Official Protocol Title:	Phase 2 Study of a Live Attenuated Measles Virus-Vectored Chikungunya Vaccine in Previously Exposed Adults
NCT number:	NCT03807843
Document Date:	23-Dec-2020

STUDY PROTOCOL

PHASE 2 STUDY OF A LIVE ATTENUATED MEASLES VIRUS-VECTORED CHIKUNGUNYA VACCINE IN PREVIOUSLY EXPOSED ADULTS

Protocol: MV-CHIK-206
WRAIR #2629
Version: 2.1
Date: 23 December 2020
IND Number: 17343

This study will be conducted in compliance with the protocol and International Conference on Harmonization Guidelines on Good Clinical Practices (ICH E6), and applicable local regulatory requirements.

Sponsor	CRO	Funding Agency
Themis Bioscience GmbH	Integrum Scientific	U.S. Department of Defense
Muthgasse 11/2 1190 Vienna Austria	302 Gallimore Dairy Road Greensboro, NC 27409 United States	Grant managed by The Geneva Foundation 917 Pacific Ave, Suite 600 Tacoma, WA 98402
PPD	PPD	PPD

CONFIDENTIAL

1 Signatures

I have read and agree to this version of the MV-CHIK-206 Protocol, entitled "Phase 2 Study of a Live Attenuated Measles Virus-Vectored Chikungunya Vaccine in Previously Exposed Adults." I am aware of my responsibilities under the Good Clinical Practice (GCP) guidelines, local regulations, and the study protocol. I agree to conduct the study according to these responsibilities.

Sponsor

PPD

Themis Bioscience GmbH

Clinical Project Manager

05-Jan-2021

Signature Date

Clinical Trial Site

PPD

San Juan Hospital Research Unit

Principal Investigator

05-Jan-2021

Signature Date

2 Protocol Synopsis

TITLE: Phase 2 Study of a Live Attenuated Measles Virus-Vectored Chikungunya Vaccine in Previously Exposed Adults
DESIGN: This will be a prospective randomized double-blind interventional clinical study. This study proposes to evaluate the safety and immunogenicity of an investigational live recombinant measles-vectored chikungunya vaccine (MV-CHIK) delivered in 2 vaccinations, 28 days apart compared with saline placebo. After providing informed consent, individuals will be screened for eligibility including verification of previous exposure to chikungunya virus. They will then be randomized in a double-blind fashion to receive either MV-CHIK or saline placebo in a 1:1 ratio. This study will be conducted in two Steps, first in up to 30 individuals ages 21 to 50. Then, pending a favorable review of the available vaccine safety data, in up to 30 individuals ages 51 to 65.
OBJECTIVES: Primary: To determine the safety of MV-CHIK administered in 2 doses separated by 28 days in previously exposed individuals. Secondary: To determine the immunogenicity by a neutralization assay of MV-CHIK administered in 2 doses separated by 28 days in previously exposed individuals.
ENDPOINTS: Primary: Incidence of solicited and unsolicited adverse events. Secondary: Immunogenicity on Days 0, 28, 56, and at the end of the study measured as geometric mean titer (GMT) of neutralizing antibodies to chikungunya. Exploratory: Changes in acute phase reactant levels after each vaccination: C-reactive Protein at 3 days and fibrinogen at 7 days post-vaccination; changes from baseline in scores on a quality-of-life survey (SF36) at 28 days after each vaccination and at the end of the study.
POPULATION: The study population is from an area with a high seroprevalence of antibodies to chikungunya (San Juan, Puerto Rico). Chikungunya exposure status will be confirmed prior to randomization. Subjects who are seronegative to chikungunya or are still under treatment for symptoms attributed to a previous chikungunya virus infection will be excluded from the study. Subjects who attribute only mild and subjective symptoms such as fatigue to previous chikungunya infection will not be excluded. Subjects with acute chikungunya infection will be excluded but may be re-screened no sooner than 3 months after resolution of their symptoms. The study will be conducted in two Steps, initially limited to those between ages 21-50. Following a favorable review of available data on vaccine safety, individuals ages 51 to 65 will be enrolled.
PHASE OF DEVELOPMENT: Phase 2.
INTERVENTION(S): MV-CHIK is a recombinant live Schwarz-strain measles-vectored vaccine expressing chikungunya virus surface proteins. It is manufactured as a clear colorless to off-white opaque liquid without excipients in a single-use glass vial and must be stored at or below -65ºC. The target dose is 5×10^5 $TCID_{50}$ per 0.4mL dose with a manufacture dependent window of ±0.5 log (1×10^5 to 1×10^6) and is delivered intramuscularly. Placebo: Intramuscular saline, 0.4mL, will be used as the placebo. Subjects will be randomized to these interventions in a 1:1 fashion.
STUDY PERIOD: The study will be conducted in two Steps. Step 1 will enroll up to 30 subjects ages 21 to 50. It is estimated that enrollment will take 6 months and the last subject in Step 1 will complete the study 13 months after study start. Step 2 will begin after a favorable review of the safety data by a Data Safety Monitoring Board (DSMB). The DSMB will convene after at least 20 volunteers in Step 1 have been vaccinated. Step 2 will enroll up to 30 subjects ages 51 to 65. It is estimated that enrollment of this group will also take approximately 6 months. The total duration of the study, from initial screening for Step 1, through DSMB review to final visit of the last subject in Step 2 is estimated to take a minimum of 24 months.

DURATION: The duration of participation for individual subjects from initial screening through the End-of-Study visit is about 9 months.
EVALUATIONS: Safety Variables: Solicited and unsolicited adverse events (AEs), serious adverse events (SAEs), and adverse events of special interest (AESIs). Immunogenicity Variable: Neutralization titers (chikungunya). Exploratory Variables: post- versus pre-vaccination C-reactive protein, fibrinogen, SF36 score.

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5 Definitions of Acronyms and Terms

5.1 Definition of Acronyms

acronym	Definition
AE	Adverse event
AESI	Adverse event of special interest
Assign-DMB	Assign Data Management and Biostatistics GmbH
BMP	Basic metabolic panel
CBC	Complete blood count
CBER	Center for Biologics Evaluation and Research of the FDA
CD46	Cluster of Differentiation-46, a cell surface molecule that confers susceptibility to measles virus infection
CDC	US Centers for Disease Control and Prevention
CDMS	Clinical Data Management System
CFR	Code of Federal Regulations
CI	Confidence Interval
CMP	Complete metabolic panel
COVID 19	Corona Virus Disease 2019
CRA	Clinical Research Associate (study monitor)
CRF/eCRF	Case report form/electronic case report form
CRO	Clinical Research Organization
CRP	C-reactive protein
DMID	Division of Microbiology and Infectious Disease, conducts and supports extramural research for the NIAID
DoD	Department of Defense
DoDI	Department of Defense Instruction
DSMB	Data Safety Monitoring Board
eCRF/CRF	Electronic Case report form/case report form
EDC	Electronic Data Capture
EIA	Immunoassay
ELISA	Enzyme-linked immunosorbent assay

EOS	End-of-study
eTMF	Electronic Trial Master File
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GmbH	Gesellschaft mit beschränkter Haftung (German for Limited Liability Company)
GMP	Good Manufacturing Practice
GMT	Geometric Mean Titer
HBsAg	Hepatitis B Surface Antigen, a test for chronic Hepatitis B infection
HCV	Hepatitis C Virus
HEENT	Head, eyes, ears, nose and throat
HHS	US Department of Health and Human Services
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (of the MRMC)
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ID	Identification
IFA	Immunofluorescence assay
IgG	Immunoglobulin class G
IM	Intramuscular
IND	Investigational New Drug application
IFNAR	Interferon-alpha receptor knock-out (a strain of mice that is susceptible to chikungunya infection)
IRB	Institutional Review Board
ITT	Intention-to-treat
IVP	Investigational vaccine product (used to indicate either MV-CHIK or placebo)
IWRS	Interactive web-based response system
LLC	Limited liability company
MCAR	Missing Completely at Random
MD	Doctor of Medicine
mL	milliliters
MedDRA	Medical Dictionary for Regulatory Activities
MMR	Measles, Mumps and Rubella vaccine
MRMC	US Army Medical Research and Materiel Command
MV-1 F4	An experimental measles-vectored vaccine for HIV infection
MV-CHIK	Measles-vectored chikungunya vaccine
NC	North Carolina
NIAID	US National Institute of Allergy and Infectious Disease
NIH	US National Institutes of Health
NLM	National Library of Medicine
OHRP	Office of Human Research Protections (of HHS)
ORP	Office of Research Protections (of the MRMC)
PAHO	Pan-American Health Organization
PI	Principal Investigator

PP	Per-protocol
PRNT, $PRNT_{50}$	Plaque-reduction neutralizing antibody test; the subscript refers to the percent of plaques reduced in order to conclude neutralization has occurred
QoL	Quality-of-life
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SF36	Standardized quality-of-life questionnaire
SL	Sociedad Limitada (Spanish for Limited Liability Company)
SME	Sponsor Medical Expert
SOC	System-organ-class
SOP	Standard Operating Procedure
SSP	Study Specific Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TBD	To be determined
TCID, $TCID_{50}$	Tissue culture infective dose; the subscript refers to the percent of cells in culture that are infected after inoculation.
UPIRTSO	Unanticipated Problem Involving Risk to Self or Others
US, USA	United States of America
VLP	Virus-like particles
WHO	World Health Organization
WHODrug	An international classification of medicines
WRAIR	The Walter Reed Army Institute of Research

5.2 Definition of Terms

Term	Definition
Adequate Contraception	A contraceptive method with a failure rate of less than 1% per year when used consistently and correctly (when applicable, as mentioned in the product label). This includes but is not limited to abstinence, combined or progestogen oral contraceptives, injectable progestogen, implants of levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches or intrauterine device or intrauterine system, vasectomy with documented azoospermia of the sole male partner or male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository) or male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream, or suppository).
Documented Azoospermia	Documented azoospermia refers to the outcome of the investigator's/designee's medical examination of the subject or review of the subject's medical history for study eligibility, as

	obtained via a verbal interview with the subject or from the subject's medical records.
Childbearing potential	All female subjects are considered to be of childbearing potential unless postmenopausal or surgically sterile and at least 3 months have passed since the sterilization procedure.
Postmenopausal amenorrhea	Amenorrhea for ≥12 months without an alternative medical cause. Permanent female sterilization procedures include tubal ligation, bilateral salpingectomy, hysterectomy, bilateral oophorectomy, or successful Essure placement.

6 Ethics

This study will be conducted in accordance with the Guidance for Industry on Good Clinical Practice (ICH Harmonised Tripartite Guideline E6 (R2); FDA CFR (21 CFR Part 50, 56, 312), and all applicable regulatory requirements.

6.1 Protocol Review Committees

This study will be conducted in compliance with Institutional Review Board (IRB) and ICH GCP Guidelines - including Title 21 Part 56 of the US Code of Federal Regulations (CFR) relating to IRBs and GCP as described in the United States Food and Drug Administration (FDA) CFR (21 CFR Part 50, 56, 312) - in accordance with applicable ICH regulations regarding clinical safety data management (E2A, E2B(R3)), and with ICH regulations regarding scientific integrity (E4, E8, E9, and E10). In addition, this study will adhere to all local regulatory requirements and requirements for data protection.

6.1.1 Institutional Review Board

Before initiating this trial, the Investigator must have written and dated approval from the IRB for the study protocol, informed consent form, any consent form updates, subject recruitment materials (e.g., advertisements), and any written information to be provided to subjects. This approval should include a statement from the IRB that these documents pass both ethical and scientific review and comply with GCP requirements. The approval letter must identify the documents and versions reviewed.

6.1.2 Human Research Protection Office

All United States Army Medical Research and Materiel Command (MRMC)-supported research involving humans, human data, human specimens, or cadavers must be reviewed for compliance with federal and Department of Defense (DoD) human subjects' protection requirements and approved by the Office of Research Protections (ORP) Human Research Protection Office (HRPO) prior to initiation. The Project Oversight Agency is responsible for HRPO submissions and must ensure that documentation is complete and approvals received before trial initiation. See Section 17.4 for additional HRPO requirements.

6.2 Informed Consent

The Investigator or designee will explain the benefits and risks of participation in the study to each subject and obtain written informed consent. Written informed consent must be obtained before initiation of any study-related procedures (to include screening and administration of the investigational vaccine product (IVP)).

The informed consent form (ICF; final, version dated) must be approved by the Sponsor and the IRB and will contain all elements required by regulatory authorities and GCP in language readily understood by the subject. Each subject's original consent form, which will be personally signed and dated by the subject and by the person who conducted the informed consent discussion, will be retained by the Investigator. The Investigator will supply all enrolled subjects with a copy of their signed ICF.

The ICF may need to be revised during the study should important new information become available that may be relevant to the safety of the subjects. In this instance, approval for the revisions must be given by the IRB and existing subjects must be informed of the changes and re-consented. This is documented in the same way as previously described. In the event safety information becomes available after a subject has completed the study, an informational letter will be sent via certified mail to the subject.

7 Roles and Responsibilities

7.1 Sponsor

Themis Bioscience GmbH
Clinical Project Manager: PPD
Muthgasse 11/2
1190 Vienna
Austria
Tel: PPD
Email: PPD
Role: Vaccine development and manufacturing, fulfillment of Sponsor responsibilities as described in 21 CFR 312.50, interaction with the FDA through their Agent, clinical trials registry posting and maintenance, contracting for and funding performance of chikungunya neutralization assays, selection and reimbursement of subject matter experts for the Data Safety Monitoring Board.

7.2 Project Oversight Agency

Walter Reed Army Institute of Research - Viral Diseases Branch
503 Robert Grant Avenue

Silver Spring, Maryland 20910
USA

7.2.1 Protocol Chair

PPD
Tel: PPD
Email: PPD
Role: Serve as the central point of contact for scientific and protocol questions from the Investigator(s) and site; review monitoring reports and protocol deviations; interact on behalf of the study team with HRPO.

