Official Title: Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

ClinicalTrials.gov Identifier: NCT02562482

Document Type:

- Redacted Study Protocol, including Letters of Amendment (Informed Consent Template located in Appendix I of the Protocol Version 2.0)
- Redacted IRB-approved Version 2.0 Informed Consent from a participating site
- Redacted Statistical Analysis Plan

Document/Date:

- Protocol Version 2.0: April 15, 2016
- Protocol Version 2.0, Letter of Amendment #1: April 26, 2016
- Protocol Version 2.0, Letter of Amendment #2: June 2, 2016
- Version 2.0 April 15, 2016 IRB-approved Informed Consent
- Statistical Analysis Plan Version 1.0: September 11, 2018

Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

Protocol VRC 704

Sponsored by
National Institute of Allergy and Infectious Diseases (NIAID)
Vaccine Research Center (VRC)
Bethesda, Maryland, USA

BB-IND 14907 – held by VRC, NIAID, NIH

Version 2.0
April 15, 2016

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ABBREVIATIONS

Abbreviation	Term
AE	adverse event
ALT	alanine aminotransferase
ANSM	French National Agency for Medicines and Health Products Safety
AoU	assessment of understanding
CBC	complete blood count
CDC	Center for Disease Control
CHIKV	Chikungunya virus
CRO	Contract Research Organization
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ELISA	enzyme-linked immunosorbent assay
EU	European Union
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GMT	geometric mean titers
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
ICH	International Conference on Harmonization
IM	intramuscular
IND	Investigational New Drug
IoR	Investigator of Record / Site Principle Investigator
IRB	Institutional Review Board
ITT	Intent-to-treat
MedDRA®	Medical Dictionary for Regulatory Activities
mITT	Modified intent-to-treat
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NVITAL	NIAID Vaccine Immune T-Cell and Antibody Laboratory
PBMC	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PP	Per-protocol
PSRT	Protocol Safety Review Team
RNA	ribonucleic acid
SAE	serious adverse event
SAP	Statistical Analysis Plan
SUSAR	serious and unexpected suspected adverse reactions
VLP	virus-like particle
VRC	Vaccine Research Center

PRÉCIS

Protocol VRC 704: Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

Study Design: This is a multicenter, randomized, placebo-controlled, double-blind study to evaluate the safety and immunogenicity of a 2-injection vaccine regimen with the Chikungunya virus (CHIKV) virus-like particle vaccine (CHIKV VLP, VRC-CHKVLP059-00-VP) in healthy adults. The hypothesis is that the vaccine regimen is safe and induces a neutralizing antibody response to CHIKV. The primary objectives are to evaluate safety and tolerability of a 2-injection investigational vaccine regimen of VRC-CHKVLP059-00-VP at 20 mcg compared to placebo in healthy adults in CHIKV endemic areas. The secondary objective is to evaluate neutralizing antibody response in vaccine recipients. The exploratory objectives relate to assessing incidence of CHIKV infection in vaccine and placebo recipients, as well as antigen-specific humoral and cellular immune responses during the study.

Product Description: VRC-CHKVLP059-00-VP is an investigational VLP vaccine that consists of the E1, E2 and capsid proteins of CHIKV (strain 37997).

VRC-PBSPLA043-00-VP is sterile phosphate-buffered saline (PBS) prepared for human injection as a placebo.

Subjects: Healthy adult subjects ages 18 to 60 years old that reside in CHIKV endemic regions.

Study Plan: 400 subjects stratified by age groups 18-40 and 41-60 years will be randomized in a 1:1 ratio to vaccine or placebo. Subjects will receive 2 intramuscular (IM) injections via needle and syringe per the study schedule below:

Group	Number of Subjects	VRC 704 Study Injection Schedule	
		Day 0	Day 28(+14)
1	200	20 mcg	20 mcg
2	200	PBS	PBS
All injections will be administered IM at 0.5 mL by needle and syringe.			

Study Duration: Subjects will be evaluated at 11 clinical visits during 72 weeks of study participation as follows: Day 0 and Weeks 4, 8, 16, 24, 32, 40, 48, 56, 64 and 72.

1. INTRODUCTION

The Dale and Betty Bumpers Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) (Bethesda, MD, USA) is dedicated to translating the latest knowledge of disease pathogenesis and immunology into new vaccine strategies to provide safe and effective means to prevent and control infectious diseases. Chikungunya virus (CHIKV) is a mosquito-borne disease causing acute infection associated with severe morbidity that can persist for several weeks, months or even years. Since its discovery in Tanzania in 1952, outbreaks of CHIKV have occurred most frequently in Africa, Asia and the Indian subcontinent. In recent decades, the disease has spread to Europe and the Americas. As of January 2015, over 1,135,000 suspected cases of CHIKV and 176 CHIKV-attributable deaths have been recorded in the Caribbean islands, Latin American countries and USA [1]. There are currently no effective vaccine or therapies against CHIKV. The rapid emergence of CHIKV supports the need for a safe and immunogenic vaccine. This protocol is designed as a Phase 2 evaluation of the investigational Chikungunya virus virus-like particle vaccine (CHIKV VLP), VRC-CHKVLP059-00-VP. This candidate vaccine was evaluated in a Phase 1 study as safe and well-tolerated and observed to induce neutralizing antibody responses at levels hypothesized to be protective against CHIKV infection [2].

1.1. Chikungunya: Etiology, Disease Course and Epidemiology

CHIKV (genus *Alphavirus,* family *Togaviridae*) is an arthropod-borne viral disease with a positive, 11.8 kilobase (kb) single-stranded RNA genome encoding structural and non-structural proteins [3, 4]. The virus has three distinct clades that share a high percentage of amino acid similarity: West African, Central/East/South African, and Asian genotypes [5-7].

Initial case reports of CHIKV refer to an outbreak in Tanzania in 1952-53 [8, 9] and early outbreaks were geographically limited [10]. Prior to 2013, CHIKV outbreaks were primarily detected in regions of Africa, Asia, Europe, and the Indian and Pacific Oceans. Late in 2013, the first local transmission of CHIKV was reported in the Americas [11-14]. The virus is transmitted through infected mosquitoes, predominantly *Aedes aegypti,* in sylvatic and human-mosquito-human cycles [12, 13]. Genetic mutations in viral genome have enabled virus transmission through a new host, *Aedes albopictus,* altering CHIKV epidemiology and driving outbreaks in new regions [15-17].

CHIKV infection is associated with severe morbidity lasting 1-2 weeks, with symptoms persisting for weeks to months. The incubation period lasts 2-12 days [18]. Acute clinical manifestation involves high fever, rash, gastrointestinal complications, conjunctivitis, headache, arthralgia, nausea, fatigue and myalgia. The hallmark symptom of the infection, debilitating polyarthralgia/arthritis, is present in nearly all cases (>70%) [11, 18, 19]. Onset of alphavirus-associated arthritis begins within 2-5 days after the infecting mosquito bite, coinciding with presence of virus by polymerase chain reaction (PCR) and innate immune responses against circulating virus. The timing and onset of arthritis is not consistent with adaptive immune responses that occur around day 7-14. Persistent arthritis (3-13% of cases) has been associated with pro-inflammatory cytokines and detectable virus in the joint fluid and cells [20-23]. CHIKV-associated mortality is low; the majority of fatal cases occur in older populations with underlying medical conditions [1, 24]. Though atypical, neurological complications including encephalitis and meningoencephalitis have been seen in a growing number of cases [11, 25, 26].

There is currently no vaccine or anti-viral therapy available for the prevention or treatment of CHIKV infection. The economic losses and added stress on public health infrastructure experienced in areas of CHIKV outbreaks along with heightened potential for global spread necessitate vaccine development as a high global priority [2, 10, 27].

1.2. Rationale for Development of VRC-CHKVLP059-00-VP

CHIKV strains have high amino acid sequence homology and are antigenically related; therefore, it is possible to design a vaccine that could be cross-protective against heterologous strains [7, 27-29]. Infection with CHIKV induces long-lasting protective immunity. Both humoral and cellular immunity are detected in survivors of CHIKV infection, however, their relative contribution to protection is unknown [21].

Recent changes in the epidemiology and pathogenicity of circulating CHIKV strains prompted close attention to genetic changes in the virus [15, 16, 30] and to viral immunobiology [21, 31-33]. It was noted that persistent chronic inflammation and incapacitating arthralgia/arthritis could be observed in some infected patients for months despite a robust immune response [21]. Comprehensive study of the production of cytokines, chemokines and growth factors during acute CHIKV infection revealed a predominance of type 2 circulating cytokines, and that increase in interleukin (IL)-1β, IL-6 and decrease in RANTES (regulated upon activation, normal T-cell expressed and secreted) expression were associated with disease severity [33].

Development of a preventive CHIKV vaccine began in the early 1970s with investigation of inactivated vaccine candidates [34, 35]. Formalin-inactivated vaccine was tested in a small group of volunteers and reported to be safe and immunogenic [34]. Subsequently, a live-attenuated vaccine candidate was developed and progressed into Phase 2 clinical trials. The attenuated vaccine induced neutralizing antibody by day 28 in 98% of evaluable vaccinees (n=57), which persisted at least 12 months in 85% of vaccinees. The vaccine was assessed as safe and well-tolerated; reactogenicity reports included transient arthralgia without arthritic signs in 8% of vaccinees [36]. Immunologic interference was revealed in sequential administration of live attenuated alphavirus vaccines [37]. Additional CHIKV vaccination strategies in preclinical and clinical stages include a chimeric alphavirus vaccine candidate, recombinant measles-virus-based CHIKV vaccine, and a consensus-based DNA vaccine candidate [38-40].

The VRC has developed a CHIKV virus-like particle (VLP) candidate vaccine [2, 27]. VLPs are multi-protein structures that mimic the organization and conformation of viruses but lack the viral genome [41]. Compared to inactivated subunit vaccines, VLPs present conformational viral epitopes similar to the authentic virus [42]. VLPs are considered highly immunogenic and are known to elicit high neutralizing antibody titers with good durability [43-45]. The FDA has approved several prophylactic VLP vaccines including hepatitis B vaccines (Engerix® and Recombivax HB®, GlaxoSmithKline and Merck, respectively) and human papilloma virus (HPV) vaccines (Cervarix® and Gardasil®, GlaxoSmithKline and Merck, respectively). Several other VLP vaccine candidates, such as vaccines for influenza, Norwalk and parvovirus, are in pre-clinical testing or Phase 1 studies [41, 42].

Selective expression of the CHIKV structural proteins in mammalian cells results in formation of VLPs that resemble replication-competent alphavirus particles [27]. Immunization with CHIKV VLPs induced antibodies against envelope proteins that neutralized virus *in vitro* and protected against a systemic live virus challenge in non-human primates (NHP). Purified IgG from the

CHIKV VLP-immunized monkeys protected immune-deficient mice from a lethal virus challenge, suggesting that antibodies are an important component of a protective immune response. Preclinical animal studies demonstrate that the VLP vaccine candidate, VRC-CHKVLP059-00-VP, has the potential to elicit protective immunity in humans.

1.3. Previous Human Experience with VRC-CHKVLP059-00-VP

VRC-CHKVLP059-00-VP was assessed under VRC 311 (2011-2012), a Phase 1 open-label, dose-escalation study to evaluate safety and immunogenicity of the vaccine. Twenty-five (25) adults, 18-50 years old, received three intramuscular (IM) injections on weeks 0, 4, and 20 via needle and syringe in doses of 10 mcg (n=5), 20 mcg (n=10), or 40 mcg (n=10).

All injections were well tolerated with no reported arthralgia or fever after vaccination and no serious adverse events (SAEs). Solicited reactogenicity parameters were either none or mild; 36% of vaccinees (n=25) reported mild local reactogenicity and 40% reported mild systemic reactogenicity at least once following vaccination [2]. Neutralizing antibodies were detected in all subjects by 4 weeks after the second vaccination. A significant boost occurred after the third vaccination in all dose groups. Neutralization titers of vaccine recipients reached levels comparable to those reported after natural infection, suggesting a potentially durable protective response to the vaccine [2]. The study detected antibodies by ELISA in 80-100% of subjects in all three dose groups and geometric mean titers (GMT) were not significantly different between the groups except at week 24. The study also found no significant difference in group GMT 4 weeks after the second dose compared to 4 weeks after the third dose.

1.4. Rationale for Study Agent Dose and Schedule

Based on VRC 311 study results that all three dose levels (10 mcg, 20 mcg, and 40 mcg) were safe and well tolerated [2] and all dose levels elicited robust titers of CHIKV neutralizing antibodies as well as durable, vaccine-induced antibodies that persisted through to the final study visit 6 months after the last dose, VRC-CHKVLP059-00-VP will be evaluated in a 2-injection schedule at a dose of 20 mcg. Gaining early information about frequency, magnitude and durability of response to a schedule with two vaccinations will be informative to designing future Phase 3 efficacy studies.

1.5. Assessment of Immunogenicity

In protocol VRC 704, specimens to evaluate immunogenicity will be taken at baseline and at specified time points. The primary immunogenicity timepoint is 4 weeks after the second injection. Measurements of CHIKV-specific humoral immune responses will be assessed by neutralization antibody (NAb) assays [2].

ELISA and other exploratory assays to assess for humoral and cellular immune responses may be performed with stored samples, depending upon the most efficient use of laboratory resources to inform product development. Human leukocyte antigen (HLA) type may be obtained from stored samples if needed to assess HLA-class restricted cellular responses.

Research samples for immunogenicity assays will be processed by the NIAID Vaccine Immune T Cell and Antibody Laboratory (NVITAL) in Gaithersburg, MD, where many of the immunogenicity assays will also be performed. Immunogenicity assays may be performed by VRC laboratories in Bethesda, MD, contract laboratories, or by other research collaborators.

2. STUDY AGENTS

Study agents are manufactured under Good Manufacturing Practice.

2.1. VRC-CHKVLP059-00-VP Study Agent

VRC-CHKVLP059-00-VP is a VLP vaccine that consists of CHIKV VLPs composed of the E1, E2, and capsid proteins of the CHIKV (strain 37997). VLPs are produced in the VRC293 cells using a transient expression system. The production process consists of upstream production (cell growth and antigen expression) and downstream purification. In the first step, VRC293 cells are expanded from the working cell bank until the desired number of cells is obtained. Cells are then transfected with the expression plasmid VRC8900 that encodes for CHIKV structural proteins (capsid, E3, E2, 6K, and E1). The 6K and E3 proteins have not been specifically detected in the VLPs of the VRC-CHKVLP059-00-VP candidate. VLPs are produced as the secreted product, harvested and clarified; this culture medium is used as the starting material for the downstream purification process. Purification includes tangential flow filtration, Q-Sepharose column chromatography, diafiltration, and sterile filtration. Enveloped VLPs self-assemble and are released into the culture medium as ~65 nm particles. No replication-competent viral genetic material is incorporated in the VLP. After purification, the VLPs are diluted into a sucrose-containing citrate buffer, sterile-filtered, filled into single-dose glass vials, and stoppered to produce drug product.

CHIKV VLP drug product is a sterile aqueous buffered solution of CHIKV VLP drug substance filled into single dose glass vials at 40±10 mcg/mL. The composition of drug product is presented in Table 1. Vials have a nominal fill of 0.8 mL to allow withdrawal of 0.5 mL. More details related to vaccine preparation, quality control and preclinical studies performed before the candidate vaccine entered Phase 1 study can be found in the Investigator's Brochure.

Table 1: Quantitative Composition of the VRC-CHKVLP059-00-VP

Ingredient	Composition (per 1.0 mL)	Function
Active Substance		
CHIKV VLP	40 mcg	Antigen
Excipients		
Sucrose	74.6 mg	Buffer component
Potassium phosphate (monobasic)	0.5 mg	Buffer component
Potassium phosphate (dibasic)	1.1 mg	Buffer component
Sodium citrate (dihydrate)	7.4 mg	Buffer component

2.2. VRC-PBSPLA043-00-VP as Placebo

VRC-PBSPLA043-00-VP, a sterile phosphate buffered saline (PBS) is the placebo for the CHIKV VLP vaccine.

3. STUDY OBJECTIVES

3.1. Primary Objective:

- To evaluate the safety and tolerability of VRC-CHKVLP059-00-VP in healthy adults that reside in CHIKV endemic areas when administered intramuscularly by needle and syringe at a dose of 20 mcg compared to placebo.

3.2. Secondary Objectives:

- To evaluate the immune response to VRC-CHKVLP059-00-VP by neutralization assay at 4 weeks after the second 20 mcg dose (Study Week 8).

3.3. Exploratory Objectives:

- To evaluate humoral and cellular responses to VRC-CHKVLP059-00-VP at additional time points. CHIKV-specific responses will be assessed by antibody assays that include antigen-specific ELISA and neutralization assay.
- To compare incidence rates of CHIKV infection in vaccine and placebo recipients.
- Clinical and virus characteristics of infections will also be compared in vaccine and placebo recipients.

4. STUDY DESIGN AND CLINICAL PROCEDURES

This is a Phase 2 multicenter, randomized, placebo-controlled, double-blind study to examine the safety and immunogenicity of a 2-injection vaccine regimen (Day 0 and 28) with the CHIKV VLP vaccine or PBS placebo in healthy adults. The hypothesis is that the vaccine regimen is safe and induces a neutralizing antibody response to CHIKV. The study schema is shown in Table 2 below. The expected duration of time on the study per subject is approximately 72 weeks.

Table 2: Study Schema

Group	Number of Subjects	VRC 704 Study Injection Schedule	
		Day 0	Day 28(+14)
1	200	20 mcg	20 mcg
2	200	PBS	PBS
All injections will be administered IM at 0.5 mL by needle and syringe.			

4.1. Study Population

The study is designed for the enrollment of healthy adults. The following eligibility criteria will be used.

4.1.1. Inclusion Criteria

A subject must meet all of the following criteria:

1. 18 to 60 years old
2. Available for clinical follow-up through Study Week 72
3. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process
4. Able and willing to complete the informed consent process
5. Willing to donate blood for sample storage to be used for future research
6. In good general health, with a BMI ≤40, without clinically significant medical history, and has satisfactorily completed screening
7. Physical examination and laboratory results without clinically significant findings within the 56 days prior to enrollment

Laboratory Criteria within 56 days prior to enrollment:

8. Hemoglobin either within institutional normal limits or accompanied by site physician approval as consistent with healthy adult status
9. White blood cells either within institutional normal range or accompanied by site physician approval as consistent with healthy adult status
10. Platelets = 125,000 – 500,000/mm^3
11. Alanine aminotransferase (ALT) ≤ 1.25 x upper limit of normal (ULN)
12. Serum creatinine ≤ 1.1 x ULN based on site institutional normal range
13. Negative result on an HIV test that meets local standards for identification of HIV infection
14. Negative result on the CHIKV screening antibody assay.

Criteria applicable to women of childbearing potential:

15. Negative human chorionic gonadotropin pregnancy test (urine or serum) on day of enrollment
16. Agree to use an effective means of birth control from 21 days prior to enrollment through 12 weeks after the last study injection

4.1.2. Exclusion Criteria

A subject will be excluded if one or more of the following conditions apply:

Women Specific:

1. Planning to become pregnant during the 16 weeks after enrollment in the study

Subject has received any of the following substances:

2. Systemic immunosuppressive medications within 2 weeks prior to enrollment

3. Blood products within 16 weeks prior to enrollment
4. Immunoglobulin within 8 weeks prior to enrollment
5. Prior vaccinations with an investigational CHIKV vaccine
6. Investigational research agents within 4 weeks prior to enrollment
7. Any vaccination within 2 weeks prior to enrollment
8. Current anti-TB prophylaxis or therapy

Subject has a history of any of the following clinically significant conditions:

9. A history of immune-mediated or clinically significant arthritis
10. Serious reactions to vaccines that preclude receipt of study injections as determined by the investigator
11. Hereditary angioedema, acquired angioedema, or idiopathic forms of angioedema
12. Asthma that is unstable or required emergent care, urgent care, hospitalization or intubation during the past two years or that is expected to require the use of oral or intravenous corticosteroids
13. Diabetes mellitus (type I or II), with the exception of gestational diabetes
14. Idiopathic urticaria within the past year
15. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws
16. Malignancy that is active or history of a malignancy that is likely to recur during the period of the study
17. Seizure in the past 3 years or treatment for a seizure disorder within the last 3 years
18. Asplenia, functional asplenia or any condition resulting in the absence or removal of the spleen
19. Psychiatric condition that may preclude compliance with the protocol; past or present psychoses; or a history of suicide plan or attempt within the five years prior to enrollment
20. Any medical or social condition that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a volunteer's ability to give informed consent

4.2. Study Schedule

The study schedule is presented in the Schedule of Evaluations table in Appendix III.

During or following any visit, if there is any concern about the well-being of the subject, the study site will conduct appropriate medical evaluations by history, physical, laboratory or other indicated testing.

4.2.1. Pre-enrollment

Potential study subjects who verbally agree to discuss their medical history may complete a scripted interview questionnaire by telephone or in person that covers protocol inclusion and exclusion criteria that are based on key self-reported history information to identify potential study volunteers.

4.2.2. Screening Visit(s)

Screening for this study may be completed through a general screening protocol after signing a site-specific IRB-approved consent form to be screened or through screening consent language incorporated into the VRC 704-specific consent form. A screening segment for the study will be included in data collection to provide information on reasons for non-enrollment to begin study injections. Evaluations will be done according to eligibility criteria and clinical assessment at screening. Screening evaluations for specific eligibility criteria must be completed within the time interval specified prior to enrollment, but may be repeated as needed to confirm eligibility. Samples collected during screening may be used for assay validation and site proficiency testing.

The Assessment of Understanding (AoU) is a tool to help staff identify topics which may not be fully understood by study volunteers. As part of the screening process, the AoU should be completed and incorrect answers discussed with the subject. The AoU may be performed one time or more than one time as needed with a given subject. The timing of when to conduct the AoU is adaptable to the practices that work best for each clinical site, however it must be done within the time frame starting after initiation of screening until just prior to or during enrollment (prior to vaccination) on Day 0. Each site is encouraged to develop a site standard among their own staff for consistency of site practices in the conduct of the consent process and deployment of the AoU as part of the consent process. A subject may be re-screened prior to enrollment if there is a suspected change in health status.

4.2.3. Enrollment Visit

VRC 704 enrollment is defined as the day of first study injection and is designated Day 0. Pregnancy test results must be confirmed as negative for women of reproductive potential on day of enrollment prior to the study injection. Protocol-specific eligibility is reviewed as part of the enrollment process, but eligibility evaluations conducted during a prior screening visit within the specified window are routinely used for eligibility. Day 0 evaluations and medical history prior to the first injection are the baseline for subsequent safety assessments, except that for any evaluation not performed on Day 0, the baseline will be the screening evaluation.

4.2.4. Administration of Injections

All Day 0 injections will be administered in a blinded manner according to the randomization assignment. Neither the clinic staff nor the subjects will know whether the injection is VRC-CHKVLP059-00-VP or PBS placebo.

Prior to the first study injection, staff should recheck eligibility to ensure the volunteer is still eligible.

Before <u>each</u> injection, clinic staff must collect research and safety blood and vital signs on the subject.

All injections will be administered IM into the upper arm deltoid muscle by needle and syringe. It is recommended, but not required, to administer the first injection into the non-dominant arm and to alternate arms for the second injection. When choosing an arm for the injection, clinicians should consider whether there is an arm injury, local skin problem or significant tattoo that precludes administering the injection or that will interfere with evaluating the arm after injection.

Following the study injection, the subject will remain in the clinic for observation for at least 15 minutes. After 15 minutes, blood pressure and pulse will be taken and the injection site will be inspected for evidence of local reaction before the subject leaves the clinic. In keeping with good medical practice, acute medical care will be provided to subjects for any immediate reactions to a study injection.

4.2.5. 7-Day Diary Card and Follow-Up

Subjects will be given a "Diary Card" to use as a memory aid, on which to record the solicited signs and symptoms daily for 7 days following each injection. If the solicited parameters are recorded directly by the subject through an electronic database, the subject's electronic record will be the source for these data. The site may use a paper diary card as a source document or clinician notes obtained by telephone interview as the source for the information recorded in the study database. The solicited signs and symptoms on the diary card will include the following parameters: pain/tenderness at injection site, largest diameters of redness (erythema) and swelling (induration at injection site, highest measured temperature per day, unusually tired/feeling unwell, muscles aches (other than at injection site), arthralgia (joint pain), headache, chills, and nausea.

At least one contact at about 7 days after each study injection will be conducted to complete the review of solicited diary card information. Subjects will be asked to contact the clinic at any time following each injection if they have any concerning signs or symptoms. Events that will require clinical evaluation include rash, urticaria, arthralgia, fever of 38.5˚C (Grade 2) or higher lasting greater than 24 hours, or significant impairment in the activities of daily living. Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

4.2.6. Study Visits and Sample Collection

The schedule of visits, permitted windows for completing the visits, and evaluations performed at each visit is shown in the table in Appendix III. After Day 0, deviations from the visit windows in completing study visits are discouraged and will be recorded as protocol deviations, but are permitted, at the discretion of the PI (or designee).

4.3. Evaluation of Chikungunya Infection during the Study

4.3.1. Chikungunya Infection Diagnostic Visit

Throughout the study if a subject reports signs and symptoms of CHIKV infection, blood samples should be collected for diagnostic testing by virus detection (e.g., PCR) by the designated diagnostic laboratories. This may occur during a scheduled study visit or unscheduled visits may be conducted to complete the testing needed. The site will be informed of results as soon as possible whether the subject has a CHIKV diagnosis and should share this information with the subject. To prevent unblinding, the site will remind subjects that all testing

for CHIKV infection should only be conducted as part of the study. Unless directed by the study Sponsor, sites should not attempt to run the CHIKV diagnostic kit that was used to screen subjects for eligibility because vaccine-induced antibodies may confound this test; the diagnostic laboratories will perform the testing according to a specified plan for case identification that will not confuse vaccine-induced antibodies with infection. Sites and the diagnostic laboratories may perform tests for other pathogens of concern. Cases of CHIKV will be recorded on the CHIKV endpoint form. Refer to the Manual of Procedures for instructions on documenting cases. Repeat testing may need to be performed to confirm or to follow the course of an infection.

4.3.2. Clinical Management and Follow-Up of Chikungunya Infection

There is no curative treatment for CHIKV infections. However, VRC 704 study sites will have plans, based on CDC recommendations, by which subjects will have access to clinical care and management of CHIKV infection that can be implemented in parallel to VRC 704. Study sites will document all signs and symptoms of infection and clinical care.

Co-enrollment of a CHIKV-infected study participant in a concurrent study of treatment and management of CHIKV infection is permitted with the approval of the Protocol Chair (or designee). Treatment procedures and co-enrollments in treatment studies will be documented in the study database and available to the Protocol Safety Review Team (PSRT) through study reports. During the course of the study if a subject wants to enroll into other protocols, site staff should seek prior approval from the PSRT.

With the study participant's permission, the results of diagnostic testing and other evaluations conducted as part of the VRC 704 study may be shared with clinicians providing the medical care in order to prevent the need for participants to undergo additional blood draws or repeat testing needed for general health care management. Sites will consider blood draws performed for clinical care or co-enrollment studies when collecting research samples to avoid exceeding allowable blood draw limits.

4.4. Concomitant Medications and Procedures

Current concomitant medications are recorded in the study database at enrollment. Concomitant medications are updated in the study database if there is an occurrence of an adverse event (AE) that requires expedited reporting. If it will not endanger a subject's health, non-investigational vaccines should not be administered 14 days before or until at least 28 days after a study injection. Licensed vaccines received by the subject within 4 weeks before the first study injection administration and 4 weeks after last study injection will be recorded in the study database. Otherwise, a record of concomitant medication changes throughout the study will not be recorded in the study database.

4.5. Criteria for Discontinuing Subject Participation

4.5.1. Discontinuation of Study Injections

A subject may be discontinued from receiving study injections for the following reasons:

- Pregnancy;

- Grade 3 AE assessed as related to a study injection (with the exception that self-limited Grade 3 solicited reactogenicity does not require discontinuation of study injections);
- Grade 4 AE assessed as related to a study injection;
- Immediate hypersensitivity reaction associated with a study injection;
- CHIKV infection or other intercurrent illness that is not expected to resolve prior to the scheduled study injection assessed by study clinician to require withdrawal from the injection schedule;
- Treatment with systemic glucocorticoids (e.g., prednisone or other glucocorticoid) or other immunomodulators (other than nonsteroidal anti-inflammatory drugs [NSAIDs]), with the exception that, study injection may continue per investigator discretion if the next one occurs at least 2 weeks following completion of glucocorticoid treatment;
- The study PI assesses that it is not in the best interest of the subject to continue on the injection schedule.

4.5.2. Discontinuation from Protocol Participation

A subject may be discontinued from protocol participation for the following reasons:

- Subject voluntarily withdraws;
- Subject develops a medical condition that is a contraindication to continuing study participation;
- The Investigational New Drug (IND) sponsor or regulatory authorities stop the protocol;
- The study PI assesses that it is not in the best interest of the subject to continue participation in the study or that the subject's compliance with the study is not sufficient.

4.6. Criteria for Pausing the Study

The IoR for each site and IND sponsor will closely monitor and analyze study data as they become available and will make determinations regarding the presence and severity of AEs. The administration of study injections and new enrollments will be paused and the IND Sponsor will be promptly notified according to the following criteria:

One (or more) subject experience a **SAE** assessed by the IND Sponsor as related to study injection.

Two (or more) subjects experience the same **Grade 4** unsolicited AE assessed by the IND Sponsor as related to the study injection.

Plan for Review of Pauses and Resuming Rules:

The IND Sponsor, with participation by the PSRT, will consult with the DSMB to conduct the review and make the decision to resume or close the study. As part of the pause review, the

reviewers will also advise on whether the study needs to be paused again for any subsequent AEs of the same type.

The study injections and enrollments would resume only if review of the AEs that caused the pause resulted in a recommendation to permit further study injections and study enrollments. When indicated, safety data reports and changes in study status will be submitted to relevant regulatory authorities including the IRB in accordance with Section 5.4 and institutional policy.

5. SAFETY AND ADVERSE EVENTS

5.1. Adverse Events

5.1.1. Adverse Event Definition

An adverse event (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease temporally associated with the use of study treatment, whether or not considered related to the study treatment.

5.1.2. Adverse Event Reporting in the Study Database

The following guidelines will be used to determine whether or not an AE is recorded in the study database.

Each AE will be graded according to the table for grading severity of AEs (see Appendix IV).

Solicited AEs will be recorded in the study database separately with data collection for 7 days after both the first and second study injections as detailed in Section 4.2.4; without the collection of attribution assessments. All unsolicited AEs will be recorded in the study database from receipt of first study injection through 4 weeks after the last study injection administered. At subsequent follow-up visits, only SAEs (as defined in Section 5.2), new chronic medical conditions, and CHIKV cases will be recorded through the last study visit. Laboratory confirmed cases of CHIKV will be recorded on a CHIKV endpoints form rather than on an AE form without an attribution assessment.

5.2. Serious Adverse Events

5.2.1. Serious Adverse Event Definition

The term “Serious Adverse Event” (SAE) is defined in the 21 CFR 312.32 in terms of the following outcomes: Death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias

or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse."

