point. These results, for laboratory tests listed on the toxicity grading scale in [Appendix D](#), will be reported by severity grade according to the FDA's toxicity grading scale adjusted for harmony with Quest normal lab values.
- The number and proportion along with mean/median of subjects with detectable dengue viremia days after each vaccination including day(s) of detectable viremia and peak viremia will be described.

Humoral Immunogenicity:

The GMTs of anti-dengue neutralization antibodies at Day 0 and Day 28 after each immunization and immediately prior to the boost vaccination (Day 90 or 180) for the prime vaccination and at Days 90, 180, 270 and 360 following boost vaccination will be calculated by group with 95% CIs. The proportion of volunteers with anti-dengue neutralizing antibody titers above the assay cut-off at these time points will be calculated with 95% CIs. The proportion of volunteers with neutralizing antibody titers above the assay cut-off to 3 or 4 DENV types (trivalent or tetravalent response rates) at these time points will be calculated by group with 95% CIs.

CMI:

CMI responses will be described using ELISPOT, Cytokine flow cytometry, flow cytometry base immunophenotyping, Fluorescence-activated cell sorting, In vitro proliferation assays, antibody-dependent cellular cytotoxicity by flow cytometry, multiplexed ELISA, immune genotyping and systems biology analyses.

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Explanation
ADCC	antibody dependent cellular cytotoxicity
ADL	Activities of daily living
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
AST	aspartate aminotransferase
ATP	according to protocol
BD	Becton Dickinson
BSE	bovine spongiform encephalopathy
C	Celsius
CFC	cytokine flow cytometry
CFR	Code of Federal Regulation
CI	confidence interval
CMI	cell-mediated immunity
CTC	Clinical Trials Center, WRAIR
DENV	dengue virus
DHF	dengue hemorrhagic fever
DoD	Department of Defense
DPIV	dengue purified inactivated vaccine
eCRF	electronic case report form
ELISPOT	enzyme-linked immunospot
F	Fahrenheit
FACS	fluorescence-activated cell sorting
FDA	US Food and Drug Administration
Fox	Forkhead box
GATA	guanine-adenine-guanine-adenine-binding transcription factor
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor
GM-CSF	granulocyte macrophage colony-stimulating factor
GMT	geometric mean titer
GSK	Glaxo Smith Kline
HCV	hepatitis C virus

Abbreviation	Explanation
HIPAA	Health Insurance Portability Accountability Act
HIV	human immunodeficiency virus
HLA	human lymphocyte antigen
ICH	International Conference on Harmonisation
IM	intramuscular(ly)
IND	investigational new drug
IRB	Institutional Review Board
LAV F17/19	tetravalent dengue live-attenuated virus formulation (either 17 or 19 is referenced)
LLN	lower limit of normal
mAb	monoclonal antibody
MCP	membrane cofactor protein
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram(s)
MIG	monokine induced by gamma interferon
MIP	macrophage inflammatory protein
mL	Milliliter(s)
mm	millimeter(s)
MN	Microneutralizing
mRNA	messenger ribonucleic acid
NCT	ClinicalTrials.gov study identifier
ORA	Office of Regulated Activities
ORP HRPO	Office of Research Protections, Human Research Protection Office
PBF	Pilot Bioproduction Facility, WRAIR
PBMC	peripheral blood mononuclear cells
PI	principal investigator
PIV	tetravalent dengue virus purified inactivated vaccine
PRN	pro re nata (as needed, whenever necessary)
PSSO	Product Safety Surveillance Office, USAMRDC
RANTES	regulated on activation, normal T cell expressed and secreted
RORC	retinoic acid receptor-related orphan receptor C
RPMI	Roswell Park Memorial Institute medium
RT-PCR	reverse transcription-polymerase chain reaction
SAE	serious adverse event

Abbreviation	Explanation
SAP	statistical analysis plan
SFC	spot forming cells
SOP	standard operating procedure
SQ	subcutaneous
SUN	serum urea nitrogen
TCR	T cell receptor
TDENV	tetravalent dengue virus
TGF	transforming growth factor
TNF	tumor necrosis factor
ULN	upper limit of normal
UPIRTSO	unanticipated problems involving risks to subjects or others
URI	University of Rhode Island
USAMRDC	United States Army Medical Research and Development Command
USAMRMC	United States Army Medical Research and Materiel Command
WBC	white blood cell count
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

4. INTRODUCTION

Dengue is a mosquito-borne disease caused by serologically related but antigenically distinct dengue viruses (DENV) grouped into 4 types (DENV-1 to DENV-4) and is endemic in 100 countries in the tropical and subtropical regions of Asia-Pacific, the Americas, the Middle East, and Africa. The World Health Organization (WHO) estimates over 40% of the world's population to be at risk for dengue infection. An estimated 100 million cases of infection occur each year, resulting in 500,000 hospitalizations with estimated 2.5% mortality ([WHO Dengue 2016](#)). Other models have yielded much higher estimates of infection rates and unreported illnesses ([Bhatt-2013](#)). In recent years, dengue outbreaks have been increasing and expanding geographically. Local transmission has been reported in Europe and the continental United States (Florida, Texas, and New Mexico).

While dengue is a threat to endemic populations, travelers and military personnel deploying to endemic areas are also at significant risk. 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 ([Jansenius-2013](#)). The annual proportionate morbidity due to dengue in Southeast Asia during an epidemic is estimated to be 159 cases per 1,000 travelers. The risk is likely higher for deployed military personnel due to longer exposure periods. US military operations over the past century have experienced numerous disruptions due to dengue ([Gibbons et al-2012](#)). With continued military focus in the Pacific region and the Americas, the number of military and civilian personnel traveling to dengue endemic regions is also expected to rise significantly.

No specific treatment is available for dengue, but symptomatic and supportive measures (eg, intravenous [IV] fluid management) significantly reduce the morbidity and mortality rates. It is estimated that intensive and careful fluid management and inpatient monitoring can lower mortality rates in severe Dengue from greater than 20% to less than 1% ([WHO Dengue 2016](#)). Currently, vector control is the only method with a demonstrated, though limited efficacy in prevention. Mosquito control does not offer satisfactory control of dengue; additional prevention methods are required. No antiviral drugs are approved for clinical use, but several candidates are under evaluation at the preclinical stage. However, available data suggest that treatment at the onset of symptoms is unlikely to have significant impact on viral dynamics ([Clapham et al-2014](#)). Several vaccine candidates are at various stages of development including a Sanofi Pasteur vaccine that is approved for use in several countries ([Punee et al-2016](#)). However, this vaccine has significantly decreased efficacy in non-immune populations and concerns remain about potential disease enhancement in some groups including those previously unexposed to any dengue infection ([Punee et al-2016](#)). Therefore, it is not a suitable vaccine for the US Military population. The ideal dengue vaccine protects against all 4 serotypes of DENV and is effective in endemic and non-endemic populations. Current Challenges to dengue vaccine development include the lack of a good animal model, lack of an established correlate of protection, and an incomplete understanding of the human immune response profile after dengue infection or vaccination.

Many approved vaccines are administered in multiple doses. The first dose primes the immune system, and the second dose serves to boost the immune response. A homologous prime-boost strategy uses the same vaccine for both doses. Live-attenuated vaccines and purified inactivated vaccines can activate different parts of the human immune system ([Lu-2009](#); [Woodland-2004](#)). Using both types of vaccines in a heterologous prime-boost strategy has proven effective at

boosting cellular responses against other pathogens ([Woodland-2004](#)). A heterologous prime-boost dengue vaccine strategy was evaluated previously using 4 different vaccination schedules (WRAIR #2136). This study involved 4 groups of 20 subjects each who received either PIV/LAV or LAV/PIV on a 0, 28 day or a 0, 180 day schedule. Overall the prime-boost schedules were safe and well tolerated in all groups (unpublished data, Lin et al). Results from this study indicated the greatest immunogenicity was achieved using PIV followed by LAV. Both the 0,28 day and 0, 180 day schedule achieved greater than 95% quadrivalent seroconversion rates(unpublished data Lin et al) assessed by microneutralizing antibody titers. However, there were differences between the antibody titers and durability of antibody responses 6 months post completion of all vaccinations. The 0, 180 day immunization regimen demonstrated significantly higher mean antibody titers at 6 months post-vaccination compared to the 0, 28 day regimen (unpublished data Lin et al). The cell-mediated immune responses did not demonstrate significant differences. Overall it appears that the 0, 180 day regimen is superior at least in durability of antibody response so this regimen was selected for further evaluation. However, a shorter interval between doses would be significantly more practical for travelers and soldiers for whom this vaccine is intended. Therefore, the previously untested 0, 90 day regimen is under consideration as a means to combine the durability of the 0, 180 day regimen with a more practical dosing schedule. Potential protective efficacy of the prime-boost strategy has also been suggested by challenge in non-human primates previously as published in 2010. This study showed sterilizing immunity from challenge with dengue serotypes 1-4 in non-human primates following vaccination with a heterologous PIV/LAV prime-boost vaccination schedule ([Simmon-2010](#)). This further suggests that the immune response generated and measured in humans with this vaccination strategy will provide protective efficacy.

5. INVESTIGATIONAL PRODUCT

PIV consists of 4 purified, inactivated dengue-virus vaccines (DENV serotypes 1-4) adjuvanted with alum. LAV is a live, attenuated DENV vaccine that is composed of 4 monovalent attenuated strains (DENV serotypes 1-4 formulation F17).

[Table 1](#) presents a summary description of the investigational products.

Table 1: Investigational Product

Product Name	Adjuvanted Tetravalent Dengue Virus Purified Inactivated Vaccine (TDENV-PIV)	Tetravalent Dengue Virus Live-attenuated Vaccine (TDENV-LAV, formulation F17)
Dosage Form	Vaccine	Vaccine
Unit Dose	0.5 mL (4 µg/serotype/dose)	0.5 mL
Route of Administration	Intramuscular injection, 1-inch, 22-gauge needle	Subcutaneous injection, 5/8-inch 25-gauge needle
Physical Description	Liquid suspension	Freeze-dried cake
Manufacturer	Pilot Bioproduction Facility (PBF), Walter Reed Army Institute of Research (WRAIR)	PBF, WRAIR
Lot Number	Lot 1725	Lot 1856
Product Indication	Prevention of dengue infection	Prevention of dengue infection

5.1. Investigational Product Packaging and Labeling

TDENV-LAV and TDENV-PIV are packaged in sterile vials sealed with plug stoppers and aluminum crimps.

The investigational products are covered under separate Investigational New Drug (IND) applications. Each vial will be labeled for human administration and will include the following statement: “Caution: New Drug – Limited by Federal Law to Investigational Use.” Example labels for each of the products are provided in [Figure 1](#) and [Figure 2](#).

Figure 1: Representative Label for TDENV-LAV F17

Tetravalent Dengue Virus Vaccine, Live-attenuated, Formulation17	
BPR No.: BPR-844-01	Lot No.: 1856
Contents: 0.7 mL (Freeze-Dried)	
Caution: New drug limited by Federal (or United States) law to investigational use	
Date of Mfg.: 31 Aug 07	Storage: -15 to -30°C
Mfg. By: WRAIR, Silver Spring, MD 20910, USA	

Figure 2: Representative Label for TDENV-PIV

Tetravalent Dengue Virus Purified Inactivated Vaccine, 4 mcg/serotype/0.5 mL dose	
BPR No.: BPR-1040-00	Lot No.: 1725
Contents: 0.7 mL (Alum Adsorbed)	
Caution: New drug - Limited by Federal (or United States) law to investigational use	
Date of Mfg.: 30Sep 11	Storage: 2 - 8°C
Mfg. By: WRAIR, Silver Spring, MD 20910, USA	

5.2. Investigational Product Storage and Packaging

LAV is stored at -15°C to -30°C and PIV is stored at 2°C-8°C at the Pilot Bioproduction Facility (PBF), Walter Reed Army Institute of Research (WRAIR) and will be transported to the clinical site according to standard operating procedures (SOP G-060).

At the study site the investigator or his or her designee will be responsible for product management. The lyophilized LAV F17 to be administered to the subjects must be stored at -15 to -30°C and PIV at 2-8°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 diluent (WFI) used for reconstitution of LAV F17 will be stored at 20- 25°C. Rehydration is performed at 20- 25°C and once vial is rehydrated it is kept at 2-8°C until administration or up to 24 hours. Any temperature excursion (ie, 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.

5.3. Investigational Product Preparation

LAV F17 is provided in single dose vials and is freeze dried. A syringe must be filled to 0.7 mL with sterile water-for-injection and used for reconstitution. The vaccine vial must be swirled until the freeze-dried cake is completely dissolved.

To prepare PIV, the vial must be inverted 3 times to ensure that the alum is resuspended, before the appropriate injection volume is withdrawn with a syringe for injection.

5.4. Summary of Clinical Trials with Investigational Products

5.4.1. TDENV-LAV

Three phase 2 studies have used LAV. The first study (TDEN-001) vaccinated 86 healthy volunteers in Silver Spring, MD and was conducted between April 2006 and March 2008. This placebo-controlled, randomized, observer-blind study evaluated pre-transfection LAV and 2 post-transfection formulations including the current LAV (F17) and another designated LAV F19 given in a 0-, 6-month vaccination schedule. LAV F19 contained 6-fold lower concentration of DENV-4 than the other serotypes. LAV F17 demonstrated an acceptable safety profile based on physical examination, clinical laboratory determinations, and the lack of symptomatic viremia

during follow-up visits. There were 7 total grade 3 AEs reported over 79 received doses of vaccine. These included one of each of the following: injection site swelling, arthralgia, fatigue, headache, muscle ache and 2 reports of redness at injection site. Neither dengue-related illness nor vaccine-related serious adverse events (SAEs) were reported ([Thomas et al-2013](#)). The rate of tetravalent seropositivity among unprimed subjects, as determined by neutralizing antibody testing (MN50) after 2 doses of LAV F17, was 60%.

Two additional studies that compared LAV F17 (pre-transfection and post-transfection) and LAV F19 (post-transfection) were conducted; one in Thailand and one in Puerto Rico ([Bauer et al-2015](#) and [Watanaveeradej et al-2014](#)). Because these are dengue-endemic areas, the majority of subjects were flavivirus-primed. In the study conducted in Thailand there were no grade 3 local solicited AEs. Out of 40 subjects receiving F17 LAV, there were 7 systemic grade 3 AEs reported. These included one each of fatigue, fever, headache, nausea, pruritus, and 2 reported rashes. There were no cases of vaccine-related SAEs. Low levels of monotypic viremia were observed, but these were not associated with illness. The vaccine improved tetravalent response rates among the primed subjects, and tetravalent antibody responses were observed after 2 doses in unprimed subjects. The Puerto Rico study included multiple sites and included young children for a total 211 subjects receiving F17 LAV, 212 receiving F19 LAV and 213 receiving placebo. No related SAEs were reported, and solicited AEs did not increase from Dose 1 to Dose 2. Among the unprimed subjects, tetravalent MN50 antibody response rates and geometric mean titers (GMTs) were higher in the formulation F17 cohorts versus the formulation F19 cohorts. These studies demonstrated that the re-derived, post-transfection LAV F17 is safe and immunogenic in flavivirus-naïve and flavivirus-primed volunteers.

Table 2: Clinical Studies of LAV

Type of Study	Study No.	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Admin.	No. of Subjects	Study Population
Phase 2	TDEN-001	Observer blind, randomized, placebo-controlled	Pre-transfection LAV F17, post-transfection LAV F17, post-transfection LAV F19, placebo. -0,6 months -Subcutaneous (SQ)	86	Healthy adults (US)
Phase 2	TDEN-002	Observer blind, randomized, placebo-controlled	Pre-transfection LAV F17, post-transfection LAV F17, post-transfection LAV F19, placebo. -0,6 months -SQ	120	Healthy flavivirus-primed adults (Thailand)

Type of Study	Study No.	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Admin.	No. of Subjects	Study Population
Phase 2	TDEN-003	Observer blind, randomized, placebo-controlled, age-de-escalation	Pre-transfection LAV F17, post-transfection LAV F17, post-transfection LAV F19, placebo. -0,6 months -SQ	636	Flavivirus-primed, 12 mos to 50 years (Puerto Rico)

5.4.2. TDENV-PIV

The WRAIR PIV combined with GSK adjuvant or alum has been used in 2 Phase 1 clinical trials. Both studies evaluated PIV (1 or 4 µg) combined with alum adjuvant or one of GSK's proprietary adjuvant systems (AS01E and AS03B). Both studies are currently in the active follow-up phase. The first study population (S-11-23; NCT01666652; DPIV-001) was comprised of healthy US adults who were mostly flavivirus naïve. The second study (S-12-12; NCT01702857; DPIV-002) is being conducted in Puerto Rico, a dengue endemic area, where most subjects are dengue-primed.