7.2.2 Sponsor Medical Expert (SME)

PPD
Tel: PPD
Email: PPD
Role: Serve as the central point of contact for medical questions from the Investigator(s) and site; review de-identified (coded) reports of AEs and SAEs; draft narratives of adverse reactions that require expedited reporting to FDA for review and submission by the Sponsor. See Section 15.2.

7.2.3 Compliance Management Unit

Director: PPD
Tel: PPD
Email: PPD
Role: Assembly of kits and labels for collection and processing of blood for research purposes; storage and retrieval of sera at WRAIR for future use.

7.3 Clinical Trial Site

San Juan Hospital Research Unit
3rd Floor Room FP-04
San Juan, PR 00935
Tel: PPD
Fax: PPD
Cell phone: PPD

7.4 Clinical Research Organization

Integrum Scientific, LLC
Project Manager: PPD
302 Gallimore Dairy Road
Greensboro, North Carolina 27409
USA
Tel: PPD

Email: PPD
Role: Study Monitoring, Clinical Trial Management, Serious Adverse Event Reporting.

7.5 Data Management and Statistical Analysis

DF/Net Research, Inc.
Data Manager: PPD
Statistical Programmer: PPD
140 Lakeside Avenue, Suite 310
Seattle, Washington 98122
USA
Tel: PPD
Email: PPD
Role: Database development and maintenance, Statistical Analysis, Interim (DSMB) and Final Data Exports. This database will not contain any personally identifying information. See Section 16.3.

7.6 Institutional Review Board of Record

San Juan Hospital Institutional Review Board
Centro Medico, Bo. Monacillos, 2nd floor annex
Rio Piedras, Puerto Rico 00936
USA
Tel: PPD
Role: scientific and ethical review.

7.7 Human Research Protection Office (HRPO)

Office of Research Protections
US Army Medical Research and Materiel Command
Human Subjects Protection Scientist: PPD
1101 Wootton Parkway, Suite 200
Rockville, Maryland 20852
USA
Tel: PPD
Email: PPD
Role: Human subject's protection review and approval; ensuring adherence to ethical standards in DoD-supported research; see Section 17.4.

7.8 DoD Research Monitor

PPD
CAPT, US Navy
Navy Medical Research Command
503 Robert Grant Avenue, Room 3E19
Silver Spring, Maryland 20910

USA
Tel: PPD
Email: PPD
Role: Independent advocacy for subject safety; member of the DSMB; see Section 15.1.

7.9 Aggregate Safety Reporting

Assign Data Management and Biostatistics GmbH (Assign-DMB)
Stadlweg 23
6020 Innsbruck
Austria
Tel: PPD
Email: PPD
Role: Holder of global MV-CHIK safety database; expedited and periodic reporting to regulatory authorities of aggregate safety data from all clinical trials with MV-CHIK.

7.10 Clinical Laboratory

San Juan Hospital Laboratory (and other labs contracted or subcontracted as necessary)
Centro Medico, Bo. Monacillos, 3rd floor annex
Rio Piedras, Puerto Rico 00936
USA
Tel: PPD
Role: Safety and screening laboratory testing

7.11 Screening Immunogenicity Laboratory

Immuno Reference Lab
Ave. Munoz Rivera #562
Hato Rey, Puerto Rico 00918
USA
Tel: PPD
Fax: PPD
Role: Screening immunogenicity testing

7.12 Endpoint Immunogenicity Laboratory

Nexelis
PPD
PPD
Town/City: 525 boulv. Cartier Ouest Laval, QC
Post code: H7V 3S8
Country: Canada
Telephone number: PPD
Role: Primary endpoint immunogenicity testing.

8 Introduction

8.1 Disease Review

8.1.1 Epidemiology and Transmission

Chikungunya virus, a mosquito-borne pathogen that causes chikungunya fever, has re-emerged since 2004, spreading through tropical areas worldwide [Silva, 2018]. Limited outbreaks have been reported in parts of Europe (Italy in 2007 and 2017; France in 2010, 2014 and 2017) and the continental United States (Florida in 2014, Texas in 2015). Puerto Rico experienced a dramatic outbreak in 2014 with over 35,000 cases reported. Incidence then dropped off significantly with fewer than 10 confirmed cases in 2017 [Pan American Health Organization (PAHO) Website]. Herd immunity likely contributes to the current low incidence of disease [Staples 2009]. The seroprevalence of chikungunya in San Juan is approximately 23.5% [Simmons 2016].

Chikungunya virus is transmitted to humans by the mosquitoes *Aedes aegypti* and *Aedes albopictus*, which are common in urban tropical areas worldwide [Vazeille 2007]. Sylvatic cycles involving other Aedes species and non-human primates have also been described [Silva 2018]. Vertical transmission may occur when mothers are viremic at the time of delivery and the resulting neonatal infection is typically severe and often fatal [Gerardin 2008]. While viral RNA has been detected in breast milk and semen, human-to-human transmission by these routes has not yet been described [Silva 2018].

8.1.2 Acute Chikungunya

The French Infectious Disease Society distinguishes three successive stages of chikungunya infection: acute, post-acute, and chronic [Simon 2015]. It appears that the majority of individuals who are infected with chikungunya virus develop symptomatic disease, though this may vary with location, possibly virus strain, and study methodology [Silva 2018]. A household contact study in Puerto Rico showed that while the majority of individuals who become infected report a compatible clinical syndrome, most do not seek healthcare [Sharp 2014]. Therefore it should be assumed that the number of reported cases underestimates the actual number significantly.

Symptomatic disease that presents to healthcare providers generally occurs after an incubation period of 2 to 6 days and is characterized by the acute onset of fever and joint pain, lasting from several days to a week. Joint symptoms usually involve multiple joints in a symmetric distribution. Most commonly involved are wrists, elbows, fingers, knees, and ankles but also more proximal joints. Lower extremity joint pains can be particularly disabling, resulting in the inability to work or perform activities of daily living. Other common signs and symptoms commonly seen include rash, headache, diffuse back pain,

muscle aches, nausea, vomiting, and conjunctivitis [Weaver 2015]. Atypical presentations include hepatitis, Guillain-Barré syndrome, myocarditis, retinitis and nephritis [Rajapakse 2010].

Increases in all-cause mortality have been described in the wakes of several chikungunya epidemics [Brito 2017][Freitas 2018a]. In Puerto Rico the excess mortality was mostly attributed to chronic ischemic heart disease and diabetes mellitus [Freitas 2018b]. Therefore there is likely significant underestimation in both the number of clinical cases and the number of deaths due to chikungunya infection. The overall case fatality ratio (CFR) of acute chikungunya is likely about 1:1000 (0.1%), with most deaths occurring in neonates, adults with underlying medical conditions and older persons [Staples 2009]. Among patients admitted to one hospital in Puerto Rico for chikungunya, fatal cases tended to be older than non-fatal cases (78 versus 68 years) [Perti 2016], similar to trends reported elsewhere [Mavalankar 2008]. Studies of all-cause mortality following chikungunya epidemics have shown the greatest mortality increases occur in the older age groups defined as >60 [Beesoon 2008], >65 [Freitas 2018b], or >75 [Josseran 2006] though an excess all-cause mortality following the chikungunya epidemic in Puerto Rico was also seen in the 25-44 year age group [Freitas 2018b].

8.1.3 Chronic Chikungunya

Following the acute phase, a majority of patients continue to experience prolonged symptoms, lasting several weeks to years [Edington 2018]. Chronic chikungunya is defined by the WHO as joint pain, rigidity, or edema that continue at least 12 weeks after the onset of acute chikungunya. These symptoms may occur continuously or recurrently. [WHO 2015]. The French Infectious Disease Society defines chronic chikungunya as the absence of return to the pre-existing condition more than 3 months after the onset of chikungunya symptoms [Simon 2015]. Symptoms of chronic chikungunya range from non-specific local or generalized muscular discomfort to chronic inflammatory rheumatisms that meet diagnostic criteria for Spondyloarthritis (SA) or Rheumatoid Arthritis (RA) with positive tests for Rheumatoid Factor and Anti-Citrullinated Peptide Antibodies [Javelle 2015]. Post-chikungunya fibromyalgia is also described [Blettery 2016]. In addition to joint symptoms, several components of quality-of-life surveys are consistently lower in patients with chronic chikungunya. Fatigue, insomnia and depression that are coincident with acute infection are increasingly recognized [Elsinga 2017][Bhatia 2015].

Risk factors for chronic disease, in addition to the severity of the acute illness, are co-morbidities such as diabetes [Van Aalst, 2017] and pre-existing joint conditions such as osteoarthritis. Multiple studies have documented an increased risk of chronic chikungunya with age using cutoffs of >35 [Schilte, 2013][Yaseen, 2014], >40 [Mohd Zim, 2013],> 45 [Sissoko, 2009], >50 [Essackjee, 2013] and >60 years [Hoarau, 2010].

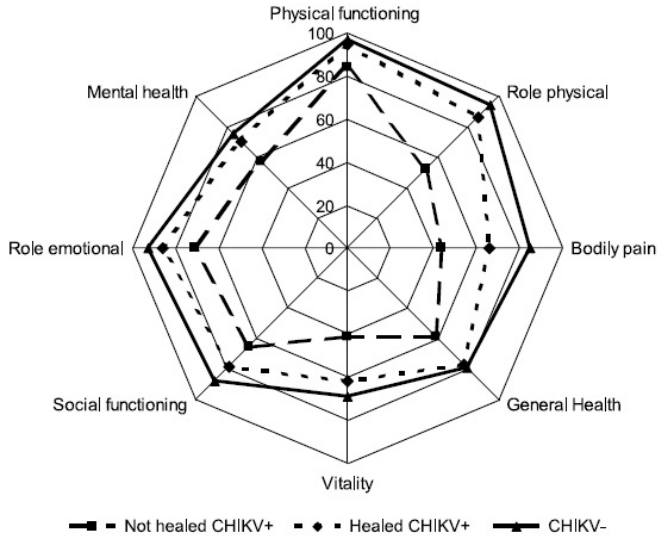

Figure 1: Quality-of-Life survey results from French soldiers stationed on La Reunion during the 2006 epidemic 2.5 years later. Note that soldiers who reported they had completely recovered ("Healed CHIKV+") continued to have detectable decreases in most QoL parameters, including Bodily pain, more than 2 years later. From [Marimoutou 2012].

The pathophysiology of chronic chikungunya is poorly understood. Some authors use the term "post-chikungunya chronic inflammatory rheumatism" (pCHIK-CIR) synonymously with chronic chikungunya [Rodriguez Morales 2017] while others use it to describe only the minority of cases that meet specific diagnostic criteria for RA, SA or undifferentiated polyarthritis who have experienced symptoms continuously since the acute infection [Simon 2015], so it is difficult to know what proportion of chronic chikungunya cases result from synovial or systemic inflammation versus other mechanisms and if these proportions vary significantly between virus strains or epidemic locations. Three mechanisms have been proposed to explain persistent symptoms: (1) persistence of infectious virus, (2) persistence of viral antigens, and (3) persistent immune activation after the virus has been cleared [Burt 2017]. For cases that meet diagnostic criteria for RA an autoimmune mechanism is suggested. However RA following chikungunya infection is distinguished by its acute onset and increased risk in older subjects while RA that is not associated with chikungunya more often has a gradual onset in middle age. Both conditions affect women more than men, have a predilection for small joints over large, and respond to treatment with methotrexate [Simon 2015]. As for other presentations of chronic chikungunya, the detection of cytokines or inflammatory markers is inconsistent [Dupuis-Maguiraga 2012] suggesting that pathogenic mechanisms vary.

8.2 Investigational Vaccine

MV-CHIK is a recombinant, live attenuated Schwarz strain measles virus-vectored vaccine in which the open reading frame of the entire structural gene of the chikungunya La Reunion strain 06-49 has been inserted [Brandler 2013]. Measles vector replication

and expression of the structural genes results in the formation of virus like particles (VLPs) *in vivo*. The advantage of VLPs is that structural proteins are expressed in their natural conformation without the risk of chikungunya replication in the host or transmission to mosquitoes. These VLPs are efficiently recognized by the immune system and elicit a high titer neutralizing antibody. In addition, live virus vectors elicit enduring cellular and humoral immune responses. A further advantage of using this platform is that the methods for producing and up-scaling production of the measles vaccine are well established. See the Investigator's Brochure for more information.

MV-CHIK is manufactured as a colorless clear to off-white opaque liquid without excipients in a glass vial and must be stored at or below -65ºC. The target dose is 5×10^5 $TCID_{50}$ per 0.4mL dose and is delivered intramuscularly. See Section 10.3 for storage and handling requirements.

8.3 Pre-Clinical Studies

8.3.1 Immunogenicity Studies

Preclinical evaluation of the MV-CHIK vaccine is described by Brandler et al [Brandler 2013]. In CD46- interferon alpha/beta receptor knockout (IFNAR) mice, which express the human measles virus receptor, CD46, MV-CHIK induced high levels of chikungunya virus neutralizing antibodies. All immunized mice were protected from lethal challenge with chikungunya virus, even after a single immunization. Passive transfer of immune sera to highly susceptible IFNAR mice conferred protection from lethal challenge with homologous and heterologous strains of chikungunya. Importantly, pre-existing immunity to measles virus did not impair protection. Immunogenicity as determined by a plaque reduction neutralization test (PRNT), has also been demonstrated in immunocompetent cynomolgus macaques receiving one or two immunizations.

8.3.2 Toxicity Studies

8.3.2.1 Biodistribution and Shedding profile

A biodistribution and shedding study for MV-CHIK was performed in cynomolgus monkeys and is described in the Investigator's Brochure. In brief, measles virus RNA was detected in the draining (iliac) lymph nodes up to 64 days post-inoculation and occasionally in a few other tissues (mandibular lymph node, tongue, popliteal lymph node and cervical lymph node), the same distribution seen with the non-recombinant parent Schwarz strain. All shedding levels were found to be at or below limit of detection (≤ 100 genome equivalents per reaction). No recombinant virus was released into the environment.