An SAE will be 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 AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

5.2.2. Reporting Serious Adverse Events to the IND Sponsor

AEs that meet SAE Reporting Requirements must be reported and submitted by the clinical site on an expedited basis to the IND Sponsor, VRC, NIAID, NIH, according to sponsor guidelines as follows:

- death
- life-threatening
- results in persistent or significant disability/incapacity
- requires unplanned inpatient hospitalization or prolongation of existing hospitalization
- is a congenital anomaly/birth defect in the offspring of a study subject
- is an important medical event that may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above

In addition, any event, regardless of severity, which in the judgment of a site investigator represents a serious AE, may be reported on an expedited basis.

A site investigator will communicate an initial SAE report within 3 days of site awareness of occurrence to VRC (IND Sponsor) through the communication methods provided by the CRO.

Any SAE entered into the study database will generate automatic email notification to the VRC Medical Officer. This email notification and/or a written report by the study site will be sent to the attention of VRC704team@nih.gov within 3 working days in order for the sponsor to comply with regulations mandating sponsor notification of expedited SAEs to the U.S. FDA.

The investigator must submit additional information as it becomes available. Personal identifying information about the subject will be removed by the site from SAE case reports before submission to the IND Sponsor.

5.2.2.1 Regulations Applicable to the French Study Sites Only

This section applies only to sites in Martinique and Guadeloupe who follow regulatory oversight by the French National Agency for Medicines and Health Products Safety (ANSM).

A site investigator will communicate an initial SAE report within 24 hours of site awareness of occurrence to:

- VRC (IND Sponsor) through the communication methods provided by the IND Sponsor, and

- The legal representative of the sponsor in the European Union (EU) through the communication method provided by him/her.

5.2.3. IND Sponsor Reporting to the FDA

It is the responsibility of the IND Sponsor to make the determination of which SAEs are "serious and unexpected suspected adverse reactions" (SUSARs) as defined in 21 CFR 312.32.

- *Suspected adverse reaction* means any AE for which there is a reasonable possibility that the drug caused the AE.
- *Unexpected Adverse Event* means an AE that is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed.

All SUSARs, as determined by the IND Sponsor, will be reported to FDA as IND Safety Reports; IND Safety Reports will be provided to all participating Investigators by the IND Sponsor or delegated CRO.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

5.3. Reporting to Site IRBs and Relevant Regulatory Agencies

Each site IoR is responsible for reporting AEs, unanticipated problems, protocol deviations and non-compliance to the site IRB and other relevant country regulatory authorities in accordance with their institutional and country requirements for reporting.

Site-specific data reports will be made available through the data management contractor to facilitate expedited and continuing review reporting requirements.

Only the IND Sponsor will submit reports to the U.S. FDA. If the IND Sponsor submits an IND Safety Report to the U.S. FDA, these will be provided to all sites with instruction as to whether or not any actions need to be taken, such as amendment of consent. Investigators must maintain documentation of compliance with actions required for IND safety reports.

5.3.1. Regulations Applicable to the French Study Sites Only

This section applies only to sites in Martinique and Guadeloupe who follow regulatory oversight by the French ANSM.

The sponsor will be responsible for reporting all Serious Adverse Reactions (SARs) to the ANSM and the Ethics Committee (CPP SOOM 4) in accordance with the local laws and regulations requirement (L. 1123-10, R. 1123-38 and following of the Public Health Code). In addition, SUSARs that are reported to the FDA as IND Safety Reports will be reported to the ANSM and the Ethics Committee (CPP SOOM 4) with 7 days or 15 days depending on the severity (according to Article R. 1123-47 of the Public Health Code). The legal representative of the sponsor in the EU will inform the ANSM of VRC 704 study pauses and the decision to resume or close the study following a study pause.

5.4. Data and Safety Monitoring Board

The PSRT (see Section 8.8), will have the primary responsibility for the real-time oversight of safety data and SAE reviews. The NIAID Intramural Data and Safety Monitoring Board

(DSMB) will review cumulative study data twice per year to evaluate safety, study conduct, and scientific validity and integrity of the trial and will be consulted if there is a study pause as required by Section 4.6 of the protocol.

The DSMB members will assess the timeliness, completeness, and accuracy of the data submitted to them for review and whether the data are sufficient for evaluation of the safety and welfare of study participants. The DSMB Executive Secretary will be provided with a sealed copy of the randomization codes needed for the DSMB review of the safety data. The DSMB will also assess the performance of overall study operations and any other relevant issues, as necessary. Following each review, the DSMB will provide its recommendations to the study sponsor, including whether the study should continue without change, be modified, or be terminated.

6. STATISTICAL CONSIDERATIONS AND SAMPLE ANALYSIS

6.1. Overview

This study is a multi-center trial to assess the safety and immunogenicity of a 2-injection schedule of VRC-CHKVLP059-00-VP or PBS placebo injections at Days 0 and 28. The primary objective relates to safety and tolerability; the secondary and exploratory objectives concern immunogenicity and incidence of Chikungunya infection.

6.2. Endpoints

6.2.1. Safety

Assessment of product safety will include clinical observation and monitoring of hematological and chemical parameters. Reactogenicity will be closely monitored for 7 days after injection and safety evaluated by clinical visits through the study duration of 72 weeks.

The following parameters will be assessed:

- Local reactogenicity signs and symptoms for 7 days after each injection
- Systemic reactogenicity signs and symptoms for 7 days after each injection
- Laboratory measures of safety through 4 weeks after last injection
- Adverse events through 4 weeks after last injection
- Serious adverse events and new chronic medical conditions throughout the study
- CHIKV infection events throughout the study

6.2.2. Immunogenicity

The principle immunogenicity endpoints are measured at Week 0 and 8 by neutralization assay. Exploratory immunogenicity endpoints are measured at Week 4, 16, 24, 48, and 72 by neutralization assay, ELISA, and other exploratory research tests.

6.3. Sample Size and Accrual

The study design is to enroll 400 healthy adult participants ages 18 to 60 that reside in CHIKV endemic areas. Randomization will equally allocate subjects to vaccine or placebo schedules with stratification by ages 18-40 and 41-60 years. The enrollment plan does not include provision for replacing subjects with incomplete injections or visit schedules.

Enrollments may occur rapidly at more than one site. The data management CRO will carefully monitor study enrollment and notify all sites and the VRC Protocol Chair when the completion of enrollment is near.

The sample size for this study was not selected to test a formal null hypothesis. It reflects the number of vaccine doses that are available for the study and the desire to gain maximal information for the conduct of possible Phase 3 follow on trials. This latter consideration is particularly important as reliable estimates of CHIKV incidence in the target population, vaccine effects and logistical considerations in implementing future trials must be understood prior to a Phase III trial. Nevertheless, power computations are provided below as an indication of the probability of observing results of interest.

There are not good data to estimate the loss to follow-up rate for this study. The power calculations assume a 10% loss in both groups for the primary and secondary endpoints. Loss to follow-up for the exploratory outcomes are likely to be larger but are not included as power calculations are not included for these outcomes.

6.3.1. Power Calculations for Evaluation of Safety

The goal of the safety evaluation for this study is to identify safety concerns associated with injections of the investigational vaccine. Two sample size calculations for safety are expressed in terms of two approaches, within group and comparing two groups.

1. The ability to detect safety or reactogenicity events within each injection group:

The ability of the study to identify safety events may be expressed in terms of the probability of observing one or more event of interest (e.g., AEs) assuming a range of "true" but unknown event rates. The power to observe one or more event in a single group of 180 subjects is 83.62% when the true rate is 1.0% and 97.37% when the true rate is 2.0% (Table 3).

Table 3: Probability of Observing One or More Safety Events within a Group (n=180)

True Event Rate	Pr (1 or more events)
0.01%	1.78%
0.10%	16.48%
0.33%	45.17%
0.50%	59.43%
1.00%	83.62%
2.00%	97.37%

1. The minimum detectable difference in safety event (e.g., AEs) rates for comparing two injection groups using Fisher's exact test:

Safety event rates may vary considerably with the type of event considered therefore a wide range of possible rates is considered for comparison by vaccine group (Table 4).

Table 4: Minimum Detectable Differences in Event Rates for Power = 80% or 90% Assuming Type I Two-Tail Error Rate of 5% (n=180 per Group)

Power	Assumed Event Rate in Group 1 (n=180)	Minimum Detectable Event Rate Group 2 (n=180)	Difference in Rates
80%	0.1%	5.5%	5.4%
	1.0%	7.5%	6.5%
	5.0%	14.2%	9.2%
	25%	39.3%	14.3%
90%	0.1%	6.9%	6.8%
	1.0%	8.9%	7.9%
	5.0%	16.0%	11.0%
	25%	41.6%	16.6%

6.3.2. Power Calculations for Evaluation of Immune Responses

A secondary objective is to evaluate the humoral immunogenicity of VRC-CHKVLP059-00-VP by neutralization assay at 4 weeks after the final 20 mcg dose (Week 8). Vaccine specific neutralization antibodies are not anticipated in the non-vaccinated group. Therefore, direct comparison between the active and placebo group is of minimal value in assessing sample size. Preliminary Phase 1 neutralization data suggest that neutralization results at the visit with peak response are log-normally distributed with a mean and standard deviation on the log-scale of 7.41 and 0.67 respectively. This standard deviation is rounded up to 0.70 and two more conservative options of 1.0 and 1.5 result in 95% confidence intervals (CI) for GMT (Table 5).

Table 5: 95% Confidence Interval for GMT Neutralization among Vaccinees at Time of Peak Response (n=180 per Group)

Assume Standard Deviation on Log Scale	95% CI for GMT Neutralization Among Vaccinees (n=180) at Time of Peak Response
0.70	(1490.7, 1831.7)
1.0	(1426.6, 1914.1)
1.5	(1324.8, 2061.1)

The study is not designed to compare two groups defined by baseline covariates such as gender or age strata. However, the following table is included to provide a sense of the power available for such comparisons. The simple case of two groups (e.g., male and female), assuming equal participation (i.e., 90 subjects for each gender-vaccine group), is given in Table 6 for comparing response rates.

Table 6: Detectable Geometric Mean Ratio between Two Groups for Power = 80% or 90% Assuming Type I Two-Tail Error Rate of 5% (n=90 per Group)

Power	Standard Deviation	Detectable

	on Log Scale	Geometric Mean Ratio (n=90 per group)
80%	0.7	1.34
	1.0	1.52
	1.5	1.88
90%	0.7	1.40
	1.0	1.63
	1.5	2.08

6.4. Statistical Analysis

Study enrollment is defined in this protocol as being randomized and receiving the first study injection. All enrolled subjects will receive at least one injection and therefore will provide some safety data. The safety population will include all enrolled subjects, summarized according to the actual vaccination received.

The Intent-to-treat (ITT) population will include all enrolled subjects, analyzed according to the randomized vaccine. The Per Protocol (PP) analyses will include all subjects receiving two vaccinations within window as assigned by the randomization schedule, and not experiencing any other major protocol deviations prior to their Week 8visit. The PP analyses will also include subjects determined to be CHIKV infected prior to the receipt of their scheduled second vaccination and not experiencing any other major protocol deviations prior to their sate of infection. Subjects will be censored from the immunogenicity analysis following CHIKV infection. The Modified intent-to-treat (mITT) analyses will include all enrolled subjects with evaluable immunogenicity data at their week 8 visit, without exclusions for protocol deviations or censoring for CHIKV infection. Additional cohorts may be defined in the Statistical Analysis Plan (SAP).

No formal multiple comparison adjustments will be employed. Imputation of missing values is not planned. They may be implemented for secondary or tertiary outcomes if supported by the data and if sufficient consequence to justify their inclusion. Analysis variables include baseline, safety, reactogenicity, and immunogenicity variables for primary and secondary objective analyses, respectively. Exploratory analyses will estimate CHIKV infection rates, and evaluate additional immunogenicity assays.

In general, descriptive statistics by vaccine group will be tabulated for all variables of interest, stratified by age as is appropriate. This will include point estimates (mean, geometric mean, median or proportions) and their respective 95% CI or percentile spread. Formal comparisons will use standard methods, contingency tables for categorical variables, t-tests or non-parametric analogs for comparing means or geometric means, logistic regression and analysis of covariance for model discrete and continuous variables respectively.

6.4.1. Baseline Demographics

Baseline characteristics including demographics and laboratory measurements will be summarized by vaccine group and stratum.

6.4.2. Safety Analysis

Reactogenicity: The number and percentage of subjects experiencing each type of reactogenicity sign or symptom will be tabulated by severity. For a given sign or symptom, each subject's reactogenicity will be counted once under the maximum severity for all assessments.

Adverse Events: AEs will be coded into Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. The number and percentage of subjects experiencing each specific AE will be tabulated by severity and relationship to treatment. For the calculations in these tables, each subject's AE will be counted once under the maximum severity or strongest recorded causal relationship to treatment.

A complete listing of AEs for each subject will provide details including severity, relationship to treatment, onset, duration and outcome.

Safety Laboratory Values: Safety laboratory values will be summarized using shift tables to compare baseline and follow-up values. Shift tables will be analyzed using Generalize Estimating Equations to account for the longitudinal nature of these data. The mean change from baseline along with 95% confidence interval at each time point measured in the study will be computed. Boxplots of safety laboratory values will be generated for baseline values and for values measured during the course of the study. Each boxplot will show the 1st quartile, the median, and the 3rd quartile, with values smaller than the 1st quartile or larger than the 3rd quartiles plotted as outliers. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

6.4.3. Analysis of Immune Responses

Immunogenicity will be analyzed for a variety of cohorts defined above, and specified in the SAP. At minimum these may include ITT, PP, and mITT cohorts. Assay results will be examined to determine if their distributions should be analyzed as normal, lognormal, nominal or categorical. It may also be appropriate to dichotomize the data into "responders" and" non-responders". Immunogenicity data will be analyzed at each time point where such data is collected. Longitudinal methods will be used to estimate the nature and extent of immune response decay over time.

6.4.4. Analysis of Chikungunya Incidence

Incidence rates for confirmed CHIKV cases will be described by vaccine group by age stratum and aggregating and by combining age strata. Results will be treated both as dichotomous (infection, no infection) and as a time to event (infection) variables. Time to event will be based on the date of blood sample with the first positive laboratory test indicating CHIKV infection. For each approach, infection rates in each treatment arm and vaccine efficacy along with their corresponding 95% CI will be computed. Efficacy for dichotomized data will be computed by a simple conversion of the relative risk to efficacy. Time to infection data, accounting for censoring, will be described using Kaplan Meir curves and efficacy computed using a Cox proportional hazards model.

Analyses of CHIKV infections are exploratory. A number of definitions of incident cases and therefore efficacy will be considered. Given the exploratory nature of these analyses, no order of primacy will be defined.

Efficacy will include all cases using 2 exploratory alternatives for case inclusion:

1. all infections post vaccination 1 (Day 0),
2. all infections post vaccination 2,

Subjects not receiving vaccination 2 will be included in definition 1. The SAP will provide any necessary additional specificity and/or cohort definitions such as discussed at the beginning of section 6.4.

6.4.5. Interim Analyses

Independent Safety Reviews: The PSRT will review safety data routinely throughout the study. The study will utilize both electronic database features and reviews by designated safety review personnel to identify in a timely manner if any of the safety pause rules of the study are met. The NIAID Intramural DSMB will provide an independent safety review at scheduled intervals to coincide with their biannual meeting schedule.

In addition, the two study groups will be compared for safety, immune responses and incidence rates at 6 and 12 months after the study is closed to accrual. All subjects are expected to be enrolled by 8 months after the study opens to accrual. These analyses will not be used for early trial termination. The 6 and 12 months reviews will be used to obtain critical data to be used in the event that circumstances external to the trial require a rapid initiation of a follow-up study. Therefore no p-value adjustments will be made for interim data analyses.

Immunogenicity Review: An interim analysis of immunogenicity data will be performed after all assays up to and including Week 8 have been completed on all participants. Reports will be provided to the PI and other key investigators solely for the purpose of informing future trial-related decisions in a timely manner. The results will remain confidential and should in no way influence the conduct of the VRC 704 trial in terms of early termination or later safety or immunogenicity endpoint assessments.

6.4.6. Randomization of Treatment Assignments and Unblinding Criteria

Randomizations will be done online using an electronic randomization system. The randomization code will be prepared by the Protocol Statistician and included in the enrollment module for the trial. The randomization code will link to the treatment assignment. To decrease the potential for subject dropouts during the period between randomization and initial injection, the electronic data system will assign each subject a randomization code after the eligibility to begin Day 0 study injections has been entered into the system.

For each clinical site, randomization will be 1:1 allocation within each stratum to either the CHIKV VLP vaccine or PBS placebo. Manual back-up procedures and instructions will be provided for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable to a study site.

If an individual is screened but does not enroll into the clinical trial, screening records will be kept to document the reason the individual did not enroll.

The injections will be prepared by an unblinded site pharmacist or otherwise qualified personnel who will not be involved in any subject assessments and who will not discuss randomizations with study clinicians. The subjects, the study personnel who perform study injections and

assessments, data entry personnel at the sites, and laboratory personnel performing immunologic assays will be blinded to the treatment assignment of all injections. Subject study injection assignments will be provided to the site IoR at completion of the study for communication to the study participant. The DSMB may receive data in aggregate and presented by treatment group, but without the treatment group identified. The DSMB may be unblinded to individual study treatment assignments, as needed, to adequately assess safety issues.

If necessary, the IoR (or designee) and Protocol Chair may agree that management of an AE requires emergency unblinding of an individual subject's assignment. A designated individual at each site will be provided with a code list for emergency unblinding purposes, which will be kept in a secure place. This will be documented as a protocol deviation and the Protocol Statistician, the site IRB, and the DSMB will be notified that an early unblinding has occurred and provided with a statement explaining the medical necessity for the early unblinding.

7. PHARMACY AND INJECTION ADMINISTRATION PROCEDURES

7.1. Study Agents

This study includes one investigational vaccine and one placebo for the investigational vaccine, as follows:

- VRC-CHIKVLP059-00-VP (CHIKV VLP vaccine), 40±10 mcg/mL in a formulation buffer filled to a volume of 0.8 mL vaccine in a 3 mL glass vial. Vials contain a clear to slightly hazy solution with some small white translucent particles that may be visible, which is an isotonic, sterile solution.
- VRC-PBSPLA043-00-VP, phosphate buffered saline (PBS) as placebo control for the CHIKV VLP vaccine filled to a volume of 1.2 mL in a 3 mL glass vial. Vials contain a clear colorless isotonic sterile solution at pH 7.2.

The CHIKV VLP vaccine and PBS are prepared under good manufacturing practice by the VRC VPP and must meet lot release specifications prior to administration for use in the clinical study. The investigational study agents may only be shipped to the site pharmacy when the protocol is IRB-approved at the study site. Vials are shipped using appropriate shipping configurations.

7.2. Study Agent Presentation, Stability and Storage

7.2.1. Labels

At the time of delivery of the study agent to the pharmacy, the labels for study agents VRC-CHIKVLP059-00-VP and VRC-PBSPLA043-00-VP will have specific product information (e.g., part number, lot number, fill volume, storage temperature) included on the product vial labels. The labels will contain an Investigational Use Statement ("Caution: New Drug – Limited by Federal Law to Investigational Use") and manufacturer information.

7.2.2. Storage

VRC-CHKVLP059-00-VP: Vials of vaccine are stored until use at -45°C to -10ºC in a qualified, continuously monitored, temperature-controlled freezer. Temperature excursions that are outside of the normal allowance for the storage device will be reported per pharmacy guidelines. The excursion must be evaluated and investigated and action must be taken to restore and maintain the desired temperature limits. Pending the outcome of the investigation, the IND sponsor will notify the pharmacist if continued clinical use of the product is acceptable. Vials of vaccine are intended for single use only and should not be refrozen after thawing.

VRC-PBSPLA043-00-VP: Vials of PBS are stored until use at -45°C to -10ºC in a qualified, continuously monitored, temperature-controlled freezer. Vials are intended for single use only, and thus do not contain a preservative. They should not be refrozen after thawing.

7.3. Preparation of Study Agent for Injection

Refer to the group assignment for the study subject to select the proper vial type. The pharmacy will label the syringe prior to delivery to the clinic with the subject identifier and the date and time allowance for administration; the label on the syringe will not include the product type information. The injection must be administered within 8 hours after removing the vial from the freezer.

7.3.1. Preparation of VRC-CHKVLP059-00-VP

Stability study conducted with VRC-CHKVLP059-00-VP show that the vaccine is stable in the syringe for 8 hours refrigerated or at room temperature. The following instructions apply to aseptic preparation of the vaccine for injection:

1. Thaw the vial containing VRC-CHKVLP059-00-VP at ambient temperature (15-25ºC).
2. Keep the material at room temperature or refrigerated during the entire preparation period until administration.
3. Keep the prepared injection out of direct sunlight before administration.

Preparation will be done in a clean preparation unit with limited access. The intended volume to administer is 0.5 mL. Draw up vaccine into the syringe. Remove air bubbles from the syringe and cap it.

7.3.2. Preparation of VRC-PBSPLA043-00-VP

Remove a vial of VRC-PBSPLA043-00-VP from the freezer and thaw at ambient temperature (15-25ºC). The intended volume to administer is 0.5 mL. Draw up VRC-PBSPLA043-00-VP into the syringe. Remove air bubbles from the syringe and cap it.

7.4. Study Agent Administration

The injection must be administered within the time allowance on the syringe label using standard IM injection technique. Each study injection is 0.5 mL in volume administered intramuscularly into the deltoid muscle by needle and syringe.

7.5. Study Agent Accountability

7.5.1. Documentation

Each study site will be responsible for maintaining an accurate record of the codes, inventory, and an accountability record of the investigational study agent supplies for this study at their site. Electronic documentation as well as paper copies will be used.

7.5.2. Disposition

The empty vials and the unused portion of a vial will be discarded in a biohazard containment bag that will be incinerated or autoclaved. Any unopened vials that remain at the end of the study will be discarded at the discretion of the VRC in accordance with policies that apply to investigational agents. Partially used vials or expired prepared doses cannot be administered to other subjects nor used for *in vitro* experimental studies and will be discarded as indicated above.

8. HUMAN SUBJECTS PROTECTION

This research will be conducted in compliance with the protocol, Good Clinical Practices (GCP) guidance, and all applicable regulatory requirements.

8.1. Institutional Review Board

A copy of the protocol, proposed informed consent and any proposed advertising material will be submitted to the site IRB for review and approval.

The Site IoR will submit and, where necessary, obtain approval from the IRB for subsequent protocol amendments and changes to the informed consent document. The Site IoR is responsible for ensuring proper IRB notification of deviations from the protocol or serious AEs occurring at the site and other AE reports received from the VRC, NIAID, in accordance with the protocol and local IRB policies. The IoR will be responsible for obtaining annual IRB approval/renewal throughout the duration of the protocol. Documentation of the IRB approval and FWA number will be provided for the Sponsor's records.

8.2. Subject Recruitment and Enrollment

Subjects for this study will be recruited by the sites in accordance with their site IRB standard for recruitment practices.

8.2.1. Participation of Children

Children are not eligible to participate in this clinical trial because this study is designed for study product evaluation in adults. If the product is assessed as safe and immunogenic, other protocols designed for children may be conducted in the future.

8.3. Informed Consent

The provided template informed consent (Appendix I**)** will be used to guide development of the site-specific consent forms. Only an IRB-approved consent form will be used to consent subjects for participation in the study. The changes in the informed consent template by the site

should be approved with the VRC Program Officer before submission to respective IRB. Study sites needing to conduct the informed consent process in more than one language will follow the practices required by the site's IRB for translation and approval of the consent(s) and provide to the IND sponsor all translations of documents used for consent. Before a subject's participation in the protocol, it is the investigator's responsibility to ensure that written informed consent is obtained from the subject after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the protocol.

The acquisition of informed consent should be documented in the subject's records, as required by 45 CFR 46.117, and the informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion. An original signed informed consent form should be retained by the site and a signed copy of the consent form should be provided to the subject.

8.4. Subject Confidentiality

The investigators at each site must ensure that the subject's anonymity is maintained in reporting study results. Subjects will not be identified in any reports of this study. All records will be kept confidential to the extent provided by federal, state and local law. Medical records will be made available for review when required by authorized agencies and regulatory authorities only under the guidelines set by the U.S. Federal Privacy Act and by relevant country-specific regulatory authorities. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The authorized reviewers will review study-related records without violating the confidentiality of the subjects. Stored study research samples will be labeled by a code (such as a number) that only the site clinical study team can link to the subject. The requirement to maintain subject confidentiality and inform subjects about review of study-related records is included in the study informed consent documents.

8.5. Risks and Benefits

8.5.1. Risks of Blood Collections:

The blood collection procedures are common in routine medical practice. The risks of blood sample collection are minimal and consist of mild discomfort at the sample collection site. The procedure may cause mild pain, bruising, fainting, and, rarely, infection at the site where the blood is taken.

8.5.2. Risks of the CHIKV VLP Vaccine:

In VRC 311, the first human trial in which the CHIKV VLP vaccine was administered to 25 subjects, the local and systemic reactogenicity was no more than mild and none of the subjects reported fever. It is possible that once administered to a larger number of subjects, other potential side effects from injection may be observed similar to those seen with other vaccines. Although not previously observed with the CHIKV VLP vaccine, this includes the possibility of anaphylaxis. With vaccines in general, local reactogenicity may include pain, tenderness, redness or induration of the skin at the injection site and systemic reactogenicity may include fever, chills, muscle aches, joint aches, nausea, headache, and fatigue. These side effects will be monitored, are expected to be short term and may or may not require treatment.

In VRC 311, seven laboratory test AEs (occurring in four participants) were assessed as related to study vaccine based on temporal relationship to vaccination. The AEs included four mild transient alanine aminotransferase increases and two mild and one moderate transient neutropenia. All resolved without clinical sequelae. There was no placebo comparison group in the VRC 311 study. The comparison of AEs in placebo and vaccine recipients in the VRC 704 study should provide a more detailed safety profile for the study vaccine.

8.5.3. Other Risks:

There should be no unusual risks associated with the CHIKV VLP vaccine. The effect of this vaccine on an unborn fetus is unknown. Female subjects of child bearing potential who are heterosexually active will be required to agree to use birth control beginning 21 days prior to enrollment and continuing through 12 weeks after last study injection. Women who are pregnant will be excluded from the study. Children will not be enrolled in this study.

8.5.4. Benefits:

Study participants may have no direct benefit from participation in this study but society may benefit from knowledge gained from research on the donated specimens. This protocol is not designed to provide treatment for any condition.

8.6. Plan for Use and Storage of Biological Samples

To be eligible for this protocol, subjects must be willing to allow stored specimens to be used in the future for studying infectious diseases, immune function, vaccine responses and other medical conditions, and must also be willing to have genetic tests, including HLA typing performed. If tests performed at a study site show evidence of any acute or chronic condition, subjects will be informed of the results and advised to seek appropriate medical care for the condition. In general, testing performed at a research laboratory is not for diagnostic purposes and results will not be available to the study site or study subject.

Intended Use of the Samples/Specimens/Data:

Samples, specimens and data collected under this protocol may be used to study infectious diseases such as influenza, immune function, vaccine responses, genetic factors in immune responses, other medical conditions and for research assay validation.

Storage of Samples, Specimens and Data from Samples:

All of the stored study research samples will be labeled by a code (such as a number) that only the study site can link to the subject. Samples will be stored in secure facilities with controlled access at the sites. The NIAID Vaccine Immune T-Cell and Antibody Laboratory (NVITAL) in Gaithersburg, MD, under the direction of the VRC, NIAID, NIH (Bethesda, MD) will serve as a central repository for stored samples. Samples collected for research may be transferred for testing to the VRC/NIAID/NIH, other approved collaborators or contract laboratories. Data will be kept secure. Only approved investigators or their designees will have access to samples and data.

In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. Regulatory approval through the proper human subjects protection agency will be sought prior to any sharing of samples that constitutes human subject research. The research use

of stored, unlinked or unidentified samples may be exempt from the need for IRB review and approval. When appropriate, exemption may be obtained through the proper regulatory procedures.

8.7. Compensation

Compensation for time and inconvenience of study participation will be provided to subjects in accordance with the site-specific IRB approved plan.

8.8. Safety Monitoring

8.8.1. Protocol Safety Review Team

Each site IoR is responsible for ensuring daily review of the site's clinical safety data as it becomes available. The PSRT includes the Protocol Chair, IND Sponsor Medical Officer, and each site IoR or designee. The PSRT will review the summary study safety data reports weekly from initiation of the study until 4 weeks after all subjects have received their last study injection and then monthly until 4 weeks after all subjects have completed the last study visit in order to be certain that the investigational vaccine has an acceptable safety profile. The PSRT will be notified and convened to review any study pauses. The Protocol Chair and IND Sponsor Medical Officer will continue to monitor the cumulative study safety data reports on at least a monthly basis through completion of the last study visit.

8.8.2. Data and Safety Monitoring Board

As described in Section 5.4, the DSMB will review safety data twice per year at their regularly scheduled meetings and will have access to the randomization code.

9. ADMINISTRATION AND LEGAL OBLIGATIONS

9.1. Protocol Initiation, Amendments and Termination

Each site must receive IRB approval, approval from local country regulatory agencies, as needed, and approval of study sponsor before initiating the study at the site. All amendments will also be submitted to the site IRBs and other regulatory authorities as needed for approval. The VRC, NIAID, NIH, the U.S. FDA and other regulatory authorities reserve the right to terminate the study. Each IoR will notify the respective site IRB of the study termination in writing and provide documentation to the IND Sponsor.

9.2. Study Documentation and Study Records Retention

The site IoR will maintain a list of appropriately qualified persons to whom trial duties have been delegated. The site IoR is responsible for ensuring that staff maintains a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from the VRC, IRB, designated CRO monitor and/or applicable regulatory authorities. Elements include but are not limited to:

- Subject files containing completed informed consent forms and supporting copies of source documentation

- Study files containing the protocol with all amendments, the Investigator's Brochure, and copies of all correspondence with the IRB

In addition, all original source documentation must be maintained and readily available. The CRO is responsible for ensuring that records and documents pertaining to the conduct of this study, including CRFs, source documents, consent forms, laboratory test results, and medication inventory records, are managed per U.S. requirements. According to the U.S. FDA, study records must be retained for 2 years after the marketing application is approved for the drug; or, if an application is not approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued. Therefore, VRC, NIAID may authorize transfer or destruction of study records. No study records will be destroyed without prior authorization from VRC, NIAID.

9.3. Data Collection and Protocol Monitoring

9.3.1. Data Capture Methods

Clinical research data will be collected and recorded by the study sites in a timely fashion in a secure electronic web-based clinical data management system. Immunological testing on collected, coded blood samples may be performed in batches at central laboratories. Extracted data without subject identifiers will be sent to the statisticians for statistical analysis as needed. The final study database and statistical evaluations will be transferred to the VRC, NIAID at the study completion.

9.3.2. Source Documents and Access to Source Data/Documents

Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH-GCP, regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in the NIAID-sponsored study, each site will permit authorized representatives of the VRC, NIAID, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, medical records, laboratory reports, pharmacy records and other research records maintained for the clinical trial.

9.3.3. Protocol Monitoring

VRC, NIAID, NIH, as the IND Sponsor, or their authorized representatives are responsible for ensuring study data integrity and compliance with the protocol. Routine data monitoring and protocol compliance will be performed by the site investigators and study coordinator on an ongoing basis in accordance with the Sponsor's monitoring plan. Site investigators will allow the study monitors and the U.S. FDA (if requested) to inspect study documents or pertinent clinic records for confirmation of study data.