All 4 PIV vaccine formulations were generally well-tolerated. The Puerto Rico study had a higher incidence of solicited AEs (Day 0-7 post-vaccination) and more Grade 3 solicited AEs (none for the continental US study) compared with the US mainland study. No Grade 3 vaccine-related unsolicited AEs have been reported in either study. No vaccine-related SAEs in either study have been reported. No subjects withdrew from either study due to vaccine-related events. No unexpected adverse events or new pIMD were observed in either study.

For the US mainland study, balanced neutralizing antibody responses (MN assay and microPRN assay) were seen at Day 56. GMTs at Day 56 were robust for the AS01_E, AS03_B (1 µg), and alum adjuvanted 4-µg dose groups ([Schmidt et al](#)). Data analysis is ongoing for both studies.

Table 3: Clinical Studies of TDENV-PIV

Type of Study	IND / Study No.	Study Design and Type of Control	Test Product(s); Dosage Regimen; Route of Admin.	No. of Subjects	Study Population
Phase 1	IND 15112 DPIV-001; S-11-23	Randomized, placebo-controlled, observer-blind with 5 parallel groups. 11 visits over 13 months	Test Products: 1 µg TDENV-PIV with Alum adjuvant 4 µg TDENV-PIV with Alum adjuvant 1 µg TDENV-PIV with AS01E adjuvant 1 µg TDENV-PIV with AS03B adjuvant Placebo (saline) Regimen: Day 0 and Day 28 Route of Administration: Intramuscular	100 total; 20/group	Healthy adults in Dengue non-endemic region (mainland US)
Phase 1	IND 15112 DPIV-002; S-12-12	Randomized, placebo-controlled, observer-blind with 5 parallel groups. 11 visits over 13 months with additional follow up through 2 dengue seasons	Test Products: 1 µg TDENV-PIV with Alum adjuvant 4 µg TDENV-PIV with Alum adjuvant 1 µg TDENV-PIV with AS01E adjuvant 1 µg TDENV-PIV with AS03B adjuvant Placebo (saline) Regimen: Day 0 and Day 28 Route of Administration: Intramuscular	100 total; 20/group	Healthy Adults in Dengue endemic area (Puerto Rico)

5.4.3. PIV/LAV Prime-boost

From 2014 to 2015, 80 subjects were enrolled in an initial evaluation of the prime-boost vaccination strategy (S-13-10 IND16122). They were separated into 4 vaccine schedule groups as below:

Group 1 (n=20): LAV (Day 0), PIV 4 µg + alum (Day 28)

Group 2 (n=20): PIV 4 µg + alum (Day 0), LAV (Day 28)

Group 3 (n=20): LAV (Day 0), PIV 4 µg + alum (Day 180)

Group 4 (n=20): PIV 4 µg + alum (Day 0), LAV (Day 180)

The prime-boost regimen was well tolerated in all 4 groups. There were no grade 3 local AEs. Grade 3 related, systemic AEs occurred in small numbers. One volunteer in Group 2 reported grade 3 headache and one reported grade 3 GI upset. One volunteer in Group 3 reported grade 3 GI upset. One volunteer in Group 4 reported grade 3 fever and another volunteer in Group 4 reported grade 3 fatigue, GI upset, headache, myalgia arthralgia. There were no grade 3 systemic related AEs in Group 1. The PIV followed by LAV schedule resulted in the highest rate of seroconversion in Group 2 with 100% tetravalent seroconversion and 94% tetravalent seroconversion in Group 4 at 28 days post-vaccination. Group 4 volunteers maintained very high geometric mean antibody titers at the final 6 month post-vaccination time point. Group 2 volunteers began to show some significant degradation of antibody titers by 6 month post boost.

6. TRIAL DESIGN

6.1. Overall Study Design and Rationale

This study is a Phase 1, randomized, open-label study of the prime-boost vaccine candidates given in the prime-boost regimen previously demonstrated to have a high level of immunogenicity and immune durability: Day 0 prime (PIV) and Day 180 boost (LAV), and compare it with a previously untested schedule: Day 0 prime (PIV) and Day 90 boost (LAV) in order to define the potential tradeoff between potential immunogenicity, including cell-mediated immunity, and a more practical dosing schedule.

Heterologous Prime-boost: Compared with inactivated vaccines, live-attenuated vaccines have the advantage of inducing high-avidity antibodies and T lymphocyte responses ([Rothman-2004](#)). Disadvantages include risks to severely immunocompromised recipients and increased reactogenicity. Most approved vaccines and previous experiments with dengue vaccine candidates have used a homologous boosting strategy (repeated administration of the same antigen and carrier). While this is effective for boosting humoral responses, it is relatively inefficient for boosting cellular immunity because prior immunity tends to impair robust antigen presentation and activation of the immune system. Giving the same antigen via different antigen-delivery systems (heterologous prime-boost) can engage multiple parts of the immune system to establish a balanced immune response. Although serotype-specific neutralizing antibodies remain the most accepted method to assess post-vaccination and post-infection immunity, as the dengue community learns about the human response to natural infection and vaccination, the role of cell-mediated immunity in dengue infection is emerging ([Woodland-2004](#)).

The rationale behind this study of heterologous prime-boost vaccination is to determine if humoral and cell-mediated immunity can be optimized while reducing reactogenicity by adjusting the vaccination schedule. By conducting a thorough immunological assessment, a heterologous prime-boost dengue vaccination study may also contribute to a better understanding of the immunological profile induced by dengue infection and vaccination. The necessity of a heterologous prime-boost study is further supported by the absence of a validated correlate of protection and recent demonstration of the inability of a live attenuated tetravalent dengue vaccine (CYD, Sanofi Pasteur) to protect against a specific serotype of circulating dengue virus, despite robust neutralizing antibody response from a specific dengue serotype ([Sabchareon-2012](#)). The current study will use the re-derived LAV F17 and 4-μg PIV with alum adjuvant

similar to S-13-10. The safety risks are not expected to exceed those demonstrated previously in S-13-10.

6.2. Trial Objectives and Purpose

6.2.1. Primary Objective

- To further evaluate the safety and reactogenicity of 2 tetravalent dengue vaccine (TDENV) candidates administered in a heterologous prime boost fashion with PIV followed by LAV 180 days later
- To evaluate the safety and reactogenicity of a previously untested vaccination schedule consisting of PIV followed by LAV 90 days later

6.2.2. Secondary Objective

- Evaluate the humoral and cell-mediated immunogenicity of the PIV/LAV Day 0, 180 administration schedule and compare it to the humoral and cell-mediated immunogenicity of the PIV/LAV Day 0, 90 schedule

6.2.3. Exploratory Objectives

- To evaluate the rate of decay and durability of humoral immunity in the Day 0, 90 PIV/LAV prime-boost vaccination schedule and the Day 0, 180 PIV/LAV prime-boost vaccination schedule
- To characterize the cell-mediated immune response of the Day 0, 90 PIV/LAV prime-boost vaccination schedule compared to the 0, 180 Day PIV/LAV prime-boost vaccination schedule

6.3. Study Endpoints

6.3.1. Primary Endpoints

The primary safety and reactogenicity endpoints are as follows:

- Solicited adverse events during the 7 days follow up period after each vaccination
- Solicited adverse events during the 28 day follow up period after each vaccination
- Unsolicited adverse events during the 28 days following each vaccination
- Hematological and biochemistry abnormalities on day of vaccination and at 7 days and 28 days post each vaccination
- Occurrence of serious adverse events (SAEs) during the entire study
- Occurrence of pIMDs during the entire study
- Occurrence of medically attended AEs during the study

6.3.2. Secondary Endpoints

- Microneutralizing (MN) and PRNT dengue antibody titers before vaccination and 28 days after each vaccination for each volunteer. Additional antibody levels will be

assessed at 90, 180, 270, and 360 days following booster vaccination vaccine administration.

- GMTs of neutralizing antibodies to each DENV serotype
- Seropositivity rates for each DENV type
- Trivalent and tetravalent seroconversion rates(Defined by Ab titers >1:10 to 3 dengue virus types or 4 dengue virus types respectively)

6.3.3. Exploratory Endpoints

- CMI markers assessment by, but not limited to, the following methods:
 - Enzyme-linked immunospot (ELISPOT), flow cytometry, carboxyfluorescein succinimidyl ester, dye-dilution proliferation, Luminex, transcriptomics, proteomics, glycomics
- Decline in GMT neutralizing antibody titers from Day 28 to Day 360 following booster vaccinations
- Vaccine induced viral replication measured by DENV serotype specific RT-PCR Day 6-14(5 sequential days will be tested when possible. Only 5 tests per patient will be obtained) after LAV vaccination

6.4. Study Site

This study will be conducted at the WRAIR Clinical Trials Center (CTC) (Silver Spring, MD, USA).

6.5. Study Population

This study will enroll healthy male and healthy non-pregnant, non-breastfeeding female dengue naïve subjects between the ages of 18 and 42 (inclusive) at the time of screening.

The subjects will be residents of the Washington, DC, metropolitan area. Dengue antibody screening will be conducted. It is expected that the majority of volunteers will be flavivirus-naïve.

A maximum of 40 subjects will be enrolled. Refer to Section [12.2](#) for a statistical justification of the sample size.

6.6. Duration of Subject Participation

Each subject will participate for approximately 18 months in Group 1 or 15 months in Group 2 (360 days following final vaccine receipt).

6.7. Measures Taken to Minimize/Avoid Bias

6.7.1. Assignment

Subjects will be assigned as listed in [Table 4](#). Subjects will be randomized to the 2 groups in a 1:1 distribution. Block randomization will be used to allocate subjects to each study cohort.

Eligible subjects will be randomized before vaccination on visit 1. Each volunteer will be assigned a subject number at initial screening starting with 0001 based on time of enrollment. The subject number may be different from volunteer screening number.

6.7.2. Open-label Study

This will be an open-label study.

6.8. Route of Administration, Dosage Regimen, Treatment Period, and Justification

PIV vaccinations will be administered intramuscularly (IM) in the deltoid region of the non-dominant arm whenever possible and LAV F17 vaccinations will be administered subcutaneously (SQ) in the upper-outer triceps/deltoid area of the non-dominant arm when possible.

Previous trials with the LAV vaccine used a Day 0 and Day 180 vaccination strategy. The Day 180 homologous boost is widely used in vaccination of live-attenuated vaccines for dengue (Sanofi's CYD vaccine) and other pathogens. The gap between priming and boosting for inactivated vaccines is typically shorter. Previous clinical trials of the PIV candidates have used the Day 0 and Day 28 vaccination schedule.

Vaccination will be given according to the regimen shown in [Table 4](#).

Table 4: Vaccination Schedule

Group	No. of Subjects	Day 0	Day 90	Day 180
1	20	PIV	-	LAV F17
2	20	PIV	LAV F17	-

6.9. Investigational Product Accountability

The sponsor's representative is responsible for distributing the investigational product to the study site and has delegated this responsibility to the WRAIR CTC; however, the sponsor's representative has ultimate responsibility for product accountability. After the investigational product is distributed, the Principal Investigator is responsible for and will maintain logs of investigational product receipt, storage, reconstitution, accountability by subject, and investigational product remaining before final disposition. Product accountability logs will be maintained by the CTC. The Principal Investigator (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.

Residual vaccine will be retained separately in the CTC under controlled conditions until the end of the study or until the study monitor can perform inspections of residual vaccine during periodic monitoring visits to confirm correct dosing. Once either of the above have been met, the sponsor will be contacted to determine if the vials may be destroyed.

Once product accountability is completed by the study monitor, and with approval by the sponsor, the residual vials may be sent back to the PBF for destruction or disposed of in a rigid biohazard container at the CTC. Damaged vaccine will be stored and held until the study monitor completes product accountability. After reconciliation is complete and a transfer memorandum has been received from the sponsor, damaged vials will be returned to the PBF Known and Potential Risks and Benefits to Human Subjects

6.9.1. Risks/Discomfort to Subjects and Precautions to Minimize Risk

Outlined below are anticipated and unexpected 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.

6.9.1.1. Local Reactions

Local reactions may include pain, redness, and swelling at the injection site. Study staff including physicians will be available at follow up visits and via phone in between visits to provide advice on any appropriate treatment of any reactions that may occur.

6.9.1.2. Systemic Reactions

General reactions may include fatigue, fever, rash, pruritus, headache with potential for retro-orbital pain, photophobia, conjunctival injection, gastrointestinal symptoms (nausea, vomiting, loss of appetite diarrhea, and abdominal pain), muscle ache, joint pain, cough, transient mild to moderate leukopenia and transient mild to moderate elevation in serum alanine aminotransferase and aspartate aminotransferase. Study staff including physicians will be available at follow up visits and via phone in between visits to provide advice on any appropriate treatment of any reactions that may occur.

6.9.1.3. LAV and DPIV SAE and Grade 3 AEs

There have been no SAEs related to these vaccines in prior clinical studies. The most common AEs following vaccination have included headaches, myalgia, pruritus, fever, nausea, lymphadenopathy, rash, and arthralgia. Grade 3 symptoms in US adults following subcutaneous (SC) administration included Redness at injection site (4.5%), swelling(2.3%), arthralgia(2.3%), fatigue(2.3%), headache(2.3%), muscle aches(2.3%) ([Thomas et al-2013](#)).

Grade 3 local AEs in Thai children included redness following dose 1 and swelling following dose 2 each in 1 of 7 children vaccinated. There were no systemic grade 3 symptoms reported in this group ([Simasathien et al-2008](#)).

Among 40 Thai adults who received F17 LAV there were no grade 3 local AEs. Reported systemic grade 3 AEs included fatigue (2.5%), fever (2.5%), headache (2.5%), nausea (2.5%), pruritus (2.5%) and rash (5%) ([Watanaveeradej et al -2014](#)).

Among 636 Puerto Rican subjects including children, the most frequently reported grade 3 local symptom was redness, reported after 2.4% of F17 LAV doses and 1.9% of placebo doses respectively. Grade 3 swelling was reported after 1.4% of F17 doses and after 0.5% of placebo doses. Grade 3 pain was reported by one subject in the F17 group after dose 2 (0.2% overall per dose) and was not reported in the placebo group. Overall, the most frequently reported grade 3

general symptom was fever (4.8% F17 LAV vs. 3.8% placebo group) and Grade 3 headache (1.0% F17 LAV vs. 2.9% placebo group). Other solicited grade 3 general symptoms were reported with a very low incidence (<0.2% of doses) ([Bauer et al-2015](#)).

Grade 3 related adverse events with dengue inactivated vaccine have been only rarely reported. In DPIV-001 there was one grade 3 related fever among all volunteers receiving 2 doses and one report of grade 3 myalgias among one volunteer receiving 3 doses. Among Puerto Rican recipients in the DPIV-002 trial, grade 3 solicited general AEs ranged between 0% and 2.5% for most AEs. Grade 3 headache was reported following 5% of AS03-adjuvanted vaccine doses, and grade 3 myalgia was reported following 10% of AS01-adjuvanted vaccine doses. No vaccine-related SAEs have been reported in either study.

Among 80 volunteers previously vaccinated using the prime-boost strategy there were 11 grade 3 adverse events. There were only 5 individuals who experienced grade 3 related adverse events. Two of these were in the 0, 28 Day PIV/LAV groups and included one grade 3 headache and one grade 3 GI upset. In the 0, 180 Day PIV/LAV there was one individual who experienced grade 3 fever and another individual who experienced grade 3 fatigue, headache, myalgia, arthralgia and GI upset. One individual in the 0, 180 Day LAV/PIV group experienced grade 3 GI upset. Overall, fatigue, fever, headache, muscle aches were commonly reported among the PIV/LAV group occurring in 20-50% of volunteers vaccinated with this schedule. There were no SAEs and no pIMDs in this prime-boost (WRAIR 2136) trial (Lin et al. unpublished data).

Therefore the vaccine reactions that have been observed are sufficiently mild to warrant continued investigation of vaccines given they have potential to protect against the severe morbidity and mortality caused by dengue infections. Study staff including physicians will be available at follow up visits and via phone in between visits to provide advice on any appropriate treatment of any reactions that may occur in order to minimize the reactions for participating subjects.

6.9.1.4. Pregnancy

Risks to fetal development are unknown at this time. Pregnant females will be excluded from this study. Study subjects should not become pregnant for at least 3 months after the last study vaccination. The following methods of contraception are considered adequate for female subjects of childbearing potential: abstinence; oral contraceptive, either estrogen progesterone combined, or progesterone alone; injectable progesterone; implants of levonorgestrel; estrogenic vaginal ring; contraceptive patches; intrauterine device or intrauterine system; double-barrier method [condom and occlusive cap (diaphragm or cervical vault caps) with or without spermicidal agent (foam, gel, film, cream, suppository)]; male partner sterilization prior to the female subject's entry into the study, and this male is the sole partner for that subject.

6.9.1.5. Lactation

Risks to nursing infants are unknown at this time. Females who are currently breastfeeding their infant/child and plan to continue to do so during the study period will be excluded from this study. Lactating females must agree not to breast feed while in the study and for 3 months after the last study vaccination in order to be included.