In clinical trial MV-CHIK 202 (described below) real-time PCR of urine and saliva samples was performed on a subset of participants at 0 7, 10, 14, 28 and 196 days after initial

vaccination. MV-CHIK RNA was not detected in any of the samples analyzed [Reisinger 2018]. In a biodistribution study of a similar measles-vectored vaccine, MV1-F4 for HIV, vaccine RNA was detected sporadically in feces, urine and vaginal secretions but no infective virus was recovered [Lorin 2012].

8.3.2.2 Neurotoxicity

A neurovirulence study in non-human primates was performed as requested by the European Pharmacopoeia and the World Health Organization (WHO) on measles vaccine seed lots. For this purpose, the MV-CHIK seed lot (MV-CHIK master virus seed stock) was inoculated into the thalamic region of each hemisphere of cynomolgus macaques. The study concluded that the MV-CHIK vaccine did not induce unexpected clinical or histopathological evidence of involvement of the central nervous system.

8.4 Clinical Studies

MV-CHIK has been evaluated in five Phase 1 and Phase 2 clinical trials enrolling over 500 subjects. Safety data from all ongoing studies will be shared in real time with the Sponsor and communicated among all clinical trial sites to ensure that any safety signals that emerge are promptly addressed.

8.4.1 Study MV-CHIK-101

This first-in-human Phase 1 trial of MV-CHIK was conducted in 42 healthy adults (aged 30.5±7.3 years) in Austria [Ramsauer 2015]. The vaccine induced neutralizing anti-chikungunya antibodies even in the presence of pre-existing anti-measles immunity in all cohorts. The medium- and high-dose groups (7.5×10^4 and 3.0×10^5 $TCID_{50}$ respectively) induced similar levels of neutralizing antibodies, which were significantly higher than the low-dose group (1.5×10^4 $TCID_{50}$) and the MMR (Priorix®) comparator group. A second immunization at a 1- or 3-month interval boosted the neutralizing titers in all treatment groups. The seroconversion rate was 100% after the second immunization in all dose groups. Measles antibody titers were boosted in all dose groups after the first immunization. Overall, MV-CHIK induced a robust anti-chikungunya immune response. No immunological correlate of protection has been established, and the protective titer is still unknown. However, the highest dose induced the most persistent titer.

The most frequent adverse events (AEs) encountered in this study were headache, injection site pain, influenza-like illness, fatigue, nausea, nasopharyngitis, and myalgia. Joint pain (arthralgia) was reported by 27.8% (10/36) of the subjects that received MV-CHIK and in all cases was self-limited and accompanied by flu-like symptoms. The most frequent related AEs were injection site pain (18/36 [50.0%]), headache (12/36 [33.3%]), and fatigue (10/36 [27.8%]).

Seven severe AEs occurred in six subjects who received MV-CHIK. One case of severe injection site induration and one case of severe injection site erythema were reported and

evaluated as definitely related to the study treatment. Injection site pain increased with dose, which was deemed related to the high inoculation volume (1 mL) and the formulation's salt buffer content rather than to the active ingredient. Of the first nine volunteers who received the highest dose, all reported significant pain, and three experienced vasovagal syncope. After decreasing the volume of injection by splitting it into two doses, the vaccine was much better tolerated. Subsequent lots of MV-CHIK, including those to be used in this study, were produced with a much lower salt concentration in the final product and were administered in a lower volume (0.4 mL) with much better tolerability.

Three cases of severe headache after vaccination were reported; one was rated as probably related, and the other two cases were possibly related. One case of severe fever was documented and was evaluated as unlikely to be related to the study treatment. One subject with pre-existing alcohol dependence and depression attempted suicide and met the seriousness criterion “hospitalization” (severe and not related), which led to early termination. One other subject experienced a serious adverse event (SAE), a meniscus injury (moderate severity and determined to be not related).

All AEs were recovered without any sequelae by study end.

8.4.2 Study MV-CHIK 202

Protocol MV-CHIK 202 (NCT02861586) was conducted in 263 adults at four sites in Austria and Germany [Reisinger 2018]. It compared two doses (5×10^4 or 5×10^5 $TCID_{50}$) of MV-CHIK administered at two different intervals (one or six months). For each of these four cohorts subjects were randomized to receive either MV-CHIK or a licensed MMR comparator vaccine in a 6:1 ratio. In addition, two cohorts of 20 consented subjects each received the MMR either one or six months before a high-dose one month regimen of MV-CHIK to assess the effect of recently boosted immunity to measles on chikungunya immunogenicity.

MV-CHIK showed an excellent safety profile with an overall incidence and severity of solicited and unsolicited AEs comparable to the MMR. The most frequent AEs were: headache (33% with MV-CHIK, 47% with MMR); fatigue (23% MV-CHIK, 24% MMR); flu like symptoms (18% MV-CHIK, 12% MMR); nausea (8% MV-CHIK, 18% MMR); and myalgia (15% MV-CHIK, 18% MMR). Arthralgia, regarded as an adverse event of special interest, was solicited and observed in 10% with MV-CHIK and in 15% of the MMR group. Injection site reactions were higher in the MV-CHIK group with induration (16% versus 0%); and tenderness (52% versus 21%) being statistically significant. Ten individuals reported severe adverse events after receiving MV-CHIK: one injection site pain, one injection site induration, four fatigue, two headache, two flu-like symptoms and one with nausea and vomiting. All severe adverse events were resolved within 1 week. No clinically relevant abnormalities were detected in hematology or chemistry laboratory tests. No vaccine-related serious adverse events occurred.

The primary endpoint of the trial was immunogenicity on day 56 by the 50%-plaque reduction neutralization test ($PRNT_{50}$). For the high-dose one-month interval to be used in this study, 95.7% seroconverted (defined as a $PRNT_{50} > 10$) at 28 days after the second dose and 87.2% remained positive seven months later. Priming with the measles vaccine did not impair the rate of seroconversion or the GMT of the chikungunya vaccine.

8.4.3 Study DMID 15-0038

Protocol 15-0038 (NCT03028441) is sponsored by the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (NIH) and is being conducted in Georgia, Iowa and Texas. This study compared two doses of MV-CHIK (5×10^4 and 5×10^5 $TCID_{50}$) given at three different intervals (one month, three months, and six months). Each of these six cohorts contained 30 consented subjects of which 25 received MV-CHIK and 5 received saline placebo.

Study enrollment and vaccination have been completed and the clinical phase should conclude by March 2019. After enrollment of 50 subjects, a Data and Safety Monitoring Board assessment was performed and did not raise any concerns for continuation of the study. The final data will be available by June 2019.

8.4.4 Study MV-CHIK 205

Protocol MV-CHIK 205 (NCT03635086) is an observer blinded, randomized study of 60 healthy adults, ages 18 to 55 years, of the safety, tolerability and long-term immunogenicity of different formulations of MV-CHIK in healthy volunteers. This study is being conducted in the United Kingdom. The regimens under comparison are each of two injections 28 days apart of the following vaccine products:

A. two doses of 5×10^4 $TCID_{50}$ from a lyophilized formulation,
B. two doses of 1×10^5 $TCID_{50}$ in a liquid frozen formulation,
C. two doses of 1×10^5 $TCID_{50}$ in a liquid SPS® (Stabilizing and Protecting Solution), formulation,
D. two doses of 1×10^6 $TCID_{50}$ in a liquid frozen formulation, and
E. one dose of 1×10^6 $TCID_{50}$ in a liquid frozen formulation plus placebo.

Subjects will return after 6 months and 1 year to follow up the durability of the immune response against chikungunya.

Recruitment is completed. An interim analysis after all subjects have completed the primary endpoint visit will be available by mid-2019. The final data will be available by the end of 2019. The same lot of 1×10^6 $TCID_{50}$ in a liquid frozen formulation will be used in the current study. Post-manufacturing analyses have shown the actual concentration to be closer to 5×10^5 $TCID_{50}$ as described in this protocol.

8.4.5 Study MV-CHIK 204

Protocol MV-CHIK 204 (NCT03101111) is a companion to the current study and is being conducted at the University of Puerto Rico Medical Sciences Campus. This study was designed to explore the safety of the MV-CHIK vaccine in previously exposed adults aged 21 to 50 by comparing volunteers who were seropositive at baseline with those who were seronegative. The reason for studying the effect of MV-CHIK in previously exposed individuals is explained in Section 8.7. Enrollment in MV-CHIK 204 was significantly impeded following Hurricane Maria in 2017 and was stopped after vaccinating 34 of 100 planned subjects. A number of previously exposed individuals reported joint pain during the post-vaccination period, but it is not clear that these events were triggered by the vaccine. This trial has not yet been unblinded, but a thorough discussion of each AESI is appropriate here.

Five among 16 subjects who were seropositive for previous chikungunya infection at enrollment reported six AESIs:

- Subject PPD is a PPD who reported on PPD, four days after the second dose, moderate left shoulder pain. PPD took acetaminophen on 17, 18 and 19 October and ibuprofen on 20, 21 and 22 October. PPD also reported diarrhea from 21 to 23 October. PPD shoulder pain resolved on PPD. PPD PPD which may have contributed to PPD shoulder pain. However, PPD specifically commented that PPD shoulder pain was similar to what PPD experienced during PPD acute chikungunya infection. PPD C-reactive protein was 0.82 prior to initial vaccination, 1.3 prior to PPD second vaccination, and 1.18 the day prior to the onset of PPD symptoms. The investigator assessed this AESI as moderate and possibly related to the IVP.

- Subject PPD is a PPD with a past history of chikungunya infection and right knee injury. On PPD nine days after the first dose of study vaccine PPD developed bilateral knee pain following exercise and housework. PPD did not report this to the investigator until after PPD received the second dose of study vaccine. MRI revealed osteoarthritic changes in both knees and a torn meniscus on the right with effusion. PPD was referred to an orthopedic surgeon and physiatrist. PPD symptoms continue but no change in the overall clinical course of this degenerative disease is attributed to the study vaccine. PPD C-reactive protein was 0.1 mg/L prior to initial vaccination, 0.25 three days later, and 0.11 prior to second vaccination. The investigator assessed this AESI as an exacerbation of an on-going and previously unreported condition of moderate severity and unrelated to the IVP.

- Two AESIs were reported by Subject PPD, a PPD PPD PPD On PPD, 31 days after the second dose of study vaccine, PPD reported right wrist arthralgia. On PPD, 67 days after the second dose of study vaccine, PPD reported left wrist arthralgia. PPD was referred to a physiatrist

who diagnosed tenosynovitis of the extensor of the 5th finger, bilaterally. Symptoms occurred intermittently until [redacted] baseline C-reactive protein was 0.27 mg/L, 0.24 prior to second vaccination and 0.45 when [redacted] right wrist symptoms were reported about one month later. The investigator assessed both AESIs as mild, work-related and unrelated to the IVP.

- Subject [redacted] is a [redacted] who on [redacted], three days after the second dose of study vaccine, reported pain in [redacted] right foot. [redacted] had injured this foot [redacted] previously which [redacted] treated topically with Bengay® with resolution of the pain. [redacted] did not report pain on the day of [redacted] second vaccination. The pain resolved by [redacted]. [redacted] C-reactive protein was 0.63 mg/L prior to initial vaccination, 0.85 prior to second vaccination and 0.82 when [redacted] symptoms were first reported three days later. The investigator assessed this as Achilles tendinitis caused by [redacted] previous fall, moderate and unrelated to the IVP.

- Subject [redacted] is a [redacted], seropositive for chikungunya, who reported on [redacted] numerous complaints including joint pain that began on [redacted] three days after initial vaccination. These complaints were raised at a time when the subject was belligerent and demanding advanced payment for [redacted] study participation. [redacted] was suspected of drug abuse and removed from the study because of a perceived threat to study site personnel. [redacted] C-reactive protein was <0.1 mg/L prior to initial vaccination and <0.1 when [redacted] symptoms were reported. The investigator assessed [redacted] joint pain as unsubstantiated, possibly confabulated and therefore unrelated to the IVP.

In addition, three among 18 subjects in the seronegative group reported three AESIs.

- Subject [redacted] is a [redacted] seronegative for previous chikungunya infection. One day after receiving the second dose of the blinded study vaccine on [redacted], [redacted] reported pain in [redacted] right wrist [redacted]. Similar pain occurred in [redacted] left wrist on [redacted]. Wrist tenosynovitis with carpal tunnel syndrome was diagnosed and attributed to [redacted] occupation as a [redacted]. [redacted] was referred to a physiatrist and ibuprofen prescribed. The condition is reported as resolved as of [redacted]. [redacted] C-reactive protein was 0.09 mg/L prior to initial vaccination, 0.19 prior to second vaccination and 0.21 three days later. The investigator assessed this condition as mild and unrelated to the IVP.

- Subject [redacted] is a [redacted] seronegative for previous chikungunya infection. In [redacted], about five months after the second dose of the blinded study vaccine, the subject developed right (dominant) wrist tenderness after a [redacted] project that required use of a pressure [redacted]. It has improved over the subsequent four months but not fully resolved. The investigator assessed this condition as mild and unrelated to the IVP.

- Subject PPD is a PPD seronegative for previous chikungunya infection. In PPD 12 months after the second dose of the blinded study vaccine, the subject reported right wrist pain when grasping objects. PPD had broken a bone in this hand two years previously and had declined recommended surgical intervention at that time. There is no obvious reason for the exacerbation of symptoms two years after the fracture and one year after the second dose of IVP. The investigator assessed this condition as mild and unrelated to the IVP.