9.4. Language

All written information and other material to be used by subjects and investigative staff must use vocabulary and language that are readily understood.

9.5. Policy Regarding Research-Related Injuries

The study site will provide immediate medical care for any injury resulting from participation in this research. In general, the VRC, the NIH, or the Federal Government will not provide long-term medical care or financial compensation for research-related injuries.

9.6. Multi-Site Management

VRC, NIAID, NIH, as the IND Sponsor for the study, is responsible for overall management of study. Assistance in managing the study is being provided by specific Contract Research Organizations (CROs).

Each study site that enrolls study participants will have a site Investigator of Record (IoR), also referred to as Principal Investigator. An IoR is defined as an individual who is responsible for the conduct of the clinical investigation at a study site and under whose direction the test article is administered to subjects. The site IoRs have parallel roles at their respective institutions in conducting the study at their site in compliance with all applicable regulations and good clinical practices.

Publication of any study related information is governed by VRC, NIAID, NIH policies. Specifically, neither the CROs nor site personnel may submit for public presentation any meeting abstract or manuscripts without prior review and approval by VRC, NIAID, NIH.

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APPENDIX I: TEMPLATE INFORMED CONSENT FORMS

The sample informed consent forms are provided to guide development of a site-specific consent form. Only IRB-approved consent forms will be used to consent subjects for study participation.

Template Informed Consent Form for Study Participation

STUDY TITLE: VRC 704: Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

INTRODUCTION

We invite you to take part in a research study at the _____.

The study is sponsored by the National Institutes of Health (NIH) in Bethesda, MD, USA. You can decide if you want to participate in this study or not. There is no penalty or loss of benefits for choosing not to participate. Please ask questions and discuss this study with anyone you want. Take as much time as you need to decide.

[SCREENING (delete this section if site has separate screening protocol or separate screening consent)]

Before you can enroll in the investigational Chikungunya virus (CHIKV) vaccine study, you will be screened for eligibility. You will need to sign this consent form before we can do the screening.

Screening involves a physical exam and blood tests to check your general health status. If you are a woman, you will be asked about your birth control use and the possibility of your becoming pregnant while in the study. You will be tested for pregnancy if applicable. During screening, we may collect some blood to store for research. We will ask you about your general health history. You will have bloods test to check for past CHIKV infection and for HIV infection. We will ask you about medications you are taking and recent vaccinations.

We will review the screening results with you and tell you if the results show that you are not eligible to join the study. You cannot be in another research study where you receive a study product and also enroll in this study unless you discuss this and receive approval from the study clinicians.

PURPOSE OF THE VACCINE STUDY

This is a research study of an experimental vaccine. It is called the Chikungunya Virus-Like Particle vaccine or "CHIKV VLP" vaccine. The U.S. Food and Drug Administration (FDA) allows it to be used for research only. It is not known if the vaccine works. The main purpose of this study is to see if the experimental vaccine is safe, if it causes any side effects, and if people who get the vaccine make an immune response. Study sites are in areas where CHIKV outbreaks have occurred. The study will last for about 72 weeks.

CHIKV was first identified in Tanzania, Africa in 1952. CHIKV outbreaks were most common in Africa, India and Asia until 2013, when the first cases were reported in the Americas. The virus is spread by mosquitoes and causes a viral infection that can lead to fever, headache, muscle pain, tiredness, and joint inflammation or pain. The disease usually does not cause death; but the joint inflammation may be severe and may last for many months. There is currently no cure for CHIKV infection or vaccine to prevent CHIKV infection.

You are eligible to participate in this study if:

- you have completed the screening process,
- you are between 18 and 60 years old,
- you have physical exam and blood test results that meet eligibility requirements, and
- you do not have any significant medical problems as determined by your screening.

STUDY INJECTIONS

Vaccines are substances used to try to create resistance (or immunity) to a disease. About 400 people will participate in this study. About half of the participants will get two injections of the study vaccine and half will get two injections of an inactive solution (or placebo).

CHIKV VLP Vaccine: The vaccine was developed by the Vaccine Research Center (VRC) at the U.S. National Institutes of Health (NIH). It was made at the VRC Vaccine Pilot Plant in Frederick, Maryland, USA. The vaccine is made of some CHIKV proteins that combine to make a particle that looks like the outside of the virus. The body's immune system may respond to this particle. There is no virus in the vaccine so you cannot get CHIKV infection from the vaccine.

Placebo Injection: The placebo in this study is a sterile salt water solution made for injection into people. It has no vaccine in it.

STUDY PROCEDURES

If you agree to take part in the study, you will be randomly assigned (by chance, like flipping a coin) to one of two groups. Neither you nor the clinic staff will know what product you are receiving until the study is complete. You will receive two injections during the study. Half the people in this study will get the investigational CHIKV VLP vaccine and the other half will get a placebo injection on the day of enrollment. Four weeks later, you will get a second injection of the same product that you already got. We will give all study injections with a needle and syringe into your upper arm muscle. If you are a woman who is able to get pregnant, we will do a pregnancy test before each injection. The test must show that you are not pregnant before a study injection is given.

The clinic staff will observe you for at least 15 minutes after each injection. We will ask you to complete a diary card for 7 days. For the diary card, you will need to record your temperature and any symptoms you feel. You will also need to look at the injection site on your arm each day and record how it looks. The clinic staff is available to you by phone 24 hours a day to report any unexpected side effects. If you experience any concerning signs or symptoms after each injection, please contact the clinic. We will ask you to review your diary card with us about a week after each injection.

If you have any signs of sickness that interfere with your usual activities, you may need to come to the clinic for an examination before your next scheduled visit. It is very important that you follow the instructions we give you.

Your participation in the study will last for about 16 months after the first injection. You will have about 11 planned clinic visits and 2 telephone contacts. Each clinic visit will last about [1-2] hours [sites may edit length of visit per clinic standard]. Experimental vaccine studies follow a set schedule. Scheduling for your visits allows some flexibility, but it is important that you work with the staff to follow the schedule. You should try to not miss any visits.

At each visit, we will check you for any health changes or problems. We will ask how you are feeling and if you have taken any medications. At scheduled study visits, we will draw about 5-12 tubes of blood from you, depending on the visit. We may ask for urine samples. If any of your test results show a health problem, we will tell you about it as soon as possible. You might need to have extra clinic visits and laboratory tests if you have health changes that we need to check.

At any time during the study, if you think you have CHIKV infection, you must contact the study site and come in for a clinical evaluation and blood tests. We will tell you the results of testing for CHIKV infection but it may take several weeks to learn the results. It is very important for the study that you get tested for CHIKV infection at our clinic **only** and that you do not go anywhere else for CHIKV testing. There is no medication that makes CHIKV infection end faster than naturally occurs. We will tell you if you need testing for any other infections that have symptoms similar to CHIKV infection.

MONITORING OF THE STUDY

This study will be monitored by a group of physicians and scientists associated with the U.S. NIH. This group will review the study information and will pay close attention to harmful reactions.

GENETIC TESTING

Some of the blood drawn from you as part of this study will be used for genetic tests. There are many different types of genetic tests. In vaccine research, some genetic tests are done to see if immune responses to a vaccine are different in people because of genetic differences. These tests will be done in a research lab using your blood samples. Genetic tests done in a research lab will **not** be in your medical record. The samples used in these tests will **not** have your name them. In the future, genetic research tests to help understand how vaccines work may be done on your DNA using stored samples.

STORED SAMPLES

To participate in this study, you must allow us to store your blood samples for future research. We will use some of your blood samples to study your immune response to the injection. We will also use these samples for future research to learn more about CHIKV infections, vaccines, the immune system, and/or other medical conditions. Results of research tests done from stored samples will not be in your medical record or reported to you.

Labeling of Stored Samples

Your stored samples will be labeled by a code (such as a number) that only the study team can link to you. Any identifying information about you will be kept confidential to the extent

permitted by law. Despite protections, there is a small chance that information identifying you will be given to someone who should not get it.

Future Studies

In the future, other investigators at NIH or outside of NIH may wish to study your stored samples. When the study team shares your stored samples, they will be marked with a code, but will not have any identifying information on them. Some information about you, such as your gender, age, health history, or ethnicity may also be shared with other investigators. Any future research studies using your samples will be conducted in a way that protects the rights and privacy of study participants.

Your stored samples will be used only for research and will not be sold. The research done with your samples may be used to develop new products in the future but you will not receive payment for such products.

POSSIBLE STUDY RISKS

Possible **risks from the injections:** temporary stinging, pain, redness, soreness, itchiness, swelling, bruising, or a cut in the arm. There is a very small chance of infection.

Possible **risks of blood drawing:** pain, bleeding, bruising, feeling lightheaded, or fainting.

Possible **risks from genetic testing:** unintended release of information that could be used by insurers or employers; discovering a gene or HLA type that suggests risk of disease for you or your family; discovering undisclosed family relationships.

Possible **risks from any vaccine:** fever, chills, rash, aches and pains, nausea, headache, dizziness, and fatigue. Some people have allergic reactions to vaccines. These types of reactions are usually greatest within the first 24 hours after injection and typically last 1 to 3 days. Over-the-counter medicine, such as acetaminophen, will generally help relieve symptoms from injection and may be used.

Possible **risks from the CHIKV VLP vaccine:** This will be the second study of this CHIKV VLP vaccine. In the past study, 25 people received the CHIKV VLP vaccine. The most common complaints were mild pain or soreness at the injection site. About half of the people had mild symptoms like headache, nausea and feeling tired or unwell. A few people had a temporary change in a laboratory test. The vaccine does not contain any CHIKV virus. The vaccine will **not** give you CHIKV infection.

The first 25 people to receive the CHIKV VLP vaccine did **not** have any fever or joint pain after vaccination. However, there may be side effects from the study vaccine that are not common which we do not yet know about. Please tell the study staff about any side effect you think you are having. This is important for your safety.

Possible **risks during Pregnancy:** We do not know if getting the study vaccine affects a fetus. Therefore, women who can have children must use effective birth control starting at least 21 days before getting the first injection until 12 weeks after the last study injection. We will discuss effective birth control methods with you. If you are pregnant or want to become pregnant in the next 16 weeks, you cannot participate. We will ask about the outcome of any pregnancy that begins during the first 16 weeks on study.

Other Risks: It is unknown if the study injection will affect how you respond to any future CHIKV infection or to any other CHIKV vaccines that you may get in the future. We will tell you if we learn about any important new findings or serious side effects of the vaccine during the study that may affect your willingness to continue participation.

You may not donate blood while participating in an investigational vaccine study and for one year after the date of the last injection of an investigational vaccine.

POSSIBLE BENEFITS

This study is not designed to benefit you or protect you from CHIKV infection. You and others may benefit in the future from the information that we learn from the study.

COSTS OF PARTICIPATION

There are no costs to you for participating in this study. You or your health insurance will have to pay for all medical costs for medical care you receive outside this study. It is possible that you may have some expenses that are not covered by the study compensation provided.

COMPENSATION TO YOU FOR YOUR PARTICIPATION

You will be compensated [insert site IRB-approved amount] for your study participation as follows: [insert site plan]. This will be based on the number of study visits you attend and study injections you receive.

REASONS FOR REMOVING YOU FROM THE STUDY WITHOUT YOUR CONSENT

The study doctor can take you out of this study without your permission *if:*

- continuing in the study could harm you,
- you do not follow study instructions or keep appointments, or
- the study is stopped by the U.S. NIH, regulatory boards, the U.S. FDA or local regulatory authorities.

If you agree to take part in this study, it is important for you to keep all your appointments. However, if you don't want to stay in the study, you can leave at any time. You will not lose any benefits that you would have had if you had not joined the study.

If you receive the first injection but not the second injection, we will ask you to continue with your planned follow-up visits until the end of the study. It is important that we continue to monitor your health even if you do not receive the second injection.

ALTERNATIVES

This study is not designed to treat or prevent any disease. You may choose to not participate.

CONFIDENTIALITY

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Results of NIH-supported research studies may also be reported in medical journals, on internet or at scientific meetings. These reports will not have information that can identify you.

In most cases, the NIH will not release any information about your research participation without your written permission. However, if you sign a release of information form, for example, for an insurance company, the insurance company receives information from your medical record. This information might affect (either positively or negatively) if the insurance company sells insurance to you.

The U.S. Federal Privacy Act protects the privacy of your medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by regulatory agencies that supervise the study, law enforcement officials, or other authorized people. [Site/country-specific information on confidentiality may be added.]

POLICY REGARDING RESEARCH-RELATED INJURIES

The study site will provide immediate medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the investigator of record, _____. Others you may call are the Study Coordinator _____at_____. You may also call the _____ Patient Representative at _____.

Please keep a copy of this document in case you want to read it again.

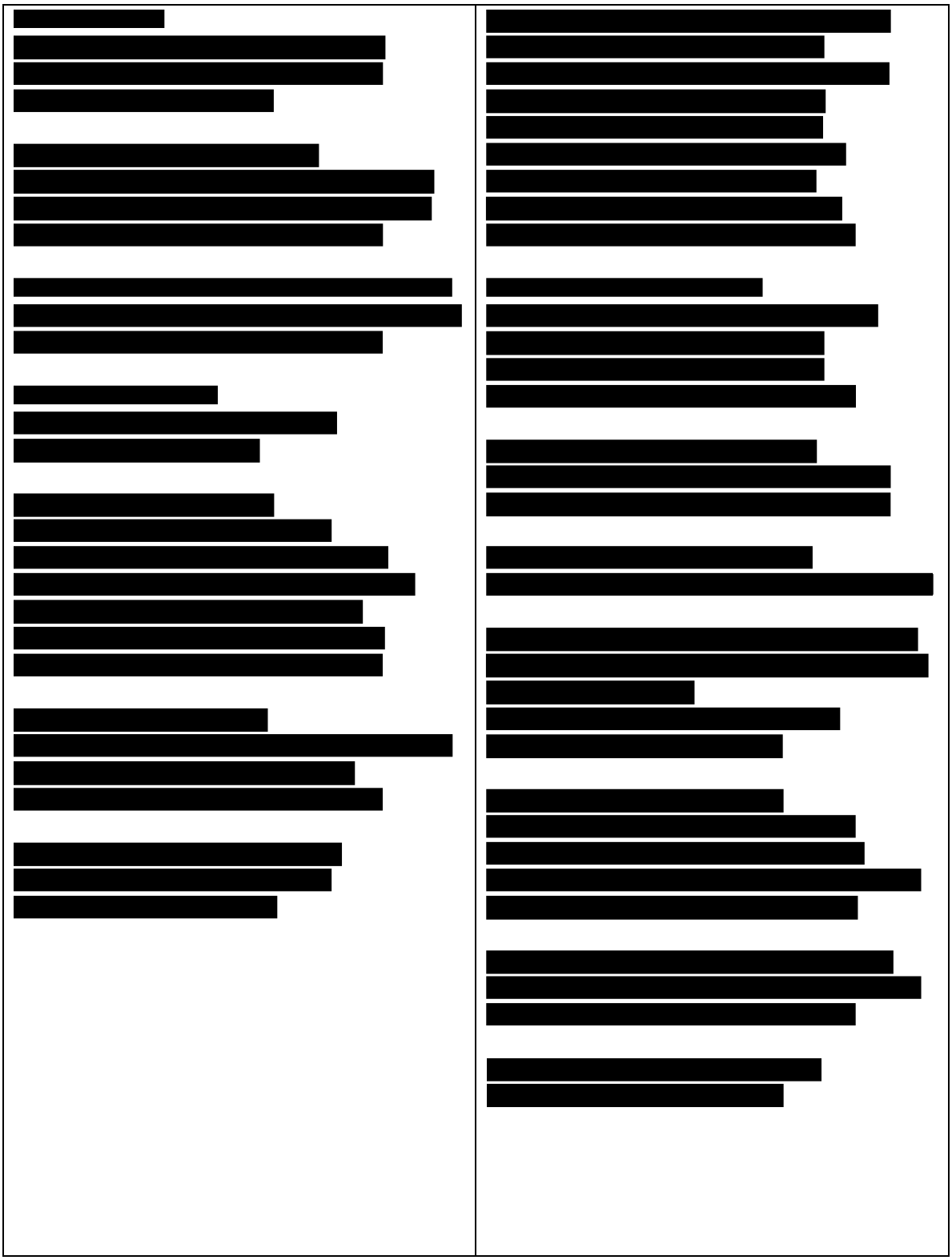
Adult Participant Consent:

I have read the explanation about this study and have been given the opportunity to discuss it and ask questions. I consent to take part in this study.

Participant Name (print)	Date and Time
Signature	

Investigator name (print)	Date
Signature	
Witness name (print)	Date
Signature	

APPENDIX II: CONTACT INFORMATION



APPENDIX III: SCHEDULE OF EVALUATIONS

VRC 704 Schedule of Evaluations															
Visit		01	02	02A	03	03A	04	05	06	07	08	09	10	11	12
Week of Study		-8 to 0	W0	W1	W4	W5	W8	W16	W24	W32	W40	W48	W56	W64	W72
[1]Day of Study		-56 to 0	D0[1]	D7	D28	D35	D56	D112	D168	D224	D280	D336	D392	D448	D504
Clinical Evaluations	[11]Tube														
[2] Informed Consent, AoU		X													
[3] Targeted physical exam		X	[X]		[X]		[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]
Targeted medical history; vital signs		X	X		X		X	X	X	X	X	X	X	X	X
[4] Study Injections			X		X[5]										
Begin 7-Day Diary Card			X		X										
Phone contact; clinic visit if indicated				X		X									
Counseling on pregnancy prevention			X		X		X								
CBC, differential, platelets	EDTA	3	3		3		3								
[6] Pregnancy test: urine (or serum)		X	X		X			X							
ALT	SST	4	4		4		4								
HIV[7], creatinine	SST	4													
Research Immunology															
Antibody assays and serum	SST	[16] [8]	40		40		40	40	40	40	40	40	40	40	40
[9] Serum for diagnostic testing	SST				[X]		[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]
[9] Plasma for diagnostic testing	EDTA				[X]		[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]	[X]
[10] PBMC and plasma	EDTA		[40]		[40]										[40]
Daily Volume (mL)		**27**	**87**	**-**	**87**	**-**	**47**	**40**	**40**	**40**	**40**	**40**	**40**	**40**	**80**
Cumulative Volume estimate (mL)		**27**	**114**	**-**	**201**	**-**	**248**	**288**	**328**	**368**	**408**	**448**	**488**	**528**	**608**

[1] Day 0=day of enrollment and (blinded) CHIKV VLP vaccine or PBS injection. Day 0 evaluations prior to first injection are the baseline for assessing adverse events subsequently. Schedule Visits 02A through 04 with respect to the last prior injection day and subsequent visits with respect to Day 0. The visit windows are: Visits 02A and 03A (+3 days); Visit 03 (+14 days); Visit 04 (±7 days); Visits 05 through 12 (±21 days).

[2] Screening evaluations may be repeated or completed over more than one visit, if needed. Screening research blood draw and consent obtained more than 56 days prior to enrollment are not required to be repeated.

[3] Screening physical exam is targeted to eligibility and includes height/weight; the [X] at other visits indicates targeted exam only if needed based on interim history.

[4] Safety and research blood and vital signs must be collected before study injections are administered. Complete BP, pulse and injection site assessment at 15 minutes or later after study injection before subject leaves the clinic.

[5] The preferred window for the second injection is +14 days. The injection may occur outside the preferred window with approval by the Protocol Chair.

[6] Confirm negative pregnancy test before administering a study injection. Reduce research blood draws to 20 mL (or eliminate) while pregnant.

[7] If a subject previously participated in an HIV vaccine study, such that they have HIV antibody from vaccine, but no evidence of HIV infection by PCR, appropriate documentation of HIV-uninfected status may be used for eligibility.

[8] Serum samples collected during screening may be used for assay validation and site proficiency testing.

[9] Collect plasma and serum to be sent for CHIKV diagnostic testing if signs or symptoms of illness or CHIKV are present. Plasma may also be collected and sent for more rapid CHIKV testing and other arbovirus testing, if clinically indicated.

[10] Only a subset of sites with PBMC processing capabilities will collect the samples for PBMC and plasma storage.

[11] Specific tube volumes and types are shown to estimate blood draw volumes. Different collection tubes may be used to meet site laboratory requirements. Tube types used for research samples must be as shown in the table or be approved by the Protocol Chair.

APPENDIX IV: ASSESSMENT OF RELATIONSHIP TO VACCINE AND ADVERSE EVENT SEVERITY GRADING

Assessment of Causality Relationship of an Adverse Event to Study Vaccine:

The relationship between an adverse event (AE) and the vaccine will be assessed by the investigator on the basis of his or her clinical judgment and the definitions below.

- **Definitely Related**. The AE and administration of study agent are related in time, and a direct association can be demonstrated.
- **Probably Related**. The AE and administration of study agent are reasonably related in time, and the AE is more likely explained by study agent than other causes.
- **Possibly Related**. The AE and administration of study agent are reasonably related in time, but the AE can be explained equally well by causes other than study agent.
- **Not Related**. There is not a reasonable possibility that the AE is related to the study agent.

For purposes of preparing data reports in which AE attributions are limited to "**Related**" or "**Not Related**", in this protocol, the "Definitely, Probably and Possibly" attributions will be mapped to the "Related" category. The definitions that apply when these two categories alone are used are as follows:

- **Related** – There is a reasonable possibility that the AE may be related to the study agent.
- **Not Related** – There is not a reasonable possibility that the AE is related to the study agent.

Grading the Severity of Adverse Events:

The FDA Guidance for Industry (September 2007): "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" is the basis for the severity grading of AEs in this protocol. Several modifications were made to the table as follows:

- "Emergency room visit" is not automatically considered a life-threatening event; these words have been removed from any "grade 4' definition where they appear in the table copied from the guidance document.
- Any laboratory value shown as a "graded" value in the table that is within the institutional normal range will not be severity graded or recorded as an AE.
- Severity grading for hemoglobin decrease on the basis of the magnitude of decrease from baseline is not applicable at the grade 1 level; only absolute hemoglobin will be used to define grade 1 decrease. Increases in hemoglobin are AEs only for values above the upper limit of normal and are graded by the systemic illness clinical criteria.
- Severity grading definition for Grade 4 local reaction to injectable product (Erythema/Redness and Induration/Swelling) included added text "requiring medical attention".
- 1 X ULN was removed from the definition for PT increase.

When not otherwise specified in the table, the following guidance will be used to assign a severity grade:

Grade 1 (Mild): No effect on activities of daily living

Grade 2 (Moderate): Some interference with activity not requiring medical intervention

Grade 3 (Severe): Prevents daily activity and requires medical intervention

Grade 4 (Life-threatening): Hospitalization; immediate medical intervention or therapy required to prevent death.

Grade 5 (Death): Death is assigned a Grade 5 severity.

Only the single AE that is assessed as the primary cause of death should be assigned "grade 5" severity.

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials
FDA Guidance - September 2007

A. Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Hospitalization
[1]Erythema/Redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis requiring medical attention
[2] Induration/Swelling	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis requiring medical attention
[3] Vital Signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
[4] Fever (°C) (°F)	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	Hospitalization for arrhythmia
[5] Bradycardia - beats per Minute	50 – 54	45 – 49	< 45	Hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	Hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	Hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	Hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

1. In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
2. Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.
3. Subject should be at rest for all vital sign measurements.
4. Oral temperature; no recent hot or cold beverages or smoking.
5. When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing Bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	Hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	Hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	Hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	Hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	Hospitalization

Systemic Illness	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	Hospitalization

B. Tables for Laboratory Abnormalities

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

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	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6

Serum*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	100 – 110 110 – 125	111 – 125 126 – 200	>125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	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

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.
***ULN" is the upper limit of the normal range.

Hematology*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) decrease from baseline value - gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) decrease from baseline value – gm/dL	not applicable	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.10 – 1.20 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

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

Dale and Betty Bumpers
VACCINE RESEARCH CENTER
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Department of Health and Human Services
Vaccines for Life™

DATE: April 26, 2016

TO: VRC 704 Study Investigators

FROM: Julie Ledgerwood, D.O, VRC 704 Protocol Chair

SUBJECT: VRC 704, Version 2.0 (April 15, 2016), **Letter of Amendment #1**

This Letter of Amendment (LoA) affects the VRC 704 Version 2.0 (April 15, 2016) protocol and must be approved by the IRB prior to implementation.

This letter and any IRB correspondence with regard to this letter will be filed in the protocol regulatory file and other pertinent files.

Purpose: The purpose of this LoA is to add (1) Dr. Grace Chen as a Protocol Co-Chair to VRC 704 and (2) [redacted] as a Scientific and Laboratory Collaborator to VRC 704 for use of study samples and stored specimens for flavivirus-related research.

Contact information is as follows:

[redacted]

This LoA has no substantive effect on the protocol plan and does not result in a change in the protocol informed consent. This update will be included in the next version of the protocol if it is amended at a future date.

Dale and Betty Bumpers
VACCINE RESEARCH CENTER
National Institute of Allergy and Infectious Diseases
National Institutes of Health
Department of Health and Human Services
Vaccines for Life™

DATE: June 2, 2016

TO: VRC 704 Study Investigators

FROM: Julie Ledgerwood, D.O, VRC 704 Protocol Chair

SUBJECT: VRC 704, Protocol Version 2.0 (April 15, 2016), **Letter of Amendment #2**

This Letter of Amendment (LoA) affects the VRC 704 protocol Version 2.0 (April 15, 2016) and must be approved by the IRB prior to implementation. This letter and any IRB correspondence with regard to this letter will be filed in the protocol regulatory file and other pertinent files.

Purpose: The purpose of this LoA is to (1) amend the Schedule of Evaluations footnote regarding the reference for scheduling study visits and (2) add a Scientific and Laboratory Collaborator.

(1) The Schedule of Evaluations footnote reads: "Schedule Visits 02A through 04 with respect to the last prior injection day and subsequent visits with respect to Day 0." With this amendment, we are changing this footnote to read: "Schedule Visits 02A through 12 with respect to the last prior injection date. Therefore, if Visit 03 does not occur exactly on Day 28, schedule all subsequent visits (03A through 12) based on the actual Visit 03 date."

This amendment is to ensure that all follow up visits are conducted in respect to the last study injection given. Therefore, if only one study injection is given, use the date of that injection to schedule all study visits.

(2) With this amendment, we are also adding [redacted] as a Scientific and Laboratory Collaborator to VRC 704 for use of study samples and stored specimens for flavivirus-related research. Contact information is as follows:

[redacted]

This LoA has no substantive effect on the protocol plan and does not result in a change in the protocol informed consent. This update will be included in the next version of the protocol if it is amended at a future date.

[redacted] Hospital
Research Unit

Informed Consent Form for Study Participation

STUDY TITLE: VRC 704: Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

INTRODUCTION

We invite you to take part in a research study at the Research Unit on [redacted] Hospital.

The study is sponsored by the National Institutes of Health (NIH) in Bethesda, MD, USA. You can decide if you want to participate in this study or not. There is no penalty or loss of benefits for choosing not to participate. Please ask questions and discuss this study with anyone you want. Take as much time as you need to decide.

Before you can enroll in the investigational Chikungunya virus (CHIKV) vaccine study, you will be screened for eligibility. You will need to sign this consent form before we can do the screening.

Screening involves a physical exam and blood tests to check your general health status. If you are a woman, you will be asked about your birth control use and the possibility of your becoming pregnant while in the study. You will be tested for pregnancy if applicable. During screening, we may collect some blood to store for research. We will ask you about your general health history. You will have bloods test to check for past CHIKV infection and for HIV infection. We will ask you about medications you are taking and recent vaccinations.

We will review the screening results with you and tell you if the results show that you are not eligible to join the study. You cannot be in another research study where you receive a study product and also enroll in this study unless you discuss this and receive approval from the study clinicians.

PURPOSE OF THE VACCINE STUDY

This is a research study of an experimental vaccine. It is called the Chikungunya Virus-Like Particle vaccine or "CHIKV VLP" vaccine. The U.S. Food and Drug Administration (FDA) allows it to be used for research only. It is not known if the vaccine works. The main purpose of this study is to see if the experimental vaccine is safe, if it causes any side effects, and if people who get the vaccine make an immune response. Study sites are in areas where CHIKV outbreaks have occurred. The study will last for about 72 weeks.

CHIKV was first identified in Tanzania, Africa in 1952. CHIKV outbreaks were most common in Africa, India and Asia until 2013, when the first cases were reported in the Americas. The virus is spread by mosquitoes and causes a viral infection that can lead to fever, headache, muscle pain, tiredness, and joint inflammation or pain. The disease usually does not cause death;

but the joint inflammation may be severe and may last for many months. There is currently no cure for CHIKV infection or vaccine to prevent CHIKV infection.

You are eligible to participate in this study if:

- you have completed the screening process,
- you are between **21** and 60 years old,
- you have physical exam and blood test results that meet eligibility requirements, and
- you do not have any significant medical problems as determined by your screening.

STUDY INJECTIONS

Vaccines are substances used to try to create resistance (or immunity) to a disease. About 400 people will participate in this study. About half of the participants will get two injections of the study vaccine and half will get two injection of an inactive solution (or placebo).

CHIKV VLP Vaccine: The vaccine was developed by the Vaccine Research Center (VRC) at the U.S. National Institutes of Health (NIH). It was made at the VRC Vaccine Pilot Plant in Frederick, Maryland, USA. The vaccine is made of some CHIKV proteins that combine to make a particle that looks like the outside of the virus. The body's immune system may respond to this particle. There is no virus in the vaccine so you cannot get CHIKV infection from the vaccine.

Placebo Injection: The placebo in this study is a sterile salt water solution made for injection into people. It has no vaccine in it.

STUDY PROCEDURES

If you agree to take part in the study, you will be randomly assigned (by chance, like flipping a coin) to one of two groups. Neither you nor the clinic staff will know what product you are receiving until the study is complete. You will receive two injections during the study. Half the people in this study will get the investigational CHIKV VLP vaccine and the other half will get a placebo injection on the day of enrollment. Four weeks later, you will get a second injection of the same product that you already got. We will give all study injections with a needle and syringe into your upper arm muscle. If you are a woman who is able to get pregnant, we will do a pregnancy test before each injection. The test must show that you are not pregnant before a study injection is given.

The clinic staff will observe you for at least 15 minutes after each injection. We will ask you to complete a diary card for 7 days. For the diary card, you will need to record your temperature and any symptoms you feel. You will also need to look at the injection site on your arm each day and record how it looks. The clinic staff is available to you by phone 24 hours a day to report any unexpected side effects. If you experience any concerning signs or symptoms after each injection, please contact the clinic. We will ask you to review your diary card with us about a week after each injection.

If you have any signs of sickness that interfere with your usual activities, you may need to come to the clinic for an examination before your next scheduled visit. It is very important that you follow the instructions we give you.

Your participation in the study will last for about 16 months after the first injection. You will have about 11 planned clinic visits and 2 telephone contacts. Each clinic visit will last about 1-2 hours. Experimental vaccine studies follow a set schedule. Scheduling for your visits allows some flexibility, but it is important that you work with the staff to follow the schedule. You should try to not miss any visits.

At each visit, we will check you for any health changes or problems. We will ask how you are feeling and if you have taken any medications. At scheduled study visits, we will draw about 5-12 tubes of blood from you, depending on the visit. We may ask for urine samples. If any of your test results show a health problem, we will tell you about it as soon as possible. You might need to have extra clinic visits and laboratory tests if you have health changes that we need to check.