6.9.1.6. 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 and/or nerve damage at the needle puncture site. All phlebotomists drawing blood in conjunction with this trial will be certified and experienced in proper phlebotomy techniques in order to minimize these risks.

6.9.1.7. Allergic Reactions and Immune Mediated Reactions

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. There also exists a theoretical chance of immune mediated events including Guillain-Barre syndrome. However, neither the PIV nor LAV used in this study has ever been associated with Guillain-Barre syndrome or any other immune mediated adverse event.

6.9.1.8. Bovine Spongiform Encephalopathy

Bovine (cow)-derived materials were used in the manufacture of the PIV and LAV vaccines in this research study. Certified, fetal bovine serum used in the manufacture of the vaccines was purchased from a company in New Zealand where herds are free from bovine spongiform encephalopathy (BSE) disease. As a result, the risk of contamination of the vaccines with BSE has been minimized.

6.9.1.9. Natural Dengue Virus Infection

Observations from natural dengue infections have shown that the greatest risk for severe dengue infection comes when a person is infected by one dengue type and then infected again, at a later time, by a different dengue type. One proposed pathophysiologic mechanism underlying severe dengue disease onset is antibody-dependent enhancement (ADE) whereby pre-existing antibodies from a previous heterotypic dengue serotype fail to protect against infection with a different serotype but rather cause enhancement of infection. It is possible, if a vaccine candidate does not protect against at least 3 dengue types after vaccination, a more severe case of dengue may develop if the subject becomes infected in the future, compared with if the subject had never been vaccinated at all. This risk is theoretical based on what we know about natural infections. In the Sanofi-Pasteur vaccine trials there was some signal towards increased rates of hospitalization in the <5 year old vaccinated subjects at Asian sites ([Halstead 2016](#)). However, other than this signal in a small segment of the vaccinated cohort, there is no clear evidence that dengue vaccines (monovalent or tetravalent) place people at increased risk for severe dengue. It is unclear if this potential increased risk is specific to Dengvaxia or whether other dengue vaccines will have similar effects. In either case, volunteers in this trial are not expected to be exposed to natural dengue virus infection since vaccination will be occurring in a non-endemic setting (USA). Cases of vaccine-induced viremia after vaccination with LAV appeared to be infrequent and were not associated with clinical symptoms or laboratory abnormalities ([Thomas-2013](#), TDEN-002, 003 study reports). Among subjects residing in dengue endemic areas who have been vaccinated with LAV, no increased risk of severe dengue or dengue hemorrhagic fever (DHF) has been identified. This study will be conducted in a dengue non-endemic region, further reducing any theoretical risk.

6.9.1.10. Unknown Risks

As with all research there is the remote possibility of risks that are unknown or that cannot be foreseen at this time. Each vaccine candidate in this study has been safely administered to human subjects. Any unknown risks identified in this study will be reported to the sponsor and research monitor.

6.9.2. Alternatives to this IND Product or Study

An alternative is to not participate in this study.

6.9.3. Intended Benefit for Subjects

There is no intended direct medical benefit for subjects participating in this study. Subjects may be protected against future dengue infections; however, this cannot be assured.

6.9.4. Risks to the Study Personnel and the Environment

The principal risks in the clinical setting include handling of needles that may be contaminated and the attendant risks including infection with hepatitis, human immunodeficiency virus (HIV), and other human pathogens. Adherence to universal precautions will reduce significantly the risk of exposure.

All biohazardous waste will be disposed of as stipulated by local, state, and federal regulations.

7. SELECTION OF SUBJECTS

It is estimated that 80 to 120 volunteers will need to be screened in order to enroll the target of 40 volunteers meeting all inclusion and exclusion criteria. Subject Inclusion Criteria

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

1. Male or female between 18 and 42 years of age (inclusive) at the time of consent
2. Able to provide written informed consent
3. Healthy as established by medical history and clinical examination and basic hematologic laboratory analysis before entering into the study
4. Able and willing to comply with the requirements of the protocol (eg, document events in memory aid, return for follow-up visits, etc.)
5. Dengue exposure naïve as established by pre-enrollment dengue antibody testing and questioning of volunteer
6. Female subject of non-childbearing potential (non-childbearing potential is defined as having either a current bilateral tubal ligation at least 3 months prior to enrollment or a history of an hysterectomy, bilateral oophorectomy, or is post-menopause(12 months or more since last menstrual period)) or Female subject of childbearing potential may be enrolled in the study, if the subject has:
 - Practiced adequate contraception for 30 days prior to vaccinations, and
 - A negative urine pregnancy test on each day of vaccination, and

- Agreed to continue adequate contraception through at least 3 months following last vaccination.

7.1. Subject Exclusion Criteria

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

1. Use of any investigational or non-registered product (drug or vaccine or device) other than the study vaccines during the period starting 30 days preceding the first dose of study vaccine and/or planned use during the study period
2. Chronic administration (defined as more than 14 days in total) of prescription immunosuppressants or other prescription immune-modifying drugs during the period starting 180 days prior to the first vaccine dose
 - For corticosteroids, this will mean prednisone ≥ 20 mg/d or equivalent
 - Inhaled and topical steroids are allowed
3. History of or active use of cancer chemotherapy or radiation therapy for the treatment of cancer
4. Receipt or planned receipt of a vaccine/product outside the study protocol within 30 days of each scheduled dose of an investigational product
5. Planned administration of any flavivirus vaccine, to include licensed vaccines for Yellow Fever or Japanese Encephalitis Virus as well as other investigational vaccines for dengue, Zika, West Nile, other flavivirus, for the entire study duration
6. Previous receipt of a foreign or investigational dengue vaccine
7. Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device)
8. Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination
9. History of, or current, auto-immune disease
10. History of any reaction or hypersensitivity likely to be triggered by any component of the vaccines or related to a study procedure (This includes hypersensitivity reactions to alum, streptomycin, neomycin, or any other flavivirus vaccine, such as Yellow Fever virus and Japanese Encephalitis virus vaccines)
11. Major congenital defects or serious chronic illness
12. History of any chronic neurological disorders or chronic and/or uncontrolled seizures
13. Acute infectious disease and/or fever (oral body temperature $\geq 100.4^{\circ}\text{F}/38.0^{\circ}\text{C}$) at the time of enrollment (a subject with a minor illness, ie, mild diarrhea, mild upper respiratory infection, etc, without fever, may be enrolled at the discretion of the investigator)

14. Acute or chronic, clinically significant pulmonary, cardiovascular, hepatic, or renal functional abnormality, as determined by history, physical examination or laboratory screening tests
15. Receipt of immunoglobulins or any blood products during the period starting 90 days preceding the first dose of study vaccine or planned receipt during the study period
16. Donated blood within 8 weeks before first scheduled investigational vaccine receipt or planned donation of blood products throughout the study period
17. History of chronic alcohol abuse and/or drug abuse that, in the opinion of the investigator, could result in poor compliance with study requirements.
18. Pregnant or breastfeeding female or female planning to become pregnant or planning to discontinue contraceptive precautions
19. A planned move to a location that will prohibit compliance with the requirements of the trial
20. Subject seropositive for hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies (anti-HCV), or human immunodeficiency virus antibodies (anti-HIV)
21. Safety laboratory test results that are outside the acceptable values at screening:
 - > 110% upper limit of normal (ULN) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, creatinine, serum urea nitrogen (SUN) and bilirubin (total and direct)
 - < 90% lower limit of normal (LLN) or > 120% ULN for hemoglobin, hematocrit and platelet count
 - < 75% LLN or > 110% ULN for total white blood cell count (WBC)

Note: Per guidance in Section 8.1, abnormal lab(s) may be repeated x1 in the screening window provided the total amount of blood drawn for all screening labs does not exceed 50 mL.
22. Active Diabetes or active peptic ulcer disease (PUD)
23. Diagnosis with Bipolar Disorder or 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 subject from meeting all requirements of the study
24. Chronic migraine headaches, defined as more than 15 headache days per month over a 3-month period of which more than 8 are migrainous, in the absence of medication over use
25. Chronic medical condition that, in the opinion of the investigator, impacts subject safety
26. Any other condition which, in the opinion of the investigator, prevents the subject from meeting all requirements of the study.
27. Do not wish to have their blood stored and used for future research

8. STUDY PROCEDURES

Detailed study procedures are provided in [Table 5](#) and [Table 6](#).

8.1. Recruitment of Subjects

Subjects will be recruited by the WRAIR CTC through the use of newspaper ads, flyers, and posters along with ads on craigslist, facebook or other social media websites. Web-based advertising (clinicaltrials.org), word of mouth, and the CTC database will be used to contact clinical trial volunteers. Potential volunteers of various ethnic backgrounds, education, and income will be residents of the Washington, DC, metropolitan area. An institutional review board (IRB)-approved recruitment script ([Appendix C](#)) will be read during all phone and face-to-face contacts with potential study subjects. All recruitment materials will be submitted to the WRAIR IRB for review and approval prior to use. CTC study subjects may also recruit new subjects. Recruitment will be by non-coercive means.

Informed consent will be obtained prior to any study procedures. 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 afforded 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 document.

The recruitment process will in general be similar for both military and civilian personnel. However, special effort will be made to ensure that military personnel are not unduly influenced or coerced into participating by individuals of superior rank or position. All contact recruiting methods (eg, oral presentations and correspondence) for military personnel will be performed either by non-military personnel or military personnel who are being observed by an independent ombudsman during the contact to ensure that no undue influence occurs. During oral presentations to groups of military personnel, this ombudsman will also act to ensure that the briefed population is not influenced or coerced by NCOs or Officers not associated with the study. Acceptable ombudsmen for this protocol include Michael Hrycyk and Joel Gonzales.

A signed copy of the informed consent will be provided to the volunteer before any study procedure is performed.

Investigators will also provide the opportunity to participate in the study to military personnel at the WRAIR and NMRC using the same IRB-approved recruitment script. The research monitor of the study will be present at the time of the study introduction and will serve as an ombudsman, except when addressing soldiers from the monitor's own branch. Additionally, no senior non-commissioned officers (NCOs) or officers from any junior enlisted soldier's chain of command will be present during the recruitment brief of junior enlisted soldiers to ensure that no undue pressure is exerted upon them.

The following procedures will be conducted no more than 90 days and no fewer than 4 days prior to study start for an individual subject. The results of these assessments will determine subject eligibility.

- Informed consent
- Medical history

- Inclusion and exclusion criteria
- Height and weight
- Demographic information
- Urine pregnancy test
- Physical exam and vital signs
- Concomitant or recent medications/ vaccination
- Blood sampling for safety laboratory analyses (30 mL)

If the investigator feels that there may be a reason that the screening tests are abnormal due to laboratory error or normal physiological variance, or a temporary self-limited condition any or all of the screening laboratory tests may be repeated once as necessary to allow an accurate determination of a potential subject's eligibility. Individuals who have minor abnormalities that are deemed not clinically significant by the physician investigator and do not meet exclusion criteria otherwise defined, may be enrolled in the study.

Subjects found to be seropositive for HIV-1 or Hepatitis B (HBV) or C (HCV) will be counseled and referred to the regular health care provider or public health clinic for further evaluation, and according to the Code of Maryland Regulations (COMAR) 10.06.01.03 C. Positive HIV, HBV and HCV results will be reported by the study team to the Maryland AIDS Administration, Department of Health and Mental Hygiene within 24 hours of being confirmed as per Health General Article 18-205.

Table 5: Study Events Schedule for Group 1: Day 0 – PIV, Day 180 – LAV

Visit	Screen	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimal Visit Day	Day -90 to -4	Day 0 PIV Prime^e	Day 7	Day 28	Day 180 LAV Boost^e	Day 186^a	Day 187^a	Day 188^a	Day 189^a	Day 190^a	Day 208	Day 270	Day 360	Day 450	Day 540
Allowed Interval for Visits (day)		N/A	±2	±5	±7	6-10 days from visit 4	7-11 days from visit 4	8-12 days from visit 4	9-13 days from visit 4	10-14 days from visit 4	26-42 days from visit 4	70- 110 days from visit 4	160- 200 days from visit 4	250- 290 days from visit 4	340- 380 days from visit 4
General Procedures															
Informed consent	•														
Randomization		•													
Medical history	•	• ^b													
Confirm inclusion/ exclusion criteria	•	•			•										
Check contraindications to subsequent vaccination					•										
Measure/record height and weight; collect demographics	•														
Urine pregnancy test, as applicable	•	•			•										
Allocate experimental vaccination number		•			•										
Receive verbal consent to vaccinate		•			•										
Vaccination		•			•										
Distribution of subject card		•													
Distribution of memory aid		•			•										

Visit	Screen	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimal Visit Day	Day -90 to -4	Day 0 PIV Prime^c	Day 7	Day 28	Day 180 LAV Boost^e	Day 186^a	Day 187^a	Day 188^a	Day 189^a	Day 190^a	Day 208	Day 270	Day 360	Day 450	Day 540
Allowed Interval for Visits (day)		N/A	±2	±5	±7	6-10 days from visit 4	7-11 days from visit 4	8-12 days from visit 4	9-13 days from visit 4	10-14 days from visit 4	26-42 days from visit 4	70- 110 days from visit 4	160- 200 days from visit 4	250- 290 days from visit 4	340- 380 days from visit 4
Return of memory aid				•							•				
Review memory aid			•	•		•	•	•	•	•	•				
Record concomitant medications/ vaccinations	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Record intercurrent medical conditions	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Safety Assessments															
Physical examination and vital signs ^f	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Prevaccination body temperature		•			•										
Blood sample for safety laboratory analyses (mL) ^e	30	10	10	10	10		10				10				
Blood sample for viral replication assay (mL)						4	4	4	4	4					
Document local and general solicited symptoms (Days 0- 28 postvaccination)		•	•	•	•	•	•	•	•	•	•				
Document unsolicited AEs (Days 0-28 postvaccination)		•	•	•	•	•	•	•	•	•	•				

Visit	Screen	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimal Visit Day	Day -90 to -4	Day 0 PIV Prime^e	Day 7	Day 28	Day 180 LAV Boost^e	Day 186^a	Day 187^a	Day 188^a	Day 189^a	Day 190^a	Day 208	Day 270	Day 360	Day 450	Day 540
Allowed Interval for Visits (day)		N/A	±2	±5	±7	6-10 days from visit 4	7-11 days from visit 4	8-12 days from visit 4	9-13 days from visit 4	10-14 days from visit 4	26-42 days from visit 4	70- 110 days from visit 4	160- 200 days from visit 4	250- 290 days from visit 4	340- 380 days from visit 4
Record SAEs		•	•	•	•	•	•	•	•	•	•	•	•	•	•
Record pregnancy		•	•	•	•	•	•	•	•	•	•	•	•	•	•
Record pIMDs		•	•	•	•	•	•	•	•	•	•	•	•	•	•
Immunogenicity Assessments															
Blood sample for neutralizing antibodies ^d	10	10		10	10						10	10	10	10	10
Blood sample for CMI assays (mL) (section 10.2)		56	16	40	40		16				40	40	40	40	40
Daily Blood Volume (mL)	40	76	26	60	70	4	30	4	4	4	60	50	50	50	50
Cumulative Blood Volume (mL)	40	116	142	202	272	276	306	310	314	318	378	428	478	528	578

^a 5 consecutive days preferred but weekends will not be included. Goal is as close as possible to get 5 consecutive draws between 6-14 days after vaccination. Visits 5-9 are intended to occur 6-14 days after the administration of LAV boost. The day numbers listed for visit day in the table above for visits after second vaccination only apply if vaccination occurs exactly on day 180. If the LAV boost is shifted back or forward as allowed per the protocol, visits 5-14 may be shifted along with the corresponding windows by the same number of days and still remain in line with the planned activities of this protocol.

^b Record changes between screening and Visit 1 in the eCRF.

^c Safety assessments include complete blood count [white blood cell count (WBC), hemoglobin (Hgb), platelet count, differential, and a calculated absolute neutrophil count (ANC)], aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum urea nitrogen (SUN), creatinine, alkaline phosphatase, total and direct bilirubin. The maximum amount of blood collected for safety assessments will be 30 mL at screening and 15 mL at other study visits. Day 0 safety laboratory tests will not be used to determine eligibility.

^d Initial blood neutralizing antibody testing will include flaviviruses: dengue, zika, yellow fever, west nile virus, Japanese encephalitis virus. Only positive results for dengue will be used as exclusionary criteria. The others will only help to define previous exposures and potential differences in immune response to vaccination

^e Vital signs on vaccination days will be taken prior to dosing and at least 30 minutes post dose, to include heart rate, blood pressure, temperature, and respiratory rate.

^f At time of screening a complete physical exam will be performed. At subsequent visits a focused physical exam will be performed based upon any subject reported symptoms. If no symptoms are reported at follow up visits no additional physical exam may be performed if not indicated based on investigator discretion.