In summary, individuals previously exposed to chikungunya reported more joint pain (six events, one of which was considered possibly related to the IVP) than seronegative individuals (three events, none of which was considered possibly related to the IVP) during MV-CHIK 204. However the temporal relationship of these events to the IVP in pre-exposed subjects varied widely (3 and 9 days after the first; 3, 4, 31 and 67 days after the second vaccination) and in each case other causative or exacerbating factors were identified. The C-reactive protein levels do not suggest an inflammatory or immunopathogenic basis for these adverse events. The study has not yet been unblinded, so it is unknown which of these subjects received MV-CHIK versus MMR. It should be noted that this study design, in which previously exposed individuals were compared with previously unexposed individuals, may have been intrinsically biased. It has been observed that individuals who report being infected by chikungunya, even those who report having fully recovered, continue to report symptoms at a higher rate than their uninfected peers (figure 1) [Marimoutou 2012]. Therefore a higher rate of adverse events including joint pain should be expected in seropositive subjects independent of the study intervention. Lessons learned from MV-CHIK 204 have been incorporated into the design of MV-CHIK 206.

8.5 Military Relevance

This study will be conducted in a non-military population of healthy volunteers up to age 65, expanding on a previous study that went up to age 50. This simulates a portion of the deploying force: More than 5% of service members who deployed to Iraq or Afghanistan between 2001 and 2010 (over 100,000 people) were age 50 or older [Rutherford, 2013]. The number and proportion of DoD civilians and contractors in combat zones over the age of 50 varies, but is also considerable. However, the population for the proposed study was primarily chosen, not to simulate a military population, but to scrutinize the potential for immunopathogenicity that any vaccine containing chikungunya antigens will have to address [Yang, 2017]. A vaccine that is not safe in this age group does not meet the public health need, is not commercially viable and is therefore unlikely to ever be available to the Warfighter.

8.6 Potential Benefits

The potential benefit of MV-CHIK vaccination for those who have not previously been infected would be protection against chikungunya infection if the vaccine is proven to be

efficacious. A boost in measles immunity may be an additional benefit. For this study, in which all subjects have previously been infected with chikungunya and presumably therefore have lifelong immunity, there is no individual benefit to participation. However the information to be gained will be vital to informing the consent of future participants in clinical trials conducted in areas of on-going transmission.

8.7 Potential Risks

8.7.1 General

Potential risks that are frequently associated with any vaccine include injection site reactions such as edema, induration and erythema, transient local pain or tenderness. Adverse systemic events include mild to moderate headache, myalgia, arthralgia, flu-like symptoms, or fatigue. In addition, vaccines are reported to induce allergic and anaphylactic reactions at a rate of about 1 per million [Kelso 2012].

8.7.2 MV-CHIK specific

As described above, AEs previously reported with MV-CHIK include injection site pain, headache, fatigue, and joint pain or arthralgia. See Section 8.4.4 for a discussion of adverse events encountered in previously exposed individuals.

8.8 Clinical Study Rationale

The rationale for this study is to collect additional data on vaccine safety in individuals previously exposed to chikungunya virus. Although natural infection with chikungunya is thought to confer lifelong immunity, self-reporting of previous chikungunya infection is not reliable, particularly in areas where clinically similar arboviruses circulate. Previously exposed persons will therefore inevitably receive the vaccine when field efficacy studies are conducted and when the vaccine is ultimately licensed for use in areas of ongoing transmission. Because older individuals are at higher risk for developing chronic chikungunya, the risk of post-vaccination immunopathology may also be greater. Therefore this study will be conducted in two Steps in which individuals over the age of 50 will not be enrolled until after a DSMB favorably reviews the accumulated safety data in younger subjects.

There are two reasons to characterize the safety of MV-CHIK in previously exposed persons at this stage of product development. First, some of the mechanisms of chronic chikungunya appear to be immunopathogenic responses to chikungunya antigens. Any vaccine containing chikungunya antigens is therefore hypothetically capable of triggering or exacerbating such symptoms. MV-CHIK has been shown to elicit both neutralizing humoral and cellular immune responses [unpublished data], the latter of which is associated with signs of chronic chikungunya in animal models [Lum 2015]. Because this live measles vectored vaccine bypasses anti-chikungunya immunity and results in *in vivo* production of chikungunya antigens, pathogenic responses to these antigens may result.

The second reason for assessing vaccine safety in previously exposed persons now is less tangible but possibly more important. The fact that many of the manifestations of chikungunya infection are common and subjective (depression, fatigue, muscle and joint aches) there is a high risk that individuals who develop these symptoms for any reason will be inclined to attribute them to the vaccine. A very similar situation occurred with, LYMErix™, a vaccine for Lyme disease licensed by Smith Kline Beecham in the late 1990s. Lyme disease, similar to chikungunya, is associated with many chronic subjective complaints (depression, fatigue, muscle and joint aches). LYMErix was shown to be safe and effective in clinical trials. The presumed mechanism of action is that vaccine-induced antibodies are ingested by the vector and transmission is blocked without inducing any inflammation in the human host. Nevertheless, this vaccine acquired a reputation for causing arthritis. Despite the unanimous support of an FDA panel convened to address questions of vaccine safety, public confidence dissipated, demand dropped and the vaccine was withdrawn from the market in 2002 [Poland 2011]. Many experts feel that the prospects for any other vaccine for this particular disease are bleak [Shen 2011][Plotkin 2011], citing issues that could easily arise following the licensure of any vaccine for chikungunya.

One issue underlying the commercial failure of LYMErix was that it was not approved for use in the age group at highest risk of disease, namely those under the age of 15. As a result, the public health impact of the vaccine was less perceptible and the tide of public opinion was more easily turned. For chikungunya, the greatest public health impact will be in older individuals living in areas of on-going transmission. Therefore studies specifically looking at vaccine safety in older adults are required prior to licensure and this study is required to appropriately inform the consent of older participants in those studies.

A second, more important issue underlying the failure of LYMErix was a lack of consumer confidence in the vaccine industry and the vaccine licensure process. According to a Senior Science Policy Advisor at the National Vaccine Program Office, the “key lesson learned from the LYMErix development story is that science and innovation are inadequate, by themselves, to assure that the public health value of a vaccine is realized… Communication, education, and transparency throughout the development and licensure process results in a deeper understanding of the vaccine. This understanding helps to inform attitudes about the vaccine not only among those individuals who are most vulnerable to Lyme disease but also among all of the other stakeholders who share a partnership role in vaccine development, licensing, distribution, and administration” [Shen 2011].

Applying this lesson to chikungunya vaccine development requires acknowledgement of the high commercial risk of developing any vaccine against chikungunya and addressing this risk early and often. It is entirely possible that MV-CHIK will induce some mild and transient symptoms in previously exposed persons. And there will inevitably be

individuals destined to develop inflammatory arthritis or depression whose onset of symptoms will coincide with the receipt of a vaccine. If this occurs with a vaccine whose path to licensure has included a cautious, deliberate and stepwise demonstration of vaccine safety in groups at hypothetical risk, subject matter experts will be in a position to lead public health authorities and consumers in an accurate assessment of the risks and benefits of vaccination. This clinical trial is, therefore, on the critical path to both licensure and the long-term commercial viability of MV-CHIK.

9 Investigational Plan

9.1 Overall Study Design

This will be a prospective randomized double-blind interventional clinical trial to evaluate the safety and immunogenicity of MV-CHIK in two intramuscular injections 28 days apart versus placebo. Consented study subjects will be screened for baseline seropositivity to chikungunya virus where seropositivity is defined by a colorimetric capture ELISA (Novatec Immundiagnostica GmbH, Dietzenbach, Germany) as absorbance units exceeding that of the negative control by greater than 10%. Seropositive subjects are then randomized to receive either MV-CHIK (the experimental vaccine) or saline (the placebo) in a blinded fashion in a 1:1 ratio. One dose level (5×10^5 $TCID_{50}$) of MV-CHIK will be studied. Subjects will be followed for safety and immunogenicity for 24 weeks after completing the series. Because rheumatologic symptoms of chikungunya infection are more likely, more severe and more persistent in older patients, this study will initially be limited to adults aged 21 to 50 years of age (Step 1). After at least 20 volunteers in Step 1 have been vaccinated, a DSMB will review the safety data and, with their recommendation, the study will proceed to Step 2 and enroll subjects aged 51 to 65 years. The study schematic is shown as Figure 2.

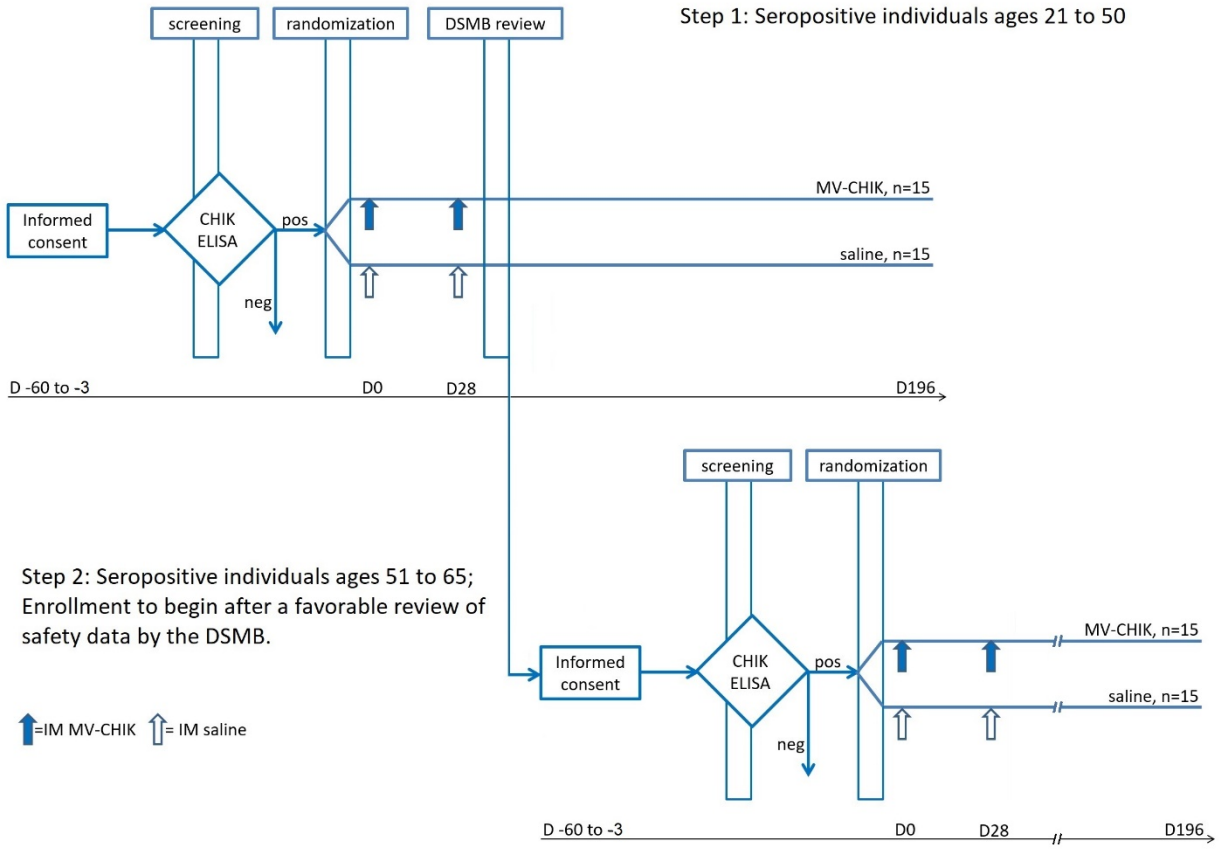

Figure 2: Study schematic

The trial will be registered online at the National Library of Medicine (NLM) public registry: http://www.ClinicalTrials.gov.

9.2 Study Objectives and Endpoints

9.2.1 Primary Objective

The Primary Objective of this study is to determine the safety of MV-CHIK administered in two injections separated by 28 days in previously exposed individuals. The following end-points will be used to assess safety in MV-CHIK versus placebo recipients:

- solicited AEs (fever, fatigue, headache, malaise, myalgia, nausea/vomiting, joint pain or injection site itching, pain/tenderness, erythema/redness or induration/swelling occurring within 7 days of vaccination)
- unsolicited AEs including the above signs or symptoms occurring more than 7 days after vaccination, clinically significant abnormal safety laboratory results, vital signs, and physical examination findings
- Solicited and unsolicited AEs of grade 2 and higher.

9.2.2 Secondary objectives

The Secondary Objective of this study is to determine the Immunogenicity of MV-CHIK in previously exposed individuals. The following end-points will be used to assess immunogenicity in MV-CHIK versus placebo recipients:

- Fold-increase from Day 0 in geometric mean titer (GMT) of neutralizing antibodies to chikungunya on Days 28, 56, and at the end of the study.

9.2.3 Exploratory Objectives

As Exploratory Objectives, this study will assess the relationship of Quality-of-Life scores and acute phase reactants with adverse events experienced post-vaccination. The following end-points will be used to assess these:

- Change in serum C-reactive protein levels from pre-vaccination to three days post-vaccination in recipients of MV-CHIK versus placebo.
- Changes in plasma fibrinogen from pre-vaccination to seven days post-vaccination in recipients of MV-CHIK versus placebo.
- Changes from baseline in Quality-of-Life survey (SF36) scores at four weeks post-vaccination and at the end of the study in recipients of MV-CHIK versus placebo.

9.3 Study Population

The study will be conducted in Puerto Rico where a chikungunya epidemic occurred in 2014 but where transmission is now negligible. Individuals will be screened until 30 subjects are vaccinated for each Step. Subjects still under treatment for symptoms attributed to a previous chikungunya virus infection will be excluded from the study. Subjects who attribute only mild and subjective symptoms such as fatigue to previous chikungunya infection may be eligible at investigator discretion; subjects with acute chikungunya infection will be excluded but may re-screen no sooner than 3 months after symptoms (other than mild subjective symptoms not requiring treatment) have resolved. This study will measure, but not control for, baseline Quality-of-Life using the SF36 questionnaire. The seroprevalence of chikungunya in San Juan is approximately 23.5% [Simmons 2016]. Up to 500 subjects may be screened for each Step but it is hoped that existing databases of seropositive study volunteers will allow enrollment to be completed much more efficiently.

Subjects will not be randomized unless all inclusion and no exclusion criteria, including lab test results, are met. Subjects who fail to meet criteria because of an identifiable temporary condition or who cannot be randomized within the 60-day screening window may repeat the screening evaluation up to two times after the initial screening (total of three) at the discretion of the Investigator.