At any time during the study, if you think you have CHIKV infection, you must contact the study site and come in for a clinical evaluation and blood tests. We will tell you the results of testing for CHIKV infection but it may take several weeks to learn the results. It is very important for the study that you get tested for CHIKV infection at our clinic only and that you do not go anywhere else for CHIKV testing. There is no medication that makes CHIKV infection end faster than naturally occurs. We will tell you if you need testing for any other infections that have symptoms similar to CHIKV infection. People who get CHIKV infection may have swollen joints, rashes or other signs of infection. We ask your permission to take a photo. You may refuse to have photos taken. The photos will be used only for study purposes.

I agree to have photos taken if I get CHIKV infection.

_____ Yes _____ No Date: _____

MONITORING OF THE STUDY

This study will be monitored by a group of physicians and scientists associated with the U.S. NIH. This group will review the study information and will pay close attention to harmful reactions.

GENETIC TESTING

Some of the blood drawn from you as part of this study will be used for genetic tests. There are many different types of genetic tests. In vaccine research, some genetic tests are done to see if immune responses to a vaccine are different in people because of genetic differences. These tests will be done in a research lab using your blood samples. Genetic tests done in a research lab will **not** be in your medical record. The samples used in these tests will **not** have your name them. In the future, genetic research tests to help understand how vaccines work may be done on your DNA using stored samples.

STORED SAMPLES

To participate in this study, you must allow us to store your blood samples for future research. We will use some of your blood samples to study your immune response to the injection. We

will also use these samples for future research to learn more about CHIKV infections, vaccines, the immune system, and/or other medical conditions. Results of research tests done from stored samples will not be in your medical record or reported to you.

Labeling of Stored Samples

Your stored samples will be labeled by a code (such as a number) that only the study team can link to you. Any identifying information about you will be kept confidential to the extent permitted by law. Despite protections, there is a small chance that information identifying you will be given to someone who should not get it.

Future Studies

In the future, other investigators at NIH or outside of NIH may wish to study your stored samples. When the study team shares your stored samples, they will be marked with a code, but will not have any identifying information on them. Some information about you, such as your gender, age, health history, or ethnicity may also be shared with other investigators. Any future research studies using your samples will be conducted in a way that protects the rights and privacy of study participants.

Your stored samples will be used only for research and will not be sold. The research done with your samples may be used to develop new products in the future but you will not receive payment for such products.

POSSIBLE STUDY RISKS

***Possible* risks from the injections:** temporary stinging, pain, redness, soreness, itchiness, swelling, bruising, or a cut in the arm. There is a very small chance of infection.

***Possible* risks of blood drawing:** pain, bleeding, bruising, feeling lightheaded, or fainting.

***Possible* risks from genetic testing:** unintended release of information that could be used by insurers or employers; discovering a gene or HLA type that suggests risk of disease for you or your family; discovering undisclosed family relationships.

***Possible* risks from any vaccine:** fever, chills, rash, aches and pains, nausea, headache, dizziness, and fatigue. Some people have allergic reactions to vaccines. These types of reactions are usually greatest within the first 24 hours after injection and typically last 1 to 3 days. Over-the-counter medicine, such as acetaminophen, will generally help relieve symptoms from injection and may be used.

***Possible* risks from the CHIKV VLP vaccine:** This will be the second study of this CHIKV VLP vaccine. In the past study, 25 people received the CHIKV VLP vaccine. The most common complaints were mild pain or soreness at the injection site. About half of the people had mild symptoms like headache, nausea and feeling tired or unwell. A few people had a temporary change in a laboratory test. The vaccine does not contain any CHIKV virus. The vaccine will **not** give you CHIKV infection.

The first 25 people to receive the CHIKV VLP vaccine did **not** have any fever or joint pain after vaccination. However, there may be side effects from the study vaccine that are not common which we do not yet know about. Please tell the study staff about any side effect you think you are having. This is important for your safety.

***Possible* risks during Pregnancy:** We do not know if getting the study vaccine affects a fetus. Therefore, women who can have children must use effective birth control starting at least 21 days before getting the first injection until 12 weeks after the last study injection. We will discuss effective birth control methods with you. If you are pregnant or want to become pregnant in the next 16 weeks, you cannot participate. We will ask about the outcome of any pregnancy that begins during the first 16 weeks on study.

Other Risks: It is unknown if the study injection will affect how you respond to any future CHIKV infection or to any other CHIKV vaccines that you may get in the future. We will tell you if we learn about any important new findings or serious side effects of the vaccine during the study that may affect your willingness to continue participation.

You may not donate blood while participating in an investigational vaccine study and for one year after the date of the last injection of an investigational vaccine.

POSSIBLE BENEFITS

This study is not designed to benefit you or protect you from CHIKV infection. You and others may benefit in the future from the information that we learn from the study.

COSTS OF PARTICIPATION

There are no costs to you for participating in this study. You or your health insurance will have to pay for all medical costs for medical care you receive outside this study. It is possible that you may have some expenses that are not covered by the study compensation provided.

COMPENSATION TO YOU FOR YOUR PARTICIPATION

We will reimburse you for the cost of meals and travelling of your study visits. You will be compensated with $50.00 per visit. If you attend to all visits, you may receive up to $600.00. This will be based on the number of study visits you attend and study injections you receive.

REASONS FOR REMOVING YOU FROM THE STUDY WITHOUT YOUR CONSENT

The study doctor can take you out of this study without your permission *if:*

- continuing in the study could harm you,
- you do not follow study instructions or keep appointments, or
- the study is stopped by the U.S. NIH, regulatory boards, the U.S. FDA or local regulatory authorities.

If you agree to take part in this study, it is important for you to keep all your appointments. However, if you don't want to stay in the study, you can leave at any time. You will not lose any benefits that you would have had if you had not joined the study.

If you receive the first injection but not the second injection, we will ask you to continue with your planned follow-up visits until the end of the study. It is important that we continue to monitor your health even if you do not receive the second injection.

ALTERNATIVES

This study is not designed to treat or prevent any disease. You may choose to not participate.

CONFIDENTIALITY

A description of this clinical trial will be available on *http://www.ClinicalTrials.gov*, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

Results of NIH-supported research studies may also be reported in medical journals, on internet or at scientific meetings. These reports will not have information that can identify you.

In most cases, the NIH will not release any information about your research participation without your written permission. However, if you sign a release of information form, for example, for an insurance company, the insurance company receives information from your medical record. This information might affect (either positively or negatively) if the insurance company sells insurance to you.

The U.S. Federal Privacy Act protects the privacy of your medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by regulatory agencies that supervise the study, law enforcement officials, or other authorized people.

POLICY REGARDING RESEARCH-RELATED INJURIES

The study site will provide immediate medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the investigator of record, [redacted]. Others you may call are the Study Coordinator, [redacted]. You may also call the Institutional Review Board for your rights as a research participant at [redacted].

Please keep a copy of this document in case you want to read it again.

Adult Participant Consent:

I have read the explanation about this study and have been given the opportunity to discuss it and ask questions. I consent to take part in this study.

Participant Name (print)	Date and Time
Signature	

Investigator name or representative (print)	Date
Signature	
Witness name (print)	Date
Signature	

IRB Institutional Review Board [redacted] Hospital

Approved [redacted]
(Chairperson (or Alternate))
19 May 2016
Date

STATISTICAL ANALYSIS PLAN

Protocol VRC 704

Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

ClinicalTrials.gov Identifier: NCT02562482

Version 1.0

DATE: 11 SEP 2018

Prepared and distributed by:
The Emmes Corporation
Rockville, Maryland USA

Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

Protocol Number Code:	VRC 704
Development Phase:	Phase 2
Products:	VRC-CHKVLP059-00-VP, VRC-PBSPLA043-00-VP (Phosphate Buffered Saline)
Form/Route:	IM injection
Indication Studied:	Chikungunya
Sponsor:	Vaccine Research Center National Institute of Allergy and Infectious Diseases National Institutes of Health
Protocol Chair:	[redacted]
Medical Monitor:	[redacted]
Biostatistician:	[redacted]
Clinical Trial Initiation Date:	18 November 2015
Clinical Trial Completion Date:	06 March 2018
Date of the Analysis Plan:	11 September 2018
Version Number:	1.0

SIGNATURE PAGE

SPONSOR: Vaccine Research Center
National Institute of Allergy and Infectious Diseases
National Institutes of Health

STUDY TITLE: Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

PROTOCOL NUMBER: VRC 704

Protocol Chair:
Signed: [redacted]

[redacted]

The Emmes Corporation:

Signed: [redacted]

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ABBREVIATIONS

Abbreviation	Term
AE	adverse event
ALT	alanine aminotransferase
AoU	assessment of understanding
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CHIKV	Chikungunya virus
CRO	Contract Research Organization
DNA	deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GMT	geometric mean titers
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
ICH	International Conference on Harmonization
IM	Intramuscular
IND	Investigational New Drug
IoR	Investigator of Record
IRB	Institutional Review Board
ITT	Intent-to-treat
MedDRA®	Medical Dictionary for Regulatory Activities
mITT	Modified intent-to-treat

Abbreviation	Term
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NVITAL	NIAID Vaccine Immune T-Cell and Antibody Laboratory
PBMC	peripheral blood mononuclear cells
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDL	Primary Diagnostic Laboratory
PI	Principal Investigator
PP	Per-protocol
PSRT	Protocol Safety Review Team
RNA	ribonucleic acid
SAE	serious adverse event
SAP	Statistical Analysis Plan
SDCC	Statistical Data Coordinating Center
SUSAR	serious and unexpected suspected adverse reaction
VLP	virus-like particle
VRC	Vaccine Research Center

1 PREFACE

This Statistical Analysis Plan (SAP) for "Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults" describes and expands upon the statistical information presented in protocol VRC 704.

This document describes all planned analyses and provides reasons and justifications for these analyses. It also includes sample tables, listings, and figures planned for the final analyses. Regarding the final analyses and Clinical Study Report (CSR), this SAP follows the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines, as indicated in Topic E3 (Structure and Content of Clinical Study Reports), and more generally is consistent with Topic E8 (General Considerations for Clinical Trials) and Topic E9 (Statistical Principles for Clinical Trials). The structure and content of the SAP provides sufficient detail to meet the requirements identified by the FDA and ICH, while all work planned and reported for this SAP will follow internationally accepted guidelines published by the American Statistical Association and the Royal Statistical Society for statistical practice.

This document contains four sections: (1) a review of the study design, (2) general statistical considerations, (3) comprehensive statistical analysis methods for efficacy and safety outcomes, and (4) a list of proposed tables and figures. Any deviation from this SAP will be described and justified in protocol amendments and/or in the CSR, as appropriate. The reader of this SAP is encouraged to also review the study protocol for details on conduct of the study and the operational aspects of clinical assessments.

2 INTRODUCTION

The Dale and Betty Bumpers Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) (Bethesda, MD, USA) is dedicated to translating the latest knowledge of disease pathogenesis and immunology into new vaccine strategies to provide safe and effective means to prevent and control infectious diseases. Chikungunya virus (CHIKV) is a mosquito-borne disease causing acute infection associated with severe morbidity that can persist for several weeks, months or even years. Since its discovery in Tanzania in 1952, outbreaks of CHIKV have occurred most frequently in Africa, Asia and the Indian subcontinent. In recent decades, the disease has spread to Europe and the Americas. In January 2015, over 1,135,000 suspected cases of CHIKV and 176 CHIKV-attributable deaths had been recorded in the Caribbean islands, Latin American countries and USA [1]. There are currently no effective vaccines or therapies against CHIKV. The rapid emergence of CHIKV supports the need for a safe and immunogenic vaccine. This protocol is designed as a Phase 2 evaluation of the investigational Chikungunya virus virus-like particle vaccine (CHIKV VLP), VRC-CHKVLP059-00-VP. This candidate vaccine was evaluated in a Phase 1 study as safe and well-tolerated and observed to induce neutralizing antibody responses at levels hypothesized to be protective against CHIKV infection [2].

2.1 Purpose of the Analyses

This is a Phase 2 multicenter, randomized, placebo-controlled, double-blind study to examine the safety and immunogenicity of a 2-injection vaccine regimen (Day 0 and 28) with the CHIKV VLP vaccine or PBS placebo in healthy adults. Note, not all analyses for endpoints included in the protocol are detailed in this plan. This statistical analysis plan details the analyses pertaining to the primary and secondary outcomes. The exploratory immunogenicity endpoint of humoral and cellular immune response as measured by NAb, ELISA and other assays at Week 4, 16, 24, 48, and 72 may not be performed. The only immunogenicity data expected is humoral and cellular immune response as measured by NAb at Week 0 and 8 and ELISA at screening. Also, interim analyses included in the protocol are not detailed in this plan, including the analysis of immune responses and CHIKV incidence rates at 6 and 12 months after the study is closed to accrual.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

Primary Objective:

- To evaluate the safety and tolerability of VRC-CHKVLP059-00-VP in healthy adults that reside in CHIKV endemic areas when administered intramuscularly by needle and syringe at a dose of 20 mcg compared to placebo.

Secondary Objectives:

- To evaluate the immune response to VRC-CHKVLP059-00-VP by neutralization assay at 4 weeks after the second 20 mcg dose (Study Week 8).

Exploratory Objectives:

- To evaluate humoral and cellular responses to VRC-CHKVLP059-00-VP at additional time points. CHIKV-specific responses will be assessed by antibody assays that include antigen-specific ELISA and neutralization assay.
- To compare incidence rates of CHIKV infection in vaccine and placebo recipients.
- To compare clinical and virus characteristics of infections in vaccine and placebo recipients.

3.2 Endpoints

3.2.1 Primary Endpoints

Safety

Assessment of product safety will include clinical observation and monitoring of hematological and chemical parameters. Reactogenicity will be closely monitored for 7 days after injection and safety evaluated by clinical visits through the study duration of 72 weeks.

The following parameters will be assessed:

- Local reactogenicity signs and symptoms for 7 days after each injection
- Systemic reactogenicity signs and symptoms for 7 days after each injection
- Laboratory measures of safety through 4 weeks after last injection
- Adverse events through 4 weeks after last injection
- Serious adverse events and new chronic medical conditions throughout the study
- CHIKV infection events throughout the study

3.2.2 Secondary Endpoints

Immunogenicity

- Humoral immune response as measured by neutralization antibody (NAb) assay at baseline (Day 0) and at 4 weeks after second injection (Week 8).

3.2.3 Exploratory Endpoints

Immunogenicity

- Humoral responses to VRC-CHKVLP059-00-VP at additional time points assessed by antibody assays that include antigen-specific ELISA and neutralization assay.

Chikungunya Infection Incidence

- Confirmed Chikungunya infections by positive PCR results for CHIKV at the end of the study.
- Cases of illness clinically consistent with Chikungunya infection confirmed or not by a positive PCR at the end of the study.
- Clinical characteristics of infections based on standard of care parameters for CHIKV disease. Virus characteristics of infections measured by signs and symptoms.

3.3 Study Definitions and Derived Variables

The baseline value will be defined as the last value obtained prior to the first injection of study product on Day 0.

Individual neutralization antibody (NAb) assay results will be reported as a titer. The lower limit of detection for the NAb assay is 15 reciprocal serum dilution and the lower limit of confidence is 30 reciprocal serum dilution. Values below the limit of detection will be imputed as 15 if not already imputed prior to analysis. For analysis, the arithmetic mean of repeated results for each sample will be computed and used as the response for all subsequent calculations.

Fold-rise (NAb) is a measure that describes how much the NAb titer changes from an initial to a final value. The fold-rise is calculated as the ratio of the final value to the initial value.

Positive response rate (NAb) will be measured as the proportion of subjects with a positive response, defined as a reciprocal serum dilution ≥ 30.

4 INVESTIGATIONAL PLAN

4.1 Overall Study Design and Plan

VRC 704 is a Phase 2 multicenter, randomized, placebo-controlled, double-blind parallel group study as summarized in **Table 1** below. It examines the safety and immunogenicity of a 2-injection vaccine regimen (Day 0 and 28) with the CHIKV VLP vaccine or PBS placebo in healthy adults (N=200 per group). Subjects will be randomly assigned in a 1:1 allocation to active vaccine or placebo control using a randomized block design stratified by age within each study site. The expected duration of time on the study per subject is approximately 72 weeks.

Table 1: Study Schema

Group	Number of Subjects	VRC 704 Study Injection Schedule	
		Day 0	Day 28(+14)
1	200	20 mcg CHIKV VLP	20 mcg CHIKV VLP
2	200	PBS	PBS
All injections will be administered IM at 0.5 mL by needle and syringe.			

The Protocol Safety Review Team (PSRT) will review safety data routinely throughout the study. The study will utilize both electronic database features and reviews by designated safety review personnel to identify in a timely manner if any of the safety pause rules of the study are met. The NIAID Intramural DSMB will provide an independent safety review at scheduled intervals to coincide with their biannual meeting schedule.

No formal statistical interim analysis intended to terminate the trial early for futility or efficacy is planned. Therefore, no p-value adjustments will be made.

4.2 Study Design Issues

The design for this study is a simple parallel group double-blind randomized control using PBS as the control. Following screening, eligible subjects are enrolled; enrollment includes subject randomized to a treatment assignment and administration of the first injection based on the randomization assignment. Subjects who are randomized but whom do not receive a study injection (for example a subject who is randomized but then decides not to participate prior to receiving the Day 0 injection) will not contribute to the analysis cohorts.

4.3 Selection of Study Population

The study is designed for the enrollment of healthy adults. The following eligibility criteria will be used.

Inclusion Criteria

A subject must meet all of the following criteria:

1. 18 to 60 years old
2. Available for clinical follow-up through Study Week 72
3. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process
4. Able and willing to complete the informed consent process
5. Willing to donate blood for sample storage to be used for future research
6. In good general health, with a BMI ≤40, without clinically significant medical history, and has satisfactorily completed screening
7. Physical examination and laboratory results without clinically significant findings within the 56 days prior to enrollment

Laboratory Criteria within 56 days prior to enrollment:

8. Hemoglobin either within institutional normal limits or accompanied by site physician approval as consistent with healthy adult status
9. White blood cells either within institutional normal range or accompanied by site physician approval as consistent with healthy adult status
10. Platelets = 125,000 – 500,000/mm^3
11. Alanine aminotransferase (ALT) ≤ 1.25 x upper limit of normal (ULN)
12. Serum creatinine ≤ 1.1 x ULN based on site institutional normal range
13. Negative result on an HIV test that meets local standards for identification of HIV infection
14. Negative result on the CHIKV screening antibody assay.

Criteria applicable to women of childbearing potential:

15. Negative human chorionic gonadotropin pregnancy test (urine or serum) on day of enrollment
16. Agree to use an effective means of birth control from 21 days prior to enrollment through 12 weeks after the last study injection

Exclusion Criteria

A subject will be excluded if one or more of the following conditions apply:

Women Specific:

1. Planning to become pregnant during the 16 weeks after enrollment in the study

Subject has received any of the following substances:

2. Systemic immunosuppressive medications within 2 weeks prior to enrollment
3. Blood products within 16 weeks prior to enrollment
4. Immunoglobulin within 8 weeks prior to enrollment
5. Prior vaccinations with an investigational CHIKV vaccine
6. Investigational research agents within 4 weeks prior to enrollment
7. Any vaccination within 2 weeks prior to enrollment
8. Current anti-TB prophylaxis or therapy

Subject has a history of any of the following clinically significant conditions:

9. A history of immune-mediated or clinically significant arthritis
10. Serious reactions to vaccines that preclude receipt of study injections as determined by the investigator
11. Hereditary angioedema, acquired angioedema, or idiopathic forms of angioedema
12. Asthma that is unstable or required emergent care, urgent care, hospitalization or intubation during the past two years or that is expected to require the use of oral or intravenous corticosteroids
13. Diabetes mellitus (type I or II), with the exception of gestational diabetes
14. Idiopathic urticaria within the past year
15. Bleeding disorder diagnosed by a doctor (e.g. factor deficiency, coagulopathy, or platelet disorder requiring special precautions) or significant bruising or bleeding difficulties with IM injections or blood draws
16. Malignancy that is active or history of a malignancy that is likely to recur during the period of the study
17. Seizure in the past 3 years or treatment for a seizure disorder within the last 3 years
18. Asplenia, functional asplenia or any condition resulting in the absence or removal of the spleen
19. Psychiatric condition that may preclude compliance with the protocol; past or present psychoses; or a history of suicide plan or attempt within the five years prior to enrollment

20. Any medical or social condition that, in the judgment of the investigator, is a contraindication to protocol participation or impairs a volunteer's ability to give informed consent

4.4 Subject Discontinuations

A subject may be discontinued from receiving study injections for the following reasons:

- Pregnancy;
- Grade 3 AE assessed as related to a study injection (with the exception that self-limited Grade 3 solicited reactogenicity does not require discontinuation of study injections);
- Grade 4 AE assessed as related to a study injection;
- Immediate hypersensitivity reaction associated with a study injection;
- CHIKV infection or other intercurrent illness that is not expected to resolve prior to the scheduled study injection assessed by study clinician to require withdrawal from the injection schedule;
- Treatment with systemic glucocorticoids (e.g., prednisone or other glucocorticoid) or other immunomodulators (other than nonsteroidal anti-inflammatory drugs [NSAIDs]), with the exception that, study injection may continue per investigator discretion if the next one occurs at least 2 weeks following completion of glucocorticoid treatment;
- The study PI assesses that it is not in the best interest of the subject to continue on the injection schedule.

A subject may be discontinued from protocol participation for the following reasons:

- Subject voluntarily withdraws;
- Subject develops a medical condition that is a contraindication to continuing study participation;
- The Investigational New Drug (IND) sponsor or regulatory authorities stop the protocol;
- The study PI assesses that it is not in the best interest of the subject to continue participation in the study or that the subject's compliance with the study is not sufficient.

4.5 Treatments

4.5.1 Treatments Administered

The active vaccine under study is 20 mcg dose of VRC-CHKVLP059-00-VP, an investigational VLP vaccine that consists of the E1, E2 and capsid proteins of CHIKV (strain 37997). The control is VRC-PBSPLA043-00-VP, sterile phosphate-buffered saline (PBS) prepared for human injection. Subjects will receive 2 intramuscular (IM) injections via needle and syringe.

4.5.2 Method of Assigning Subjects to Treatment Groups (Randomization)

For each clinical site, randomization will be 1:1 allocation within each stratum of age group to either the CHIKV VLP vaccine or PBS placebo. The randomization sequence was generated in SAS using permuted blocked randomization with randomly selected block sizes of two or four.

Randomizations will be done online using an electronic randomization system. The randomization code was prepared by the Protocol Statistician and included in the enrollment module for the trial. The randomization code will link to the treatment assignment. To decrease the potential for subject dropouts during the period between randomization and initial injection, the electronic data system will assign each subject a randomization code on Day 0 after confirmation of eligibility to begin Day 0 study injections has been entered into the system. Manual randomization back-up procedures and instructions will be provided for use in the event that the site temporarily loses access to the Internet or the online enrollment system is unavailable to a study site.

If an individual is screened but does not enroll into the clinical trial, screening records will be kept to document the reason the individual did not enroll.

4.5.3 Blinding and Conditions for Unblinding

The injections will be prepared by an unblinded site pharmacist or otherwise qualified personnel who is not involved in any subject assessments and who will not discuss randomizations with blinded study staff. The subjects, study personnel who perform study injections and assessments, data entry personnel at the sites, and laboratory personnel performing immunologic assays will be blinded to the treatment assignment of all injections. Subject study injection assignments will be provided to the site Principal Investigator (PI) at completion of the study for communication to the study participants. The DSMB may receive data in aggregate and presented by treatment group during the study, but without the treatment group identified. The DSMB may be unblinded to individual study treatment assignments, as needed, to adequately assess safety issues.

If necessary, the site PI (or designee) and Protocol Chair may agree that management of an AE requires emergency unblinding of an individual subject's assignment following the first injection. This will be documented as a protocol deviation and the Protocol Statistician, the site IRB, and the DSMB will be notified that an early unblinding has occurred and provided with a statement explaining the medical necessity for the early unblinding. For further details on unblinding in the study, consult the Study Blinding Plan.

4.6 Safety and Immunogenicity Variables and Chikungunya Infection Incidence

4.6.1 Safety

Solicited AEs will be recorded in the study database with data collection for 7 days after both the first and second study injections. All unsolicited AEs will be recorded separately in the database from receipt of first study injection through 4 weeks after the last study injection administered. At subsequent follow-up visits, only serious adverse events (SAEs) and new chronic medical conditions will be recorded through the last study visit.

4.6.2 **Immunogenicity**

The principle immunogenicity endpoint is measured 4 weeks post second injection (Week 8) by neutralization assay.

4.6.3 **Chikungunya Infection Incidence**

The primary variable for this analysis is PCR-confirmed CHIKV illness. Reports of clinical illness with or without PCR confirmation will also be provided. Throughout the study if a subject reports signs and symptoms of CHIKV infection, blood samples should be collected for diagnostic testing by virus detection (e.g., PCR) by the Primary Diagnostic Laboratory (PDL). This may occur during a scheduled study visit or unscheduled visits may be conducted to complete the testing needed. CHIKV cases will be recorded through the last study visit. Laboratory confirmed cases of CHIKV will be recorded on a CHIKV endpoints form rather than on an AE form without an attribution assessment.

5 SAMPLE SIZE CONSIDERATIONS

The study design is to enroll 400 healthy adult participants ages 18 to 60 that reside in CHIKV endemic areas. Randomization will equally allocate subjects to vaccine or placebo schedules with electronic stratification by ages 18-40 and 41-60 years. The enrollment plan does not include provision for replacing subjects with incomplete injections or visit schedules.

Enrollments may occur rapidly at more than one site. The sample size for this study was not selected to test a formal null hypothesis, rather it reflects the number of vaccine doses that are available for the study and the desire to gain maximal information for the possible conduct of Phase 3 follow on trials. There are not good data to estimate the loss to follow-up rate for this study. The power calculations assume a 10% loss in both groups for the primary and secondary endpoints.

5.1 Power Calculations for Evaluation of Safety

The goal of the safety evaluation for this study is to identify safety concerns associated with injections of the investigational vaccine. Two sample size calculations for safety are expressed in terms of two approaches, within group and comparing two groups.

1. The ability to detect safety or reactogenicity events within each injection group:

The ability of the study to identify safety events may be expressed in terms of the probability of observing one or more event of interest (e.g., AEs) assuming a range of "true" but unknown event rates. The power to observe one or more event in a single group of 180 subjects is 83.62% when the true rate is 1.0% and 97.37% when the true rate is 2.0% (Table).

Table 2: Probability of Observing One or More Safety Events within a Group (n=180)

True Event Rate	Pr (1 or more events)
0.01%	1.78%
0.10%	16.48%
0.33%	45.17%
0.50%	59.43%
1.00%	83.62%
2.00%	97.37%

2. The minimum detectable difference in safety event (e.g., AEs) rates for comparing two injection groups using Fisher's exact test:

Safety event rates may vary considerably with the type of event considered therefore a wide range of possible rates is considered for comparison by vaccine group (Table).

Table 3: Minimum Detectable Differences in Event Rates for Power = 80% or 90% Assuming Type I Two-Tail Error Rate of 5% (n=180 per Group)

Power	Assumed Event Rate in Group 1 (n=180)	Minimum Detectable Event Rate Group 2 (n=180)	Difference in Rates
80%	0.1%	5.5%	5.4%
	1.0%	7.5%	6.5%
	5.0%	14.2%	9.2%
	25%	39.3%	14.3%
90%	0.1%	6.9%	6.8%
	1.0%	8.9%	7.9%
	5.0%	16.0%	11.0%
	25%	41.6%	16.6%

5.2 Power Calculations for Evaluation of Immune Responses

A secondary objective is to evaluate the humoral immunogenicity of VRC-CHKVLP059-00-VP by neutralization assay at 4 weeks after the second 20 mcg dose (Week 8). Vaccine specific neutralization antibodies are not anticipated in the non-vaccinated group. Therefore, direct comparison between the active and placebo group is of minimal value in assessing sample size. Preliminary Phase 1 neutralization data suggest that neutralization results at the visit with peak response are log-normally distributed with a mean and standard deviation on the log-scale of 7.41 and 0.67 respectively. This standard deviation is rounded up to 0.70 and two more conservative options of 1.0 and 1.5 result in 95% confidence intervals (CI) for geometric mean titers (GMT) (Table).

Table 4: 95% CI for GMT Neutralization among Vaccinees at Time of Peak Response (n=180 per Group)

Assume Standard Deviation on Log Scale	95% CI for GMT Neutralization Among Vaccinees (n=180) at Time of Peak Response
0.70	(1490.7, 1831.7)
1.0	(1426.6, 1914.1)
1.5	(1324.8, 2061.1)

The study is not designed to compare two groups defined by baseline covariates such as gender or age strata. However, the following table is included to provide a sense of the power available for such comparisons. The simple case of two groups (e.g., male and female), assuming equal participation (i.e., 90 subjects for each gender-vaccine group), is given in Table for comparing response rates.

Table 5: Detectable Geometric Mean Ratio between Two Groups for Power = 80% or 90% Assuming Type I Two-Tail Error Rate of 5% (n=90 per Group)

Power	Standard Deviation on Log Scale	Detectable Geometric Mean Ratio (n=90 per group)
80%	0.7	1.34
	1.0	1.52
	1.5	1.88
90%	0.7	1.40
	1.0	1.63
	1.5	2.08

6 GENERAL STATISTICAL CONSIDERATIONS

6.1 General Principles

All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation, median, maximum and minimum. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical measures. In general, all data will be listed, sorted by site, treatment and subject, and when appropriate by visit number within subject. All summary tables will be structured with a column for each treatment in the order (Experimental, Control) and will be annotated with the total population size relevant to that table/treatment, including any missing observations.

Summary statistics for discrete data will include estimates and confidence intervals for binomial data; contingency tables, along with summary analyses such as tests of independence. Two-by-two tables may also include summaries of risk differences or risk ratios as is appropriate along with their confidence intervals.

Tabulations will be used extensively to summarize the data and will include summary statistics. For continuous data, tables will include measures of central tendency such as the mean, geometric mean, or median, and measures of dispersion such as the standard deviation or inter-quartile range. Tables of discrete data will include proportions and their confidence intervals.

Unless noted, statistical analyses will use standard methods as described below.

6.2 Timing of Analyses

This study is addressing an emerging and potentially expanding epidemic of Chikungunya. Therefore, at any time during the study an **unplanned** analysis may be required. There is no intention to use such an analysis to modify the study design. The extent of unblinding (e.g., group versus individual level and parameters examined) and the distribution of results will be limited to the maximal extent compatible with necessary use of the results. For example, site investigators will not receive interim results without an explicit request from the PI. Absent any possible safety concerns in a blinded review of the results, unblinded analyses will focus on immune responses and CHIKV incidence. The PSRT may also request an unblinding of the safety data at any time if, in their opinion, data internal or external to the trial requires such a review.

If any interim analyses are conducted, a separate analysis plan will be established to further describe the nature of the analyses and presentation of data. While these interim results may be used for design of future trials they will not be used to modify the design of this protocol.

Final analyses and the production of a CSR will occur when all subjects have completed their follow-up or have otherwise been prematurely censored, their data is cleaned within the limitations of practical follow-up and the database is locked.