Table 6: Study Events Schedule for Group 2: Day 0 – PIV, Day 90 – LAV

Type of Contact	Screen	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimal Visit Day	Day -90 to -4	Day 0 PIV Prime^e	Day 7	Day 28	Day 90 LAV Boost^e	Day 96^a	Day 97^a	Day 98^a	Day 99^a	Day 100^a	Day 118	Day 180	Day 270	Day 360	Day 450
Allowed Interval for Visits (day)		N/A	±2	±5	±7	6-10 days from visit 4	7-11 days from visit 4	8-12 days from visit 4	9-13 days from visit 4	10-14 days from visit 4	26-42 days from visit 4	70- 110 days from visit 4	160- 200 days from visit 4	250- 290 days from visit 4	340- 380 days from visit 4
General Procedures															
Informed consent	•														
Randomization		•													
Medical history	•	• ^b													
Confirm inclusion/exclusion criteria	•	•			•										
Check contraindications to subsequent vaccination					•										
Measure/record height and weight; collect demographics	•														
Urine pregnancy test, as applicable	•	•			•										
Allocate experimental vaccination number		•			•										
Vaccination		•			•										
Distribution of subject card		•													

Type of Contact	Screen	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimal Visit Day	Day -90 to -4	Day 0 PIV Prime^c	Day 7	Day 28	Day 90 LAV Boost^c	Day 96^a	Day 97^a	Day 98^a	Day 99^a	Day 100^a	Day 118	Day 180	Day 270	Day 360	Day 450
Allowed Interval for Visits (day)		N/A	±2	±5	±7	6-10 days from visit 4	7-11 days from visit 4	8-12 days from visit 4	9-13 days from visit 4	10-14 days from visit 4	26-42 days from visit 4	70- 110 days from visit 4	160- 200 days from visit 4	250- 290 days from visit 4	340- 380 days from visit 4
Distribution of memory aid		•			•										
Return of memory aid				•							•				
Review memory aid			•	•		•	•	•	•	•	•				
Record concomitant medications/ vaccinations	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Record intercurrent medical conditions	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Safety Assessments															
Physical examination and vital signs ^f	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
Prevaccination body temperature		•			•										
Blood sample for safety laboratory analyses (mL) ^d	30	10	10	10	10		10				10				
Blood sample for viral replication assay (mL)						4	4	4	4	4					
Document local and general solicited symptoms (Days 0-28 postvaccination)		•	•	•	•	•	•	•	•	•	•				
Document unsolicited AEs (Days 0-28 postvaccination)		•	•	•	•	•	•	•	•	•	•				
Record SAEs		•	•	•	•	•	•	•	•	•	•	•	•	•	•
Record pregnancy		•	•	•	•	•	•	•	•	•	•	•	•	•	•

Type of Contact	Screen	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Optimal Visit Day	Day -90 to -4	Day 0 PIV Prime^c	Day 7	Day 28	Day 90 LAV Boost^c	Day 96^a	Day 97^a	Day 98^a	Day 99^a	Day 100^a	Day 118	Day 180	Day 270	Day 360	Day 450
Allowed Interval for Visits (day)		N/A	±2	±5	±7	6-10 days from visit 4	7-11 days from visit 4	8-12 days from visit 4	9-13 days from visit 4	10-14 days from visit 4	26-42 days from visit 4	70- 110 days from visit 4	160- 200 days from visit 4	250- 290 days from visit 4	340- 380 days from visit 4
Record pIMDs		•	•	•	•	•	•	•	•	•	•	•	•	•	•
Immunogenicity Assessments															
Blood sample for neutralizing antibodies ^d	10	10		10	10						10	10	10	10	10
Blood sample for CMI assays (mL) (section 10.2)		56	16	40	40		16				40	40	40	40	40
Daily Blood Volume (mL)	40	76	26	60	70	4	30	4	4	4	60	50	50	50	50
Cumulative Blood Volume (mL)	40	116	142	202	272	276	306	310	314	318	378	428	478	528	578

^a 5 consecutive days preferred but weekends will not be included. Goal is as close as possible to get 5 consecutive draws between 6-14 days after vaccination.

Visits 5-9 are intended to occur 6-14 days after the administration of LAV boost. The day numbers listed for visit day in the table above for visits after second vaccination only apply if vaccination occurs exactly on day 90. If the LAV boost is shifted back or forward as allowed per the protocol, visits 5-14 may be shifted along with the corresponding windows by the same number of days and still remain in line with the planned activities of this protocol.

^b Record changes between screening and Visit 1 in the eCRF.

^c Safety assessments include complete blood count [white blood cell count (WBC), hemoglobin (Hgb), platelet count, differential, and a calculated absolute neutrophil count (ANC)], aspartate aminotransferase (AST) and alanine aminotransferase (ALT), serum urea nitrogen (SUN), creatinine, alkaline phosphatase, total and direct bilirubin. The maximum amount of blood collected for safety assessments will be 30 mL at screening and 15 mL at other study visits. Day 0 safety laboratory tests will not be used to determine eligibility.

^d Initial blood neutralizing antibody testing will include flaviviruses: dengue, zika, yellow fever, west nile virus, Japanese encephalitis virus. Only positive results for dengue will be used as exclusionary criteria. The others will only help to define previous exposures and potential differences in immune response to vaccination.

^e Vital signs on vaccination days will be taken prior to dosing and at least 30 minutes post dose, to include heart rate, blood pressure, temperature, and respiratory rate.

^f At time of screening a complete physical exam will be performed. At subsequent visits a focused physical exam will be performed based upon any subject reported symptoms. If no symptoms are reported at follow up visits no additional physical exam may be performed if not indicated based on investigator discretion.

8.2. Informed Consent

The informed consent process and document will be reviewed and approved by the WRAIR IRB and sponsor's representative prior to initiation of the study. The consent document contains a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. The consent document indicates that by signature, the subject permits witnessing of applicable study procedures by the sponsor's representative, as well as access to relevant medical records by the sponsor's representative and by representatives of the US Food and Drug Administration (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 WRAIR IRB and the ORP.

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 subject before any study-related procedures are initiated for that subject. The consent process will follow the WRAIR CTC SOP (UWD-C 206). All potential subjects will receive a formal briefing in the CTC by an investigator. The investigators will present the protocol in lay terms to individual subjects. The briefing will describe the rationale for and the key features of the study, the nature of subject participation, inclusion and exclusion criteria, and potential risks and benefits to the subject. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the principal investigator. This briefing will involve a combination of standardized presentation materials and spontaneous interaction between investigator and potential subjects. All standardized materials will have been approved by the WRAIR IRB prior to their use. No subject should grant consent until questions have been answered to his/her satisfaction. The subject will be informed that the study products are investigational vaccines and not licensed by the FDA for commercial use, but permitted to be used in this clinical research. Informed consent includes the principle that it is critical the subject be informed about the potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary,
- Subjects may withdraw from participation at any time,
- Refusal to participate involves no penalty, and
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

Should the protocol be modified, the subject consent document must be revised to reflect the changes to the protocol. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the subject. The original consent document must be retained by the investigator as part of the study records. Each subject will receive a copy of the signed informed consent document. At each vaccination visit, inclusion and exclusion criteria will be reviewed and verbal consent to receive the vaccine will be obtained.

All subjects will be required to sign an Information Sheet and indicate whether or not they are a federal employee (Active Duty or Civilian) when they participate in research as human subjects to demonstrate that they have received the compensation policies for federal employees for research subjects. Military subjects will also need to submit a signed statement of supervisor's approval.

The subject will be informed that a description of this clinical trial will be available on <http://www.ClinicalTrials.gov>.

8.3. Study Visits

Refer to [Table 5](#) through [Table 6](#) for study procedures for each of vaccination Groups 1 and 2.

8.4. Subject ID Cards

A subject card will be provided to each subject once they are enrolled in the study. This card will include the address and telephone number of the main contact for information about the clinical study. In an emergency situation, this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator. Subjects will be instructed to keep the subject card in their possession at all times.

8.5. Memory Aids

Memory aids will be provided to each subject after each vaccination to record body temperature through 7 days post vaccination and any solicited AEs occurring within 28 days. After 7 days post vaccination subjects will only be asked to record a temperature if they feel feverish. The subject will be instructed to bring the completed memory aid to the investigator at the 7 day for review and at Day 28 day for final review. Memory aids will be reviewed by an investigator at these visits and any symptoms or signs recorded by the subject on their memory aid will be discussed for clarification.

Collection and verification of completed memory aid will be done during discussion with the subject. The investigator will review the memory aid with the subject, to ensure that all information is accurate. The investigator will have latitude in determination of severity through questioning of the subject. Any unreturned memory aid will be sought from the subject through telephone call(s) or any other convenient methods.

If a subject does not return the memory aid or the memory aid is returned incomplete, the study staff will verbally solicit the information on pain, general symptoms, inter-current illnesses, and concomitant medications during the study visit and record them in the study record. If the subject fails to fill out part of the memory aid or fails to return it he or she will not be asked to fill out the data retrospectively. Instead, after discussion any recalled adverse events will be recorded in the subject's chart per their recollection at the time of the visit. Temperature, redness, and induration will be considered lost data and documented as a protocol deviation since they require real time measurement by the study subject. If a subject's memory aid is lost and not returned a protocol deviation will be noted. Upon completion of 28 days post vaccination, the memory aids will be returned and placed into the subject's chart for inclusion as part of the subject's source documents.

8.6. Biological Samples

All sample testing will be done in accordance with the consent of the individual subject.

Samples collected under this protocol will be used to conduct protocol-related safety and immunogenicity evaluations.

Collected samples may also be used in other assays, for test improvement or test development of analytical methods related to the study vaccine and its constituents or the disease under study to allow a more reliable measurement of the vaccine response. Under these circumstances, additional testing on the samples may be performed by the sponsor outside the scope of this protocol.

Any study requiring future use of these biological samples will require IRB approval. In addition, a subject may decide at any point during the duration of the study to withdraw consent for the future use of his/her samples. Should a subject withdraw consent for the future use of his or her samples, the samples will be destroyed after the conclusion of this study.

Collected samples will be stored until exhausted through use in laboratory testing, unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent.

8.7. Concomitant Medications or Vaccinations

This protocol places no restrictions on rescue medications; the investigator will recommend medication for symptomatic relief, if necessary.

When contacted by the investigator, the subject will be questioned about any medications taken and vaccinations received. All concomitant medication and (non-study) vaccinations, with the exception of vitamins and dietary supplements, are to be recorded in the eCRF.

Concomitant medication administered prophylactically in anticipation of reaction to the vaccination and any medication intended to treat an AE will also be recorded. A prophylactic medication is a medication administered in the absence of any symptom and in anticipation of a reaction to the vaccination (eg, an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring). The use of prophylactic medications is discouraged.

Prescription immunosuppressants or other prescription immune-modifying drugs or cancer chemotherapy drugs will not be permitted during the study. Any volunteer who initiates treatment with medications of this nature will be excluded from any further vaccinations. Inhaled or topical corticosteroids will be allowed and do not require exclusion from vaccination or enrollment.

FDA approved vaccinations may proceed during the study but subjects will be asked about these at each visit and they will be recorded in the subject's chart. Subjects will be asked not to receive any vaccinations within 30 days before or after any study vaccinations if possible.

8.8. Procedures for Monitoring Subject Compliance

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

electronic CRF. The study termination eCRF will be completed, with the reason for withdrawal specified. Study termination procedures include recording: date of last contact, major reason for termination, concomitant vaccination and medication, AEs, SAEs, pregnancy, study conclusion and signature of the investigator.

9. DOSE ADJUSTMENT AND STUDY TERMINATION PROCEDURES

9.1. Contraindications to Subsequent Vaccination

The following events constitute absolute contraindications to the second planned vaccination. If any of these events occur during the study, the subject must not receive additional doses of vaccine but may continue other study procedures at the discretion of the investigator.

- Hypersensitivity reaction, including anaphylaxis, following the administration of the first dose of study vaccine.
- Pregnancy
- Autoimmune disease or pIMD
- Discovery of any health condition which, in the investigator's opinion, places the subject at increased risk from receipt of further investigational product; or discovery of a change in the subject's status which renders him/her unable to comply with protocol-mandated safety follow-up
- Initiation of new immunosuppressive or immune modulating drug or therapy
- Noncompliance that requires withdrawal from the study or precludes subsequent vaccination

The investigational product(s) can be administered to subjects with a minor illness (ie, mild diarrhea, mild upper respiratory infection, etc) without fever (fever is considered to be oral body temperature $\geq 37.5^{\circ}\text{C}/99.5^{\circ}\text{F}$). If the subject has fever, with or without a minor illness, at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the specified time window or withdrawn at the discretion of the investigator.

Subjects may be eliminated from the according to protocol (ATP) cohort for immunogenicity if, during the study, they incur a condition that has the capability of altering their immune response or are confirmed to have an immunodeficiency condition.

9.2. Safety Criteria for Stopping Doses

If any of the events listed in [Table 7](#) or [Table 8](#) occurs, administration of investigational product will be discontinued for all subjects in all groups until a thorough review of the event is undertaken by the study team and the sponsor. Specifically, the primary investigator, the DOD research monitor and the sponsor's safety physician will discuss the event to determine the likely cause. If after review, it is determined that the event(s) is unlikely related to vaccination and/or it is determined after discussion that this event does not represent a significant or unexpected risk to research subjects then vaccinations may be resumed.

Table 7: Adverse Event Study Hold Rules

Event	Number or Percentage of Subjects ^a
If one subject dies or experiences any SAE related to the investigational products	≥ 1
If one subject is withdrawn from the study (by investigator request) following a Grade 3 AE that cannot reasonably be attributed to another cause	≥ 1
Any local or general solicited AE leading to hospitalization, or fever >40°C (104°F) that cannot reasonably be attributed to another cause or necrosis at the injection site within the 28-days (Days 0-28) post-vaccination period	≥ 1
Subjects reporting any Grade 3 solicited local AE in an investigational group, within the 28-day post-vaccination period	≥ 30% of vaccines so far and ≥ 3 subjects
Subjects reporting any Grade 3 solicited general AE in an investigational group, within the same system organ class, that cannot reasonably be attributed to another cause, within the 28-day post-vaccination period	≥ 2
Subjects reporting any Grade 3 unsolicited AE in an investigational group, within the same system organ class, that cannot reasonably be attributed to another cause, within the 28-day (Days 0-27) post-vaccination period	≥ 2
Subjects reporting any ≥ Grade 3 abnormality within the same system organ class in pre-specified hematological, biochemical laboratory parameters in an investigational group, that cannot reasonably be attributed to another cause, within the 28- day post-vaccination period	≥ 2

^a The number or percentage (as marked) of subjects who must experience the event for the study to be put on hold until review can be performed by the PI, research monitor and sponsor to determine appropriate follow-up actions.

Table 8: Laboratory Assay Study Hold Rules

Test	Analyte	Grade ≥ 3
Serum chemistry	ALT	> 5 x ULN
	AST	> 5 x ULN
	Alkaline phosphatase	> 3 x ULN
	Creatinine	> 2 x ULN
Hematology/Complete blood count (CBC)	White Blood Cells	> 20,000 or <1,500 cell/mm ³
	Neutrophils	< 1,000 cell/mm ³
	Lymphocytes	< 500 cell/mm ³
	Eosinophils	> 5,000 cell/mm ³
	Hemoglobin female	< 9.5 g/dL or
	Hemoglobin male	< 10.5 g/dL or
	Platelet count	< 100,000 cell/mm ³

9.3. Study Termination Criteria

The PI, research monitor, sponsor's representative, the WRAIR IRB, or the FDA may stop or suspend the use of these products or this study at any time. This would result in a halt to any further vaccinations unless or until a later determination is made to restart the study. Any

previously vaccinated subjects would still be followed for safety for at least 180 days following receipt of any vaccine product.

9.3.1. Subject Withdrawal Criteria

Each subject may withdraw consent at any time during the study without penalty. Counseling about the subject'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 subject will be provided.

Subjects who are withdrawn because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn due to an SAE/AE until resolution of the event.

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

- A subject fails to comply with study procedures
- A subject's safety or health may be compromised by further participation (eg, an AE or SAE resulting in a safety concern)

9.3.2. How to Withdraw Subjects

If a subject withdraws, the investigator will make a reasonable effort to determine the reason for the withdrawal from the study and to complete termination procedures. Telephone calls, registered letters, and email correspondence are considered reasonable effort. For subjects leaving the study, a targeted examination may be performed, if medically indicated and if permitted by the subject.

When a subject withdraws due to an AE or is withdrawn by the PI due to an AE, the sponsor's safety office (the United States Army Medical and Research Development Command (USAMRDC), Office of Regulated Activities (ORA), Product Safety Surveillance Office (PSSO)) must be notified within 24 hours via email (usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil) and investigators must follow specific policy regarding the timely reporting of AEs and SAEs to the WRAIR IRB (Section [11.7.1.2](#)). In all cases, the PI will make a reasonable effort to complete study termination procedures.