9.3.1 Inclusion Criteria

Subjects MUST satisfy all of the following entry criteria before they will be allowed to participate in the study:

1. Previous infection with chikungunya as verified by a serum immunoassay.
2. Age appropriate for the Step being conducted:
    a. ≥21 to ≤50 years on the day of enrollment for Step 1.
    b. ≥51 to ≤65 years on the day of enrollment for Step 2.
3. Able to provide informed consent.
4. Available and accessible for the duration of the trial.
5. Able and willing to comply with all requirements of the study.
6. For women of childbearing potential, willing to practice adequate contraception (see Definition of Terms) for the duration of the study. This is similar to recommendations following MMR vaccination [McLean 2013] but for a longer duration (six months versus one month) due to the higher concentration of measles virus and the lack of reproductive or developmental toxicology data on MV-CHIK.
7. Medical history and physical examination findings are considered normal or not clinically significant in the opinion of the Investigator, which includes resolution of any arthralgias that may have occurred during prior chikungunya infection, as well as the absence of synovitis.
8. Laboratory values are considered normal or not clinically significant in the opinion of the Investigator. If laboratory screening tests are out of the normal reference range and of potential clinical significance, the test(s) may be repeated up to 2 times (a total of 3 per screening evaluation) at the discretion of the Investigator, and the repeat values and their potential clinical significance will be used to determine eligibility.
9. History of immunity to measles. For persons born after 1957, this will be established by a history of compliance with vaccination policies that included measles vaccination or known vaccination as an adult at least one month before they are randomized. Volunteers born before 1957 will be presumed to have immunity to measles based on natural exposure in accordance with CDC guidelines [McLean 2013].

9.3.2 Exclusion Criteria

If any of the following apply, the subject MUST NOT be enrolled in the study:

1. Taking medication or other treatment for unresolved symptoms attributed to a previous chikungunya virus infection.
2. Prior receipt of any investigational chikungunya or other alphavirus vaccine. To date, no alphavirus vaccines have been commercially available in the United States.
3. Recent infection:

- self-limited upper respiratory infections until afebrile without medication for >1 week;
- chikungunya unless/until asymptomatic (other than mild subjective symptoms not requiring treatment) for >3 months;
- non-recurrent upper respiratory or urinary tract infections successfully treated with antibiotics, until asymptomatic for 1 month after full antibiotic course has been completed.

4. History of an acute allergic or anaphylactic reaction to any vaccine.
5. History of an immunosuppressive disorder (such as HIV infection, Common Variable Immune Deficiency), chronic infection (such as chronic hepatitis B or C), autoimmune disease (such as rheumatoid arthritis, systemic lupus erythematosus (SLE), autoimmune thyroid disease), or any medical condition that, in the opinion of the Investigator, could lead to an atypical immune response to the vaccine.
6. History of moderate or severe non-traumatic arthritis or arthralgia within 3 months of the Screening Visit.
7. Recent (within 30 days), current or anticipated use of any immunosuppressive or immune modifying medication including corticosteroids (excluding nasal, ophthalmic, and other topical preparations).
8. Other vaccination or planned vaccination within 4 weeks of either study dose (within 2 weeks for seasonal influenza vaccine).
9. Receipt or planned receipt of blood products including immunoglobulins within 120 days of the Screening Visit.
10. Pregnant or lactating or planning pregnancy during the trial.
11. Known alcohol or other substance abuse that in the opinion of the Investigator affects the ability or willingness of the subject to understand and comply with the study protocol.
12. Participation in another clinical study within the past 30 days in which the subject was exposed to an investigational product (pharmaceutical product or placebo or device) or planned participation in another interventional clinical study while participating in this study.
13. Relevant history of any medical condition that, in the opinion of the Investigator, may interfere with the safety of the subject or aims of the study.
14. History of neoplastic disease (excluding successfully treated non-melanoma skin cancer or cervical intraepithelial neoplasia) within the past 5 years or a history of any hematological malignancy.
15. Behavioral or psychiatric disease or cognitive impairment that in the opinion of the Investigator affects the ability or willingness of the subject to understand and comply with the study protocol.
16. Non-consent to storage of blood specimens for future research.
17. Persons in direct relationship with the Sponsor or its contracted service providers, the CRO or its subcontractors, the Investigator, or study site staff. Direct relationship includes first degree relatives or dependents (children,

spouse/partner, siblings or parents), as well as employees (site or Sponsor). Employees of the San Juan City Hospital not directly employed by the Research Unit will not be excluded.

9.3.3 Randomization

Initially, thirty (30) Step 1 subjects will be randomized at Visit 1, Day 0 using a Clinical Data Management System (CDMS). Subsequent to DSMB review, another thirty (30) Step 2 subjects will be enrolled and randomized at their Visit 1, Day 0. Visit 1 occurs after Screening (Visit S, Day -60 to -3) and includes enrollment, randomization and vaccine administration. At Visit 1 subjects will be randomized after enrollment and before vaccine administration. Subjects in each Step will be assigned to one of two groups, as shown in Table 1. Block randomization of appropriate size will accommodate balanced enrollment in ratio of 1:1 into each of the two treatment groups for the planned 30 Step 1 and 30 Step 2 subjects.

Table 1: Treatment Assignments

Step	N	Treatment Assignment	Study Days of Administration
1	15	MV-CHIK 5×10^5 $TCID_{50}$, 0.4mL IM injection	0, 28
	15	Saline, 0.4mL IM injection	0, 28
2	15	MV-CHIK 5×10^5 $TCID_{50}$, 0.4mL IM injection	0. 28
	15	Saline, 0.4mL IM injection	0, 28

The list of randomized treatment assignments will be prepared by statisticians at DF/Net Research. The blinded randomization number will be provided through the randomization module in the data management system. The designated unblinded site personnel will be provided with the unblinded treatment key, which links the randomization number to the actual treatment assignment and should be securely stored.

Instructions for use of the data management system for randomization will be included in the Pharmacy Manual. The Pharmacy Manual will also detail the manual back-up randomization procedures in the event that the site temporarily loses access to the internet or the randomization module is unavailable. All documentation of the randomization procedure and output will be maintained by DF/Net until the end of the study.

9.3.4 Blinding

Vaccine administration will be double-blind, with the Investigator, study coordinator, all study personnel involved in assessing AEs and the subject being unaware of the treatment assignment. To achieve this, the thawed MV-CHIK vaccine and saline will be given the same wait time after procurement to allow both temperature equalization and non-discrepant wait times between treatments. Furthermore, only designated site

personnel (pharmacist, etc.), unblinded CRAs and the unblinded biostatistician (at DF/Net) will have access to the treatment assignments.

Unblinding will occur after the last subject's last visit (Visit 10) and database lock, though the safety data may be unblinded upon Sponsor or DSMB request.

9.4 Withdrawal of Subjects

9.4.1 Criteria for Subject Withdrawal from the Study

In accordance with the Declaration of Helsinki and applicable regulations, a subject has the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the physician or the institution.

Subjects may also be withdrawn from the study if the Investigator determines that the subject should be withdrawn due to any unforeseen circumstance that may affect the safety of the subject, staff or the integrity of the study.

The reason for early withdrawal will be recorded in the clinical records and the electronic case report form (eCRF). All subjects who are withdrawn prematurely from the study will undergo an early termination visit (see Section 11.12). However, it is acknowledged that a subject who is withdrawn from the study, might refuse to provide a reason for withdrawal and/or might refuse to return for an early termination visit.

9.4.2 Criteria for Discontinuing Individual Subjects from Further Vaccination

The following, in addition to the Exclusion Criteria listed above, are specific criteria for discontinuing individual subjects from further vaccination, but not from completing scheduled follow-up assessments, unless the subject is explicitly withdrawn from the study:

- Anaphylaxis within 24 hours after administration of the study vaccine
- Generalized urticaria within 72 hours after administration of the study vaccine
- An SAE or AESI that is considered to be related to the study vaccine and, in the opinion of the Investigator, is likely to recur with repeat dosing.
- A grade 3 or greater systemic or injection site AE that is considered to be related to the study vaccine that lasts longer than 3 days
- A grade 3 or greater laboratory abnormality that is considered to be related to the study vaccine or that has not resolved prior to the next scheduled study vaccination

If a subject withdraws consent or is discontinued from further vaccination or study participation because of an adverse event, appropriate measures to treat the subject will

be taken, and the IRB, Sponsor, SME, DoD Research Monitor and HRPO will be notified immediately.

For safety reasons, all subjects who received at least one dose of study medication should not be withdrawn due to issues arising from the SARS-CoV-02 pandemic. It is recommended that subjects withdrawals only occur under the existing conditions prescribed in the clinical protocol.

If a subject received only a single vaccination prior to the SARS-CoV-02 pandemic and were unable to return for the second vaccination due to research site closure, a second vaccination will not be given. The subject will receive a follow up telephone call to elicit general health status, new and existing adverse events and concomitant medications. The subject will be followed through all remaining visits per the original schedule of events.

During a quality control check of the MV-CHIK TCB 001 03 18, Themis found some deficiencies in the prevention and detection of microbial and particulate contamination. Following notification of the research site PI of the concern, subjects who received only a single vaccination will not receive a second vaccination and will continue to be followed for remaining scheduled visits through Day 196.

9.4.3 Evaluations at Withdrawal

For any subject who is withdrawn before completing all study visits, the Investigator should:

1. Perform an EOS visit (see Section 11.12). This assessment should be performed no later than 14 days after withdrawal/discontinuation.
2. Complete all appropriate eCRF pages, providing the date of and explanation for the subject's withdrawal/discontinuation.
3. When indicated, arrange for appropriate follow-up and/or alternative medical care of the discontinued subject.

If the subject fails to attend a scheduled follow-up or early termination visit, there will be at least two attempts to contact the subject via telephone and two written communications. If these receive no reply the subject will be considered lost to follow-up.

9.4.4 Replacement of Subjects

Once vaccinated, subjects will not be replaced.

9.5 Premature Termination or Suspension of the Study

The study may be temporarily suspended or prematurely terminated at any time if in the best interests of subjects and justified on either medical or ethical grounds. The Sponsor,

Investigator, IRB or DoD Research Monitor, acting in the interest of the study volunteers, are each empowered to suspend the study. Written notification, documenting the reason for study suspension, will be provided by the suspending party to the Sponsor, Investigator, IRB and HRPO.

9.5.1 Study Stopping Criteria

The following study stopping criteria will suspend enrollment and study vaccinations pending review of available safety data and should result in Sponsor notification of the Center for Biologics Evaluation and Research (CBER):

- Any subject experiences an SAE that is assessed as related to vaccination.
- Three or more subjects experience the same or similar grade 3 or greater adverse events or clinically significant abnormal laboratory tests that are assessed as related to the vaccination.

Regardless of how or by whom the study is suspended, the type of safety review or criteria required for study resumption will be determined by the Sponsor at the time of the suspension and will require approval by the IRB. Whether the study is resumed or terminated, adequate consideration must be given to the protection of the subjects' interests.

9.5.2 Interim Management of the Study Due to SARS-CoV-2

Due to the SARS-CoV-02 pandemic, social isolation has been imposed by the Puerto Ric0 Government which has resulted in the closure of the San Juan Research Unit. During this period of social isolation, active screening and enrollment will be suspended. It is understood, to protect subject safety and limit exposure to SARS-CoV-2 that the San Juan Research Unit may need to remain closed past the Puerto Rican Government's regulatory period. Once the San Juan Research Unit re-opens and is fully functional, screening and enrollment will be re-started.

Active subjects in the study with scheduled visits during the San Juan Research Unit closure will receive a follow up telephone call during the visit window or as soon as feasibly possible by the study staff. Once the San Juan Clinical Research Unit re-opens subjects will be scheduled for their next follow up visits.

10 Study Intervention

10.1 MV-CHIK

MV-CHIK is a recombinant live Schwarz-strain measles-vectored vaccine expressing chikungunya virus structural proteins. It is manufactured as a colorless clear to off-white opaque liquid without excipients and stored in a single-use glass vial at or below -65ºC.

The target dose is 5×10^5 $TCID_{50}$ per 0.4mL dose and is delivered intramuscularly. The specific lot to be used in this study has been quantified as 6.34×10^5 $TCID_{50}$ per 0.4mL dose.

MV-CHIK was manufactured under responsibility of Themis Bioscience and will be supplied as a frozen liquid. MV-CHIK/drug substance was manufactured, quality control checked and released in accordance with Good Manufacturing Practice (GMP) PPD PPD PPD The drug substance PPD PPD and manufacturing, quality control, and release was performed in accordance with GMP. No excipients were added. Full details on manufacturing are given in the "Master File Type II – Chemistry, Manufacture and Control of Chikungunya Virus-Recombinant Measles Virus (Schwarz Strain)-Vectored Vaccine", MF #17143).

The single use vials will be removed from the freezer and sit at room temperature for at least 30 minutes before 0.4mL is drawn into a syringe. The vaccine will be injected IM immediately or the syringe will be placed in the refrigerator for up to one hour and injected not later than 90 minutes after the vial was removed from the freezer. For more details see the Pharmacy Manual.

The MV-CHIK vaccine will be shipped as a hazardous material and labeled as live attenuated measles vaccine expressing foreign antigens on dry ice. The biosafety level (BSL) / Risk Group is 2.

During a quality control check of the MV-CHIK TCB 001 03 18, Themis found some deficiencies in the prevention and detection of microbial and particulate contamination. A medical assessment has been completed including review of health changes for the study. There have been no serious health changes attributed to the vaccine to date. Non-serious health changes have been observed and appear consistent with vaccine administration. There is no evidence that these health changes have resulted from transmission of infectious agent and/or injection of particulate matter and the risk of harm is reduced by the close follow-up of study participants. The MV-CHIK TCB 001 03 18 has been segregated from clinical supplies and will be maintained under the labeled storage conditions until it is returned per the sponsor's instructions.