6.3 Analysis Populations

The **Safety** population will include all enrolled subjects, summarized according to the actual vaccination received.

The **Per Protocol (PP)** analyses will include all subjects receiving two injections within window as assigned by the randomization schedule, and not experiencing any other major protocol deviations prior to their week 8 visit. Major protocol deviations include ineligibility, product administration deviations, and primary endpoint missing data. The subjects will be analyzed based on product actually received, regardless of randomization assignment. If subjects receive study vaccine for one visit and placebo for the other, then they will be included in the PP population until they receive the second injection or are otherwise removed from the PP population. The PP analyses will also include subjects determined to be CHIKV infected prior to the receipt of their scheduled second injection and not experiencing any other major protocol deviations prior to their state of infection. Subjects will be censored from the immunogenicity analysis following PCR-confirmed CHIKV infection. Immunogenicity and infection rates will both be examined for the PP population.

The **Intent-to-treat (ITT)** population will include all enrolled subjects, analyzed according to the randomized treatment. The ITT population will be used to analyze immunogenicity and infection rates.

The **Modified intent-to-treat (mITT**) analyses will include all enrolled subjects that received at least one product administration with non-missing immunogenicity data at their Week 8 visit, analyzed according to the study product(s) received. This definition will exclude subjects with protocol deviations related to possible dosing errors. The mITT population will only be used for immunogenicity analyses.

Subjects excluded from Immunogenicity (or CHIKV Incidence) analyses are listed in Appendix III, **Listing 16.2.3.**

6.4 Covariates and Subgroups

This study is not powered to perform formal subgroup analyses. Age stratum (18-40, 41-60 years) and site will be used to define subgroup analyses for descriptive purposes.

6.5 Missing Data

Imputation of missing values is not planned. Imputation may be implemented for secondary or tertiary outcomes if supported by the data and if sufficient consequence to justify their inclusion.

6.6 Interim Analyses and Data Monitoring

The PSRT will review safety data routinely throughout the study. The NIAID Intramural DSMB will provide an independent safety review at scheduled intervals to coincide with their biannual meeting schedule.

Interim analyses are mentioned above in **Section 6.2** and are also covered and detailed elsewhere in the VRC 704 Interim Analysis Plan dated 19 January 2018.

Note that these analyses will not be used for early trial termination. Therefore, final p-values will not be adjusted for interim analyses.

6.7 Multicenter Studies

Data will be pooled across all clinical sites. Center effects are not anticipated because the sites are using standardized procedures for vaccination and assessment of solicited and unsolicited adverse events, and the study relies on central laboratories for the assessment of immunogenicity and clinical efficacy endpoints.

Nevertheless, to guard against the potential for regional differences, the primary analysis will also be presented by site.

6.8 Multiple Comparisons/Multiplicity

This study is not designed to test a specific hypothesis. Rather it is intended to obtain sufficient data to design and implement future efficacy trials. Absent the testing of a formal primary hypothesis there is no requirement for multiple comparison adjustments.

7 STUDY SUBJECTS

7.1 Subject Disposition

Table 14.1.1 (Appendix I), will present a summary of the reasons that subjects were screened but not enrolled.

The composition of analysis populations, including reasons for subject exclusion, by treatment arm, is presented in **Table 14.1.2, Appendix I.**

The disposition of subjects in the study will be tabulated by site and treatment group (**Table 14.1.3, Appendix I**). The table shows the total number of subjects screened, enrolled, receiving at least 1 dose, receiving at least 2 doses, discontinued dosing or terminated from study follow-up and the number completing the study. CHIKV infection status and subject disposition will be tabulated by site and treatment group (**Table 14.1.4, Appendix I**). The table shows the number and percentages of subjects who had a PCR-confirmed CHIKV infection, those with a clinical CHIKV infection (both further split by when infection occurred), and those uninfected.

A flowchart showing the disposition of study subjects, adapted from the CONSORT Statement [3] will be included **(Figure 14.1.1, Appendix II).** This figure will present the number of subjects screened, enrolled, lost to follow-up, and analyzed, by treatment arm where applicable.

A listing of subjects who discontinued dosing or terminated from study follow-up and the reason will be included **in Listing 16.2.1 (Appendix III).**

7.2 Protocol Deviations

A summary of subject-specific protocol deviations will be presented by the reason for the deviation, the deviation category, and treatment group for all subjects **(Table 14.1.5).** Deviations that are considered major in the categories of ineligibility, product administration, and primary endpoint missing data will be reviewed for possible subject exclusion from the per protocol population. All subject-specific protocol deviations and non-subject-specific protocol deviations will be included in Appendix III as data listings (**Listings 16.2.2.1 and 16.2.2.2, respectively)**.

8 SAFETY EVALUATION

8.1 Demographic and Other Baseline Characteristics

Summaries of age, gender, ethnicity, and race will be presented by treatment group overall and by site **(Tables 14.1.6.1-14.1.6.4, Appendix I).** Ethnicity is categorized as Hispanic or Latino, Not Hispanic or Latino, or Unknown/Not reported.

In accordance with NIH reporting policy, subjects may self-designate as belonging to more than one race or may refuse to identify a race, the latter reflected in the eCRF as "No" to each racial option. The race categories to be used are: American Indian or Alaska Native; Asian; Native Hawaiian or Other Pacific Islander; Black or African American; White; Unknown. Individual subject listings **(Appendix III)** will be presented for all demographics **(Listing 16.2.4.1);** pre-existing medical conditions (**Listing 16.2.4.2);** vital signs and oral temperature **(Listing 16.2.9.1);** and concomitant medications (**Listing 16.2.10).**

8.1.1 Concurrent Illnesses and Medical Conditions

All current illnesses and past pre-existing medical conditions will be Medical Dictionary for Regulatory Activities (MedDRA®) coded using MedDRA dictionary version 19.1 or higher.

Summaries of subjects' pre-existing medical conditions will be presented by treatment group and age stratum (**Table 14.1.7, Appendix I**).

Individual subject listings will be presented for all medical conditions **(Listing 16.2.4.2, Appendix III**).

8.1.2 Prior and Concurrent Medications

Current concomitant prescription medications are recorded in the study database at enrollment. Concomitant medications are updated in the study database if there is an occurrence of an adverse event (AE) that requires expedited reporting. If it will not endanger a subject's health, non-investigational vaccines should not be administered 14 days before or until at least 28 days after a study injection. Licensed vaccines received by the subject within 4 weeks before the first study injection administration and 4 weeks after last study injection will be recorded in the study database. Otherwise, a record of concomitant medication changes throughout the study will not be recorded in the study database.

All concomitant medications taken during the study will be recorded on the source documentation.

A by-subject listing of concomitant medication for the safety population use will be presented in **Table 14.3.6, Appendix I.**

Individual subject listings will be presented for all concomitant medications (**Listing 16.2.10, Appendix III**).

8.2 Measurements of Treatment Compliance

All subjects were to receive two doses of study product administered in the clinic. The number of doses of study product administered to subjects will be presented by treatment group and by site as part of the subject disposition table **(Table 14.1.3, Appendix I).**

Table 14.1.8 (Appendix I) presents compliance with the dosing schedule, i.e., the number of subjects who received first dose, by treatment arm and by site. **Listing 16.2.5 (Appendix III)** presents individual subject listings for treatment compliance and dosage administration.

8.3 Adverse Events

The severity of an adverse event (AE) is defined as: Grade 1 (Mild), Grade 2 (Moderate), Grade 3 (Severe), Grade 4 (Life-threatening), and Grade 5 (Death). Only the single AE that is assessed as the primary cause of death should be assigned "Grade 5" severity.

Relation of an AE to the study product is defined as Definitely Related, Probably Related, Possibly Related, and Not Related. For purposes of preparing data reports in which AE attributions are limited to "Related" or "Not Related", in this protocol, the "Definitely, Probably and Possibly" attributions will be mapped to the "Related" category.

AEs will be coded into MedDRA preferred terms.

When calculating the incidence of AEs (i.e., on a per subject basis), each subject will only be counted once and any repetitions of AEs within a subject will be ignored; the denominator will be the total population size. All AEs reported will be included in the summaries and analyses.

8.3.1 Solicited Events and Symptoms

Solicited AEs will be recorded in the study database with data collection for 7 days after both the first and second study injections, without the collection of attribution assessments.

Following the study injection, the subject will remain in the clinic for observation for at least 15 minutes. After 15 minutes, blood pressure and pulse will be taken and the injection site will be inspected for evidence of local reaction before the subject leaves the clinic.

Subjects will be given a diary card to use as a memory aid, on which to record the solicited signs and symptoms daily for 7 days following each injection. The site may use a paper diary card as a source document or clinician notes obtained by telephone interview as the source for the information recorded in the study database. The solicited signs and symptoms on the diary card will include the following parameters: pain/tenderness at injection site, largest diameters of redness (erythema) and swelling (induration) at injection site, highest measured temperature per day, unusually tired/feeling unwell, muscle/body aches (other than at injection site), arthralgia (joint pain), headache, chills, and nausea.

At least one contact at about 7 days after each study injection will be conducted to complete the review of solicited diary card information. Subjects will be asked to contact the clinic at any time following each injection if they have any concerning signs or symptoms. Events that will require clinical evaluation include rash, urticaria, arthralgia, fever of 38.5˚C (Grade 2) or higher lasting more than 24 hours, or significant impairment in the activities of daily living. Additionally, other clinical concerns may prompt a study visit based on the judgment of a study clinician.

The proportion of subjects reporting at least one solicited AE will be summarized by dose, treatment group and age stratum for each solicited adverse event, any systemic symptom, any local symptom, and any symptom. The 95% CI calculated using Clopper-Pearson methodology from a binomial distribution will be presented **(Table 14.3.1.1, Appendix I).**

For each solicited systemic and local event, any systemic event, any local event, and any solicited event, the maximum severity over 7 days after each dose will be summarized for the safety population by treatment group and age stratum. The number and percentage of subjects reporting each event will be summarized by the maximum severity and treatment group, separately for each dose and over all doses. For each event the denominator is the number of subjects with non-missing data for the specific event **(Table 14.3.1.2, Appendix I).**

The number and proportion of subjects reporting a solicited AE will be summarized pre-dose and for each day post-dose for each injection and for all injections combined both in a summary table by treatment group and age stratum **(Table 14.3.1.3, Appendix I)** and graphically in a bar chart **(Figure 14.3.1.1, Appendix II).** A comparison of the event rate of solicited adverse events for each treatment group between dose 1 and dose 2 will be presented by age stratum (**Tables 14.3.1.4-14.3.1.5, Appendix I**).

Solicited AEs by subject will be presented in **Listing 16.2.7.1, Appendix III.**

8.3.2 Unsolicited Adverse Events

All unsolicited AEs will be recorded in the study database from receipt of first study injection through 4 weeks after the last study injection administered. At subsequent follow-up visits, only SAEs and new chronic medical conditions will be recorded through the last study visit.

The proportion of subjects reporting at least one unsolicited AE will be summarized by MedDRA system organ class and preferred term for each injection, age stratum, and treatment group and over all injections. Denominators for percentages are the number of subjects who received the injection being summarized. A 95% CI will be presented along with a Fisher's exact test comparing the treatment groups and testing the difference in the proportion of subjects reporting an unsolicited AE for each MedDRA system organ class and preferred term **(Table 14.3.1.6, Appendix I).**

Unsolicited AEs by subject will be presented in **Listing 16.2.7.2, Appendix III.**

The following summaries for unsolicited AEs will be presented by MedDRA system organ class (SOC), preferred term, dose, age stratum, treatment group, and site:

- *Subject level summary of severity and relationship to study product* ***(Tables 14.3.1.7.1-14.3.1.7.2, Appendix I);***
- *Subject incidence of AEs over time (Days 1-7, Days > 7)* ***(Tables 14.3.1.8.1-14.3.1.8.2, Appendix I);***
- *Total frequency of AEs over time (Days 1-7, Days > 7)* ***(Table 14.3.1.9, Appendix I);***
- *Subject listing of non-serious AEs of moderate or greater severity* ***(Table 14.3.2.2, Appendix I);***
- *Bar chart of non-serious AEs by severity and MedDRA SOC* ***(Figure 14.3.1.2, Appendix II);***
- *Bar chart of non-serious AEs by severity* ***(Figure 14.3.1.3, Appendix II);***
- *Bar chart of non-serious AEs by relationship to study product and MedDRA SOC* ***(Figure 14.3.1.4, Appendix II);***

- *Bar chart of non-serious AEs by relationship to study product* ***(Figure 14.3.1.5, Appendix II).***

8.4 Deaths, Serious Adverse Events and other Significant Adverse Events

For reported deaths, SAEs, and new onset chronic medical conditions, the following listings will be presented:

- Deaths and SAEs **(Table 14.3.2.1, Appendix I);**
- New Onset Chronic Medical Conditions **(Table 14.3.2.3, Appendix I)**.

These tables will be presented in terms of the following variables: Subject ID, Age (years), AE Description, Severity, AE Onset Date/End Date, Last Dose Received/Days Post Dose, Effect on Injection Schedule, Reason Reported as an SAE, Relationship to Treatment, Outcome, and Duration of Event (days).

8.5 Pregnancies

For subjects who become pregnant during the first 16 weeks on study, every attempt will be made to follow them to completion of pregnancy to document the outcome, including information regarding any complications with pregnancy and/or delivery. The pregnancy outcomes are captured by the pregnancy eCRF. Pregnant subjects are discontinued from receiving the study injections.

A table summarizing the total pregnancies, number of live births, and number of spontaneous abortions, elective abortions or stillbirths by treatment will be presented (**Table 14.3.7, Appendix I**). In addition, a listing of pregnancies and outcomes will be presented (**Listing 16.2.11, Appendix III**).

8.6 Clinical Laboratory Evaluations

Safety laboratory parameters evaluated from serum will include only the liver function test measuring ALT. Hematology parameters include hemoglobin, white blood cells (WBC), red blood cells (RBC), hematocrit, mean corpuscular volume (MCV), platelets, and absolute values for lymphocytes, neutrophils, eosinophils, monocytes and basophils. Sites enter the absolute values for the laboratory parameters and the system calculates percentage values. Laboratory safety evaluations are scheduled at week 4, 8, 16, 24, 32, 40, 48, 56, 64, and 72. Clinical laboratory results by subject will be presented in **Listings 16.2.8.1 – 16.2.8.2, Appendix III.**

As a note, the lab data for site PRCTRC were initially entered as percentage values but, in June 2016, the site switched to reporting absolute values with their respective units. This caused some discrepancy between calculated values in the database and data collection forms at the site. This could influence precision and will be considered as lab data are reported.

Only results for scheduled routine visits will be included in these analyses. In particular, laboratory results obtained at visits other than Week 4 and 8 for diagnosis of AEs will not be included.

Safety laboratory evaluations will be graded as follows: mild (grade 1), moderate (grade 2), severe (grade 3), or life-threatening (grade 4).

Safety laboratory results will be tabulated for each measure by study visit, treatment group, age stratum and grade **(Table 14.3.4.1, Appendix I)**. The mean change from baseline along with 95% CI at each time point measured in the study will be computed **(Table 14.3.4.2, Appendix I).** Boxplots of safety laboratory values will be generated for baseline values and for values measured during the course of the study. Each boxplot will show the 1st quartile, the median, and the 3rd quartile, with values smaller than the 1st quartile or larger than the 3rd quartiles plotted as outliers. If appropriate, horizontal lines representing boundaries for abnormal values will be plotted.

Post dose 1 measures may also be summarized using shift tables to compare baseline and follow-up values **(Tables 14.3.4.4.x – 14.3.4.5.x, Appendix I).** Shift tables may be analyzed using Generalized Estimating Equations to account for the longitudinal nature of these data, as follows:

1) **For laboratory parameters with two categories: Normal and abnormal (Binary response)**

   a. For each parameter, the shift table displays the number of subjects who are categorized as normal or abnormal at baseline cross tabulated with normal or abnormal results at the post-vaccination visit.

   b. A GEE for the binary responses above is fitted and used for parameter estimation

   - Observation (y_{ij}, X_{ij}) for each subject $i = 1, \dots, n$ and repeated measurement time point $j = 1, \dots, J$
   - dependent variable $y_{ij} = \begin{cases} 1 & abnormal \\ 0 & normal \end{cases}$, independent variable $X_{ij} = (group_{ij}, time_{ij}, group_{ij} * time_{ij})$;
   - probability of abnormal is $p_{ij} = \Pr(y_{ij} = 1)$;
   - model: $g(p_{ij}) = X'_{ij}\beta$ using the logit link;
   - GEE: $\sum_{i=1}^{n} \left(\frac{\partial P_i}{\partial \beta}\right) V_i^{-1}(\alpha)(Y_i - P_i)=0$ where $V_i(\alpha)$ is the working covariance matrix;
   - Implementation: PROC GENMOD (SAS) using SUBJECT option in the REPEATED statement to identify the cluster variable
   - Output: point estimates and 95% CI for the individual levels of the GROUP, TIME and GROUP*TIME variables, and Type-3 SAS output for the parameter significance;
   - Other analysis and outputs: Odds ratio with associated 95% CI of observing abnormality (from the fitted logistic model). Implemented with LSMEANS statement in PROC GENMOD.

2) **For laboratory parameters with four categories: Grade 1, Grade 2, Grade 3, and Grade 4 (Ordinal response)**

   a. For each parameter the shift table displays the number of subjects who are categorized as grade 1, grade 2, grade 3, or grade 4 at baseline cross tabulated with grade 1, grade 2, grade 3, or grade 4 results at the post-vaccination visit.

b. A GEE model for the ordinal responses above are fitted and used for parameter estimation

- Observation (Y_{ij}, X_{ij}) for each subject $i = 1, \dots, n$ at time point$j = 1, \dots, J$. $Y_{ij} = (y_{ij1}, y_{ij2})$is the ordinal response variable with 3 ordered categories;
- Cumulative probability $p_{ijk} = \Pr(y_{ijk} = 1: in\ lower\ k\ category) = \Pr(Y_{ij} \leq k), k = 1,2;$
- Proportional Odds model$\begin{cases} g(p_{ij1}) = \mu_1 + X'_{ij}\beta \\ g(p_{ij2}) = \mu_2 + X'_{ij}\beta \end{cases}$ using the logit link;
- $X'_{ij}\beta = \sim\beta_1 t_{ij} + \beta_2 group_{ij} + \beta_3 t_{ij} * group_{ij}$
- GEE: $\sum_{i=1}^{n} \left(\frac{\partial P_i}{\partial \beta}\right) V_i^{-1}(\alpha)(Y_i - P_i)$=0 where$P_i = E(Y_i)$ $V_i(\alpha)$ is the working covariance matrix;
- Output: estimated of two intercepts from the two fitted logistic models; point estimates and 95% CI for the individual levels of the GROUP, TIME and GROUP*TIME variables, and Type-3 SAS output for the parameter significance (chi-Square test);

The study is not powered to detect differences by vaccine group, therefore the absence of statistically significantly differences should not be interpreted that no such differences exist.

Individual clinical laboratory results for hematology and chemistry will be presented in **Listing 16.2.8.1 – 16.2.8.2, Appendix III.** Laboratory summary statistics are presented in **Table 14.3.4.2, Appendix I.** Box plots for laboratory results are presented in **Figures 14.5.1 -- 14.5.x, Appendix II.** A listing of abnormal laboratory results is presented in **Table 14.3.4.3, Appendix I.**

8.7 Vital Signs and Physical Evaluations

Vital signs measurements include temperature, pulse, and systolic and diastolic blood pressure. The assessment of vital signs is planned at week 0, 4, 8, 16, 24, 32, 40, 48, 56, 64 and 72. The physical exam is only required at the screening visit and may be performed at subsequent visits if needed based on interim history. These optional physical exam evaluations are not captured in the data system.

Vital signs are tabulated by visit, age stratum, and treatment group. Vital signs will be summarized over time graphically using box plots for subjects in the Safety population (**Table 14.3.5, Appendix I**, **Figure 14.4.1-14.4.x, Appendix II, Listing 16.2.9, Appendix III**).

9 IMMUNOGENICITY EVALUATION

Specimens to evaluate immunogenicity will be taken at baseline and at specified time points. The primary immunogenicity timepoint is 4 weeks after the second injection. Measurements of CHIKV-specific humoral immune responses will be assessed by neutralization antibody (NAb) assays. A listing of individual titer levels and the corresponding planned and actual collection dates are presented in **Appendix III, Listing 16.2.6.**

At some sites, positive baseline Week 0 neutralization antibody assay results were reported. ELISA response data, captured as part of the screening eligibility process, have been received by the Sponsor from the laboratory. When raw ELISA data is received, baseline ELISA responses will be assessed in relation to neutralization antibody data and results will be reported. For further information, a separate analysis plan will be created to elaborate on specific analyses to be performed.

The principle immunogenicity endpoint is measured at Week 8 by neutralization assay.

Descriptive analyses will be presented by study day, age group, and treatment group, aggregated over all study sites and by site. If none or a very small number of placebo group subjects have a neutralization response, their results will only be reported for the positive response rate summaries and omitted from other analyses. This approach is taken to achieve brevity and easier understanding of results when the control group has no meaningful responses.

Summaries include:

- GMT and their confidence intervals **(Table 14.2.1, Appendix I)**
- Geometric mean ratio (GMR) of results obtained at baseline (Week 0) to results obtained at Week 8 and its 95% CI. **(Table 14.2.1, Appendix I)**
- Positive response rates, as defined by an assay-specific post dose response, and their exact 95% CI **(Table 14.2.1, Appendix I)**
- Reverse cumulative distribution of the neutralization assay results **(Figure 14.2.1, Appendix II)**
- Boxplots of titer values **(Figure 14.2.2, Appendix II).**

These summaries will be repeated for all exploratory research tests used to measure exploratory immunogenicity endpoints at the timepoints for which there are data.

10 ANALYSES OF CHIKUNGUNYA INFECTION INCIDENCE AND TIME TO EVENT

Analyses of CHIKV infections are exploratory. A few definitions of incident cases exist and therefore efficacy will be considered in:

- cases for which CHIKV is confirmed by a positive PCR
- cases with symptoms consistent with CHIKV but not necessarily confirmed by a positive PCR

Efficacy will include all cases using 2 exploratory alternatives for case inclusion:

1. all infections post dose 1 (Day 0),
2. all infections post dose 2.

Subjects not receiving dose 2 will be included in definition 1.

The analysis of efficacy is divided into two parts which are described below, using the intent-to-treat cohort.

Clinical cases as well as PCR confirmed cases are listed in **Listing 16.3.1, Appendix III.** Individual time to event data post dose one and post dose two are listed in **Listing 16.3.2 -16.3.3, Appendix III.**

10.1 Description of Observed Events:

- Incidence and number of clinical cases of CHIKV
  - Inclusion 1: All cases post dose 1 (Day 0)
  - Inclusion 2: All cases post dose 2. Subjects not receiving dose 2 will be considered for inclusion 1.
- Incidence and number of PCR confirmed cases of CHIKV
  - Inclusion 1: All cases post dose 1 (Day 0)
  - Inclusion 2: All cases post dose 2. Subjects not receiving dose 2 will be considered for inclusion 1.

For each scenario, incidence rates and number of cases will be described by treatment group by age stratum and aggregating and by combining age strata. Results are presented in **Tables 15.1.1-15.1.4 in Appendix I.**

10.2 Analysis of Vaccine Efficacy:

- Time to occurrence of clinical CHIKV infection
  - Inclusion 1: All cases post dose 1 (Day 0)
  - Inclusion 2: All cases post dose 2. Subjects not receiving dose 2 will be considered for inclusion 1.
- Time to occurrence of PCR confirmed CHIKV infection
  - Inclusion 1: All cases post dose 1 (Day 0)
  - Inclusion 2: All cases post dose 2. Subjects not receiving dose 2 will be considered for inclusion 1.

Time to event will be based on the date of onset of the clinical symptom (for clinical cases) or on blood sample with the first positive laboratory test indicating CHIKV infection (for PCR confirmed cases).

Time to occurrence post dose one and post dose two will be calculated for clinical CHIKV infections as well as PCR confirmed CHIKV infections as follows:

Post first injection:

- For subjects with infection: First date of diagnosis of clinical CHIKV infection minus date of first injection + 1. These subjects will not be censored.
- For subjects with no infection: Date of study completion or date of early termination minus date of first injection + 1. These subjects will be censored at their date of study completion or date of early termination.

Post second injection:

- For subjects with infection: First date of diagnosis of clinical CHIKV infection minus date of second injection + 1. These subjects will not be censored.
- For subjects with no infection: Date of study completion or date of early termination minus date of second injection + 1. These subjects will be censored at their date of study completion or date of early termination.
- Subjects that do not receive the second dose are excluded.
- Subjects that develop CHIKV before second dose are excluded.

The principal exploratory analysis consists of a comparison between the vaccine and placebo groups of the time to occurrence of infection after first dose and after second dose. The magnitude of vaccine effect will be estimated in terms of vaccine efficacy (95% CI) obtained from Cox proportional hazard model. Statistical testing will be performed using Log-rank test. (**Tables 15.2.1-15.2.4, Appendix I**)

The secondary exploratory analysis consists of an adjusted survival for age and site using a Log-rank test. In addition to univariate adjustments, multivariate adjustment will be performed in a single model using Cox regression for time to infection after first dose and after second dose. Estimates of vaccine efficacy adjusting for baseline characteristics will be displayed **in Tables 15.3.1-15.3.4, Appendix I**. Kaplan-Meier survival curves will be plotted by group, as presented in **Figures 15.1.1-15.1.4 Appendix III.**

10.3 Sample SAS Code for Analysis:

Notations

Timevar = the variable that gives the time to occurrence

Censor_var = the variable defining whether timevar is censored

Trtmnt_group = treatment group where 0 = Placebo and 1=VRC-CHIKVLP059-00-VP;

Cox Proportional Hazard

SAS Code for Cox Regression to obtain Vaccine Efficacy and 95% CI for Vaccine Efficacy:

```
Proc phreg;
        Model timevar*censor_var(0)=trtmnt_group (and any covariates)/rl;
        Ods output parameterestimates=ests(keep=HazardRatio HRUpperCL HRLowerCL);
        Run;
```

```
Ods output RiskDiffCol1=rdc1;

Run;
```

Calculate the following from the hazard ratios/estimates obtained from SAS

Vaccine Efficacy = 1 – Hazardratio;

Lower 95% CI= 1 – HRUpperCL which is the same as 1 – exp(estimate + 1.96*stderr)

Upper 95% CI= 1 – HRLowerCL which is the same as 1 - exp(estimate – 1.96*stderr*)*

Log-Rank Test

SAS Code for Log-Rank Test to obtain p-value:

```
Proc lifetest;
time timevar*censor_var(0);
strata trtmnt_group;
id pid;
Ods output homtests = tests(where=(test="Log-Rank") keep=test probchisq);
Run;
```

Obtain p-value from SAS: p-value=probchisq;

11 REPORTING CONVENTIONS

P-values ≥0.001 and ≤0.999 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001"; p-values greater than 0.999 will be reported as "> 0.999". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Proportions will be presented as two decimal places; values <0.01 will be presented as "<0.01". Percentages will be reported to the nearest whole number; values < 1% will be presented as "<1". Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients), will be reported to 3 significant figures.

12 TECHNICAL DETAILS

SAS version 9.3 or above will be used to generate all tables, figures and listings.

13 REFERENCES

1. WHO. *Chikungunya*. 2015 [cited; Available from: http://www.who.int/mediacentre/factsheets/fs327/en/.]
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3. Schulz Kenneth F, Altman Douglas G, Moher David. *CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials.* BMJ 2010; 340:c332

14 LISTING OF TABLES, LISTINGS AND FIGURES

Table, figure, and listing shells are presented in Appendices I, II and III respectively.

Statistical Analysis Plan

Appendix I: Table Mock - Ups

for

Protocol VRC 704

Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

Version 1.0

Prepared and distributed by:
The Emmes Corporation
Rockville, Maryland

Table of Contents

Section 14.1 Demographic Data

TABLE 14.1.1:
Ineligibility Summary of Screen Failures by Site

Inclusion/Exclusion Criterion	Number of Times Item Marked Ineligible*						
	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	All Sites
Number of subjects failing any eligibility criterion	x						
Laboratory findings	x						
CHIKV Positive or Indeterminate	x						
Physical exam findings	x						
Medical history	x						
Other	x						

*More than one criterion may be marked per subject.

Note: If subject is positive or indeterminate for at least one result from the IgG and IgM CHIKV tests, then they will be counted as CHIKV Positive or Indeterminate.

TABLE 14.1.2:
Analysis Populations by Treatment Group

[Implementation note: All percentages should use N as the denominator, where N is the total number of subjects defined in column headers.

More than one criterion may be marked per subject.]

Analysis Populations		CHIKV (N=X)		Placebo (N=X)		All Subjects (N=X)	
		n	%	n	%	n	%
Population 1, Per Protocol	Subjects included in PP	x	x.x	x	x.x	x	x.x
	Any reason for exclusion						
	Vaccination out of window						
	Major protocol deviation prior to Week 8 visit						
	Error in second vaccination						
Immunogenicity Only	PCR-confirmed CHIKV infection						
Population 2, Intent to Treat							
Immunogenicity	Subjects included in ITT						
	Immunogenicity data not available						
Infection Rates	Subjects included in ITT (no exclusions)						
Population 3, Modified Intent to Treat							
Immunogenicity Only	Subjects included in mITT						
	Any reason for exclusion						
	Immunogenicity data not available at Week 8 visit						
	Received no product administrations						
	Protocol deviation related to dosing error						

TABLE 14.1.3:
Subject Disposition by Site and Treatment Group

Subject Disposition	Site 1 CHIKV (N=X)		Site 1 Placebo (N=X)		Site 2 CHIKV (N=X)		Site 2 Placebo (N=X)		Site 3 CHIKV (N=X)		Site 3 Placebo (N=X)		Site 4 CHIKV (N=X)		Site 4 Placebo (N=X)		Site 5 CHIKV (N=X)		Site 5 Placebo (N=X)		Site 6 CHIKV (N=X)		Site 6 Placebo (N=X)		All Sites CHIKV (N=X)		All Sites Placebo (N=X)		All Sites All Subjects (N=X)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Screened	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
Enrolled and Received First Vaccination																														
Received Second Vaccination Remaining In PP Population [a]																														
Completed- Per Protocol[b]																														
Lost to Per Protocol Before Vaccination Two																														
Lost to Per Protocol At or After the Time of Vaccination 2																														
Completed ITT Cohort																														

[a]Refer to Listing 16.2.1 for reasons subjects discontinued or terminated early.

[b]Refer to Listing 16.2.3 for reasons subjects are excluded from the per protocol population.