If a subject meets withdrawal conditions for a concomitant medication violation or noncompliance, the reason should clearly be stated in the source document and the study termination eCRF.

9.3.3. Data Collected for Withdrawn Subjects

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.

9.3.4. Replacement of Subjects

Subjects who withdraw or are withdrawn after the first vaccination will not be replaced. However, subjects who withdraw or are withdrawn prior to the first vaccination will be replaced.

9.3.5. Follow-up for Withdrawn Subjects

The investigator will make every effort to follow all subjects who are withdrawn/withdraw, after receiving vaccine, regardless of the reason, until the end of the study period.

If administration of the investigational products is discontinued for a subject it will be annotated in the record but the subject may still be followed for safety assessments if they are amenable.

10. IMMUNOGENICITY ASSESSMENTS

10.1. Geometric Mean Titers and Seroconversion

Assessment of neutralizing antibodies (NAb) against DENV type 1-4 will be performed by a qualified microneutralizing antibody assay as previously described ([Putnak et al.-2008](#)) or flow cytometry-based neutralization assay as previously described ([Kraus et al.-2007](#)) at WRAIR ([Table 9](#)). The 50% neutralization (NT50) titers will be reported by both assays. Seropositivity and seroconversion rates for each DENV serotype and GMTs will be evaluated. Samples will be used to generate data within the scope of this protocol.

Characterization of homotypic and heterotypic NAb will be evaluated using an established assay for depletion of DENV-specific antibodies from human sera. The antibody depletion method will be carried out as described previously ([de Alwis et al.-2012](#)). Purified DENV or BSA control protein will be adsorbed onto 4 μ m Polybead polystyrene microspheres following the manufacturer's instructions (Polysciences, Inc.). The polystyrene microspheres will be washed 3 times with 0.1M borate buffer (pH 8.5) and will be incubated with purified DENV or BSA control protein in borate buffer overnight at room temperature. The control and DENV-adsorbed beads will be blocked 3 times with BSA (10 mg/ml) in borate buffer for 30 min at room temperature and washed 6 times with PBS. Human immune sera will be depleted of DENV-specific antibody by incubation with virus-adsorbed beads for 2 hrs at 37°C with gentle mixing. Sequential rounds of antibody depletion will be performed and the depleted and undepleted sera will be tested by ELISA for determining successful removal of the desired DENV-specific antibodies. ELISA binding of human sera to purified DENV 1-4 will be measured as described previously ([de Alwis et al.-2012](#)).

The potential of sera to mediate antibody-dependent enhancement (ADE) will be assessed using an established in vitro ADE assay. Two-fold serial dilutions of heat-inactivated sera will be mixed with an equal volume of virus (sufficient to achieve approximately 15% infection of 5×10^4 K562-DC-SIGN cells) and incubated for 1 hr at 37°C. This mixture will be added to a 96-well plate containing medium (RPMI-1640 supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine (200mM), and 1% non-essential amino acids (10 mM)) with 5×10^4 K562 cells per well in duplicate and incubated 18-20 hrs overnight in a 37°C, 5% CO₂, humidified incubator. Following overnight incubation, the cells will be fixed, permeabilized and immunostained with flavivirus group-reactive mouse monoclonal antibody 4G2, and secondary polyclonal goat anti-mouse IgG PE-conjugated antibody (catalog no. 550589, BD Biosciences, San Jose, CA). The percent infected cells will be quantified on a BD Accuri C6 Plus flow cytometer (BD Biosciences, San Jose, CA).

Table 9: Humoral Immunity Assessments (Antibody Determination)

System	Component	Method	Unit	Cut-off	Laboratory
Serum	Neutralizing antibody against DENV types 1-4	DENV neutralization assay in-house (Vero cells)	The titer giving 50% reduction in viral infection	MN50 \geq 10	WRAIR ^a
Serum	Neutralizing antibody against DENV types 1-4	DENV neutralization assay in-house (human U937-DC-SIGN)	Reciprocal serum dilution giving 50% reduction in viral infection	NT50 \geq 40	WRAIR VDB

^a WRAIR laboratory refers to Viral Diseases Branch or (503 Robert Grant Avenue, Silver Spring, MD 20910)

10.2. Cell-Mediated Immunity (CMI) Assessment

The Viral Disease Branch (VDB) Immunology Laboratory will assess vaccine-induced cell-mediated immunity (CMI) using a variety of assay platforms, as outlined below. Samples for CMI testing include peripheral blood mononuclear cells (PBMC) and serum/plasma collected contemporaneously and will be utilized fresh or after cryopreservation. Samples will be released for testing at the discretion of the PI and according to VDB Compliance Management Unit (CMU) procedures. All assays for CMI will be performed as exploratory studies using appropriate SOPs developed in the laboratory or other methods consistent with those in the published literature. PBMC will be assessed for markers of cellular activation and maturation as well as antigen-specific cytokine production. Cell subsets of interest (for example, activated T cells) may be sorted by fluorescence-activated cell sorting (FACS) and subjected to systems analysis for nucleic acid and protein expression profiling. Serum/plasma will be analyzed for potential biomarkers of acute phase reactant (protein) content as well as antigen-specific antibodies involved in NK cell function. CMI assays will be performed on pre-vaccination samples and at various time points post-vaccination. Pre-vaccination samples will serve as a control for all assays performed and hence a larger blood draw is scheduled at Day 0 (day of first vaccination).

Assays for assessment of vaccine-induced CMI will include:

- ELISPOT – for T cell function and B cell antibody secretion
- Cytokine Flow Cytometry (CFC) – for T cell function and phenotype
- Flow cytometry-based Immunophenotyping – for profiling of cell subset activation and phenotype
- Fluorescence-activated cell sorting (FACS) – to identify specific cell subsets of interest for sorting and downstream detailed analysis
- *In vitro* proliferation assays – for T cell function
- Antibody-dependent Cellular Cytotoxicity (ADCC) by flow cytometry – for antibody-dependent NK cell function

- Multiplexed enzyme linked immunosorbent assay (ELISA) – for cytokine expression
- Immune Genotyping
- Systems Biology Analyses

10.2.1. CMI Assays and Assay Systems

10.2.1.1. ELISPOT

Vaccine-induced T cell responses will be assessed using a standard ELISPOT assay for expression of IFN-gamma and/or other cytokines of interest, following SOP #UWF-D-085. Antigens for the T cell ELISPOTs will include overlapping peptide pools matching select DENV-derived proteins and when possible epitope mapping will be performed using individual peptides.

B cell ELISPOTs will be performed to identify DENV-specific antibody-secreting cells, following SOP #UWF-D-099. The antigens used for coating plates will be either a crude preparation of virus from cell culture, the vaccine itself, recombinant proteins or other related antigens.

10.2.1.2. Cytokine Flow Cytometry (CFC)

For T cell function and *in vitro* activation studies, cryopreserved PBMC will be thawed and immediately stimulated with select DENV-derived peptide pools in the presence of protein transport inhibitors prior to staining for a variety of functional and/or cytokine markers, following SOP #UWF-D-079.

10.2.1.3. Flow Cytometry-based Immunophenotyping and Fluorescence-activated Cell Sorting (FACS)

Flow cytometry will be used to comprehensively assess cellular activation and maturation in all lymphoid and myeloid cell lineages in PBMC, following SOP #UWF-D-084. In select cases for which the minimal epitope has been identified, HLA/peptide tetramer reagents may be used to assess in-depth the phenotype of antigen-specific cells. After staining, sub-populations of interest may be sorted using FACS and subjected to subsequent systems biology analyses.

10.2.1.4. In vitro proliferation assays

Cryopreserved PBMC will be thawed and immediately stained with a cell-tracking dye (eg, CFSE) followed by stimulation with select DENV-derived peptide pools and staining by flow cytometry, following SOP #UWF-D-083.

10.2.1.5. Antibody-dependent Cellular Cytotoxicity (ADCC)

Serum samples will be used to determine 50% and end-point titers of vaccine-induced antibodies that mediate ADCC. All assays will be conducted using cell lines and methodology developed in the VDB Immunology Laboratory.

10.2.1.6. Multiplexed Enzyme Linked Immunosorbent Assay (ELISA)

Commercially available multiplexed bead-based ELISAs, such as Luminex-based assays, will be used to measure numerous acute phase proteins, chemokines and cytokines circulating in the serum/plasma of vaccinees. Culture supernatants from lymphocyte proliferation assays may also be tested.

10.2.1.7. Immune Genotyping

Samples may be subject to limited genetic testing (genotyping) for certain genes and alleles (of these genes) that are of immunological relevance. This testing could include genes of any of the following loci: human leukocyte antigen (HLA) gene family, killer-cell inhibitory receptor (KIR) gene family, and genes that code for immunoglobulins (antibodies) and T cell receptors (TCRs). The results of these tests will be exclusively used for research (NOT diagnostic) purposes and will allow coordinated analysis of particular genotypes with immune phenotypes and responses characterized herein.

10.2.1.8. Systems Biology Analyses

The transcriptomic profile of purified subpopulations of PBMC from vaccines will be characterized to identify molecular signatures of vaccine-induced immunity. Such analysis can be performed using a variety of approaches, including microarrays and total RNA sequencing (RNA-Seq).

Microarray data analysis: The Human Transcriptome Array 2.0 (Affymetrix) will be used to determine differentially expressed genes and differences in the transcript isoforms from each of the cell populations. As biological pathways are multifaceted and multiple genes/proteins are involved, vaccine induced differential expression of genes would be identified and evaluated by gene array analysis.

Total RNA Sequencing (RNA-Seq): cDNA libraries will be prepared from purified, FACS-sorted subpopulations of cells for high-throughput sequencing using Illumina HiSeq and/or MiSeq instruments.

11. SAFETY ASSESSMENT

Safety monitoring will be conducted throughout the study; therefore safety concerns will be identified by continuous review of the data by the PI, clinic staff, clinical monitor, research monitor, and sponsor safety office (ORA PSSO).

A Data and Safety Monitoring Board (DSMB) is not required for this study.

Research Monitor

In accordance with the DoD Instruction (DoDI) 3216.02, all DoD-conducted research studies determined to be greater than minimal risk [as defined by 32 CFR 219.102(i)] require the appointment of an independent research monitor by the IRB.

Research monitors shall:

1. Discuss research progress with the PI, interview subjects, consult on individual cases, or evaluate suspected adverse reaction reports on behalf of the IRB.

2. Perform, at the direction of the IRB, oversight functions (eg, observe recruitment, enrollment procedures, and the consent process for individuals, groups or units; oversee study interventions and interactions; review monitoring plans and unanticipated problems involving risks to subjects or others (UPIRTSO) reports; and oversee data matching, data collection, and analysis).
3. Promptly report discrepancies or problems to the IRB.
4. Have the authority to stop a research study in progress, remove individual subjects from a study, and take whatever steps are necessary to protect the safety and well-being of research subjects until the IRB can assess the research monitor's report.
5. Review all UPIRTSOs, SAEs/SUSARs, unanticipated problems, and all subject deaths, and provide an unbiased written report of the events promptly to the sponsor's safety office (USAMRDC ORA PSSO). The Research Monitor will provide an unbiased report for all UPIRTSOs, related SAEs, and all subject deaths promptly (within 48 hours) to the WRAIR IRB by email (usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil) or facsimile (301) 319-9961 and they will provide a written report of these events within 10 working days to the WRAIR IRB.

USAMRDC ORA PSSO: The sponsor's safety office (ORA PSSO) is responsible for coordinating and integrating the review of safety data regarding The Surgeon General, Department of the Army (TSG-DA)-sponsored products. This office reviews each SAE reports for medical consistency, accuracy, and completeness and follows each event until it is satisfactorily resolved. The USAMRDC safety pharmacovigilance physician, as delegated by the sponsor, evaluates all safety cases and provides the final determination on expectedness and relatedness to the product, and whether expedited reporting is warranted, per current FDA regulation and guidance.

11.1. Specification of Safety Assessments

11.1.1. Demographic Data

Demographic data, to include age, sex, race, and ethnicity will be collected, by study personnel as part of the screening process.

11.1.2. Physical Examination, Vital Signs, and Medical History

Physical examinations will be conducted by study investigator(s) or other qualified study personnel. The physical examination of the subject will include assessment of body temperature and resting vital signs: systolic/diastolic blood pressure and heart rate after at least 10 minutes of rest. Any pre-existing conditions or signs and/or symptoms will be noted, and medical history, height, and weight will be documented. Vital signs recorded after vaccination that are respectively above or below FDA scale vital sign ranges will be entered as adverse events if appropriate within the clinical context within which they occur and within the context of previously established normal range of each individual subject. Grading of abnormal vital signs will be guided by the ranges established by the FDA toxicity scale for abnormal vital signs.

11.1.3. Clinical Laboratory Assessments

Clinical laboratory assessments will be performed by Quest laboratories according to standard operating procedure. Hematology assessments include a complete blood count: WBC, Hgb, platelet count, differential, and ANC. Blood chemistry assessments include AST, ALT, BUN, creatinine, alkaline phosphatase, total and direct bilirubin.

Abnormal laboratory findings or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE or SAE (Section 11.1.5). Only abnormal lab results determined to be clinically significant will be recorded as AEs or SAEs respectively. Abnormal laboratory results that are both clinically significant and not clinically significant will be graded per the modified FDA toxicity criteria in [Appendix D](#) (if the laboratory test is listed on that scale) for the Sponsor's PSSB safety surveillance. Abnormal lab results will be judged by investigators to be clinically significant if they meet one or both of the following criteria:

6. The abnormality suggests a disease and/or organ toxicity that is new or has worsened from baseline.
7. The abnormality is of a degree that requires additional active management, eg, change of dose, discontinuation of the drug, close observation, more frequent follow-up assessments, or further diagnostic investigation.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected before the first vaccination will be documented on medical history as pre-existing conditions and will be reported as AEs or SAEs only if the pre-existing conditions are worse following the vaccination.

Clinically significant abnormal laboratory findings or other abnormal assessments that are detected after the first vaccination during the study will be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

Clinically significant laboratory abnormalities will be followed up until they have returned to normal or to baseline, stabilized with the probability that it will become chronic, or a satisfactory explanation has been provided. Additional information (including but not limited to laboratory results) relevant to the subsequent course of such abnormalities noted for any subject must be made available to the study monitor.

The sponsor may request that the investigator perform or arrange for the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of an SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognized follow-up period, the investigator will provide a copy of any post-mortem findings, including histopathology, to the USAMRDC PSSO (Safety Office).

11.1.4. Post-vaccination Viremia

Assessment of viremia will be conducted at WRAIR using quantitative RT-PCR or by detecting viable virus through culture in vero cells. Based on results from WRAIR 2136 RNAemia appears to occur most frequently between d]Days 7-14. However, it is often very transient. We will attempt to capture the greatest number of positive samples by testing daily between 6 and 14

days following vaccination with LAV. Sequential days of testing will be encouraged as much as possible but weekend testing will be excluded due to logistical constraints. Testing for viremia will occur for a total of 5 days per subject. Viremia is not expected after PIV vaccinations so viremia testing will occur only after LAV vaccinations. RNAemia will be reviewed to assess for correlation with solicited and unsolicited adverse events along with subsequent immune responses elicited by vaccination.

11.1.5. Definition of Adverse Events

An ADVERSE EVENT (AE) is any untoward medical occurrence associated with the use of the investigational product in humans, whether or not considered related to the investigational product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal (investigational) product.

SUSPECTED ADVERSE REACTION means any adverse event for which there is a reasonable possibility that the vaccine caused the adverse event. For the purposes of IND safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the vaccine 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 study product.

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

- Local solicited adverse events:
 - Pain at injection site
 - Redness at injection site
 - Swelling at injection site
- General solicited adverse events:
 - Fatigue
 - Fever
 - Headache
 - Gastrointestinal symptoms (nausea, vomiting, diarrhea, and/or abdominal pain)
 - Joint pain
 - Muscle aches
 - Rash

Solicited symptoms will be recorded by subjects on memory aids from the day of vaccination (Day 0) through the end of Day 27 after each vaccination. Redness and swelling will be measured in millimeters, using a metric ruler provided by the CTC, and the greatest surface diameter of each will be recorded. Body temperature (oral route preferred) will be recorded in the evening, using a thermometer provided by the CTC. Actual temperatures will only be recorded in the eCRF if they are abnormal (per grading in [Table 11](#)). Volunteers will only be

asked to record daily temperatures for the first 7 days following each vaccination. Following that they will be asked to record a temperature only if they feel feverish for Days 8-28. If multiple temperatures are recorded by a volunteer in a single day, the highest temperature measured on that day will be recorded in the eCRF.

UNSOLICITED AEs are all other adverse events not listed as a solicited AE, occurring from the day of vaccination through 28 days after vaccination.