10.2 Placebo

Saline placebo will be kept in a monitored refrigerator (2-8º C). When ready for use, the single use vial will be transferred to room temperature for at least 30 minutes before 0.4 mL is be drawn into a syringe and injected IM immediately or the syringe will be returned to the refrigerator and injected not later than 60 minutes after drawing it up. For more details see the Pharmacy Manual.

10.3 Handling of the Investigational Vaccine Products

10.3.1 Labelling

Both MV-CHIK and the saline placebo will be supplied as a liquid in single-use vials. The vials and carton labels will include the following information:

- Product manufacturer name (carton)
- Sponsor name (carton)
- Vaccine name (carton and vial)
- Dosage form (carton and vial)
- Concentration of vaccine (carton and vial)
- Lot (batch) number (carton and vial)
- Route of administration (carton and vial)
- Storage conditions (carton and vial)
- "For clinical trial use only" (carton and vial)

The target dose at the time of manufacture was 1×10^6 (±0.5log) $TCID_{50}$, which appears as the concentration of vaccine on the label. The vaccine lot to be used in this study has been quantified as 6.34×10^5 $TCID_{50}$. This value is in the same range as some cohorts in other clinical trials of this vaccine (5×10^5 (±0.5log) $TCID_{50}$), including the companion study, MV-CHIK 204. With the exception of the vaccine label, this latter value is used throughout the documentation for this study to clarify which cohorts in other studies received the directly comparable dose.

10.3.2 On-site storage

MV-CHIK will be stored on-site in monitored freezers at or below -65ºC.

All supplies of MV-CHIK and saline placebo will be accounted for in accordance with GCP. An individual study IVP accountability record will be kept for each subject, and the Investigator will keep and maintain accurate records of the disposition of all IVP (MV-CHIK and placebo) received during the study. These records should include the amounts and dates supplies were received, administered to the subject, or returned to the Sponsor. If errors or damages in the vaccine supply shipments occur, the Investigator should contact the Integrum project manager immediately for information on how the error or damage will be handled. Copies of the investigational vaccine accountability records will be provided by the Investigator for inclusion in the Trial Master File after database lock. The Clinical Research Associate (CRA) will periodically check the supplies of IVP held by the Investigator or pharmacist to verify accountability of all IVP used.

The Investigator will only approve administration of the IVP to the identified subjects of this study, according to the procedures described in this protocol. After the end of the

study, all unused IVP and all containers can be destroyed on site as long as proper documentation is provided. If destruction on site is not possible or at the discretion of the Sponsor, investigational vaccine and all containers may be returned to the Sponsor for destruction. The Sponsor or CRO designee will verify that a final report of IVP accountability is prepared and maintained in the Investigator's file and the electronic Trial Master File (eTMF).

11 Study Events by Visit

The Schedule of Events (Table 2) shows which procedures/assessments are to be performed at each scheduled visit for the screening, vaccination, and follow-up periods. Prior to conducting any procedures, the subject will provide informed consent (see Section 6.2).

Table 2: Schedule of Events

	Screening	**Vaccination Period**				**Post-Vaccination Follow-up Period**					
	Day -60 to Day-3	**Day 0**	**Day 3 (-1,+2 days)**	**Day 7 (+5 days)**	**Day 28 (-3,+7 days)**	**Day 31 (3 [-1,+2] days post dose 2)**	**Day 35 (7 [+5] days post dose 2)**	**Day 56 (±7 days)**	**Day 84 (± 7 days)**	**Day 140 (±14 days)**	**Day 196 (±14 days) EOS visit**
Visit	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Informed consent	X										
Randomization		X									
Clinical Assessments											
Medical & medication history	X										
SF36 Questionnaire		X			X			X			X
Physical examination	X										
Directed physical examination		X	X	X	X	X	X	X	X	X	X
Vital signs [a]	X	X	X	X	X	X	X	X	X	X	X
Dispense diary [b]		X			X						
Collect and/or review diaries			X	X	X	X	X	X			
Review concomitant medications		X	X	X	X	X	X	X	X	X	X
Interim history/adverse events		X	X	X	X	X	X	X	X	X	X
Review inclusion/exclusion criteria	X	X			X						
Laboratory Assessments [c]											
Serology: chikungunya [d], HBsAg, anti-HCV, anti-HIV-1 and -2	X										
Complete blood count [e]	X	X		X	X		X				
Complete metabolic panel [e]	X							X			
Basic metabolic panel [e]		X	X		X	X					
Urinalysis (clean catch)	X										
Pregnancy test [f]	X	X			X						X
Neutralizing antibody to chikungunya		X			X			X			X
Sera for future immunogenicity studies [g]		X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X		X	X		X			

	Screening	Vaccination Period				Post-Vaccination Follow-up Period					
	Day -60 to Day-3	Day 0	Day 3 (-1,+2 days)	Day 7 (+5 days)	Day 28 (-3,+7 days)	Day 31 (3 [-1,+2] days post dose 2)	Day 35 (7 [+5] days post dose 2)	Day 56 (±7 days)	Day 84 (± 7 days)	Day 140 (±14 days)	Day 196 (±14 days) EOS visit
Visit	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Fibrinogen		X		X	X		X				
Ferritin	X										
Vaccination											
Blinded vaccination		X			X						

Abbreviations: AE, adverse event; EOS, end of study; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus.
Note: See Section 9.4.3 for Early Termination Visit procedures/assessments.
a: To include body temperature, pulse rate, systolic and diastolic blood pressure.
b: The paper subject diary will assess solicited local injection site reactions (erythema/redness, induration/swelling, itching, and pain/tenderness) after each injection for up to 7 days. Systemic signs and symptoms (fever, fatigue, headache, malaise, myalgia, nausea/vomiting, and joint pain) will also be solicited for 7 days. The diary will include a section for recording unsolicited AEs and concomitant medications.
c: The cumulative total of blood drawn in this study is approximately 300 mL per subject.
d: Determine chikungunya exposure status using an enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin G (IgG).
e: See Section 12.5 for individual tests performed.
f:Urine pregnancy testing will be done on women of childbearing potential at Screening, on vaccination days and at the EOS visit.
g: On Day 0, an 80-mL blood sample will be collected for sera; on other collection days, 10 mL will be collected for sera. These samples will be shipped to the Viral Diseases Branch of WRAIR for storage pending future testing and analysis. Refer to the Investigator Site File for sample preparation, handling, and shipping instructions.

11.1 Screening Visit S: Day -60 to -3

At the time of screening the following assessments/procedures will be performed:

- Medical and medication history
- Physical examination to include vital signs
- Review of inclusion/exclusion criteria
- Chikungunya serology to determine the subject's serostatus (seropositive or seronegative) using enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin G (IgG)
- Hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), anti-HIV-1/2 serologies
- Complete blood count
- Comprehensive Metabolic Panel
- Urinalysis (clean catch)
- Ferritin
- Pregnancy test (urine, women of child bearing potential only)

11.2 Randomization Visit 1: Day 0

The following assessments/procedures will be performed:

- Pregnancy test (urine, women of childbearing potential only)
- Review of inclusion/exclusion criteria
- Directed physical examination and vital signs
- Interim history
- Concomitant medication review
- SF36 Questionnaire
- Baseline immunogenicity test samples

  - Neutralizing antibody to chikungunya
  - Sera for future immunogenicity analyses (from 80 mL of whole blood)
- Complete blood count
- Basic metabolic panel
- Acute phase reactants (C-reactive protein and fibrinogen)
- Randomization (via CDMS) to receive either MV-CHIK or Placebo
- Vaccine administration (IM) in a blinded fashion
- Monitor for at least 30 minutes post-vaccination
- Dispense diary and instruct on its use

11.3 Visit 2: Day 3 (-1,+2 days)

The following assessments/procedures will be performed:

- Focused physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Diary review
- Basic metabolic panel
- Sera for future immunogenicity analyses (from 10cc of whole blood)
- Acute phase reactant blood sample (C-reactive protein only)

11.4 Visit 3: Day 7 (+5 days)

The following assessments will be evaluated:

- Focused physical examination and vital signs
- Interim history/adverse events

- Concomitant medication review
- Diary review
- Complete blood count
- Sera for future immunogenicity analyses (from 10 mL of whole blood)
- Acute phase reactant blood sample (fibrinogen only)

11.5 Visit 4: Day 28 (-3,+7 days)

The following assessments/procedures will be performed:

- Focused physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- SF36 Questionnaire
- Collect and review first diary
- Pregnancy test (urine, women of childbearing potential only)
- Review of inclusion/exclusion criteria
- Immunogenicity Test Samples
  - Neutralizing antibody to chikungunya
  - Sera for future immunogenicity analyses (from 10mL whole blood)
- Complete blood count
- Basic metabolic panel
- Acute phase reactants (C-reactive protein and fibrinogen)
- Vaccine administration in a blinded fashion
- Monitor for at least 30 minutes post-vaccination
- Dispense second diary

11.6 Visit 5: Day 31 (3 [-1,+2] days post dose 2)

This visit is not required unless or until after the subject receives Dose 2.

The following assessments/procedures will be performed:

- Focused physical examination and vital signs
- Interim history
- Concomitant medication review
- Diary review
- Basic metabolic panel
- Sera for future immunogenicity analyses (from 10 mL whole blood)
- Acute phase reactant (C-reactive protein only)

11.7 Visit 6: Day 35 (7 [+5] days post dose 2)

The following assessments will be performed:

- Focused physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Diary review
- Complete blood count
- Sera for future immunogenicity analyses (from 10 mL of whole blood)
- Acute phase reactant blood sample (fibrinogen only)

11.8 Visit 7: Day 56 (±7 days)

The following assessments/procedures will be performed:

- Focused physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Collect and review diary
- SF36 Questionnaire
- Immunogenicity Test Samples
    - Neutralizing antibody to chikungunya
    - Sera for future immunogenicity analyses (from 10 mL of whole blood)
- Complete blood count
- Comprehensive metabolic panel
- Acute phase reactants (C-reactive protein only)

11.9 Visit 8: Day 84 (± 7 days)

The following assessments/procedures will be performed:

- Focused physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Immunogenicity Test Samples
    - Sera for future immunogenicity analyses (from 10mL whole blood)

11.10 Visit 9: Day 140 (± 14 days)

The following assessments/procedures will be performed:

- Brief physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Immunogenicity Test Samples
  - Neutralizing antibody to chikungunya
  - Sera for future immunogenicity analyses (from 10mL whole blood)

11.11 Visit 10: Day 196 (± 14 days) EOS visit

The following assessments/procedures will be performed:

- Focused physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- SF36 Questionnaire
- Pregnancy test (urine, women of childbearing potential only)
- Immunogenicity Test Samples
  - Neutralizing antibody to chikungunya
  - Sera for future immunogenicity analyses (from 10mL whole blood)

11.12 Early Termination Visit

The early termination visit should occur within 14 days of subject withdrawal.

The following assessments/procedures will be performed:

- Focused physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Pregnancy test (urine, women of childbearing potential only)

- Collect and review the diary if termination is prior to Day 56
- SF36 Questionnaire
- Immunogenicity Test Samples
  - Neutralizing antibody to chikungunya
  - Sera for future immunogenicity analyses (from 10mL whole blood)

11.13 Study Visits During SARS-CoV-2 Pandemic

Due to the SARS-CoV-02 pandemic, social isolation has been imposed by the Puerto Rico Government which has resulted in the closure of the San Juan Research Unit. Active subjects in the study with scheduled visits during the San Juan Research Unit closure period will receive a follow up telephone call during the visit window or as soon as feasibly possible by the study staff. At the time of the telephone call the following information will be elicited:

- general health status,
- new and existing adverse events,
- concomitant medications.

Data collected will be recorded on a Subject Encounter Source Document and entered into the study database. Subjects will be reminded to follow current country protocol if experiencing symptoms of COVID-19 and to follow up at the hospital emergency room for medical emergencies.

Clinical activities which will not be completed during the San Juan Research Unit closure include the following:

- Focused physical exam and vital signs
- Blood and urine collection for protocol scheduled tests
- SF 36 Questionnaire
- Review and collection of diary

Once the San Juan Clinical Research Unit re-opens subjects will be scheduled for their follow up visits.

For Day 196 visits which were not conducted due to social isolation, subjects will be invited to return to the research site for the following:

- Completion of the SF-36
- Blood collection neutralizing antibody to chikungunya and sera for future use studies.

12 Methods of Assessment

During each study visit, the Investigator will maintain progress notes in the subject's study records to document all significant observations.

12.1 Vital Signs

Vital signs will be assessed by the Investigator or a qualified designee. Clinically significant abnormal findings after the IVP is administered will be reported as AEs as determined by the Investigator.

Systolic and diastolic blood pressure will be assessed by sphygmomanometer measurement after the subject has been in a supine or sitting position for at least 5 minutes. On vaccination days, the blood pressure will be taken prior to injecting the vaccine. Pulse and temperature will also be assessed.

The measurement of vital signs may be repeated at the discretion of the Investigator as needed for improved accuracy or to monitor the subject for safety reasons.

12.2 Physical Examination

A complete physical examination will be performed by a clinical Investigator at the Screening Visit and will include the following: general appearance, head, ears, eyes, nose, throat (HEENT), neck, skin, musculoskeletal (especially joints and movement), cardiovascular system, respiratory system, abdominal system and nervous system (with an assessment of the reflexes, motor and sensory nerve assessment, sensory checks of the extremities and mental status assessment).

A focused physical examination will be performed at all subsequent visits during the study and will include mental status, musculoskeletal (joints and movement) and any additional systems as per the Investigator's judgment.

Findings at the Screening Visit and before the first dose of IVP will be recorded as medical history. Findings after the first dose of IVP is administered will be recorded as AEs.

12.3 Medical History

A complete medical history will be taken by a clinical Investigator at the screening visit with a view to identifying past, chronic or recurring problems that would result in exclusion from the study. In particular, any recalled symptoms of chikungunya infection will be documented.