TABLE 14.1.4:
Chikungunya Infection Subject Status by Site and Treatment Group

Subject Disposition	Site 1 CHIKV (N=X)		Site 1 Placebo (N=X)		Site 2 CHIKV (N=X)		Site 2 Placebo (N=X)		Site 3 CHIKV (N=X)		Site 3 Placebo (N=X)		Site 4 CHIKV (N=X)		Site 4 Placebo (N=X)		Site 5 CHIKV (N=X)		Site 5 Placebo (N=X)		Site 6 CHIKV (N=X)		Site 6 Placebo (N=X)		All Sites CHIKV (N=X)		All Sites Placebo (N=X)		All Sites All Subjects (N=X)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Enrolled	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
ChikV infection confirmed by PCR																														
ChikV infection prior to second injection																														
Received second injection and not ChikV infected																														
ChikV infected post second injection																														
Completed follow-up in PP Cohort without infection																														
Lost to follow-up and not known to be infected																														
Completed ITT Cohort without ChikV infection																														
Clinical ChikV infection (confirmed or not by PCR)																														
ChikV infection prior to second injection																														
Received second injection and not ChikV infected																														
ChikV infected post second injection																														

Subject Disposition	Site 1 CHIKV (N=X)		Site 1 Placebo (N=X)		Site 2 CHIKV (N=X)		Site 2 Placebo (N=X)		Site 3 CHIKV (N=X)		Site 3 Placebo (N=X)		Site 4 CHIKV (N=X)		Site 4 Placebo (N=X)		Site 5 CHIKV (N=X)		Site 5 Placebo (N=X)		Site 6 CHIKV (N=X)		Site 6 Placebo (N=X)		All Sites CHIKV (N=X)		All Sites Placebo (N=X)		All Sites All Subjects (N=X)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Completed follow-up in PP Cohort without infection																														
Lost to follow-up and not known to be infected																														
Completed ITT Cohort without ChikV infection																														

TABLE 14.1.5:
Distribution of Subject-Specific Protocol Deviations by Category and Treatment Group

Category	CHIKV (N=X)		Placebo (N=X)		All Subjects (N=X)	
	# of Subj.	# of Dev.	# of Subj.	# of Dev.	# of Subj.	# of Dev.
Eligibility/enrollment						
Product administration schedule						
Follow-up visit schedule						
Protocol procedure/assessment						
Product administration						
Blinding policy/procedure						

TABLE 14.1.6.1:
Distribution of Sex, Ethnicity, Race, and Language by Site

Demographic Category	Characteristic	Site 1 (N=X)		Site 2 (N=X)		Site 3 (N=X)		Site 4 (N=X)		Site 5 (N=X)		Site 6 (N=X)		All Subjects (N=X)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sex	Male	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Female														
	Unknown or Not Reported														
Ethnicity	Hispanic or Latino	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Not Hispanic or Latino														
	Unknown or Not Reported														
Race	American Indian or Alaska Native	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Asian														
	Native Hawaiian or Other Pacific Islander														
	Black or African American														
	White														
	Unknown														
Language	English	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	French														
	Spanish														
	Haitian Creole														
	Antillean Creole														

TABLE 14.1.6.2:
Age Statistics (in Years) by Site

Statistic	[Site 1] (N=X)	[Site 2] (N=X)	[Site 3] (N=X)	[Site 4] (N=X)	[Site 5] (N=X)	[Site 6] (N=X)	All Subjects (N=X)
Mean	x.x	x.x					x.x
Standard Deviation	x.x	x.x					x.x
Median	x.x	x.x					x.x
Minimum	X	X					x
Maximum	X	X					x

TABLE 14.1.6.3:
Distribution of Sex, Ethnicity, Race, and Language by Treatment Group

Demographic Category	Characteristic	CHIKV (N=X)		Placebo (N=X)		All Subjects (N=X)	
		n	%	n	%	n	%
Sex	Male	x	x.x	x	x.x	x	x.x
	Female						
Ethnicity	Unknown or Not Reported Hispanic or Latino Not Hispanic or Latino Unknown or Not Reported	x	x.x	x	x.x	x	x.x
Race	American Indian or Alaska Native Asian Native Hawaiian or Other Pacific Islander	x	x.x	x	x.x	x	x.x
	Black or African American White						
	Unknown						
Language	English French	x	x.x	x	x.x	x	x.x
	Spanish						
	Haitian Creole						
	Antillean Creole						

TABLE 14.1.6.4:
Age Statistics (in Years) by Treatment Group

Statistic	CHIKV (N=X)	Placebo (N=X)	All Subjects (N=X)
Mean	x.x	x.x	x.x
Standard Deviation	x.x	x.x	x.x
Median	x	X	x
Minimum	x	X	x
Maximum	x	X	x

TABLE 14.1.7:
Distribution of Subjects with Pre-Existing Medical Conditions by MedDRA® System Organ Class and Treatment Group by Age Stratum

Age Stratum (years)	MedDRA® System Organ Class	CHIKV (N=X)		Placebo (N=X)		All Subjects (N=X)	
		n	%	n	%	n	%
18-40	Any SOC	x	x.x	x	x.x	x	x.x
	[SOC 1]						
	[SOC 2]						
41-60	Any SOC						
	[SOC 1]						
	[SOC 2]						

Note: This table presents number and percentage of subjects. A subject is only counted once per SOC.

TABLE 14.1.8:
Dates of First Treatment by Site and Treatment Group

Month and Year	[Site 1] CHIKV (N=X)	[Site 1] Placebo (N=X)	[Site 2] CHIKV (N=X)	[Site 2] Placebo (N=X)	[Site 3] CHIKV (N=X)	[Site 3] Placebo (N=X)	[Site 4] CHIKV (N=X)	[Site 4] Placebo (N=X)	[Site 5] CHIKV (N=X)	[Site 5] Placebo (N=X)	[Site 6] CHIKV (N=X)	[Site 6] Placebo (N=X)	All Sites CHIKV (N=X)	All Sites Placebo (N=X)	All Sites All Subjects (N=X)
November 2015	x	x	x	x	x	x	x	x	x	x	X	x	x	x	x
December 2015															
…															

Section 14.2 Immunogenicity Data

TABLE 14.2.1:
Geometric Mean Titer (GMT), Geometric Mean Ratio, and Positive Response Rates of the [specific strain/assay] Results with 95% Confidence Intervals at Baseline and Week 8 by Site and Treatment Group <<Age Stratum>>

	Baseline		Week 8						
Treatment Group	N	GMT (95% CI)	N	GMT (95% CI)	Comparison of GMT at Week 8 (p-value)	Geometric Mean Ratio (95% CI)	P-value comparison of GMR	Positive Response Rates (%) at Week 8 (95% CI)^	P-value comparison of Positive Response Rates*
Overall									
CHIKV VLP 20 mcg	x	xx.x (xx.x, xx.x)	x	xx.x (xx.x, xx.x)	x.xxx	x.xx (x.xx, x.xx)	x.xxx	xx.x (xx.x, xx.x)	x.xxx
PBS									
Site 1 [repeat for all sites]									
CHIKV VLP 20 mcg									
PBS									

GMT = Geometric Mean Titer, CI = Confidence Interval, GMR = Geometric Mean Ratio
^Positive immune response defined as [positivity criteria here]
*p-value from Fisher's exact test

Section 14.3 Safety Data

TABLE 14.3.1.1:
Number and Percentage of Subjects Experiencing Solicited Events with 95% Confidence Intervals by Symptom, Dose, Treatment Group, and Site <<Age Stratum>>

[Implementation note: Repeat rows for each site like the example at the end of the table for Site 1]

	Post Dose 1 CHIKV (N=X)			Post Dose 1 Placebo (N=X)			Post Dose 2 CHIKV (N=X)			Post Dose 2 Placebo (N=X)			Post Either Dose CHIKV (N=X)			Post Either Dose Placebo (N=X)		
Symptom	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**	**n**	**%**	**95% CI**
Overall																		
Any Symptom	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x	x.x	x.x, x.x
Any Systemic Symptom																		
Nausea																		
Chills																		
Headache																		
Malaise [Unusually Tired/Feeling Unwell]																		
Myalgia [Muscle/Body Aches]																		

TABLE 14.3.1.1: Number and Percentage of Subjects Experiencing Solicited Symptoms with 95% Confidence Intervals by Symptom, Dose and Treatment Group *(continued)*

Symptom	Post Dose 1 CHIKV (N=X)			Post Dose 1 Placebo (N=X)			Post Dose 2 CHIKV (N=X)			Post Dose 2 Placebo (N=X)			Post Either Dose CHIKV (N=X)			Post Either Dose Placebo (N=X)		
Joint Pain																		
Temperature																		
Any Local Symptom																		
Pain/Tenderness																		
Erythema/Redness																		
Induration/Swelling																		
Site 1																		
Any Symptom																		
…[continue for all symptoms and sites]																		

TABLE 14.3.1.2:
Number and Percentage of Subjects Experiencing Solicited Events by Symptom, Maximum Severity, Dose, Treatment Group, and Site <<Age Stratum>>

Any Symptom

Symptom	Severity	Post Dose 1 CHIKV (N=X)		Post Dose 1 Placebo (N=X)		Post Dose 2 CHIKV (N=X)		Post Dose 2 Placebo (N=X)		Post Either Dose CHIKV (N=X)		Post Either Dose Placebo (N=X)	
		n	%	n	%	n	%	n	%	n	%	n	%
Overall													
Any Symptom	None	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Mild												
	Moderate												
	Severe												
Site 1 [repeat for all sites]													
Any Symptom	None												
	Mild												
	Moderate												
	Severe												

Severity is the maximum severity reported post dosing for each subject.

Systemic Symptoms <<Age Stratum>>

Symptom	Severity	Post Dose 1 CHIKV (N=X)		Post Dose 1 Placebo (N=X)		Post Dose 2 CHIKV (N=X)		Post Dose 2 Placebo (N=X)		Post Either Dose CHIKV (N=X)		Post Either Dose Placebo (N=X)	
		n	%	n	%	n	%	n	%	n	%	n	%
Overall													
Any Systemic Symptom	None	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Mild												
	Moderate												
	Severe												
[Systemic Symptom 1]	None												
	Mild												
	Moderate												
	Severe												
[Systemic Symptom 2]	None												
	Mild												
	Moderate												
[continue for all systemic symptoms]	Severe												
Site 1 [repeat for all sites]													
Any Systemic Symptom	None												
	Mild												
	Moderate												
	Severe												

Severity is the maximum severity reported post dosing for each subject.

Local Symptoms <<Age Stratum>>

Symptom	Severity	Post Dose 1 CHIKV (N=X)		Post Dose 1 Placebo (N=X)		Post Dose 2 CHIKV (N=X)		Post Dose 2 Placebo (N=X)		Post Either Dose CHIKV (N=X)		Post Either Dose Placebo (N=X)	
		n	%	n	%	n	%	n	%	n	%	n	%
Overall													
Any Local Symptom	None	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Mild												
	Moderate												
	Severe												
[Local Symptom 1]	None												
	Mild												
	Moderate												
	Severe												
[Local Symptom 2]	None												
	Mild												
	Moderate												
[continue for all local symptoms]	Severe												
Site 1 [repeat for all sites]													
Any Local Symptom	None												
	Mild												
	Moderate												
	Severe												

Severity is the maximum severity reported post dosing for each subject.

TABLE 14.3.1.3:
Number and Percentage of Subjects Experiencing Solicited Events by Symptom, Severity, Dose, Day Post Dosing, Treatment Group, and Site <<Age Stratum>>

[Implementation Note: If there are more than 7 days of solicited symptom data, then you may want to group together days, for example: Pre-Dose, Post-Dose through Day 2, Days 3-5, Days 6+.]

Any Symptom

[CHIKV, Post Dose 1] [Repeat this table for each group, each dose]															
Symptom	**Severity**	**Day 1 (N=X)**		**Day 2 (N=X)**		**Day 3 (N=X)**		**Day 4 (N=X)**		**Day 5 (N=X)**		**Day 6 (N=X)**		**Day 7+ (N=X)**	
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Overall															
Any Symptom	None	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Mild														
	Moderate														
	Severe														
	Not Reported														
Site 1 [repeat for all sites]															
Any Symptom	None														
	Mild														
	Moderate														
	Severe														
	Not Reported														

Severity is the maximum severity reported post dosing for each subject for each day.

Systemic Symptoms <<Age Stratum>>

[CHIKV, Post Dose 1] [Repeat this table for each group, each dose]															
		Day 1 (N=X)		Day 2 (N=X)		Day 3 (N=X)		Day 4 (N=X)		Day 5 (N=X)		Day 6 (N=X)		Day 7+ (N=X)	
Symptom	**Severity**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Overall															
Any Systemic Symptom	None	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Mild														
	Moderate														
	Severe														
	Not Reported														
[Systemic Symptom 1]	None														
	Mild														
	Moderate														
	Severe														
	Not Reported														
[Systemic Symptom 2]	None														
	Mild														
	Moderate														
	Severe														
[continue for all sys. symptoms]	Not Reported														
Site 1 [repeat for all sites]															
Any Systemic Symptom	None														

Severity is the maximum severity reported post dosing for each subject for each day.

Local Symptoms <<Age Stratum>>

[CHIKV, Post Dose 1] [Repeat this table for each group, each dose]															
		Day 1 (N=X)		Day 2 (N=X)		Day 3 (N=X)		Day 4 (N=X)		Day 5 (N=X)		Day 6 (N=X)		Day 7+ (N=X)	
Symptom	**Severity**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Overall															
Any Local Symptom	None	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Mild														
	Moderate														
	Severe														
	Not Reported														
[Local Symptom 1]	None														
	Mild														
	Moderate														
	Severe														
	Not Reported														
[Local Symptom 2]	None														
	Mild														
	Moderate														
	Severe														
[continue for all local symptoms]	Not Reported														
Site 1 [repeat for all sites]															
Any Local Symptom	None														

Severity is the maximum severity reported post dosing for each subject for each day.

TABLE 14.3.1.4:
Number and Percentage of Subjects Experiencing Solicited Events for Dose 1 Compared with Dose 2 by Treatment Group

Systemic Symptoms <<Overall/Site, Age Stratum>>

Treatment Group	Dose 1	Dose 2 – No Symptoms (N=X)	Dose 2 – Mild or Greater Symptom (N=X)	Dose 2 – Total (N=X)
CHIKV	No Symptoms (N=X)	x (x.xx)	x (x.xx)	x (x.xx)
	Mild or Greater Symptom (N=X)			
	Total (N=X)			
Placebo	No Symptoms (N=X)			
	Mild or Greater Symptom (N=X)			
	Total (N=X)			

Local Symptoms <<Overall/Site, Age Stratum>>

Treatment Group	Dose 1	Dose 2 – No Symptoms (N=X)	Dose 2 – Mild or Greater Symptom (N=X)	Dose 2 – Total (N=X)
CHIKV	No Symptoms (N=X)	x	x	x
	Mild or Greater Symptom (N=X)			
	Total (N=X)			
Placebo	No Symptoms (N=X)			
	Mild or Greater Symptom (N=X)			
	Total (N=X)			

TABLE 14.3.1.5:
Comparison of the Proportion of Subjects Experiencing Solicited Events by Treatment Group and Site <<Age Stratum>>

Symptom	Statistic	CHIKV Post Either Dose (N=X)	Placebo Post Either Dose (N=X)
Overall			
Any Symptom	Proportion	x.xx	x.xx
	95% CI (for proportion)	x.xx, x.xx	x.xx, x.xx
	Difference	x.xx	x.xx
	95% CI (for difference)	x.xx, x.xx	x.xx, x.xx
[Symptom 1]	Proportion		
	95% CI (for proportion)		
	Difference		
[continue for all symptoms]	95% CI (for difference)		
Site 1 [repeat for all sites]			
Any Symptom	Proportion		
	95% CI (for proportion)		
	Difference		
	95% CI (for difference)		

TABLE 14.3.1.6:
Number and Percentage of Subjects Experiencing Unsolicited Adverse Events with 95% Confidence Intervals by MedDRA® System Organ Class, Preferred Term, Treatment Group and Dose Number <<Age Stratum>>

Post Dose 1 [Repeat for Dose 2 and Either Dose]

MedDRA® System Organ Class	MedDRA® Preferred Term	CHIKV (N=X)			Placebo (N=X)			Comparison of % subjects experiencing event (p-value)[1]
		n	%	95% CI	n	%	95% CI	
Overall								
Any SOC	Any PT	x	x.x	x.x, x.x	x	x.x	x.x, x.x	x.xxx
[SOC 1]	Any PT							
	[PT 1]							
	[PT 2]							
[SOC 2]	Any PT							
	[PT 1]							
[continue for all SOC/PT]	[PT 2]							
Site 1 [repeat for all sites]								
Any SOC	Any PT							

Note: This table presents number and percentage of subjects. A subject is only counted once per PT/time point.
1. p-values from Fisher's exact test.

TABLE 14.3.1.7.1:
Number and Percentage of Subjects Experiencing Unsolicited Adverse Events by MedDRA® System Organ Class and Preferred Term, Severity, Relationship, Treatment Group, and Site <<Age Stratum>>

CHIKV [Repeat for Placebo] (N=X)

				Severity						Relationship to Treatment			
		Any Incidence		Mild		Moderate		Severe		Not Related		Related	
MedDRA® System Organ Class	MedDRA® Preferred Term	n	%	n	%	n	%	n	%	n	%	n	%
Overall													
Any SOC	Any PT	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
[SOC 1]	Any PT												
	[PT 1]												
	[PT 2]												
[SOC 2]	Any PT												
	[PT 1]												
[continue for all SOC/PT]	[PT 2]												
Site 1 [repeat for all sites]													
Any SOC	Any PT												

Note: This table presents number and percentage of subjects. A subject is only counted once per PT and is summarized according to their highest severity and closest relationship.

TABLE 14.3.1.7.2:
Number and Percentage of Subjects Experiencing Related Unsolicited Adverse Events by MedDRA® System Organ Class and Preferred Term, Severity, Treatment Group, and Site <<Age Stratum>>

CHIKV [Repeat for Placebo] (N=X)

				Severity					
		Any Incidence		Mild		Moderate		Severe	
MedDRA® System Organ Class	MedDRA® Preferred Term	n	%	n	%	n	%	n	%
Overall									
Any SOC	Any PT	x	x.x	x	x.x	x	x.x	x	x.x
[SOC 1]	Any PT								
	[PT 1]								
	[PT 2]								
[SOC 2]	Any PT								
	[PT 1]								
[continue for all SOC/PT]	[PT 2]								
Site 1 [repeat for all sites]									
Any SOC	Any PT								

Note: This table presents number and percentage of subjects. A subject is only counted once per PT and is summarized according to their highest severity.

TABLE 14.3.1.8.1:
Number and Percentage of Subjects Experiencing Unsolicited Adverse Events by MedDRA® System Organ Class and Preferred Term, Day Post Dosing, Treatment Group, and Site <<Age Stratum>>

CHIKV [Repeat for Placebo] (N=X)

[Implementation Note: Day x-y interval should correspond to period of collection for solicited symptoms, in this case Day 1-7. Day y-z interval should be up until subsequent dose, in this case Days > 7.]

		[Day 1-7] Post Dose 1		[Day 8-28] Post Dose 1		[Day 1-7] Post Dose 2		[Day 8-28] Post Dose 2		Anytime Post Either Dose	
MedDRA® System Organ Class	**MedDRA® Preferred Term**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Overall											
Any SOC	Any PT	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
[SOC 1]	Any PT										
	[PT 1]										
	[PT 2]										
[SOC 2]	Any PT										
	[PT 1]										
[continue for all SOC/PT]	[PT 2]										
Site 1 [repeat for all sites]											
Any SOC	Any PT										

Note: This table presents number and percentage of subjects. A subject is only counted once per PT/Time point.

TABLE 14.3.1.8.2:
Number and Percentage of Subjects Experiencing Related Unsolicited Adverse Events Within 7 Days Post Dosing by MedDRA® System Organ Class and Preferred Term, Dose, Treatment Group, and Site <<Age Stratum>>

CHIKV [Repeat for Placebo] (N=X)

[Implementation Note: Day x-y interval should correspond to period of collection for solicited symptoms, if applicable. In this case, that is Days 1-7.]

		[Day 1-7] Post Dose 1		[Day 1-7] Post Dose 2		Within y Days Post Either Dose	
MedDRA® System Organ Class	**MedDRA® Preferred Term**	**n**	**%**	**n**	**%**	**n**	**%**
Overall							
Any SOC	Any PT	x	x.x	x	x.x	x	x.x
[SOC 1]	Any PT						
	[PT 1]						
	[PT 2]						
[SOC 2]	Any PT						
	[PT 1]						
[continue for all SOC/PT]	[PT 2]						
Site 1 [repeat for all sites]							
Any SOC	Any PT						

Note: This table presents number and percentage of subjects. A subject is only counted once per PT/Time point.

TABLE 14.3.1.9:
Number of Unsolicited Adverse Events by MedDRA® System Organ Class and Preferred Term, Day Post Dosing, Treatment Group, and Site <<Age Stratum, Overall/Site>>

CHIKV [Repeat for Placebo] (N=X)

[Implementation Note: Day x-y interval should correspond to period of collection for solicited symptoms, if applicable. Day y-z interval should be up until subsequent dose.]

		[Day 1-7] Post Dose 1	[Day 8-28] Post Dose 1	[Day 1-7] Post Dose 2	[Day 8-28] Post Dose 2	Anytime Post Either Dose
MedDRA® System Organ Class	**MedDRA® Preferred Term**	**# of Events**	**# of Events**	**# of Events**	**# of Events**	**# of Events**
Overall						
Any SOC	Any PT	x	x	x	x	x
[SOC 1]	Any PT					
	[PT 1]					
	[PT 2]					
[SOC 2]	Any PT					
	[PT 1]					
[continue for all SOC/PT]	[PT 2]					
Site 1 [repeat for all sites]						
Any SOC	Any PT					

Note: This table presents number of events.

TABLE 14.3.2.1:
Listing of Deaths and Serious Adverse Events

[Implementation note: If event is ongoing at end of study, indicate as "Ongoing" in the "Duration" column.

If there are no comments for an event, populated "Comments" row with "None."]

Part 1

Subject ID	Age (Years)	Treatment Group	AE Number	Adverse Event	Adverse Event Onset Date	Associated with Dose #	# of Days Post Associated Dose	Adverse Event End Date	Duration (Days)	Reason Reported as an SAE	Severity

Part 2

Subject ID	Adverse Event	Relationship to Study Treatment	Effect on Injection Schedule	Subject Discontinued Due to AE	Outcome	Comments

TABLE 14.3.2.2:
Listing of Non-Serious, Unsolicited, Moderate or Severe Adverse Events

[Implementation note: If there are no comments for an event, populated "Comments" row with "None."]

Part 1

Subject ID	Age (Years)	Treatment Group	AE Number	Adverse Event	Adverse Event Onset Date	Associated with Dose #	# of Days Post Associated Dose	Adverse Event End Date	Duration (Days)	Severity	Relationship to Study Treatment

Part 2

Subject ID	Adverse Event	Effect on Injection Schedule	Subject Discontinued Due to AE	Outcome	Comments

TABLE 14.3.2.3:
Listing of New Onset Chronic Medical Conditions

[Implementation note: These conditions are met when AD12SAEA = '3' (Persistent/significant disability/incapacity) and/or AD12OTCM = '3' (Ongoing/persistent condition)]

Part 1

Subject ID	Age (Years)	Treatment Group	AE Number	Adverse Event	Adverse Event Onset Date	Associated with Dose #	# of Days Post Associated Dose	Adverse Event End Date	Duration (Days)	Severity	Relationship to Study Treatment

Part 2

Subject ID	Adverse Event	Effect on Injection Schedule	Subject Discontinued Due to AE	Outcome	Comments

TABLE 14.3.4.1:

Distribution of Laboratory Results by Parameter, Severity, Study Day, and Treatment Group <<Age Stratum, Overall/Site>>

[Implementation: Repeat this table for the Placebo group]

Lab. Param	Severity	Baseline CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)		Week 40 CHIKV (N=X)		Week 48 CHIKV (N=X)		Week 56 CHIKV (N=X)		Week 64 CHIKV (N=X)		Week 72 CHIKV (N=X)		Max Severity Post Baseline CHIKV (N=X)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Any parameter	Normal	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
ALT	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
WBC	Normal																								

Lab. Param	Severity	Baseline CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)		Week 40 CHIKV (N=X)		Week 48 CHIKV (N=X)		Week 56 CHIKV (N=X)		Week 64 CHIKV (N=X)		Week 72 CHIKV (N=X)		Max Severity Post Baseline CHIKV (N=X)	
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
RBC	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
Hemoglobin	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
Hematocrit	Normal																								

Lab. Param	Severity	Baseline CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)		Week 40 CHIKV (N=X)		Week 48 CHIKV (N=X)		Week 56 CHIKV (N=X)		Week 64 CHIKV (N=X)		Week 72 CHIKV (N=X)		Max Severity Post Baseline CHIKV (N=X)	
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
MCV	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
Platelets	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								

Lab. Param	Severity	Baseline CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)		Week 40 CHIKV (N=X)		Week 48 CHIKV (N=X)		Week 56 CHIKV (N=X)		Week 64 CHIKV (N=X)		Week 72 CHIKV (N=X)		Max Severity Post Baseline CHIKV (N=X)	
Neutrophil	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
Lymphocyte	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
Monocyte	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								

Lab. Param	Severity	Baseline CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)		Week 40 CHIKV (N=X)		Week 48 CHIKV (N=X)		Week 56 CHIKV (N=X)		Week 64 CHIKV (N=X)		Week 72 CHIKV (N=X)		Max Severity Post Baseline CHIKV (N=X)	
	Grade 4																								
Eosinophil	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
Basophil	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								
	Grade 3																								
	Grade 4																								
Other	Normal																								
	Outside Normal Range																								
	Grade 1																								
	Grade 2																								

Lab. Param	Severity	Baseline CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)		Week 40 CHIKV (N=X)		Week 48 CHIKV (N=X)		Week 56 CHIKV (N=X)		Week 64 CHIKV (N=X)		Week 72 CHIKV (N=X)		Max Severity Post Baseline CHIKV (N=X)	
	Grade 3																								
	Grade 4																								

Note: The "Max Post Baseline" column includes the maximum severity experienced by each subject at any time point post baseline, including unscheduled assessments.
All subjects will be counted once per symptom by time point at the maximum severity.

TABLE 14.3.4.2:
Laboratory Summary Statistics by Parameter, Study Day, and Treatment Group <<Age Stratum, Overall/Site>>

[Implementation Note: For calculated fields (Mean, SD, Median), decimal place should be the format in which the data were collected + 1 extra place. For Min, Max, decimal place should be in the same format in which the data were collected.

Repeat this table for the Placebo group]

Laboratory Parameter	Statistic	Baseline CHIKV (N=X)	Week 4 CHIKV (N=X)	Week 8 CHIKV (N=X)	Week 16 CHIKV (N=X)	Week 24 CHIKV (N=X)	Week 32 CHIKV (N=X)	Week 40 CHIKV (N=X)	Week 48 CHIKV (N=X)	Week 56 CHIKV (N=X)	Week 64 CHIKV (N=X)	Week 72 CHIKV (N=X)
ALT	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										
ALT Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
WBC	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										

	Min, Max	xx.x, xx.x										
WBC Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
RBC	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										
RBC Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Hemoglobin	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										

Hemoglobin Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Hematocrit	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										
Hematocrit Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
MCV	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										

MCV Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Platelets	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										
Platelets Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Neutrophil	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										

Neutrophil Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Lymphocyte	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										
Lymphocyte Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Monocyte	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										

Monocyte Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Eosinophil	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										
Eosinophil Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Basophil	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										

Basophil Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									
Hematocrit	N	x										
	Mean	xx.x										
	Standard Deviation	xx.x										
	Median	xx.x										
	Min, Max	xx.x, xx.x										
Hematocrit Change from Baseline	N	N/A	x									
	Mean (95% CI)	N/A	xx.x									
	Standard Deviation	N/A	xx.x									
	Median	N/A	xx.x									
	Min, Max	N/A	xx.x, xx.x									

TABLE 14.3.4.3:
Listing of Abnormal Laboratory Results

[Implementation Note: This listing is included in the table shells document, as it is included in the body of the CSR.
This listing only includes abnormal laboratory results. A complete listing of all laboratory results is included in the listings document.
In the Laboratory Parameter column, indicate the units after the parameter, e.g., Hemoglobin (g/dL).
This listing is not color-coded, but the severity should be included in parentheses after the result, e.g., 16.2 (mild).]

Subject ID	Treatment Group	Sex	Age (years)	Date Specimen Collected	Laboratory Parameter (Units)	Result (Severity)	Relationship to Treatment	Action Taken with Study Treatment	Subject Discontinued Due to Result?

TABLE 14.3.4.4.x:

Shift Table for <<binary-graded laboratory parameter>> by Treatment Group Comparing Pre-Dose One Measure to Post-Dose One Measures <<Overall/Site>>

	CHIKV (N=X) Baseline			Placebo (N=X) Baseline		
Pre- Dose One[1]	**Normal**	**Abnormal**	**Total**	**Normal**	**Abnormal**	**Total**
<<Post Dose One Visit 1>>						
Normal	x	x	x [row total]	x	x	x
Abnormal						
Total	[column total]		[sum of 4 blocks, should be N]			
<<Post Dose One Visit 2>>						
Normal						
Abnormal						
Total						
Continue for all Post Dose One visits						

1. Footnote defines when pre-dose measurement was taken

TABLE 14.3.4.5.x:

Shift Table for <<ordinal-graded laboratory parameter>> by Treatment Group Comparing Pre-Dose One Measure to Post-Dose One Measures <<Overall/Site>>

	CHIKV (N=X) Baseline					Placebo (N=X) Baseline				
Pre-Dose One[1]	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Total**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Total**
Grade 1	x	x	x	x	x	x	x	x	x	x
Grade 2										
Grade 3										
Grade 4										
Total										
Continue for all Post Dose One visits										

1. Footnote defines when pre-dose measurement was taken

TABLE 14.3.5:
Distribution of Vital Signs by Day Post Dosing, Grade, and Treatment Group <<Age Stratum, Overall/Site>>

[Implementation Note: If an assessment has a grading scale that includes grading for both high and low, then include one row for each severity for high and low.
If data is not collected, enter 'N/A' for entry. (For example, Temperature at Post-Injection)

Repeat this table for Placebo group]

Vital Signs Assessment	Severity	Pre-injection (Baseline) CHIKV (N=X)		Post-injection CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)	Week 40 CHIKV (N=X)	Week 48 CHIKV (N=X)	Week 56 CHIKV (N=X)	Week 64 CHIKV (N=X)	Week 72 CHIKV (N=X)	Max Severity Post Baseline CHIKV (N=X)	
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Any Assessment	Normal	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x	x	x.x
	Mild																				
	Moderate																				
	Severe																				
	Potentially Life Threatening																				
Systolic BP	Normal																				
	Mild																				
	Moderate																				
	Severe																				
	Potentially Life Threatening																				

Vital Signs Assessment	Severity	Pre-injection (Baseline) CHIKV (N=X)		Post-injection CHIKV (N=X)		Week 4 CHIKV (N=X)		Week 8 CHIKV (N=X)		Week 16 CHIKV (N=X)		Week 24 CHIKV (N=X)		Week 32 CHIKV (N=X)	Week 40 CHIKV (N=X)	Week 48 CHIKV (N=X)	Week 56 CHIKV (N=X)	Week 64 CHIKV (N=X)	Week 72 CHIKV (N=X)	Max Severity Post Baseline CHIKV (N=X)	
Diastolic BP	Normal																				
	Mild																				
	Moderate																				
	Severe																				
	Potentially Life Threatening																				
Pulse	Normal																				
	Mild																				
	Moderate																				
	Severe																				
	Potentially Life Threatening																				
Temperature (°C)	Normal																				
	Mild																				
	Moderate																				
	Severe																				
	Potentially Life Threatening																				

TABLE 14.3.6:
Number and Percentage of Subjects with Concurrent Medications by Treatment Group <<Age Stratum>>

Medication	CHIKV (N=X)		Placebo (N=X)		All Subjects (N=X)	
	n	%	n	%	n	%
	x	x.x	x	x.x	x	x.x

TABLE 14.3.7:
Number of Pregnancies by Outcome and Treatment Group

	CHIKV (N=X)	Placebo (N=X)	All Subjects (N=X)
Total pregnancies	x	x	x
Live births			
Spontaneous abortions			
Elective abortions			
Ectopic pregnancies			
Stillbirths			

TABLE 15.1.1:

Incidence of Clinical Cases of CHIKV Post Dose One by Treatment Group and Stratified by Age

Age Stratum	CHIKV			Placebo		
	n	%	95%CI	n	%	95%CI
18-40	x	xx	xx-xx	x	xx	xx-xx
41-60						
Aggregate (18-60)						

Note: Subjects not receiving dose two are included in this table.
n = number of subjects with CHIKV symptoms (clinical cases)
% = n divided by the number of subjects receiving dose one
95%CI = 95% Clopper-Pearson confidence interval.