A SERIOUS ADVERSE EVENT (SAE)/SERIOUS SUSPECTED ADVERSE REACTION is an AE or suspected adverse reaction that, in the view of either the PI or medical monitor, results in any of the following outcomes:

- death
- a life-threatening adverse event
 - An adverse event or suspected adverse reaction is considered “life-threatening” if, in the view of either the investigator or sponsor, its occurrence places the patient or subject 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.
- Inpatient hospitalization or an event associated with the investigational product that results in hospitalization or prolongation of hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- Important Medical Events when meeting the below criteria:

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 and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

An UNEXPECTED ADVERSE EVENT/UNEXPECTED SUSPECTED ADVERSE REACTION is an AE or suspected adverse reaction that is not listed in the investigator’s brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. 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 study products or as anticipated from the pharmacological properties of the study product, but are not specifically mentioned as occurring with the particular study product under investigation.

11.1.6. Unanticipated Problems Involving Risks To Subjects Or Others

Federal regulations require that UPIRTSOs be promptly reported to the IRB within the IRB specified time frame. These events encompass a broader category of events than SAEs and may include issues such as problems with loss of control of subject data or the investigational product; adverse psychological reactions; or breach of confidentiality. Risks to others (eg, study personnel) must also be reported.

The WRAIR IRB requires prompt reporting of UPIRTSOs (using the Deviation/Unanticipated Problem Report Form) to the HSPB (within 48 hours) upon becoming aware of the event by telephone, facsimile, or email, and recording it in the study unanticipated problem log (Deviation/Unanticipated Problem Report Form). A written report is required to be submitted by the PI to the HSPB within 10 working days of knowledge of the event.

All other unanticipated problems should be included in the deviation/unanticipated problem log and reported to the IRB as part of the protocol continuing review, as per the SOP UWZ-C-618, Continuing Review and Continuation Determinations.

Unanticipated problems involving risks to subjects or others 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 subject population;
- Related or possibly related to a subject's participation in the study; and
- Suggests that the study places subjects or others at a greater risk of harm than was previously known or recognized.

The IRB and the ORP will evaluate the PI's and research monitor's reports to determine whether a given incident, experience or outcome constitutes an unanticipated problem involving risk to subjects or others and, in coordination with the sponsor's safety office, ensure reporting of the unanticipated problems involving risk to subjects or others to the appropriate investigative sites and regulatory offices, as applicable.

11.1.7. Potential Immune-mediated Diseases

Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune etiology. AEs that need to be recorded and reported as pIMDs include those listed in [Table 10](#). The investigator will exercise his/her medical and scientific judgment in deciding whether other immune-mediated diseases have an autoimmune origin (ie, pathophysiology involving systemic or organ-specific pathogenic auto antibodies) and should also be recorded as a pIMD.

11.2. Relationship to Investigational Product

The investigator 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. A determination of ‘related to investigational product’ or ‘not related to investigational product’ will be made along with a narrative and supporting documentation. The determination that an AE is ‘not related to investigational product’ will be made if the AE is clearly not related to the administration of the study treatment, another cause of the event is most plausible, a clinically plausible temporal sequence is inconsistent with the onset of the event and the study treatment, and/or a causal relationship is considered biologically implausible. The determination that an AE is ‘related to the investigational product’ will be made if the AE is temporally related to the administration of the study treatment, a causal relationship is considered biologically plausible, and there is no alternative cause that provides a more likely explanation.

Table 10: List of Potential Immune-mediated Diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
Cranial nerve disorders, including paralyses/paresis (eg, Bell’s palsy), and neuritis (eg, optic neuritis)	Systemic lupus erythematosus Scleroderma (including, CREST syndrome and morphea)	Psoriasis Vitiligo Raynaud’s phenomenon Erythema nodosum Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) Cutaneous lupus erythematosus Alopecia areata Lichen planus Sweet’s syndrome
Multiple sclerosis (including variants)	Systemic sclerosis Dermatomyositis Polymyositis Antisynthetase syndrome Rheumatoid arthritis, Juvenile chronic arthritis, (including Still’s disease) Polymyalgia rheumatica Reactive arthritis Psoriatic arthropathy Ankylosing spondylitis (including undifferentiated spondyloarthritides) Relapsing polychondritis Mixed connective tissue disorder	
Transverse myelitis		
Guillain-Barré syndrome, (including Miller Fisher syndrome and other variants)		
Other demyelinating diseases (including acute disseminated encephalomyelitis)		
Myasthenia gravis (including Lambert-Eaton myasthenic syndrome)		
Non-infectious encephalitis/ encephalomyelitis		
Neuritis (including peripheral neuropathies)		
Narcolepsy		
Liver disorders	Gastrointestinal disorders	Metabolic diseases
Autoimmune hepatitis	Crohn’s disease	Autoimmune thyroiditis (including Hashimoto thyroiditis)

Primary biliary cirrhosis	Ulcerative colitis	Grave's or Basedow's disease
Primary sclerosing cholangitis	Ulcerative proctitis	Diabetes mellitus type I
Autoimmune cholangitis	Celiac disease	Addison's disease
Vasculitides	Others	
Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis.	Autoimmune hemolytic anemia	
Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.	Autoimmune thrombocytopenia	
	Antiphospholipid syndrome	
	Pernicious anemia	
	Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulo-nephritis, and mesangioproliferative glomerulonephritis)	
	Uveitis	
	Autoimmune myocarditis/cardiomyopathy	
	Sarcoidosis	
	Stevens-Johnson syndrome	
	Sjögren's syndrome	
	Idiopathic pulmonary fibrosis	
	Goodpasture syndrome	

11.3. 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. Solicited adverse events will be graded according to the scale shown in [Table 11](#). All other events will be graded according to the scale in [Table 12](#). The eCRF for AEs will reflect only the highest severity for continuous days an event occurred.

If a subject is evaluated in an emergency room for non-life threatening illness or symptoms (ie, 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 subject's clinical signs and symptoms.

Clinically significant abnormal laboratory values will be graded by study investigators based upon a modified FDA toxicity scale ([Appendix D](#)). All abnormal lab values will graded (based

on table in [Appendix D](#)) upon entry into the database for PSSO safety surveillance. The tables in [Appendix D](#) were modified to come into harmony with established laboratory normal values per Quest Diagnostics guidelines. That is, changes were made to the FDA toxicity scale to insure that no lab values are marked as “normal” per the Quest Diagnostics laboratory standards but are considered “abnormal” by the grading scale which could create a conflict or possibly missed laboratory related adverse events.

As defined by the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice (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 subject/event outcome or action criteria usually associated with events that pose a threat to a subject’s life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. In addition to the severity scales listed below any adverse event that is deemed by the investigator to be potentially life threatening will be graded as Grade 4 and any adverse event that results in death will be graded as Grade 5.

Table 11: Solicited Adverse Event Intensity Scales

Adverse Event	Intensity Grade	Parameter
Pain at injection site	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities.
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Fever ^a	1	37.5°C – 38.4°C (99.5°F – 101.1°F)
	2	38.5°C – 38.9°C (101.2°F – 102.0°F)
	3	≥39.0°C (102.1°F)
Redness at injection site	1	Greatest surface diameter ≥ 25 to 50 mm
	2	Greatest surface diameter ≥ 51 to 100 mm
	3	Greatest surface diameter ≥ 101 mm
Swelling at injection site	1	Greatest surface diameter ≥ 25 to 50 mm
	2	Greatest surface diameter ≥ 51 to 100 mm
	3	Greatest surface diameter ≥ 101 mm
Headache	1	Mild: Headache that is easily tolerated
	2	Moderate: Headache that interferes with normal activity
	3	Severe: Headache that prevents normal activity
Fatigue	1	Mild: Fatigue that is easily tolerated
	2	Moderate: Fatigue that interferes with normal activity
	3	Severe: Fatigue that prevents normal activity

Adverse Event	Intensity Grade	Parameter
Gastrointestinal symptoms (nausea, vomiting, diarrhea and/or abdominal pain)	1	Mild: Gastrointestinal symptoms that are easily tolerated
	2	Moderate: Gastrointestinal symptoms that interfere with normal activity
	3	Severe: Gastrointestinal symptoms that prevent normal activity
Joint pain	1	Mild: Joint pain that is easily tolerated
	2	Moderate: Joint pain that interferes with normal activity
	3	Severe: Joint pain that prevents normal activity
Muscle aches	1	Mild: Muscle aches that is easily tolerated
	2	Moderate: Muscle aches that interferes with normal activity
	3	Severe: Muscle aches that prevents normal activity
Rash	1	Eruption covering <30% of total body surface area and not limiting ADLs
	2	Eruption covering <50% of total body surface area and/or limiting ADLs
	3	Eruption covering >50% of total body surface area and/or limiting self-care

^a The preferred route for recording body temperature in this study is oral.

Table 12: Intensity Scale for All Other Adverse Events

Grade	Description
1 (mild)	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	An AE which is sufficiently discomforting and interferes with normal everyday activities.
3 (severe)	An AE which prevents normal, everyday activities (ie, prevent attendance at work/school) and would necessitate the administration of corrective therapy

11.4. Assessment of Outcome

Outcome of any non-serious AE occurring within 28 days post-vaccination (ie, unsolicited AE) or any SAE/pIMD reported during the entire study will be assessed as:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered /resolved with sequelae
- Fatal (SAEs only)

11.5. Duration of Follow-Up of Subjects after 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 safety office (ORA PSSO) using the Serious Adverse Event Report Form.

Investigators are not obligated to actively seek SAEs in former subjects; 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 safety office as defined in Section 12.7.1.

Adverse events will otherwise be followed either until resolution or until end of study at which time the current status of the adverse event will be recorded as described in Section 12.4.

11.6. Recording Adverse Events

AEs and SAEs will be assessed at all study visits. AEs will be recorded on the eCRF only on day of vaccination through to 28 days following each vaccination respectively. SAEs will be recorded at all study visits. These AEs and SAEs will be documented in the source records, and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. It should be noted that the form for collection of SAE information is not the same as the AE eCRF. When the same data are collected on both forms, the forms must be completed in a consistent manner. For example, the same event term should be used on both forms. New or updated information will be recorded in the originally completed CRF using

Good Documentation Practices. The investigator will submit any updated SAE data to the sponsor's safety office (ORA PSSO) within 24 hours of receipt of the information.

When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to investigational product, severity, and other possible etiologies. When an event has not resolved by study closure, it will be documented on the AE eCRF as "not recovered/not resolved."

11.7. Reporting Adverse Events

The PI will report all AEs to USAMRDC, the WRAIR IRB, and the USAMRMC ORP 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 in the Clinical Services Support Division.

11.7.1. Reporting Serious and Unexpected Adverse Events

Contact information for reporting SAEs is provided in [Table 13](#). **Email reporting of SAE should be sent to:** usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil.

11.7.1.1. Reporting to the Sponsor

All SAEs must be reported promptly (within 24 hours) to the sponsor's safety office (ORA PSSO) per 21 CFR 312.64, whether or not the event is considered related to study product using a sponsor-approved Serious Adverse Event Form. Further, the investigator should comply with relevant study site standard operating procedures (SOP) on reporting SAEs.

The minimum information that the investigator will provide to the ORA PSSO is specified in Table 14. The sponsor's representative may request additional information for purposes of the study.

In order to comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 calendar days, investigators must submit additional information as soon as it is available. The sponsor's representative will report unexpected SAEs associated with the use of the vaccine to the FDA as specified at 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy regarding the timely reporting of SAEs to the research monitor, the WRAIR IRB.

Reporting to the sponsor's safety office does not fulfill the investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The PI will notify the WRAIR IRB, and the research monitor.

Table 13: Study Contacts for Reporting Serious Adverse Events and Unanticipated Problems Involving Risk to Patients or Others

Sponsor's Safety Office	US Army Medical Research and Development Command Office of Regulated Activities Product Safety Surveillance Office ATTN: MCMR-UMR 1430 Veterans Drive Fort Detrick, MD 21702-5009 Fax: 301-619-7790 Telephone: 301-619-1106 Email: usarmy.detrick.medcom-usamrmc.mbx.sae-reporting@mail.mil
Institutional Review Board	503 Robert Grant Avenue Silver Spring, MD 20910-7500 Phone: 301-319-9940 Fax: 301-319-9961 Email: usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil
Research Monitor	LCDR Tida Lee MD Naval Medical Research Center(NMRC) 503 Robert Grant Avenue Silver Spring, MD 20910-7500 Telephone: Fax: Email: tida.k.le.mil@mail.mil

Table 14: SAE Information to be Reported to the Sponsor's Safety Office

Notification Method	Information to be Provided
Email or Telephone (within 24 hours of knowledge of event)	IND number, sponsor study number, name of the investigational product, and investigator name and contact number Subject identification number SAE term, description, onset date, date(s) of investigational product administration, severity, relationship to investigational product, and subject's current status
AND Email or Fax	Cover sheet or letter Sponsor-approved Serious adverse event report form Concomitant medication case report form or a list of concomitant medications taken within 30 days of the event Discharge summary or Medical record progress notes including pertinent laboratory/diagnostic test results

11.7.1.2. Reporting to the IRB

Serious Adverse Events Reporting:

Serious Adverse Events (SAEs) that are related and all deaths should be promptly (within 48 hours) reported by the investigator to the WRAIR IRB. Immediate and prompt notifications should be in the form of email, phone, or fax. A written report for each reportable SAE and deaths will be provided to the WRAIR IRB within 10 working days of the initial notification. A summary of all Serious Adverse Events reported for the study will be provided in the continuing review reports.

The investigator should not wait to receive additional information to fully document the event before notifying the HSPB/WRAIR IRB of a related serious adverse event. As new information is obtained, updated reports should be submitted, and if available, should include copies of relevant hospital case records, autopsy reports, and other documents where applicable.

Unanticipated Problems Reporting:

All unanticipated problems involving risk to subjects or others will be promptly (within 48 hours) reported by phone, by email, or by fax to the WRAIR IRB. A complete written report will follow the initial notification within 10 working days. In addition to the methods above, the complete report may also be physically delivered to the address of the WRAIR HSPB. All unanticipated problems reported during the study will be summarized in the continuing review reports.

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

11.7.2. Reporting Additional Immediately Reportable Events to the Sponsor's Safety Office and WRAIR IRB

11.7.2.1. Pregnancy

Each pregnancy must be reported within 24 hours of identification **on the Pregnancy Report Form** by email or fax to the sponsor's safety office (ORA PSSO). Subject pregnancy will also be promptly reported to the WRAIR IRB within 48 hours by phone, fax, or email.

Subjects who become pregnant after Day 0 will be followed to term, and the following information will be gathered for outcome: type and date of delivery, Apgar scores, health status of the mother and child including the child's sex, 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.

11.7.2.2. AE-related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported within 24 hours of identification by email or fax to the sponsor's representative via the safety mailbox. Report the withdrawal to the WRAIR IRB within 48 hours by phone, fax, or email.

11.7.2.3. Additional Immediately Reportable Event

pIMDs must be reported to the WRAIR IRB and sponsor's safety office (ORA PSSO) within 24 hours after the diagnosis of a new pIMD or exacerbation of a pre-existing pIMD (serious or non-serious) are made known to the primary investigator or representative.

11.7.2.4. 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 as soon as study investigators become aware to the WRAIR IRB and the USAMRMC ORP and the sponsor's representative.

11.7.3. IND Annual Report and Final Clinical Study Report

11.7.3.1. IND Annual Report

The PI will contribute to the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to USAMRDC. Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. The sponsor's representative will notify the PI of the due date with sufficient time for the PI to assemble the required information.

11.7.3.2. 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 USAMRDC for review and approval. USAMRDC will use this report to prepare the final clinical study report for submission to the FDA.

12. STATISTICS

12.1. Description of Statistical Methods

Detailed statistical procedures, listings, table shells and figures will be provided in a separate statistical analysis plan (SAP). The SAP will be finalized before 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,
- Indication of whether the comparisons will be one-tailed or two-tailed (with justification of the choice) and the level of significance to be used,

- Identification of whether any adjustments to the significance level or the overall p value will be made to account for any planned or unplanned subgroup analyses or multiple testing,
- 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.

12.1.1. Analysis Addressing the Primary Study Objective

The primary objective is the safety and reactogenicity of the 2 vaccine candidates in a heterologous prime-boost strategy in the 27 days after each vaccination, inclusive of the day of vaccination. The percentage of subjects reporting any AE within 28 days (0-27) following vaccination and their 95% confidence interval (CI) will be computed using the Wilson score or exact (Clopper-Pearson) methods by groups according to the type of AE, intensity, and relationship to vaccination. The occurrence of related SAEs, PIMDs, and medically attended AEs will be reported descriptively..

12.1.2. Analysis Addressing the Secondary Study Objective

Seropositivity rates for each DENV type along with trivalent and tetravalent antibody responses as determined by MN50 cutoff titer will be calculated with 95% CI using the Wilson score or exact (Clopper-Pearson) methods for each vaccination group. Antibody titers will be summarized as GMTs, with the 95% CI, and reverse cumulative curves. A DENV-primed subject is defined with a MN50 titer cutoff of ≥ 10 .