Interim medical history will be reviewed at subsequent visits to identify any adverse events and to verify eligibility on vaccination days.

12.3.1 Subject Diaries

In order to facilitate a discussion between the investigator and the subject, diaries will be dispensed on each vaccination day and reviewed and collected according to the study events schedule.

Diary Day 0 – 7 entries:

- The following solicited injection site adverse reactions will be assessed and entered: erythema/redness, induration/swelling, itching, and pain/tenderness after each injection (See Appendix 1 for the Toxicity Grading Scale, Table A, Local Reactions to Injectable Product).
- Solicited systemic symptoms to be recorded are fatigue, headache, malaise, myalgia, nausea/vomiting and joint pain. (See Appendix 1, Toxicity Grading Scale, Appendix Table B, Solicited Systemic Adverse Events).
- This diary will also include a section for recording temperature daily and if the subject feels feverish and entering unsolicited AEs and concomitant medications.

Diary Day 8 – 28 entries:

- This diary will include a section for recording temperature if the patient feels feverish and entering unsolicited AEs and concomitant medications.

12.3.2 Medication History

Concomitant medications will be reviewed to identify any interventions that resulted from adverse events and to verify eligibility on vaccination days. Medications will be coded by WHODrug. The number and percentage of subjects receiving each category of medication will be summarized by IVP administered.

12.4 Quality-of-Life Surveys

The SF36 Quality-of-Life survey has been used in several studies to assess longitudinal function in patients with a history of chikungunya infection. [Marimoutou 2012] [Marimoutou 2015][Rodriguez-Morales 2017]. This tool was developed by the RAND Corporation and is in the public domain. Non-English versions of the SF36 are widely used but have not been validated. The use of SF36 scores in this study will be exploratory. The test will be given to subjects to self-administer on study days 0, 28, 56 and at the end of the study. Scoring of results will be done in accordance with RAND Corporation instructions. See Appendix 2.

12.5 Routine Laboratory Tests of Vaccine Safety

In accordance with routine practice in early phase clinical trials, several clinical lab tests of a general nature will be monitored according to the schedule indicated in the Study Events Schedule. Venous blood samples will be taken by a trained phlebotomist or qualified designee. Subjects will be provided with instructions on submission of clean-catch urine specimens. Additional and repeat laboratory safety testing may be performed at the discretion of the Investigator.

12.5.1.1 Complete Blood Count (CBC)

white blood cell count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), hemoglobin, hematocrit, platelet count.

12.5.1.2 Basic Metabolic Panel (BMP)

glucose, sodium, potassium, carbon dioxide, chloride, urea nitrogen, and creatinine.

12.5.1.3 Comprehensive Metabolic Panel (CMP)

BMP analytes plus calcium, albumin, total protein, ALP (alkaline phosphatase), ALT (alanine amino transferase), AST (aspartate aminotransferase), and bilirubin.

12.5.1.4 Ferritin

Ferritin may be used to indicate iron-deficiency anemia. Ferritin will be checked at baseline as a means of identifying subjects who may benefit from iron supplementation during the study. For use of serum ferritin as an acute phase reactant see Section 12.6.2.3.

12.5.1.5 Urinalysis

protein, glucose, hemoglobin, erythrocytes, and leukocytes. The screening urinalysis can be repeated twice if necessary to ensure there is no clinically significant underlying medical condition. No other urine samples will be collected except at the discretion of the Investigator.

12.5.1.6 Pregnancy Testing

Pregnancy will be determined by evaluation of β-human chorionic gonadotrophin (HCG) in urine for all women of childbearing potential. All female subjects are considered of childbearing potential unless postmenopausal or surgically sterile and at least 3 months have passed since the sterilization procedure. Postmenopausal is defined as amenorrhea for ≥12 months without an alternative medical cause. Permanent female sterilization procedures include tubal ligation, bilateral salpingectomy, hysterectomy, bilateral oophorectomy, or successful Essure placement. Subjects with a positive pregnancy test will be excluded from further vaccinations and study-related blood draws.

The Investigator will inform the Sponsor immediately of any case of pregnancy during the study and collect information on any female subject who becomes pregnant while participating in this study. If the subject consents, she will continue to be followed and the outcome of the pregnancy will be documented.

12.6 Samples for Future Research

At the end of the study, the site(s) will ship any remaining sera to the Viral Diseases Branch of WRAIR for storage and/or future research. The blood samples will be de-identified, shipped and stored in accordance with applicable regulations and approved Study Specific Procedures (SSPs). For samples stored at WRAIR, protection of subject confidentiality during any future research with the stored specimens will be guaranteed by labeling samples with a unique tracking number to protect confidentiality.

Personnel at the WRAIR Viral Diseases Branch will not know any personally identifying information corresponding to the subject ID code. Any further research will be done in

accordance with a WRAIR IRB-approved laboratory protocol(s). Specimens will be retained under the supervision of the Compliance Management Unit of the Viral Diseases Branch, WRAIR. No identifying information will be available for use in the reporting or publication of any results. Refer to the Investigator Site File for more detailed information.

Samples from subjects who withdraw consent for future use will be destroyed after all protocol-specified assays, including immunogenicity assays, are completed.

12.6.1 Serological Tests

12.6.1.1 Chikungunya serology

A commercial laboratory will test subjects for chikungunya antibodies at baseline. The IgG antibody test will be performed via EIA or ELISA to determine the subject's pre-vaccination chikungunya serostatus and will be interpreted according to the laboratory's guidance.

12.6.1.2 HBsAg, anti-HCV, anti HIV-1 and -2

Baseline screening for HBsAg, anti-HCV, anti-HIV-1/2 will require one blood sample to be sent to the local laboratory for analysis. Subjects incidentally found to be positive to any of these viruses will be notified in a manner consistent with local practice and will be excluded from study participation.

12.6.1.3 Neutralizing Antibody to Chikungunya

Subjects will be tested for neutralizing anti-chikungunya virus antibodies in order to determine the response to vaccination. Immunogenicity samples will be sent for testing (Days 0, 28, 56, and 196) to NEOMED Labs. The assay will be an immunofocus microneutralization assay based on chikungunya strain 181/25.

12.6.1.4 Future immunogenicity testing

Subjects will be required to agree to the future use of blood specimens as specified in the ICF. On Day 0, 80mL of sera will be collected for future immunogenicity testing. This large volume will provide baseline samples for future studies comparing results at multiple time points post-vaccination. In addition, these samples may be used in passive transfer studies as a control for naturally acquired immunity. On other sera collection days, 10-mL blood will be collected for future immunogenicity studies. These samples will be stored at WRAIR.

12.6.2 Acute phase reactants

Chikungunya infection is associated with both acute and chronic joint symptoms. Different pathophysiological mechanisms appear to cause these symptoms in different people, with one possible mechanism related to an aberrant host immune response to the virus or virus antigens [Javelle, 2015]. Because of this possibility, safety studies of vaccines that contain chikungunya antigens in previously exposed subjects are necessary before large scale efficacy studies or vaccination campaigns can be conducted in areas of on-going transmission. In the current study, joint pain will be solicited as an adverse event, and non-traumatic joint pain or stiffness that persists for more than 24 hours or is associated with objective findings of effusion or soft tissue swelling will be considered an AESI. However, assessing joint symptoms is complicated by the fact that they can be subjective and are regularly reported in the placebo arms of vaccine studies [Steere, 1998]. Arthralgia(s) were reported by nearly 20% of adult volunteers in a clinical trial of a conjugated meningococcal vaccine, yet post-licensure studies have not shown this to be a significant side effect [Sanofi Pasteur, 2016] suggesting that soliciting this symptom in early phase clinical trials can be misleading. To add clarity and objectivity to the assessment of arthralgia reported in this trial, and to help determine if joint symptoms that study participants report are due to an immunological or inflammatory mechanism, acute phase reactants will be included in the laboratory tests assessed routinely to monitor participant safety.

12.6.2.1 C-reactive Protein

C-reactive protein (CRP) is a blood biomarker signifying an inflammatory process. CRP is produced in the liver, and levels increase in response to inflammation. CRP levels are frequently used to monitor rheumatologic diseases [NCCCC, 2009] and also correlate with viremia in acute chikungunya infection [Anfasa, 2017]. Even in the absence of arthralgia or infection, CRP increases after influenza vaccination [Liuba, 2007]. The rationale for routinely testing CRP in this study is to provide Investigators with an objective assessment of systemic inflammation to aid in assigning causality to arthralgia(s) or other potentially inflammatory symptoms. The timing of collection (3 days post-vaccination) was chosen based on a study of CRP levels following an inactivated (influenza) vaccine [McDade 2015].

Because so little is known about how CRP responds to vaccination, and because it can be expected that values will be increased at Day 3 post-vaccination, abnormal lab values will not be considered AEs. However, levels that are particularly high or more persistent

compared to other study participants may be considered by the Investigators as evidence that the vaccine or other inflammatory stimulus plays a causal role in the development of some AEs. Blood samples will be obtained on Days 0, 3, 28, 31, and 56 and at additional time points in individual subjects at the discretion of the Investigators.

12.6.2.2 Fibrinogen

Fibrinogen has not been shown to correlate with chronic chikungunya [Hoarau 2010] but has been shown to increase mildly following vaccination with other live [van der Beek 2002] and inactivated [Liuba 2007] vaccines. Collection of these samples for this study is exploratory. The timing of collection (7 days post-vaccination) was chosen based on a study of fibrinogen levels following another live (Yellow Fever) vaccine [Verschuur, 2004]. Blood samples will be obtained on Days 0, 7, 28, 35, and 56 and at additional time points at the discretion of the investigators.

12.6.2.3 Ferritin

Ferritin is a blood cell protein containing iron that increases in inflammatory conditions. Ferritin has been shown to correlate with chronic arthralgia in chikungunya infection [Anfasa, 2017]. Prospective collection of ferritin was performed in MV-CHIK 204 without any clear benefit. In this study, ferritin will be collected at baseline so that, at the discretion of the Investigator, future values can be checked and compared.

13 Adverse Event Reporting

Adverse event (AE) definitions and reporting procedures provided in this protocol comply with current 21 CFR Part 312. An AE is any untoward medical occurrence temporally associated with the use of an IVP that does not necessarily have a causal relationship with the IVP. An AE can therefore be any unfavorable or unintended sign, abnormal laboratory finding, symptom, or disease whether or not considered related to the IVP.

Adverse events will be monitored throughout the entire study. The Investigator will review the subject diary (if applicable) and ask subjects at each visit if they have experienced any untoward effects since the last study visit. All AEs will be recorded on the corresponding eCRF page: a description of the event, severity, time of occurrence, duration, any action (e.g., treatment or follow-up tests) and the outcome should be provided along with the Investigator's assessment of the relationship to the IVP.

Adverse events will be recorded from the time subjects receive their first Day 0 vaccination through the last study follow-up visit on Day 196 (±14 days). If known, the name of the illness should be recorded, in preference to the listing of individual signs or symptoms.

13.1 Severity Assessment

Adverse events must be graded as being mild, moderate, or severe and their approximate duration given. Definitions of severity are as follows:

Mild: an AE that does not interfere with normal activities;
Moderate: an AE that is sufficiently discomforting to interfere with normal activities;
Severe: an AE that is incapacitating or prevents normal activities.

Even if the Investigator judges there is no relationship to the IVP, all AEs must be recorded in the eCRF.

See Appendix 1 for grading scales to be used for local reactions to injectable product (Table A), solicited systemic adverse events (Table B), unsolicited systemic adverse events (Table C), and vital signs (Table D). For clinical laboratory test results outside of the normal range the FDA toxicity scale will be used to assign AE severity. The Investigator will assign the grade of severity and document in the eCRF.

13.2 Causality Assessment

The Investigator is obligated to assess the relationship between the IVP and the occurrence of each AE/SAE. The Investigator will use clinical judgment to determine the relationship. Alternative plausible causes, underlying medical conditions, concomitant therapy, other risk factors, and the temporal relationship of the event to the IVP will be considered and investigated. The Investigator will also consult the Investigator's Brochure in the determination of his/her assessment.

All solicited local (injection site) AEs will be considered causally related to vaccination.
Causality of all other AEs should be assessed by the Investigator using the following question: "Is there at least a reasonable possibility that the AE may be related to the IVP?"

NO: The AE is not causally related to administration of IVP. There are other, more likely causes and administration of the IVP is not suspected to have contributed to the AE.
YES: There is at least a reasonable possibility that the IVP may be related to the AE.

13.3 Serious Adverse Events

13.3.1 Definition of a Serious Adverse Event

A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that fulfills the following criteria:

- results in death;
- is life threatening;
- requires hospital admission or prolongation of an on-going hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital abnormality/birth defect;
- is an important medical event not captured by the preceding criteria but which may require medical intervention to prevent one of the preceding outcomes.

Events associated with hospitalization for the following will not be considered SAEs:

- Evaluation or treatment of a pre-existing or recurring condition as long as the condition:
  - is recorded in the subject's medical history as documented in the eCRF (e.g., degenerative disease)
  - has not worsened in severity or frequency during the subject's exposure to the IVP
  - has not required a change in treatment management during the subject's exposure to the IVP
- Treatment that is elective or due to a pre-existing condition.

13.3.2 Reporting of Serious Adverse Events

Themis will be responsible for reporting of SAEs to the regulatory authorities as required. The Investigator is responsible for reporting SAEs to the DoD Research Monitor and the

IRB as required and maintaining all IRB correspondences on file at the site and in the eTMF. Refer to the Investigator Site File for full details of the procedures adopted and approved by responsible parties, in brief:

- Any SAE that occurs to any subject after entering into intervention in this study through the last study follow-up visit on Day 196 (±14 days) must be reported by the Investigator to the SME, DoD Research Monitor, CRO and Sponsor regardless of whether or not the SAE is considered related to the IVP,
- SAEs that occur after the last study follow-up visit and that are deemed to be related to the IVP must also be reported by the Investigator to the Sponsor. If possible, the SME, DoD Research Monitor, and CRO should also be notified.
- All subjects with SAEs must be followed for outcome.