TABLE 15.1.2:

Incidence of Clinical Cases of CHIKV Post Dose Two by Treatment Group and Stratified by Age

Age Stratum	CHIKV			Placebo		
	n	%	95%CI	n	%	95%CI
18-40	x	xx	xx-xx	x	xx	xx-xx
41-60						
Aggregate (18-60)						

n = number of subjects with CHIKV symptoms (clinical cases)
% = n divided by the number of subjects receiving dose two and free of infection when receiving dose two
95%CI = 95% Clopper-Pearson confidence interval.

Additional Tables using the same format as 15.1.1 and 15.1.2

TABLE 15.1.3:

Incidence of PCR Confirmed Cases of CHIKV Post Dose One by Group and Stratified by Age

TABLE 15.1.4:

Incidence of PCR Confirmed Cases of CHIKV Post Dose Two by Group and Stratified by Age

TABLE 15.2.1:

Primary Exploratory Analysis – Vaccine Efficacy for Time to Occurrence of Clinical CHIKV Infection Post Dose One

	CHIKV N= Placebo N=		
	V.E.	95% CI for V.E.	p-value
Primary Analysis: Unadjusted	x.xx	xx.xx - xx.xx	x.xxx

N=number of subjects in the specific treatment group that received first dose

V.E. =Vaccine efficacy obtained from Cox Proportional Hazard model

95%CI = 95% confidence interval obtained from Cox Proportional Hazard model

p-value = result of comparison of Kaplan-Meier survival curves between groups by Log-rank test.

TABLE 15.2.2:

Primary Exploratory Analysis – Vaccine Efficacy for Time to Occurrence of Clinical CHIKV Infection Post Dose Two

	CHIKV N= Placebo N=		
	V.E.	95% CI for V.E.	p-value
Primary Analysis: Unadjusted	x.xx	xx.xx - xx.xx	x.xxx

N=number of subjects in the specific treatment group that received second dose
V.E. =Vaccine efficacy obtained from Cox Proportional Hazard model
95%CI = 95% confidence interval obtained from Cox Proportional Hazard model
p-value = result of comparison of Kaplan-Meier survival curves between groups by Log-rank test.

Additional Tables using the same format as 15.2.1 and 15.2.2

TABLE 15.2.3:

Primary Exploratory Analysis – Vaccine Efficacy for Time to Occurrence of PCR Confirmed CHIKV Infection Post Dose One

TABLE 15.2.4:

Primary Exploratory Analysis – Vaccine Efficacy for Time to Occurrence of PCR Confirmed CHIKV Infection Post Dose Two

TABLE 15.3.1:

Secondary Exploratory Analysis - Vaccine Efficacy for Time to Occurrence of Clinical CHIKV Infection Post Dose One with Adjustment for Baseline Characteristics

	CHIKV N= Placebo N=		
	V.E.	95% CI for V.E.	p-value
Secondary Analysis: Multivariate adjustment for age, race, site	xx.x	xx.x – xx.x	x.xxx
Univariate Adjustments Age (18-40, 41-60) Race (White/Non-White) Site (1-6)	 xx.x xx.x xx.x	 xx.x – xx.x xx.x - xx.x xx.x – x.xx	 x.xxx x.xxx x.xxx

N=number of subjects in the specific treatment group that received first dose

V.E. =Vaccine efficacy obtained from Cox Proportional Hazard model

95%CI = 95% confidence interval obtained from Cox Proportional Hazard model

p-value = for the multivariate adjustment, it is the result of comparison of Kaplan-Meier survival curves between groups by Log-rank test.

For univariate adjustment, it is the result of comparison of Kaplan-Meier survival curves between groups stratified by the adjusted variables by Log-Rank test.

TABLE 15.3.2:

Secondary Exploratory Analysis - Vaccine Efficacy for Time to Occurrence of Clinical CHIKV Infection Post Dose Two with Adjustment for Baseline Characteristics

	CHIKV N= Placebo N=		
	V.E.	95% CI for V.E.	p-value
Secondary Analysis: Multivariate adjustment for age, race, site	xx.x	xx.x – xx.x	x.xxx
Univariate Adjustments Age (18-40, 41-60) Race (White/Non-White) Site (1-6)	 xx.x xx.x xx.x	 xx.x – xx.x xx.x - xx.x xx.x – x.xx	 x.xxx x.xxx x.xxx

N=number of subjects in the specific treatment group that received second dose

V.E. =Vaccine efficacy obtained from Cox Proportional Hazard model

95%CI = 95% confidence interval obtained from Cox Proportional Hazard model

p-value = for the multivariate adjustment, it is the result of comparison of Kaplan-Meier survival curves between groups by Log-rank test.

For univariate adjustment, it is the result of comparison of Kaplan-Meier survival curves between groups stratified by the adjusted variables by Log-Rank test.

Additional Tables using the same format as 15.3.1 and 15.3.2

TABLE 15.3.3:

Secondary Exploratory Analysis – Vaccine Efficacy for Time to Occurrence of PCR Confirmed CHIKV Infection Post Dose One with Adjustment for Baseline Characteristics

TABLE 15.3.4:

Secondary Exploratory Analysis – Vaccine Efficacy for Time to Occurrence of PCR Confirmed CHIKV Infection Post Dose Two with Adjustment for Baseline Characteristics

Statistical Analysis Plan

Appendix II: Figure Mock- Ups

for

VRC 704

Study Title:

Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

Version 1.0

Prepared and distributed by:
The Emmes Corporation
Rockville, Maryland

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FIGURE 14.1.1:
CONSORT Flow Diagram By Age Stratum

[Implementation Note: In the SAP, include a blank diagram with the possible reasons why subjects may be excluded from analyses. The reasons for exclusion should be in line with the SAP text.]

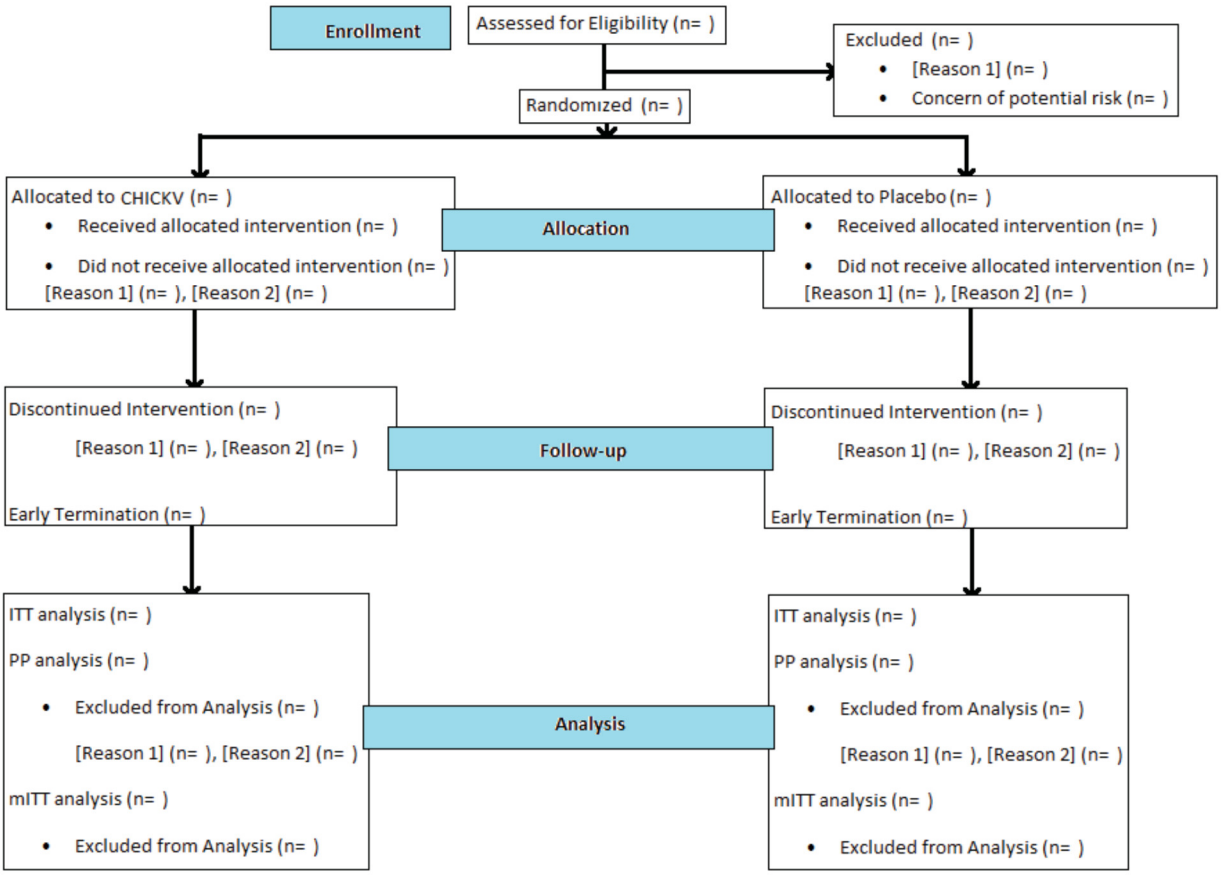
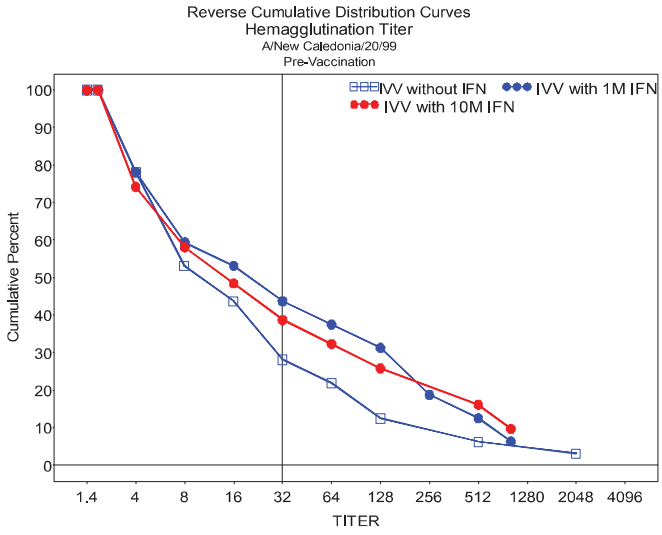
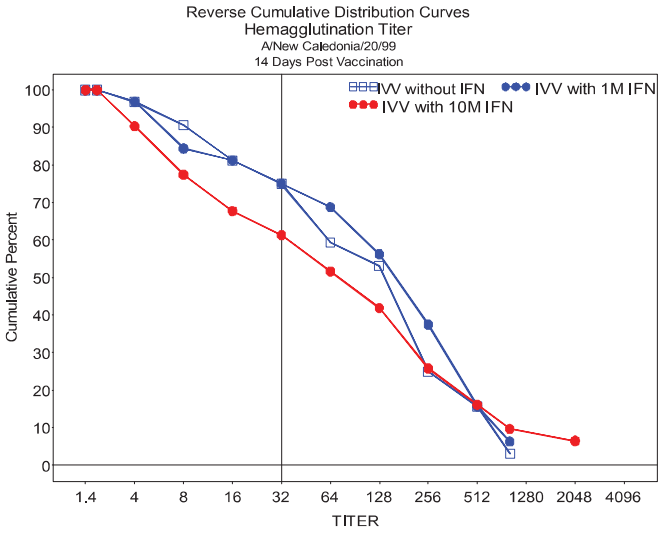


FIGURE 14.2.1:
Reverse Cumulative Distribution of the [specific assay] by Study Day, Site, and Treatment Group <<Age Stratum>>

Note: These are illustrative curves. The actual curves compare CHIKV vaccine and placebo. For neutralization (the primary immunogenicity endpoint), the time periods to be used are Days 0, 28 and 56.





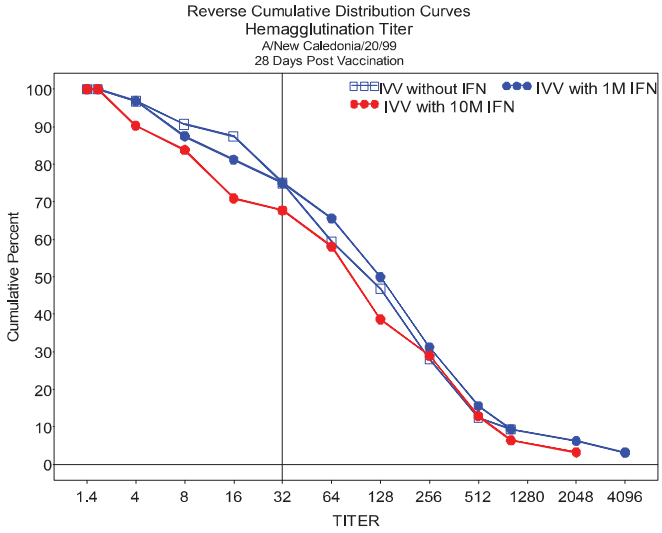


FIGURE 14.2.2:
Boxplots with Overlaid Scatter Plots Summarizing the [specific assay] by Study Day, Site, and Treatment Group <<Age Stratum>>

Note: Y-axis label will change with specific assay and x-axis will change based on time points available in the data.

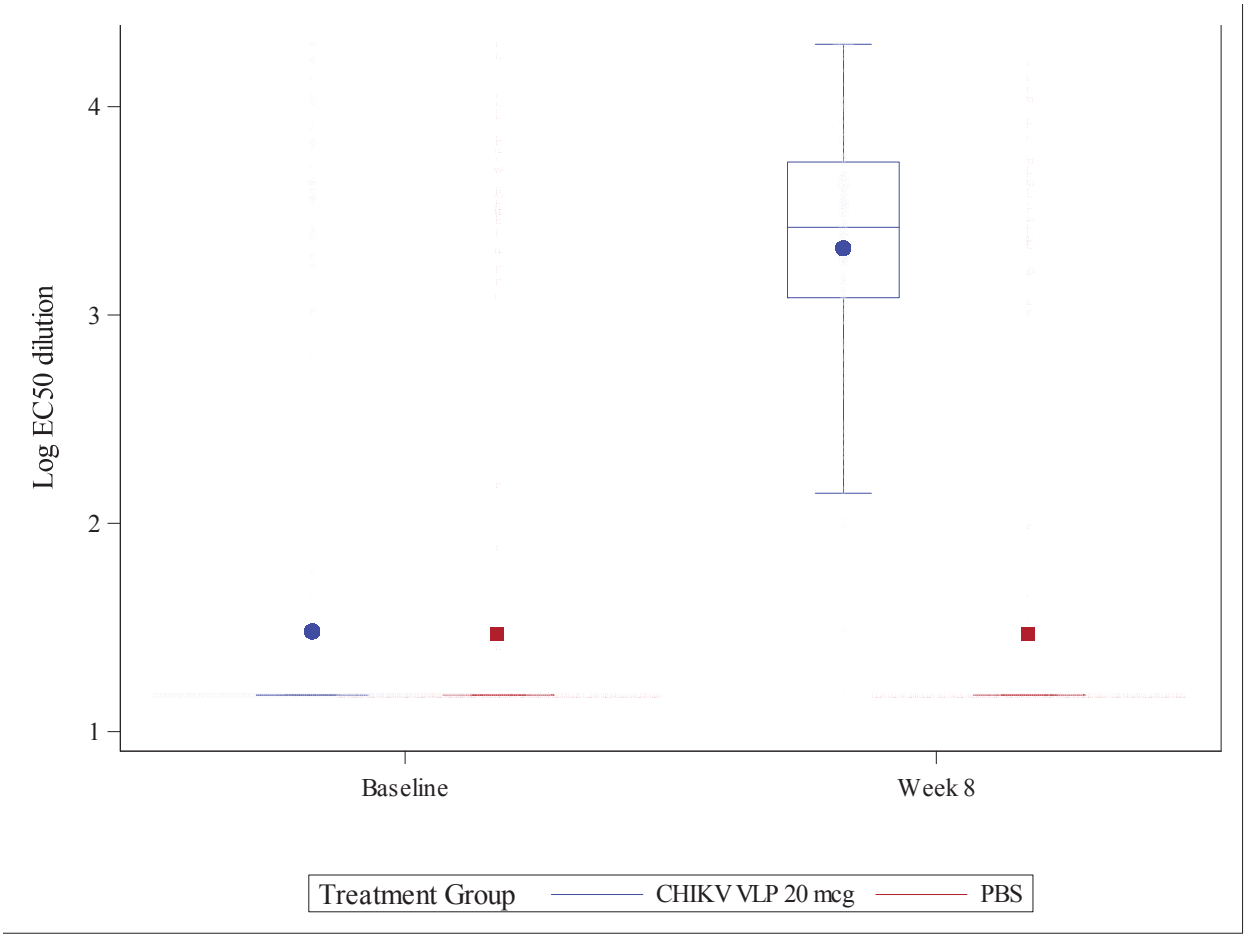


FIGURE 14.3.1.1:
Maximum Severity per Subject by Days Post Treatment

CHIKV, Dose 1 [Repeat for Placebo Group and Dose 2] (N=X)

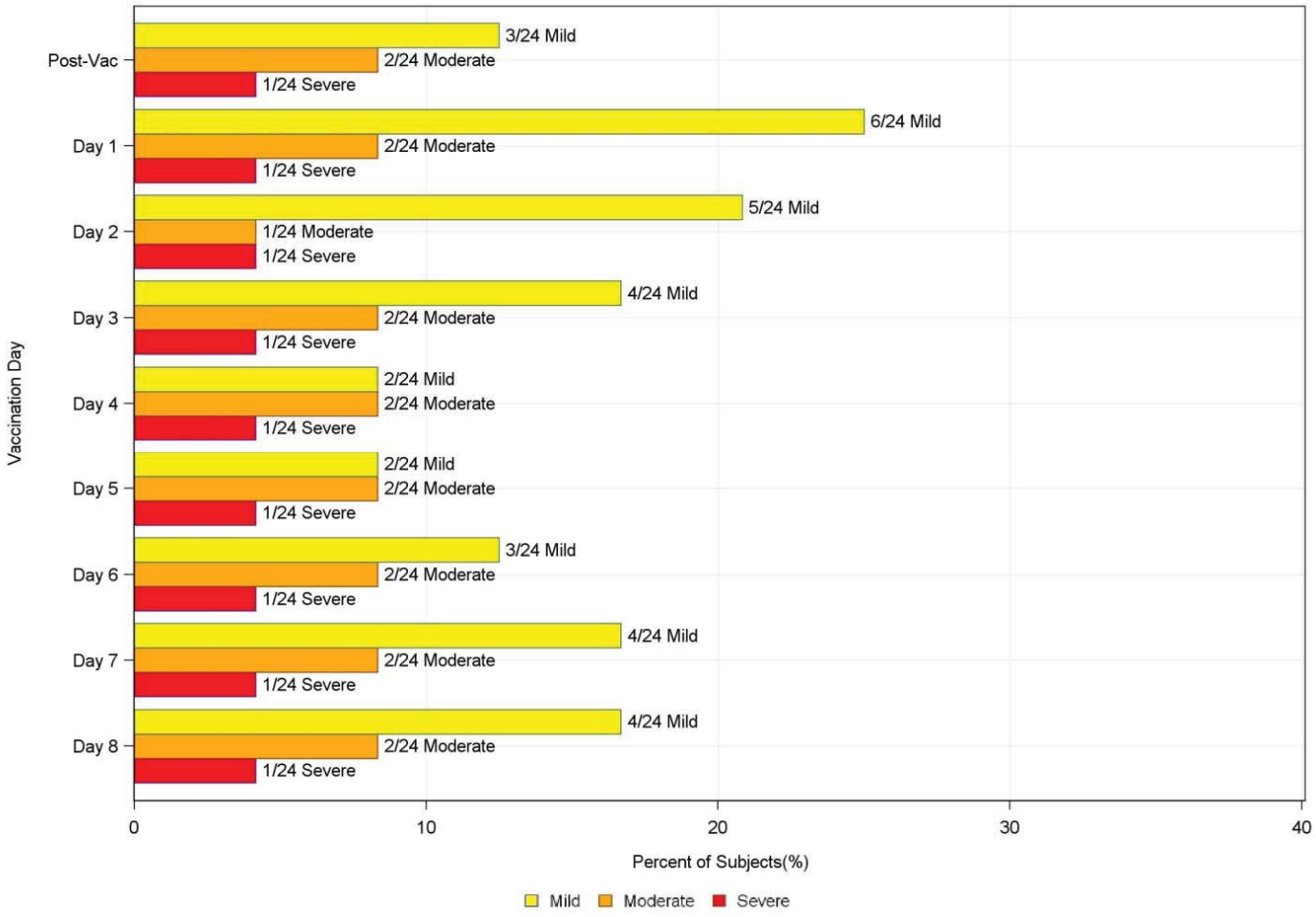


FIGURE 14.3.1.2:

Number and Severity of Non-Serious Adverse Events by MedDRA® System Organ Class

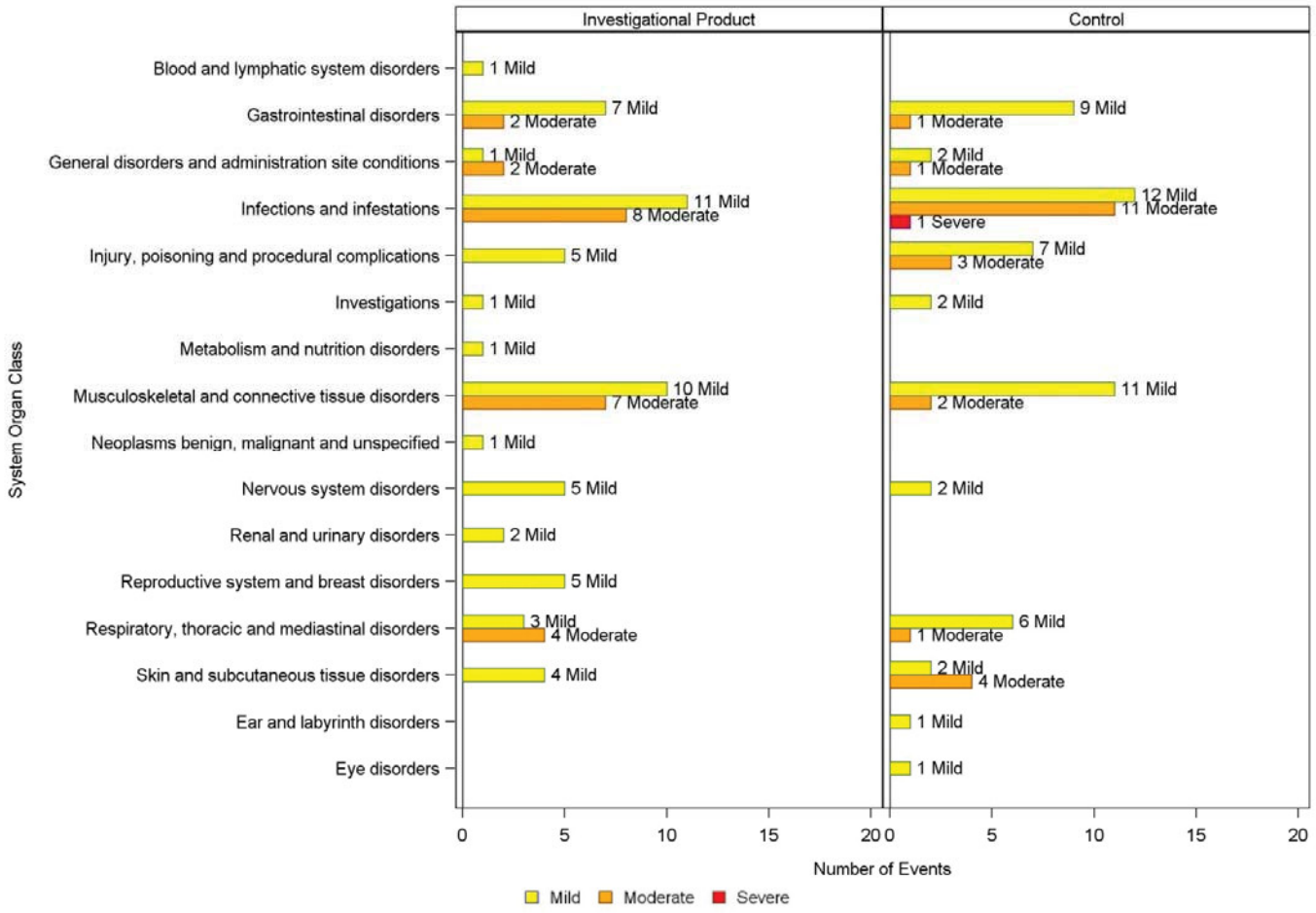


FIGURE 14.3.1.3:
Non-Serious Adverse Events by Severity

CHIKV [Repeat for Placebo Group] (N=X)

[Implementation Note: This figure includes all unsolicited adverse events.]

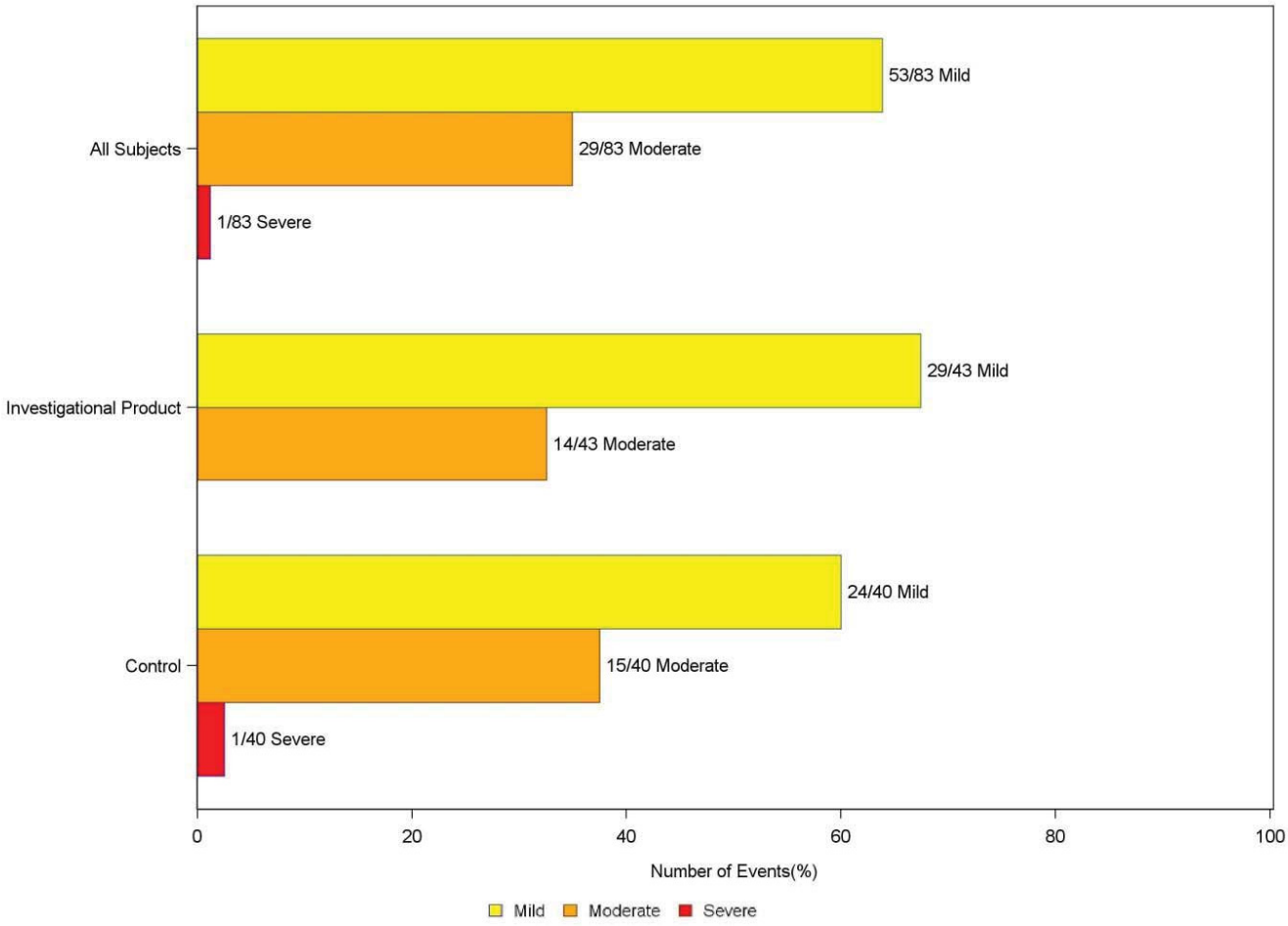
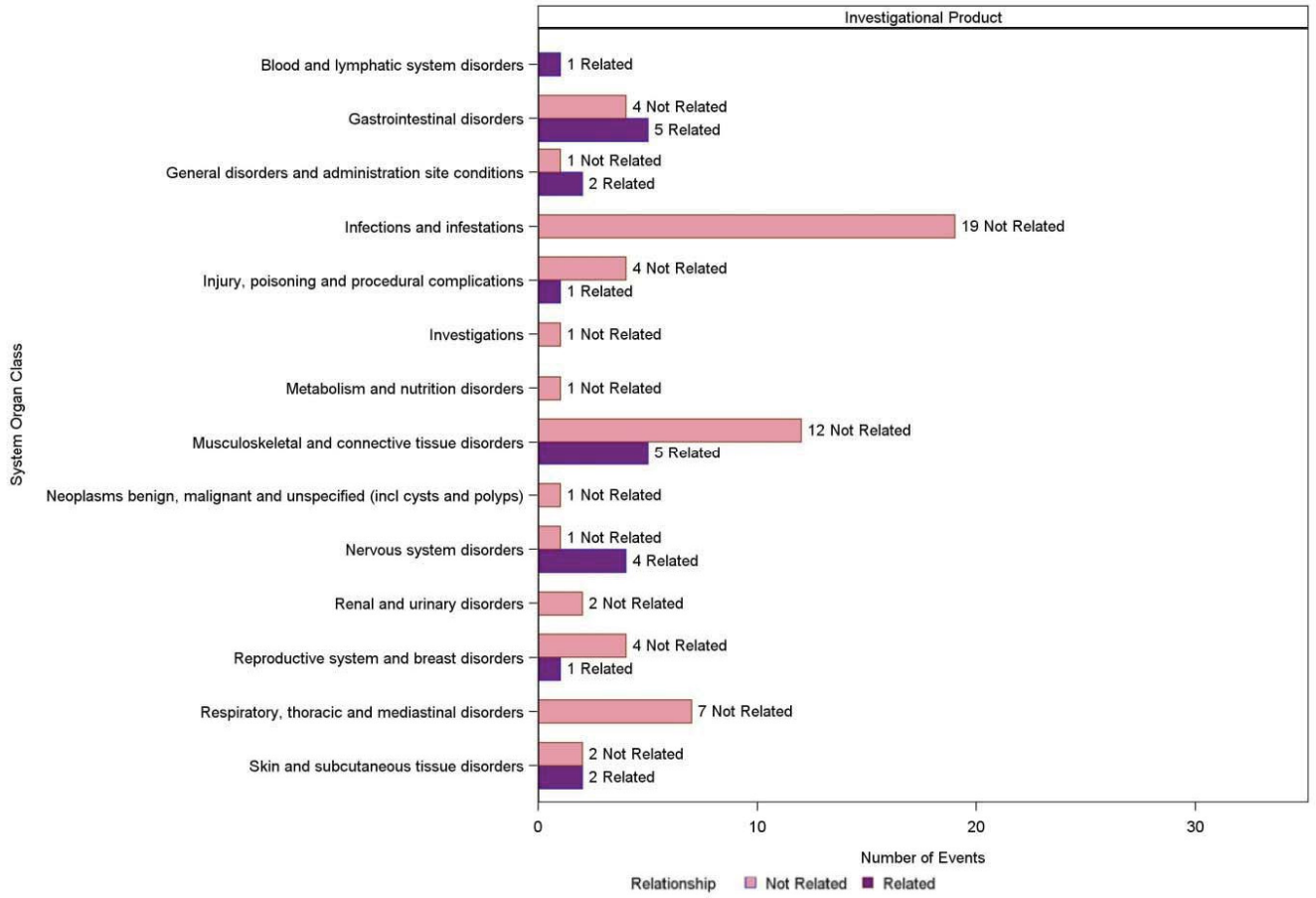


FIGURE 14.3.1.4:
Number and Relationship of Non-Serious Adverse Events by MedDRA® System Organ Class

CHIKV [Repeat for Placebo Group] (N=X)

[Implementation Note: This figure includes all unsolicited adverse events.]