Additionally the cell-mediated immune (CMI) response to the 2 tetravalent dengue vaccine candidates in a heterologous prime-boost schedules will be assessed. CMI markers will be evaluated by, but not limited to, the following methods: ELISPOT, flow cytometry, CFSE dye-dilution proliferation, Luminex, transcriptomics, and proteomics. For adaptive B and T cell response assessments statistical analysis will be performed using GraphPad Prism 6, Stata, or other well established software packages. Non-parametric tests will be used as the default to compare effects between different test and control groups. Where appropriate parametric statistical tests (eg, T-tests, ANOVA) will be applied to normally distributed data. P values less than 0.05 will be considered significant. Standard deviations and 95% confidence intervals will also be measured. Corrections for multiple comparisons will be made where necessary and appropriate. For the multiplex bead-based assays and RNA expression studies proprietary and specialty software will be used for analysis.

12.1.3. Analysis Addressing Exploratory Objective

Analysis of type specific antibody levels rate of decay will be described and compared between the 0, 180 Day PIV/LAV and 0, 90 Day PIV/LAV regimens. The percentage of subjects with a measurable dengue RNAemia by DENV type will be tabulated after each dose of LAV for each study group and quantitative RNAemia levels will also be compared. Safety Analyses

Safety analysis will include data collected from all subjects who received at least one vaccination. Adverse event data will be listed individually (including intervention and outcome) and summarized by body system and preferred terms within a body system for each treatment group. Serious and/or unexpected AEs will also be discussed on a case-by-case basis. For the tabulation of the AEs by body system, a subject will be counted only once in a given body system. For example, a subject reporting nausea and diarrhea will be reported as one subject, but the symptoms will be listed as 2 separate AEs within the class. Therefore the total number of AEs reported within a body system may exceed the number of subjects within the body system reporting AEs. The safety analysis will be included in the final study report.

12.1.4. Clinical Laboratory Data Analyses

For hematology and serum chemistry tests, the mean, mean change, median, median change along with standard deviations, and range of all values for each test for each treatment group at baseline and for the final 'on therapy' values will be printed in a summary table. Another table will be prepared displaying the numbers of subjects in each treatment group who had values below, within, and above the normal range at baseline and at the final visit.

These tables will be reviewed by the PI or research monitor to evaluate whether any significant trends in laboratory values occurred. The PI or research monitor will also review the urinalysis data by inspecting the laboratory data tabulations, but no summary tables of these will be prepared.

The cut-off value used to determine seropositivity for DENV neutralizing antibodies titers in a DENV-primed subject is defined by a MN50 titer cutoff of ≥ 10 or via flow cytometry based antibody neutralization assay $NT50 \geq 40$. A seronegative subject for a specific DENV type is a subject whose titer for that DENV type is below the cut-off value while a seropositive subject is a subject whose titer for that DENV type is greater than or equal to the cut-off value.

Tetavalent seropositivity rate is defined as the percentage of subjects who are seropositive to all 4 DENV types.

Trivalent seropositivity rate is defined as the percentage of subjects who are seropositive to 3 DENV types.

The GMT calculations are performed by taking the anti-log of the mean of the log 10 titer transformations. Antibody titers below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation. For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced but will be noted.

For the analysis of unsolicited adverse events/serious adverse event/concomitant medication, subjects who did not report an event will be considered as subjects without an event. In case of significant non-compliance of study procedures for reporting symptoms, the analysis plan will be reassessed to ensure more accurate reporting of study data by further analysis.

For the analysis of solicited symptom, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the total vaccinated cohort

will include only subjects/doses with documented safety data (ie, symptom screen/sheet completed).

12.1.5. Schedule of Analyses

There will be 2 separate database locks that will occur at the visit level with analyses occurring after each one.

Once all data for all subjects have been collected and entered into the database up to and including Visit 10 (28 days post final vaccination) the database will be locked/frozen for all data up to Visit 10 and these data will be analyzed as described above for both groups.

After the database lock/freeze for data up to Visit 10, data will continue to accrue for only those events subsequent to Visit 10. The database will be locked/frozen for all events subsequent to Visit 10 after the remainder of the data have been collected and entered into the database. These data will be analyzed as described above for both groups after this final database lock.

The analyses performed at these 2 time points will be used to generate the final clinical study report.

12.2. Planned Enrollment and Reason for Sample Size

The sample size of 40 subjects is appropriate to further define safety profile for PIV/LAV formulations in healthy adult population living in a non-endemic region and to perform further evaluation of immunogenicity in this population. No sample size calculation in relation to a specific objective was performed.

Table 15 shows the precision of the 95% CI that a sample size of 20 subjects per group will provide for varying frequency rates of AEs using the Wilson score or exact (Clopper-Pearson) method. Major differences in terms of safety and reactogenicity between the vaccination schedules are not expected and this study is not powered to detect small differences in reactogenicity. Differences in immunogenicity in terms of GMTs and antibody level durability will be compared between the 0, 180 Day PIV/LAV and 0, 90 Day PIV/LAV and 95% CI will be compared for statistical significance. Given these represent continuous variables and up to 10 fold differences in antibody titers were seen between some groups and timepoints in WRAIR 2136, statistically significant differences in immunogenicity results are expected if such differences exist and are in fact substantial. Using all PIV-LAV antibody titers at six months for all dengue strains, the pooled standard deviation of the log-transformed antibody titers was 0.049. Based on this standard deviation and a sample size of 20 subjects per group, the smallest detectable difference between groups that can be observed with 80% power and 95% confidence is 10.7% increase (a difference of 0.0444 in the log₁₀-transformed means). At 90% power this increases to a 12.6% increase (a difference of 0.0444 in the log₁₀-transformed means). Assuming a two-fold increase in antibody titers is the minimal potentially clinically significant difference, there is greater than 99.9% power to detect this difference under the current design.

Table 15: Precision for the 95% Confidence Interval for Varying Frequency Rates (N=20)

Number of Subjects with Events / Number of Vaccinated Subjects	Frequency of Subjects with Event	95% CIs for the Frequency of Subjects with Event
1/20	5.00%	0% - 14.60%
2/20	10.00%	0% - 23.10%
5/20	25.00%	6.00% - 44.00%
10/20	50.00%	28.10% - 71.90%

12.3. Level of Significance to be Used

Statistical significance will be defined with an alpha of 0.05 and/or lack of overlapping values when 95% CIs are calculated.

12.4. Statistical Criteria for the Termination of the Trial

12.4.1. Stopping Rules

There are no statistical criteria for study termination in this uncontrolled clinical trial. Adverse event and laboratory assay study hold rules are presented in Section 9.2.

12.5. Accounting for Missing, Unused, and Spurious Data

Non-analyzable data will be documented in the deviations.

12.6. Procedures for Reporting Deviations from the Original Statistical Plan

Any planned changes from the original statistical plan will be described in an amendment to the protocol and/or the SAP as necessary. Deviations from the SAP will be documented in the final clinical study report.

12.7. Selection of Subjects to be Included in Analyses

12.7.1. Total Vaccinated Population

The total vaccinated population will include all subjects who received at least one vaccination. While those who do not receive both vaccinations in this heterologous prime-boost study will be monitored for safety signals, only those who received 2 dengue vaccinations will be included in analysis of immunological endpoints.

12.7.2. According to Protocol Population

The according to protocol (ATP) population for analysis of immunogenicity will include all subjects who receive both doses of study vaccine according to protocol and:

- Meet all eligibility criteria

- Are seronegative to all 4 DENV types before vaccination
- Comply with the procedures and intervals defined in the protocol
- Did not present with a potentially confounding medical condition

The ATP population will be defined at each analysis time point and subjects will be included based on their available data up to that time point. Subjects will not be retrospectively removed from the ATP population based on data from later time points.

The following criteria should be checked at each visit subsequent to the first vaccination. If any become applicable during the study, it will not require withdrawal of the subject from the study but may determine a subject's ability to be evaluated in the ATP analysis.

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccine during the study period.
- Chronic administration (defined as more than 14 days) of immunosuppressants or other immune-modifying drugs during the study period. For corticosteroids, this will mean prednisone ≥ 20 mg/d or equivalent. Inhaled and topical steroids are allowed.
- Administration of a vaccine not foreseen by the study protocol during the period starting 30 days before dose 1 and ending 30 days after dose 2.
- Administration of immunoglobulins and/or any blood products during the study period.
- Subject incurs a condition that has the capability of altering their immune response (ie, flavivirus vaccination) or is confirmed to have an immunodeficiency condition.

A detailed, comprehensive list of reasons for elimination from ATP analyses will be established at the time of data cleaning.

13. ETHICS

13.1. Compliance Statement

The study will be conducted according to the protocol and in accordance and in compliance with ICH GCP, Belmont Principles, DoD requirements (32 CFR 219, DoDI 3216.02), and 21 CFR 50, 56, and 312. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guideline and clinic site SOPs. Roles and responsibilities of study staff are presented in [Appendix A](#).

13.2. Ethics Review

The study is based on adequately performed laboratory and animal experimentation and will be conducted under a protocol reviewed by the WRAIR IRB. The study is to be conducted by scientifically and medically qualified persons. The IRB will determine whether the benefits of the study are in proportion to the risks. The rights and welfare of the subjects will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; the results to be reported will be accurate; subjects 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.

13.2.1. Review/Approval of Study Protocol

Before a clinical study can be initiated, the study protocol and other required documents will be submitted to the following departments in the order listed for review and/or approval, with the final review by the FDA:

- Integrated Product Team
- WRAIR Scientific Review Committee
- Sponsor's protocol review board
- WRAIR Commander
- WRAIR IRB
- Sponsor's Representative (acting for The Surgeon General of the Army)
- USAMRMC Commanding General, if applicable

Enrollment in this protocol may not begin until all approvals have been obtained, and the formal authorization letter is received by the PI from the sponsor's representative.

13.2.2. Confidentiality

In this research, the subject's health information will be collected and used to conduct the study; to monitor the subject'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 subject has the right to see and receive a copy of his/her information.

Representatives of the TSG as the IND sponsor, USAMRDC as the sponsor's representative, the WRAIR IRB and the ORP, the DoD, and the FDA are authorized 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 authorized to witness the applicable study procedures to assure the safety of subjects.

Each subject's personally identifiable healthcare information will be kept confidential to the extent possible but confidentiality cannot be guaranteed. Subject identification numbers will be used to facilitate confidentiality in discussing subject specific information during and after the study.

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

13.2.3. Compensation for Participation

Subjects will receive compensation for their participation in this study. Study volunteers will be provided a check, reloadable debit card, gift card or cash from the WRAIR CTC as compensation for visits.

Subjects will receive \$50 for the screening visit and \$130 for each of the scheduled 14 study visits once enrolled. Blood draws are scheduled to occur at every visit. For non-active duty military and non-federal employees, the maximum compensation is \$2210 if all visits are kept. Under 24 USC 30, active duty personnel and federal employees will receive \$50 for the screening visit and \$50 for each study visit involving blood draws during duty or work hours. If the visit occurs during off-duty hours or non-work hours, the subject will be eligible to receive the full compensation of \$130. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available to the subjects participating in this clinical trial. Subjects will not be compensated for any missed visits during the study. Subjects will be compensated \$50 for each unscheduled visit if medically necessary and blood draw occurs. Subjects will be offered a \$200 bonus on their last visit in addition to the \$130 standard visit amount if they have made all of their study visits within the required time ranges. This is to encourage participants to stay in the study and complete all visits in this study which will run a total of 18 months.

Compensation for non-military, non-federal employees and military or federal employee on leave or non-working hours:

Screening (\$50) + Visits 1-14 (\$130 each x 12 = \$1560) + Vaccination visits (2x \$200) + completion bonus (\$200) = \$2210 total

Compensation for military during duty or federal employees during work hours:

Screening (\$50) + Visits 1-14 (\$50 each x 14 = \$700) = \$750 total

There will be \$25 referral bonus paid when recruiting another volunteer into the study. A subject is eligible for this referral bonus even if he or she is excluded at screening. Also note that active duty personnel or federal employees may only receive the completion bonus at their final visit if this visit occurs while on approved leave or outside duty hours. Medical Care for Research-Related Injury

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

If a subject is injured because of participation in this research and is a DoD healthcare beneficiary (eg, active duty in the military, military spouse or dependent), the subject is entitled to medical care for that injury within the DoD healthcare system, as long as the subject remains a DoD healthcare beneficiary. This care includes, but is not limited to, free medical care at Army hospitals or clinics.

If a subject is injured because of participation in this research and is not a DoD healthcare beneficiary, the subject is entitled to medical care for that injury at an Army hospital or clinic; medical care charges for care at an Army hospital or clinic will be waived. The subject is also entitled to care for that injury, but such care for that injury at other DoD (non-Army) hospitals or clinics may be limited by time, and the subject's insurance may be billed. It cannot be determined in advance which Army or DoD hospital or clinic will provide care. If the subject

obtains care for research-related injuries outside of an Army or DoD hospital or clinic, the subject or the subject's insurance will be responsible for medical expenses.

For DoD healthcare beneficiaries and non-DoD healthcare beneficiaries: Transportation to and from hospitals or clinics will not be provided. No reimbursement is available for incurred medical expenses to treat research-related injuries. No compensation is available for research-related injuries.

Subjects are not waiving any legal rights. If a subject believes that they have sustained a research-related injury, they should contact the Principal Investigator (PI). If they have any other questions regarding research-related injuries, they should contact the PI.

13.3. Biological Sample Handling and Analysis

All sample testing will be done in line with the consent of the individual subject.

Samples collected under this protocol will be used to conduct protocol-related safety and immunogenicity evaluations.

Collected samples may also be used in other assays, for test improvement or test development of analytical methods related to the study vaccine and its constituents or the disease under study to allow a more reliable measurement of the vaccine response. Under these circumstances, additional testing on the samples may be performed by the sponsor outside the scope of this protocol.

Genetic testing may be performed on these samples only after specific informed consent is acquired for such testing and IRB approval is obtained.

In order to be enrolled in the study, the subject must agree to allow their samples to be used for future studies.. This is because we cannot predict what future studies will be most useful and cannot confidently enumerate all studies that need to be performed in this protocol. We anticipate that future laboratory analysis will be necessary and so we ask subjects to consent to this use as well. However, a subject may decide at any point during the duration of the study to withdraw consent for the future use of his/her samples. Should a subject withdraw consent for the use of his or her samples, the samples will be destroyed.

Collected samples will be stored until exhausted through use in this or future laboratory studies, unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. Samples will not be sold for profit or used as a component in a commercial product being sold for profit.

13.4. Specimen Storage and Labeling

The Viral Diseases Branch (VDB), Compliance Management Unit (CMU) shall manage (log-in, track, and issue) specimens via the FreezerWorks® database. Specimens shall be physically stored in VDB CMU-controlled storage units based on temperature requirements [ie, -80±10°C, vapor-phase liquid nitrogen (-130°C or below), etc]. All storage units used to store specimens for this study shall be access-controlled and environmentally-monitored on a 24/7 basis. VDB CMU will also generate and manage specimen labels via a combination of the Zebra Designer Pro®, FreezerWorks®, and Microsoft® Access software. Specimens will be labeled in accordance with the template shown in [Appendix B](#). Study primary investigator and sub-investigators along with

VDB immunologists and microbiologists will have access to these specimens and may obtain these specimens for testing through request to the VDB CMU.

14. DATA HANDLING AND RECORDKEEPING

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 transcribed 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.

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 in the USAMRDC ORA. All updates to the data management plan must be approved before study close-out and database lock.

Any missing, unused or obviously spurious data will be rectified through review of the corresponding primary source documents.

14.1. Inspection of Records

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

Subjects' 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. The consent document indicates that by signature, the subject permits access to relevant medical records by the sponsor's representative and by representatives of the FDA.

Upon a subject's termination from the trial, completed eCRFs will be ready and available for on-site review by the sponsor's representative or the designated representative within 14 days after receipt of the subject's data.

14.2. Retention of Records

The PI must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval, or if not approved for 2 years following the discontinuance of the investigational product for investigation. 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.

Completed, monitored eCRFs will be stored in a secure location by the sponsor's representative or designee. A copy of each completed eCRF will be retained by the investigator. Subject charts will be maintained in a secure location in the CTC during the study with access permitted only to the PI and the project coordinator. Upon completion of the clinical phase, these records will be stored in a locked cabinet within the CTC, which also can be accessed by only the clinical coordinator or PI, until moved to the secure archives in Building 500 after study closeout.

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

It is the policy of the USAMRMC that data sheets are to be completed for all subjects participating in research (Form 60-R, Volunteer Registry Data Sheet). The data sheets will be entered into this Command's Volunteer Registry Database. The information to be entered into this confidential data base includes the subject's name, address, and Social Security Number; study title; and dates of participation. The intent of this data base is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that USAMRMC can exercise its obligation to ensure research subjects are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years. The Volunteer Registry Database is a separate entity and is not linked to the study database.

15. QUALITY CONTROL, QUALITY ASSURANCE, AND DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Subjects will be identified on eCRFs by a unique subject 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 subject identification number will be used if it becomes necessary to identify data specific to a single subject. Representatives of USAMRMC, the sponsor's representative, the WRAIR IRB and/or the USAMRMC ORP, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research. Personal identifiers will be removed from photocopied medical and research records.

15.1. Study Monitoring

Study monitoring will be the responsibility of the USAMRDC ORA. 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 subjects 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 subject has completed the study. A report of monitoring observations will be provided to the PI (for corrective actions) and members of the Sponsor's Representative team.

15.2. Audits and Inspections

Authorized representatives of the sponsor, the FDA, the independent ethics committee, the WRAIR Institutional Review Board, or other regulatory agencies may visit the site to perform audits or inspections, including source data verification, and review the regulatory files. 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. Auditing of the clinical trial may be conducted at any time during the study.

The investigator should contact the sponsor's representative and Office of Research Protections, Human Research Protection Office (ORP HRPO) immediately if contacted by a regulatory agency about an inspection.

Auditing may also be performed by independent personnel designated by the Office of Quality Management, USAMRDC. Audit findings will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

15.3. Institutional Review Board

As the IRB of record, the WRAIR IRB 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 subject consent form and recruitment materials, must be maintained by the PI or representative or designee and made available for inspection. The PI is responsible for preparing and submitting continuing review reports to the WRAIR IRB before the established continuing review date or at least on an annual basis, whichever comes first. All deviations, unanticipated problems, and SAEs occurring within the reporting period should be summarized in the continuing review reports submitted to the WRAIR IRB. The PI will submit a closeout report to the WRAIR IRB once the study is complete.

15.4. Protocol Suspensions or Discontinuation

The study may be suspended or discontinued at any time by the Sponsor, WRAIR IRB, US FDA, or other government agencies, as part of their duties to ensure that research participants are protected. Any suspensions (to include continuing review lapses), clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the Sponsor, or Regulatory Agencies will be promptly (within 48 hours) reported to the WRAIR IRB by phone (301-319-9940), by email (usarmy.detrick.medcom-wrair.mbx.hspb@mail.mil), or by facsimile (301-319-9961). Such a change in study status should also be reported to the sponsor's representative as well.

16. PROTOCOL MODIFICATIONS AND DEVIATIONS

16.1. Protocol Modifications

All amendments and modifications will be reviewed and approved by the WRAIR IRB prior to implementation. Any protocol amendment will be agreed upon and approved by the sponsor's representative prior to submission to the WRAIR IRB and administrative review by NMRC. Any modification or amendment that could potentially increase risk to subjects must be submitted to the FDA prior to implementation. The informed consent document will be revised to concur with any significant amendment that directly affects volunteers, and will also be reviewed and approved with the amendment. New subjects enrolled in the study will be consented with the most recent approved consent documents. Subjects already enrolled in the study will be informed about the revision and, depending on the impact of the amendment, may be asked to re-consent. This will be accomplished by repeating the consent process with the revised consent document with attention given to the changes, or it may be done using an addendum consent that states the revision or new information. The new documents will be signed, placed in the study record, and signed copies will be given to the subject.

16.2. Protocol Deviation Procedures

A protocol deviation is defined as any occurrence involving a procedure that did not follow the study protocol, applicable procedures, and/or regulatory requirements. All staff involved in the conduct of a clinical trial will be aware of the specific protocol requirements for completing study visits and notify the PI in the event of any breach of protocol according to the following procedures. Deviations are recorded in the source documents and reported as per requirements.

Notify the PI as soon as possible if they are not present when the deviation is discovered.

Document in the volunteer's study chart all protocol deviations directly involving or affecting the volunteer, and document a thorough explanation of the circumstances leading up to the deviation. The requirement and timeline for reporting protocol deviations to the WRAIR IRB is determined by the categorization of the deviation: major or minor.

Major deviations are departures from protocol that have a potential to affect the rights of the research participant, to increase the risk to the participant, to change the willingness of the volunteer to continue participation, or to compromise the integrity of the study data in such a way that the study objectives may not be achieved. Major deviations that occur will be reported to the WRAIR IRB within 48 hours of identification and recorded in the deviation study log, and a written report submitted within 10 working days of the PI becoming aware of the deviation. All reports will be submitted with a cover memo naming the protocol, description of the event, summary of any harm to study participant(s) and steps to prevent further deviations. A summary of the major deviations occurring within the reporting period should also be included in the continuing review reports. Major deviations will be reported immediately to the sponsor's representative as well.

Minor deviations are routine departures that typically involve a volunteer's failure to comply with the protocol (Example: missing scheduled visits). Minor deviations that occur will be reported to the WRAIR IRB in the Continuing Review Report(s).

Knowledge of any instances of serious or continuing non-compliance with the regulations or requirements will be reported immediately to the WRAIR IRB.

17. PUBLICATION POLICY

All data collected during this study will be used to support this IND. All data may be published in the open medical or military literature with the identity of the subjects protected, once the final clinical study report has been submitted to the FDA. Anyone desiring to publish or present data obtained during the conduct of the study will conform to WRAIR/USAMRDC policies and then forward the publication for review to the Commander, USAMRDC or designee and usarmy.detrick.medcom-usamrmc.list.clearances@mail.mil prior to submission.

17.1. Initial Analysis

An initial analysis is planned to review data through to 28 days post vaccination for both Group 1 and 2. These data will be analyzed following initial database lock/freeze as detailed in the statistics section above and will then be available for presentation and/or publication as needed following approval through the standard WRAIR/USAMRDC publication approval process.

18. LIST OF REFERENCES

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19. APPENDICES

APPENDIX A. ROLES AND RESPONSIBILITIES

- Principal Investigator
 - Complies with protocol and all Federal and local regulations and policies and has ultimate responsibility for the conduct of the study and submission of the study report.
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Supervises sub-investigators and protocol nurse coordinator
 - Trains study staff
 - Screens individuals and reviews all of the screening tests to determine eligibility of person to participate in the study. Investigator or designee makes final eligibility decision.
 - Permits auditing, institutional monitoring (US Army Medical Research and Development Command) and inspection by the US Food and Drug Administration
 - Assures adequate resources, time, and patient population to meet study requirements
 - Provides medical care for adverse events
 - Documents protocol deviations
 - Reports all serious adverse events to the regulatory bodies (WRAIR IRB) and sponsor's representative (US Army Medical Research and Development Command)
 - Reports unanticipated problems and regulatory audits to the IRB
 - Delegates investigational product accountability and administration
 - Evaluates AEs for diagnosis, relationship, and severity,
 - Ensures documentation and safety reporting
 - Ensure that he/she has sufficient time to conduct and complete the study
 - Ensures he/she has adequate staff and appropriate facilities that are available for the duration of the study and to ensure that other studies do not divert essential subjects or facilities away from the study at hand
 - Submits an up-to-date curriculum vitae and other credentials (e.g. medical license number in the United States) to the Sponsor and, where required, to relevant authorities
 - Acquires the normal ranges for laboratory tests performed locally and, if required by local regulations, obtains the laboratory licenses or certifications

- Informs the IRB of any non-protocol specified laboratory testing that may occur on stored serum samples left over after protocol specified laboratory testing is completed (e.g., clinical tests performed in the evaluation of a subject's medical problem or additional specific immunologic evaluations as they become available)
 - Prepares and maintain adequate case histories designed to record observations and other data pertinent to the study
 - Conducts the study in compliance with the protocol and any amendments
 - Cooperates with representatives of USAMRDC in monitoring the study and in resolution of queries about the data
- Sub-investigators
 - Comply with Good Clinical Practices and Belmont Principles
 - Qualified by training, education and experience
 - Assist Principal Investigator in conduct of study
 - Assist in supervising study staff
 - Review all of the screening tests to determine the eligibility of individuals to participate in the study (Principal investigator or designee makes final eligibility decision)
 - Evaluate adverse events
 - Screen individuals for protocol participation (physical examination and medical history)
- Study Coordinator(s)
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified by training, education and experience, and holds a current, unrestricted nursing license
 - Delegates investigational vaccine accountability to licensed nursing personnel
 - Reviews all of the screening tests
 - Schedules follow-up appointments
 - Collects information on adverse events
 - Accurately and promptly completes case report forms
 - Trains staff on protocols/protocol changes
 - Documents protocol deviations
 - Stores IND product in temperature controlled and monitored units
 - Signs out IND product to the investigational product preparer

- Verifies and records administration of investigational products to study participants
- Other Study Staff
 - Comply with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Recruit study participants with IRB approved materials
 - Collect pre- and post-vaccination vital signs
 - Contact study participants to collect adverse event data
 - Schedule follow-up appointments
 - Follow-up on non-compliant study participants
 - Ensure efficient clinic flow
 - Perform subject specific training
- Study Administrative Support
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Maintains essential clinical trial documents
 - Hosts visits of auditors, monitors and inspectors
 - Coordinates routing of all protocol activity
 - Maintains and updates all study staff curriculum vitae and Good Clinical Practices training certificates
 - Supports IND product accountability
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Assists with vaccine accountability, storage and vial destruction
 - Issues vaccine to clinic
- Research Monitor
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Serves as a safety advocate for study participants
 - Reviews all severe adverse events, serious adverse events, unanticipated problems, protocol violations and annual reports

- Discuss research progress with the PI, interview participants, consult on individual cases, or evaluate suspected adverse reaction reports on behalf of the IRB
 - Perform, at the direction of the IRB, oversight functions (e.g., observe recruitment, enrollment procedures, and the consent process for individuals, groups or units; oversee study interventions and interactions; review monitoring plans and UPIRTSO reports; and oversee data matching, data collection, and analysis)
 - Promptly report discrepancies or problems to the IRB
 - Have the authority to stop a research study in progress, remove individual participants from a study, and take whatever steps are necessary to protect the safety and well-being of research participants until the IRB can assess the research monitor's report.
- Data Manager
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Ensures the accuracy, quality, and integrity of the data:
 - Performs MedDRA and World Health Organization (WHO) coding
 - Reviews electronic case report forms (eCRF) for consistency and clarity, generates and resolves data queries and provides analysis and feedback to PI, protocol nurse coordinator and protocol team
 - Runs discrepancy report and quality control check reports and distributes to the protocol nurse coordinator to review for data accuracy against the eCRF
 - Oversees the data entry process for the study
 - Manages and maintains the database
- Statistician
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Statistically analyzes the verified data according to the protocol, statistical analysis plan and any amendments in place
 - Provides documentation of statistical findings to the principal investigator for incorporation into required reports
- Internal Quality Assurance
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience

- Reviews study procedures, staff training and study documentation to ensure safety and accuracy in accordance with internal procedures
- Regulated Studies Laboratory Staff and Research Investigators
 - Complies with Good Clinical Practices, Good Laboratory Practices and Belmont Principles
 - Qualified through training, education and experience
 - Analyzes study participants sera for immune response to vaccines
- Clinical Laboratory Staff
 - Complies with Good Clinical Practices and Belmont Principles
 - Qualified through training, education and experience
 - Performs all phlebotomy for the study
 - Processes samples for biochemistry, hematology, urinalysis, hepatitis panel, human immunodeficiency virus, and β -HCG analysis on study participants
 - Performs urine pregnancy test

APPENDIX B. LABELS

APPENDIX C. RECRUITMENT SCRIPT

When called by a potential subject, please make introductions and answer any questions that they may have concerning the study. If the potential subject is still interested, please read the following statement:

THANK YOU FOR CALLING ABOUT INFORMATION ON VOLUNTEERING TO PARTICIPATE IN “A PHASE 1, RANDOMIZED, OPEN-LABEL, SINGLE-CENTER, COMPARISON OF HETEROLOGOUS PRIME-BOOST VACCINATION SCHEDULES OF TETRAVALENT DENGUE VIRUS PURIFIED INACTIVATED VACCINE (PIV) AND TETRAVALENT DENGUE VIRUS LIVE ATTENUATED VACCINE (LAV) IN HEALTHY ADULTS IN A NONENDEMIC REGION”.

We appreciate your interest. I will provide you some information that will help you determine if you can participate in the study.

Alternatively, the information below can be emailed to potential subjects:

I would like to share some information with you regarding an upcoming clinical trial entitled:

“A Phase 1, Randomized, Open-label, Single-center, Comparison of Heterologous Prime-Boost Vaccination Schedules of Tetravalent Dengue Virus Purified Inactivated Vaccine (PIV) and Tetravalent Dengue Virus Live Attenuated Vaccine (LAV) in Healthy Adults in a Nonendemic Region”.

Background:

This study involves 2 experimental dengue vaccines. Dengue is a common infection affecting residents and travelers to many areas of the world, including Southeast Asia, Central America, South America, and the Caribbean. It is caused by a virus and is transmitted by a mosquito. Dengue can cause fever, tiredness, and even severe bleeding or death. It can pose a threat to military operations; therefore, the military is trying to develop a vaccine to protect against dengue.

This study will look at the effects of combining 2 experimental dengue vaccines. This is not the first time that either of these dengue vaccines has been used in humans nor the first time that they have been used together. It will take place at a clinic-style research facility in Silver Spring, Maryland, and is sponsored by The Surgeon General, Department of the Army. The vaccines will be given in your upper arm using a needle, and blood samples will be collected to look at your body’s response. The goals of this study are to determine if the vaccines are safe when used together and to see how your body responds to the vaccines. One of the vaccines is a live-attenuated, or weakened, vaccine, just like the chickenpox or measles vaccines. The other is an inactivated or killed vaccine, like the flu vaccine or polio vaccine.

Duration:

This study will last 18 months. One or 2 clinic visits are required to see if you qualify for the study. If you are accepted into the study, you will receive 1 dose of each of the vaccines. All volunteers will get the killed vaccine first followed by the live vaccine. After each injection, there will be follow-up visits. These visits will continue for 12 months following the last vaccination in each group. There will be a total of 14 scheduled clinic visits (not including the initial screening).

Requirements and Restrictions:

You must meet ALL of the following requirements in order to participate in this study:

- You **MUST** be **18 to 42** years of age (at the time of initial screening).
- You **MUST** be in good health and have no significant medical conditions or diseases.
- You **CANNOT** be pregnant, breastfeeding or planning to become pregnant during the study. For safety reasons, you must **ALSO** be willing to use a reliable form of birth control during the study. The effects of these vaccines have not yet been studied in infants or unborn children.
- You must have **NEGATIVE** blood tests for hepatitis B, hepatitis C and HIV.
- You **CANNOT** have ever had a dengue infection or received a dengue vaccine.
- You **MUST** have a valid state or US government issued photo ID (such as a driver's license, military ID or US Passport).
- You **MUST** be willing to attend all of the required visits over the next 18 months.
- You **MUST** be willing to refrain from participation in any other clinical studies involving investigational drugs or vaccines while participating in this study.
- You **CANNOT** have donated or received blood, blood products, or plasma within 90 days prior to starting the study or plan on donating blood or plasma during the study.

There may be other reasons why you cannot participate in this study. Those will be discussed at your initial screening visit.

Possible Risks:

There are risks associated with receiving these vaccines.

Based on experience with these and similar vaccines, mild reactions are expected to occur in some subjects. These generally include tenderness, redness, and mild swelling at the injection site. These reactions will most likely resolve on their own within a few days. You may also experience other reactions, such as headache, nausea, a low fever, rash, or mild flu-like symptoms. There may be some risks that are unknown. There is always a chance that someone may experience a severe allergic reaction, just as some people do to other vaccines or drugs, like penicillin. After each vaccination you will be observed in the clinic for a short period of time to make sure you do not have an allergic reaction, or to treat you immediately if you do.

After each vaccination you will see a physician in the clinic who will evaluate the number and type of reactions you may have.

You will be paid for your participation in this study. Please refer to the study schedule or contact one of our recruiters for more detailed compensation information.

Can I schedule an appointment for you to be seen by the research staff so that you can hear more about volunteering for this study?

APPENDIX D. MODIFIED FDA LABORATORY TOXICITY SCALES

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	147-148	149-150	150-152	> 152
Potassium – Hyperkalemia mEq/L	5.4-5.5	5.6-5.7	5.7-5.8	> 5.8
Potassium – Hypokalemia mEq/L	3.3-3.4	3.1-3.2	3.0-3.1	< 3.0
Glucose – Hypoglycemia mg/dL	60-64	52-59	45 – 51	< 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	26 – 28	29 – 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 – 1.4	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.4	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN	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
Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	10.5-11.6	9.5 – 10.4	8.0 – 9.4	< 8.0

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Hemoglobin (Male) - gm/dL	12.5 – 13.0	10.5 – 12.4	8.5 – 10.4	< 8.5
WBC Increase - cell/mm ³	10,900 – 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 – 849	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm ³	1,499 – 2,000	1,000 – 1,498	500 – 999	< 500
Eosinophils - cell/mm ³	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm ³	125,000 – 139,000	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	426 – 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)