Or ….

FIGURE 14.3.1.4: Number and Relationship of Non-Serious Adverse Events by MedDRA® System Organ Class *(continued)*

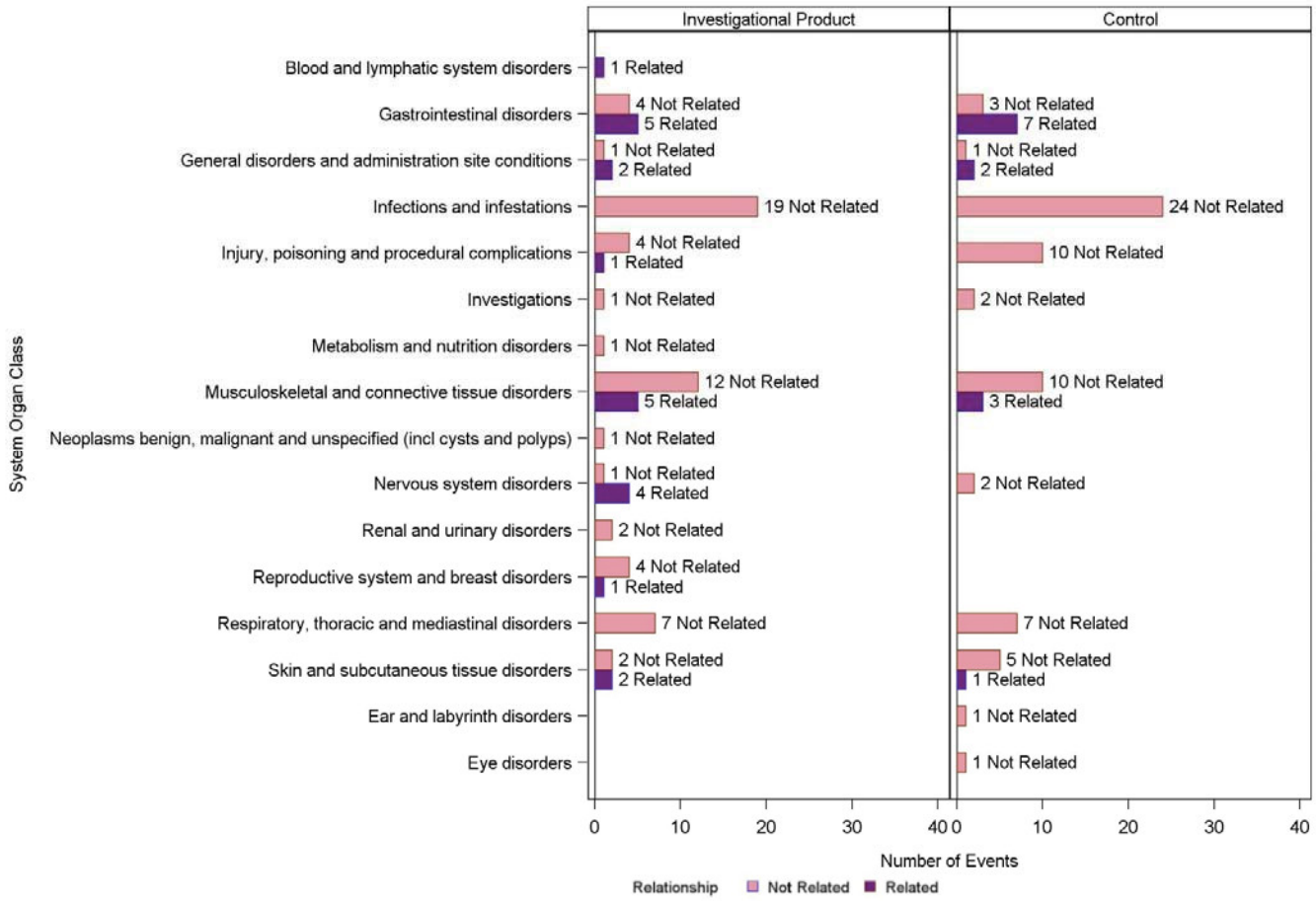


FIGURE 14.3.1.5:
Non-Serious Adverse Events by Relationship to Treatment

CHIKV [Repeat for Placebo Group] (N=X)

[Implementation Note: This figure includes all unsolicited adverse events.]

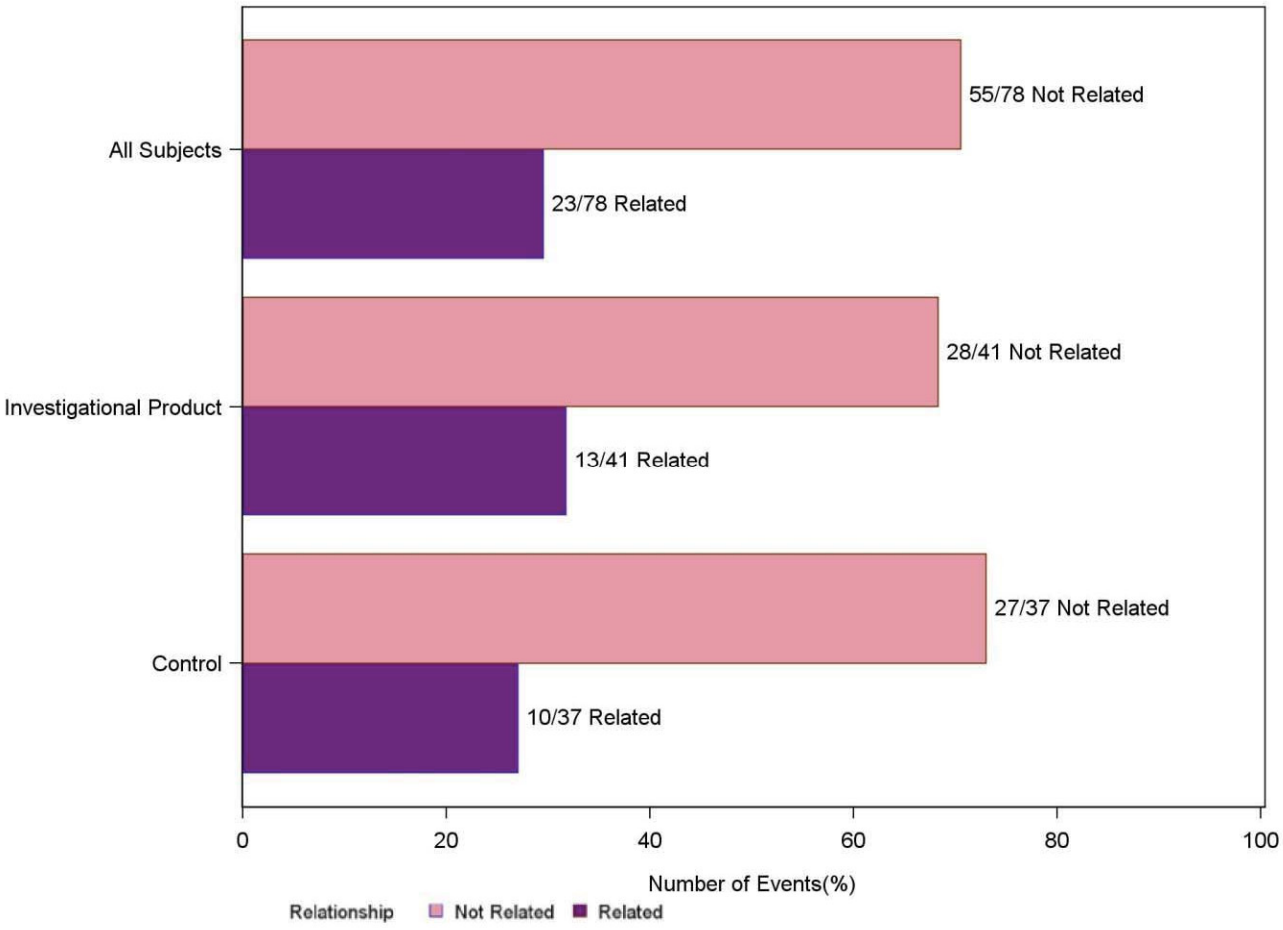


FIGURE 14.3.4.1:
Laboratory Results by Scheduled Visits: Mean Changes from Baseline by Laboratory Parameter and Treatment Group <<for Age Strata>>

CHIKV [Repeat for Placebo Group] (N=X)

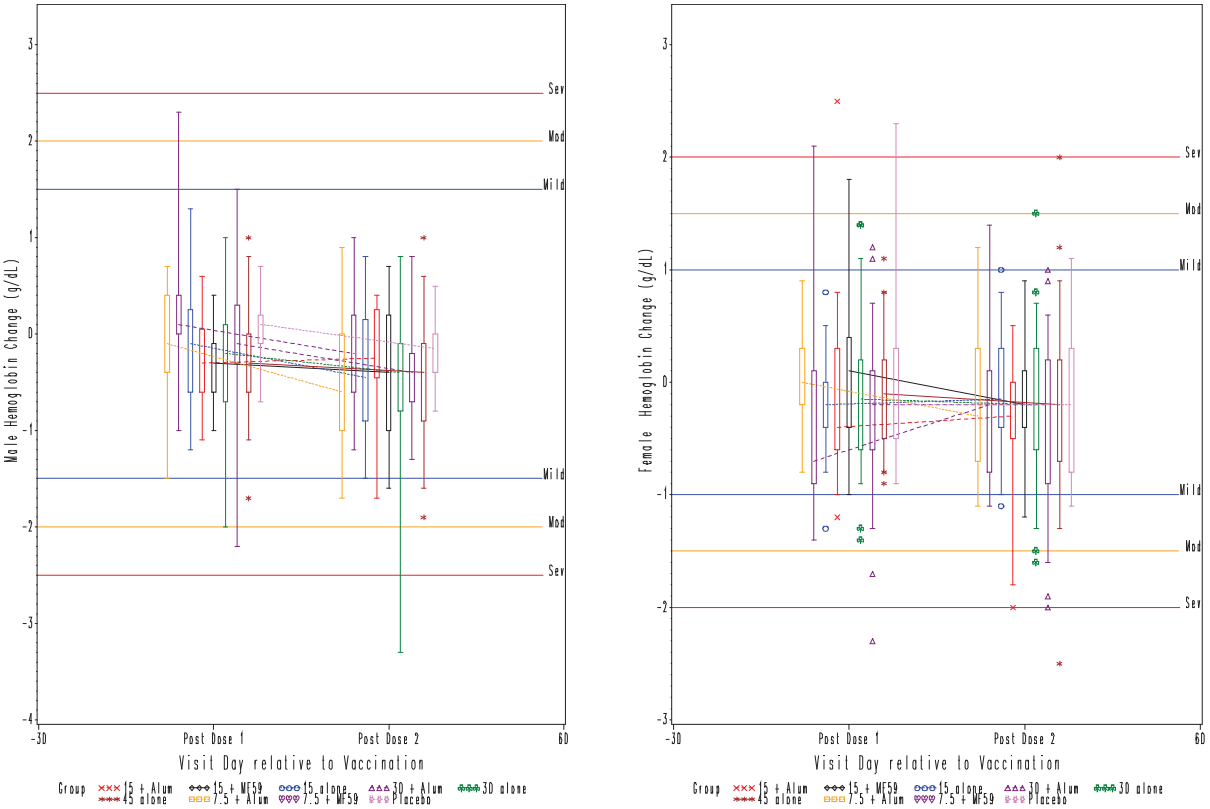
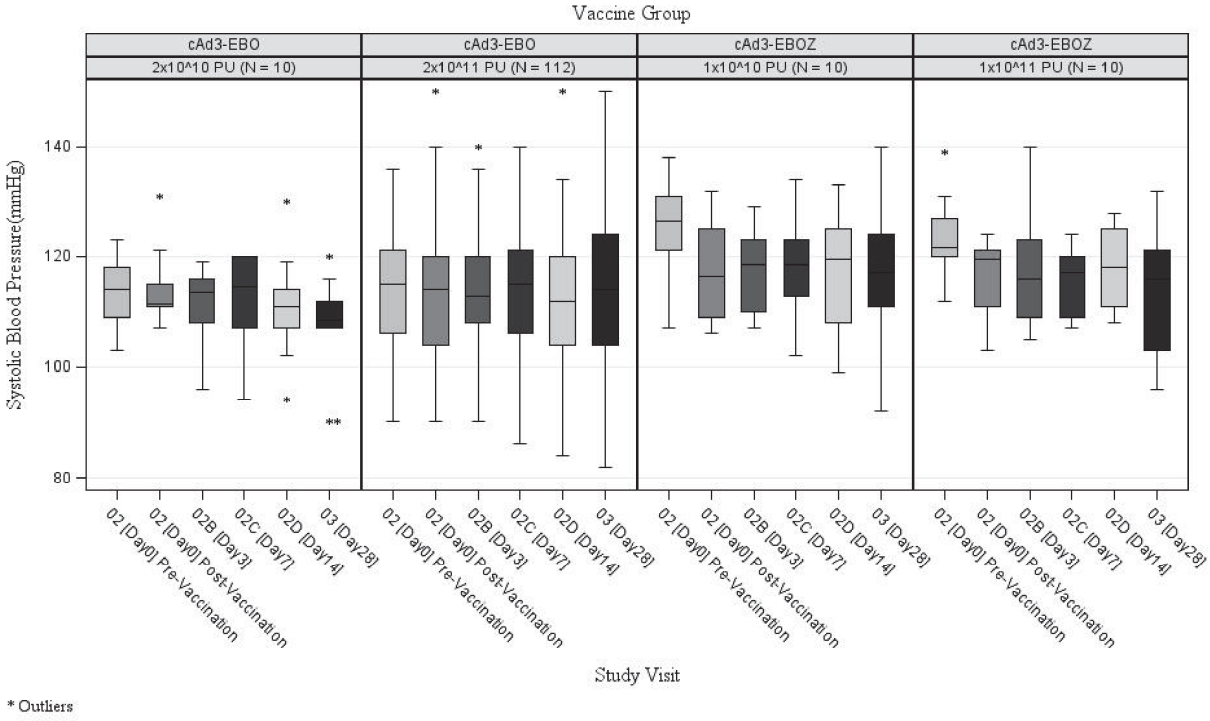


FIGURE 14.4.1-14.4.x:

Boxplot of Vital Signs by Scheduled Visits – Pulse

[Implementation Note: There will be only two treatment groups and not 4, and the visits will be updated based on available data; the box-plot will be repeated for each recorded vital sign]



* Outliers

FIGURE 14.5.1-14.5.x:

Boxplot of Laboratory Results by Scheduled Visits – Hemoglobin

[Implementation Note: There will be only two treatment groups and the visits will be updated based on available data; the box-plot will be repeated for each recorded laboratory measure]

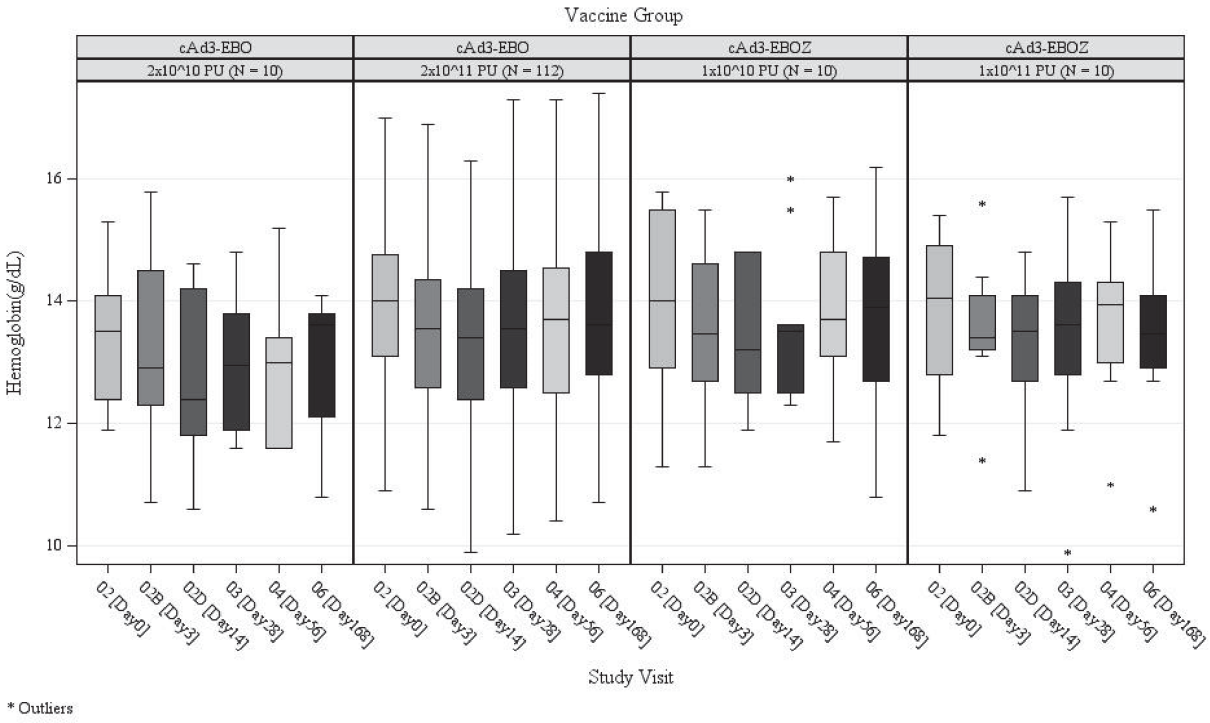
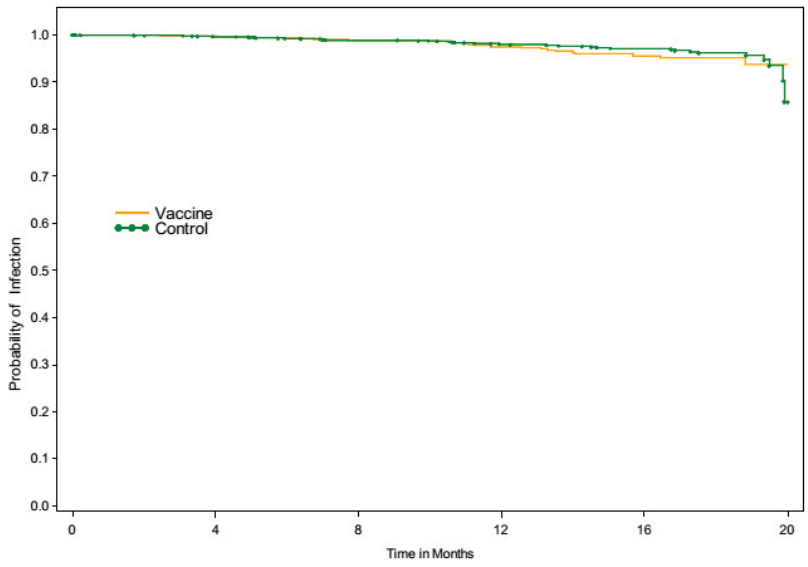


FIGURE 15.1.1:

Kaplan-Meier Curves for Time to Occurrence of Clinical CHIKV Infection Post Dose One



[Implementation notes: there will be two lines, one for each treatment group; x-axis is the time variable for time to occurrence of infection, y-axis is survival estimate from Kaplan-Meier curves; all subjects that received first dose will be considered]

FIGURE 15.1.2:

Kaplan-Meier Curves for Time to Occurrence of Clinical CHIKV Infection Post Dose Two

[Implementation notes: there will be two lines, one for each treatment group; x-axis is the time variable for time to occurrence of infection, y-axis is survival estimate from Kaplan-Meier curves; all subjects that received second dose will be considered]

FIGURE 15.1.3:

Kaplan-Meier Curves for Time to Occurrence of PCR Confirmed CHIKV Infection Post Dose One

FIGURE 15.1.4:

Kaplan-Meier Curves for Time to Occurrence of PCR Confirmed CHIKV Infection Post Dose Two

Statistical Analysis Plan

Appendix III: Listing Mock- Ups

for

Protocol VRC 704

Study Title:

Phase 2 Randomized, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of a Chikungunya Virus-Like Particle Vaccine, VRC-CHKVLP059-00-VP, in Healthy Adults

Version 1.0

Prepared and distributed by:
The Emmes Corporation
Rockville, Maryland

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LISTING 16.2.1:
Early Terminations and/or Discontinued Subjects

Subject ID	Treatment Group	Category	Reason for Early Termination or Treatment Discontinuation	Date of Termination or Discontinuation

LISTING 16.2.2.1:
Subject-Specific Protocol Deviations

[Implementation note: sort by Treatment Group, Subject ID, then Deviation Number.]

Treatment Group	Subject ID	Deviation Number	Deviation	Deviation Date	Reason for Deviation	Deviation Resulted in AE?	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Category	Deviation Resolution	Meet IRB Reporting Requirements? If Yes, Date IRB Notified	Comments

LISTING 16.2.2.2:
Non-Subject-Specific Protocol Deviations

[Implementation note: sort by site, start date.]

Site	Deviation	Deviation Date	Reason for Deviation	Deviation Resulted in Adverse Event?	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Category	Deviation Resolution	Meet IRB Reporting Requirements? If Yes, Date IRB Notified	Comments

LISTING 16.2.3:
Subjects Excluded from the Efficacy/Immunogenicity Analysis

[Implementation Note: This data in this listing should be congruent with the "Analysis Populations by Treatment Group" table (Table 14.1.2, Appendix I). The reasons included here should match the SAP text that describes who will be excluded from analyses.]

Treatment Group	Subject ID	Analyses in which Subject is Included	Analyses from which Subject is Excluded	Results Available?	Reason Subject Excluded
		[e.g., Safety, ITT, mITT, PP]	[e.g., Safety, ITT, mITT, PP]		

Note: "Yes" in the "Results available" column indicates that available data were removed from the analysis. "No" indicates that no data were available for inclusion in the analysis.

LISTING 16.2.4.1:
Demographics Data

[Implementation Note: If a subject is multi-racial, in "Race" column, note "Multiple: (list races, separated by a comma)."]

Subject ID	Treatment Group	Sex	Age at Enrollment (years)	Ethnicity	Race	Language

LISTING 16.2.4.2:
Pre-Existing Medical Conditions

Subject ID	Treatment Group	MH Number	Medical History Term	MedDRA® System Organ Class	MedDRA® Preferred Term

LISTING 16.2.5:

Compliance and/or Drug Concentration Data

[Implementation note: only use if these cases exist. Data will come from either UVD (if subject missed product administration) or special cases excel spreadsheet (not eCRFs).]

Scenario 1

Treatment Group	Subject ID	Dose Number	Actual [Dosage/Concentration] Administered

Scenario 2

Treatment Group	Subject ID	Dose(s) Missed
		[e.g., Day 3, Day 3 AM, etc.]

LISTING 16.2.6:
Individual Immunogenicity Response Data

Subject ID	Treatment Group	Assay	Collection Date	Titer

LISTING 16.2.7.1:
Solicited Events

[Implementation Note: This listing is not color-coded. To indicate severity for quantitative symptoms (e.g., temperature, measurements), include the grade in parentheses after the number, e.g., 100.7 (Mild).
We are not indicating the "side" or arm assessed. If the arm assessed was wrong (not the arm that received treatment), then note this error in a footnote in the Local Symptoms listing.
This listing includes baseline assessments in addition to post-treatment assessments.
MA uses DCA, Clinic uses VTL (only for temperature).]

Systemic Symptoms

Subject ID	Treatment Group	Dose Number	Post Dose Day	Assessment*	[Symptom 1]	[Symptom 2]	[Symptom 3]	[Symptom 4]
				MA				
				Clinic				

* MA = Data reported by subject on the Memory Aid and reviewed by clinic staff and reported in Solicited Events eCRF.
Clinic = Data collected by clinic staff during physical exam or symptom assessment (treatment administration record, in-clinic assessment, etc.)

Local Symptoms

Subject ID	Treatment Group	Dose Number	Post Dose Day	Assessment*	[Symptom 1]	[Symptom 2]	[Symptom 3]	[Symptom 4]
				MA				

* MA = Data reported by subject on the Memory Aid and reviewed by clinic staff and reported in Solicited Events eCRF.

LISTING 16.2.7.2:
Unsolicited Adverse Events

[Implementation Note: This listing includes all unsolicited adverse events.]

Part 1

Subject ID	Treatment Group	AE Number	Adverse Event	MedDRA Preferred Term	Number of Administrations	Days Since Last Admin	Severity	SAE?	Relationship to Study Treatment	Admin Date	AE Onset Date	Date of Resolution

Part 2

Subject ID	Adverse Event	Action Taken with Study Treatment	Subject Discontinued Due to AE	Outcome	Comments

Note: For additional details about SAEs, see Table: 14.3.2.1.

LISTING 16.2.8.1:
Individual Clinical Laboratory Results – Hematology

[Implementation Note: These listings (for hematology and biochemistry) include all laboratory results, scheduled and unscheduled. These listings are not color-coded, but the severity should be included in parentheses after the result for abnormal results, e.g., 16.2 (Mild). The "extra" fields that are completed for abnormal results are not included in this listing; they are included in the listing of abnormal laboratory results that is included in the table shells document.]

Subject ID	Treatment Group	Sex	Age (years)	Date Specimen Collected	[Laboratory Parameter 1 (Units)]	[Laboratory Parameter 2 (Units)]	[Laboratory Parameter 3 (Units)]

LISTING 16.2.8.2:
Individual Clinical Laboratory Results – Biochemistry

Subject ID	Treatment Group	Sex	Age (years)	Date Specimen Collected	[Laboratory Parameter 1 (Units)]	[Laboratory Parameter 2 (Units)]	[Laboratory Parameter 3 (Units)]

LISTING 16.2.9:
Vital Signs

[Implementation Note: This listing includes all vital sign assessments, scheduled and unscheduled.
These listings are not color-coded, but the severity should be included in parentheses after the result for abnormal assessments, e.g., 100.7 (Mild).]

Subject ID	Treatment Group	Date	Temperature (°C)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (beats/min)	Weight (kg)	Height (cm)	BMI

LISTING 16.2.10:
Concomitant Medications

[Implementation Note: "Medication Start Day" and "Medication End Day" are relative to enrollment (which is Day 1, day before enrollment is Day -1). For medication start dates that are > 30 days prior to enrollment, rather than use exact study days, categorize as follows:

- > 5 years prior to enrollment
- 1-5 years prior to enrollment
- 1-12 months prior to enrollment

If ongoing, display "Ongoing" in the "Medication End Day" column.
If taken for an AE, display "Yes" with the AE Number in parentheses, e.g., "Yes (7)".]

Subject ID	Treatment Group	Medication Number	Medication	Medication Start Date	Medication End Date	Indication	Taken for an AE? (AE Number)	Comments

LISTING 16.2.11:
Pregnancy Reports

[Implementation Note: Only include the "Pregnancy Number" column if a subject has more than 1 pregnancy.]

Subject ID	Treatment Group	Pregnancy Number	Start Date of Last Menstrual Period	Date Site Notified	Date of Last Study Injection	Date of Delivery or Pregnancy Termination	Pregnancy Outcome	If Live or Still Birth, Congenital Anomalies or Birth Defects?	If Live Birth, Neonatal Death?	Clinical History of Pregnancy	Therapeutic Management of Pregnancy	Hospitaliz-ations Relevant to this Pregnancy

Note: Medications taken during pregnancy are included in the Concomitant Medications Listing.

LISTING 16.3.1:

Chikungunya Endpoints Evaluations Performed

Subject ID	Illness Onset Date	Number of PCR Test	Number of Positive PCR	Clinical CHIKV Case?	Fever Severity Grade	Arthritis/ Arthralgia Severity Grade	Atypical Case?	Hospitalized?	SAE?	Illness Resolution Date	Duration of CHIK Illness (days)

LISTING 16.3.2:

Individual Time to Occurrence of Clinical CHIKV Infection Efficacy Data

Subject ID	Treatment Group	Date of Dose One	Date of Dose Two	Date of CHIKV Symptoms	Time from Dose One	Time from Dose Two
					xx.xx	xx.xx
					Censored	Censored
						N/A

Time from dose one represents the time from dose one to CHIKV infection. This can be either censored (if the subject does not develop CHIKV during the study period) or have an actual value (time from first dose to event).

Time from dose two represents the time from dose two to CHIKV infection. This can be either censored (if the subject does not develop CHIKV during the study period), have an actual value (time from second dose to event) or not applicable if the subject did not receive second dose/developed CHIKV before dose two. Subjects with N/A are not included in the analysis of this outcome.

LISTING 16.3.3:

Individual Time to Occurrence of PCR Confirmed CHIKV Infection Efficacy Data

Subject ID	Treatment Group	Date of Dose One	Date of Dose Two	Date of CHIKV PCR confirmation	Time from Dose One	Time from Dose Two
					xx.xx	xx.xx
					Censored	Censored
						NA

Time from dose one represents the time from dose one to CHIKV infection. This can be either censored (if the subject does not develop CHIKV during the study period) or have an actual value (time from first dose to event).

Time from dose two represents the time from dose two to CHIKV infection. This can be either censored (if the subject does not develop CHIKV during the study period), have an actual value (time from second dose to event) or not applicable if the subject did not receive second dose/developed CHIKV before dose two. Subjects with NA are not included in the analysis of this outcome.