All SAEs must be reported by the Investigator to the SME, CRO and Sponsor within 24 hours of learning about the event. This can be done by sending a completed Safety Report Form via email. All follow-up information pertaining to an SAE must also be reported within 24 hours of knowledge of the follow-up information.

Contact information for the Integrum Drug Safety Center (includes vaccine reporting):

Integrum Drug Safety Center
Email: PPD
Telephone: PPD

The initial report should be promptly followed by detailed, written reports, which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable. This additional information will be requested, if necessary, by the SME or CRA within 5 days of receipt of the alert report. After the initial SAE report, the Investigator is required to follow up proactively with the subject and provide further information on the subject's condition as it becomes available.

Integrum, in consultation with the SME and Sponsor, will assess initial and follow-up SAE reports from the site for expectedness, which will be determined based on the most recent edition of the Investigator's Brochure. Completed Safety Report Forms will be provided to the Sponsor for submission to regulatory authorities. The Sponsor will expedite reports of Suspected Unexpected Serious Adverse Reactions (SUSARs) as an Investigational New Drug (IND) Safety Report within the required time frame.

The Integrum safety team may collect further information for final evaluation of the SAE case and for submitting an updated Safety Report Form to the Sponsor for submission to the regulatory authorities.

See Section 17.4 for HRPO reporting requirements.

13.4 Assessment of Subjects with Adverse Events

Each subject must be carefully assessed for AEs by the Principal or designated Subinvestigator. This includes review of symptoms reported in the diary or at follow-up visits, physical findings, and laboratory results. Assessments must be made of the seriousness, severity, and relationship to the IVP. During the study all AE/SAEs should be followed to resolution or stabilization unless the event is considered by the Investigator to be unlikely to resolve or stabilize or the subject is lost to follow-up.

13.4.1 Adverse events reported by history

Throughout the duration of the study, SAEs and AESIs will be reported. See Appendix 1, Table C for the Toxicity Grading Scale: Unsolicited Systemic Adverse Events. Subject diaries will not be considered source documents, but rather memory aids. They should facilitate a discussion between the investigator and the subject, but the investigator is not compelled to regard each entry as an AE or accept the subject's assessment of severity.

13.4.2 Abnormal laboratory test results

Abnormalities in laboratory parameters that are not present at screening will, if considered clinically significant in the judgment of the Investigator, be recorded in the eCRF as AEs. All clinically significant abnormal lab values will also be assigned a severity grade by the Investigator according to the Toxicity Grading Scale for Healthy Adult Volunteers Enrolled in Preventive Vaccine Clinical Trials; FDA/CBER Guidance, September 2007. The grade will be either: 1, mild; 2, moderate; 3, severe; or 4 potentially life threatening, and will be entered in the eCRF. Abnormal laboratory tests should be repeated and followed up until they have returned to the normal range or an adequate explanation of the abnormality is found.

An exception is made for CRP and fibrinogen in which abnormal values are expected. These tests will be used to aid in assigning causality to AEs that are potentially due to inflammatory causes, but abnormal values will not be considered AEs in themselves.

13.4.3 Abnormal physical exam findings

Physical examination findings that were not present at screening and, in the judgment of the Investigator, are clinically significant will be recorded as AEs.

13.5 Adverse Events of Special Interest

13.5.1 Definition

An AESI is an adverse event of scientific and medical concern specific to the Sponsor's product or program, which requires additional monitoring and rapid communication. Such an event might warrant further investigation, including unblinding the treatment allocation of the subject, in order to better characterize and understand it.

In this study, the AESI for MV-CHIK vaccine will be defined as: non-traumatic joint pain or stiffness that persists for more than 24 hours or is associated with objective findings of joint effusion or soft tissue swelling. For the purposes of this definition, "joint" includes all structures associated with the joint including ligaments and tendons. Overuse of a joint should not be considered "traumatic" and joint pains resulting from overuse should not be excluded from this definition. To characterize these symptoms, additional serologic, immunologic, and radiographic data may need to be obtained and other etiologies ruled out. The basis for selection of persistent joint symptoms as an AESI is discussed in Section 8.7. If the Investigator has any question about a case of persistent joint symptoms being an AESI, the SME should be contacted.

13.5.2 AESI Reporting

An AESI should be reported by the Investigator to Integrum, the SME and the Sponsor within 24 hours of learning about the event by completing the Safety Report Form and sending via email.

13.6 Aggregate Safety Monitoring

Assign-DMB will review de-identified safety data generated in this and other trials of MV-CHIK in order to keep the global MV-CHIK safety database up to date and consequently to detect any emerging safety signals in a timely fashion. Assign DMB and the Sponsor will ensure that applicable information will be reported to concerned authorities and communicated to the individual sites as appropriate. Assign-DMB will

incorporate data from this and all other trials into the development safety update report (DSUR) of MV-CHIK for annual submission to regulatory authorities.

14 Statistical Analysis

Data management, analysis, and reporting will be performed by DF/net.

14.1 Database Management

An electronic CRF and electronic data capture (EDC) will be used for this study, and a Data Management Plan will be prepared. See Section 16.3.

14.2 Sample Size Estimate

A formal sample size calculation was not conducted for this study. The sample size was determined based on prior experience in evaluating the safety and immunogenicity of vaccines and is typical for early phase clinical studies. In previous studies MV-CHIK was associated with joint pain in about 4% of previously unexposed recipients. The largest study to date is MV-CHIK 202 in which 9/229 reported joint pain (95% confidence interval: 1.8 to 7.3%). A higher risk of joint pain in previously exposed individuals would be suggested if 4/15 individuals who receive MV-CHIK in either Step 1 or 2 of the current study develop joint pain (95% confidence interval: 7.8% to 55.1%) while the placebo group develops none.

It is not possible to define a statistical threshold of safety that will rule out any increased risk of this vaccine in previously exposed individuals or guarantee market acceptance of this or any vaccine for an infection associated with joint pain, as demonstrated by the experience with LYMErix (see Section 8.7). This study is not expected to provide a final answer on whether or not to continue development of this vaccine candidate, but rather contribute to the incremental gathering of information on vaccine safety. In this regard, the details of individual cases including the time course and severity of their symptoms and the associated changes in their acute phase reactants and quality of life scores, will have to be examined individually. A qualitative description of these events to include their assessed causality, severity, duration, and association with evidence of inflammation and immunopathology will provide critical data to inform the consent of participants in field trials conducted in areas of on-going transmission.

14.3 Statistical Analysis Plan

A Statistical Analysis Plan (SAP) will be written and finalized prior to any lock of the study database. The SAP will provide a detailed description of the statistical methods and expand on the details provided in the protocol. Additional analyses may be added. The SAP will include templates for the report tables, listings, and figures.

14.4 Analysis Populations

All of the analysis populations will be identified and finalized before the blind is broken for the study. The primary and exploratory analyses of safety will use the intent-to-treat (ITT) population. The per-protocol (PP) population will be used for secondary analyses of immunogenicity. Analysis of the study Steps will be conducted separately.

14.4.1 Intent-to-treat Population

The ITT population will include all subjects who receive at least one dose of vaccine and will be the population for the safety analyses.

14.4.2 Per Protocol Population

The PP population is a subset of the ITT population that includes subjects who received both doses of IVP, have at least one post-vaccination immunogenicity assessment, and do not experience a protocol deviation that would affect their evaluation for immunogenicity. The protocol deviations that affect evaluation for immunogenicity will be determined based on a blinded data review prior to database lock.

14.5 Statistical Methods

Statistical methodology including hypotheses and all analyses will be provided in the Statistical Analysis Plan (SAP). Categorical variables will be summarized using the number frequency and percentage by Step, treatment and overall of subjects falling into each category. Continuous variables will be summarized using mean, standard error or standard deviation, median, minimum, maximum, and number of subjects with observations by Step of subjects falling into each category. Continuous variables will be summarized using mean, standard error or standard deviation, median, minimum, maximum, and number of subjects with observations treatment and overall.

14.5.1 Missing Data

The intervention is not expected to have any effect on missing data. As such, any missing data will be considered Missing Completely At Random (MCAR) and will be assumed to have no effect on the analysis and no imputations will be performed. All attempts will be made to prevent missing data. An observed cases approach will be applied for all endpoints.

14.5.2 Demographic and Baseline Clinical Data

Demographics will be summarized by age (in years, at time of signing informed consent), gender and ethnicity. Baseline characteristics and medical history will also be summarized by relevant characteristics and classifications.

14.5.3 Subject Disposition

The following will be summarized categorically:

- Subjects in each of the populations.
- Subjects randomized and who received at least one dose of IVP.
- Subjects with protocol deviations and reason for deviation.
- Subjects who discontinued the study early along with the reasons for discontinuation.
- Screen failures and reasons for screen failure.
- Subjects at each study visit, and the number of subjects with important protocol deviations (defined in Section 17.2).

14.5.4 Vaccine Exposure

The number and percentage of subjects who receive only one of the IVP doses and the number and percentage of subjects who receive both of the IVP doses will be summarized.

14.5.5 Concomitant Medications

Concomitant medications will be coded by WHODrug. The number and percentage of subjects who received each category of medication will be summarized by IVP administered.

14.5.6 Adverse Events

All verbatim terms for any reported AEs will be coded to the appropriate system organ class and preferred term according to the current version of MedDRA and graded by the Investigator for severity as per the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adults Enrolled in Vaccine Clinical Trials; 2007. The number and percentage of subjects experiencing an AE (all, serious, and vaccine-related) will be reported.

All AEs will be summarized by IVP received according to the MedDRA system organ class (SOC) and preferred term. An additional summary will be provided for the incidence of all AEs by severity. Summaries will be presented for SAEs, AEs of grade 2 (moderate) and higher, IVP-related AEs, AESIs, and AEs where an action was taken. All AE data will be listed by subject, and a separate listing will include all SAEs, including any deaths on study.

Adverse events will also be summarized separately for solicited and unsolicited events. Specific local injection site reactions (erythema/redness, induration/swelling, itching, and pain/tenderness) will be solicited using the subject diary from 0 to 7 days after each injection and Investigator interviews at follow-up visits. Specific systemic AEs (fever, fatigue, headache, malaise, myalgia, nausea/vomiting and joint pain) will be solicited using the subject diary from 0 to 7 days and Investigator interviews at follow-up visits. Unsolicited AEs will be all of those excluding the preferred terms of solicited AEs. Similar summaries will be presented for solicited and unsolicited AEs as noted above.

14.5.7 Safety Laboratory Tests including Acute Phase Reactants

Clinical laboratory test results and changes from baseline will be summarized by time point. Clinically significant abnormal laboratory results will also be captured as AEs. Abnormal results of CRP, fibrinogen or ferritin will not be captured as AEs.

14.5.8 Vital signs and physical exam findings

Vital signs results (including blood pressure, heart rate, and body temperature) and changes from baseline will be summarized by time point. Abnormal vital signs will be graded (see Appendix Table D) and findings from physical examinations will be assessed for clinical significance and included in the tabular summaries and by-subject AE listings.

14.5.9 Immunogenicity

Analyses of immunogenicity will be conducted in the PP population according to IVP received. The analysis of immunogenicity will be measured as GMTs of neutralizing antibodies on Days 0, 28, 56, and at EOS. The GMT will be calculated using a mixed effects model with IVP (MV-CHIK versus placebo), visit and study Step (age group)) and treatment visit interaction as fixed factors and subject as a random effect. The analysis of variance will be fit using log10 transformed data. The estimates for the least squares means and corresponding 95% confidence intervals (CIs) will be back-transformed by taking the anti-log to obtain the GMTs and CIs.

15 Study Monitoring Procedures

The Sponsor has ethical, legal, and scientific obligations to conduct this study in accordance with established research principles and ICH GCP guidelines. As such, in order to fulfill these obligations, a DoD Research Monitor, a SME, and blinded and unblinded CRAs will be employed for the duration of the study. In addition, this study will include an interim review of data by a DSMB.

15.1 DoD Research Monitor

The DoD Research Monitor is a physician independent of the Sponsor responsible for serving as advocate for the medical safety of volunteers in accordance with Department of Defense Instruction (DoDI) 3216.02. As such, they may:

- Perform oversight functions and report their observations to the IRB or other designated officials on recruitment/enrollment procedures, the consent process, other study interventions and interactions, data matching, data collection, and analysis.
- Review ‘unanticipated problem involving risk to subjects or others’ (UPIRTSO) reports.
- Review all SAEs, deviations and other unanticipated problems and provide an independent assessment/report of these events.

The DoD Research Monitor is authorized to:

- Interview and examine subjects and their clinical data.
- Remove individual subjects from the study.
- Stop the research protocol in progress.

- Promptly report their observations and findings to the IRB or other designated official and the HRPO as required.
- Take any other steps necessary to protect the safety and well-being of human subjects until the IRB can assess their report.

15.2 Sponsor Medical Expert

The SME will review monitoring plans and serve as the central point of contact for medical questions from the site and Investigator(s). They will review all AE and SAE reports on behalf of the Sponsor using de-identified (coded) information only. They will also receive notifications from the site of any adverse events that require time-sensitive reporting to FDA or HRPO to include Serious and Unexpected Suspected Adverse Reactions (SUSARs) and adverse events that meet study stopping criteria. For SUSARs, the SME will draft a narrative of the event for the Sponsor to review prior to reporting to FDA. The SME will also communicate adverse events directly with HRPO in accordance with their requirements. The SME will not interact with study subjects nor obtain their personally identifying information.

15.3 Study Monitors

Clinical Research Associates (CRAs), both blinded and unblinded, will monitor study progress by scheduling and performing on-site study visits throughout the study.

15.3.1 Blinded Study Monitor

A blinded CRA will conduct a site initiation visit, several interim monitoring visits and a site close out visit. The blinded CRA will also communicate with the site via phone, email and formal visit confirmation and follow-up letters. The blinded CRA will be responsible for 100% source document verification of study data, to include reviewing all UPIRTSOs associated with the protocol. The CRA may escalate any critical subject safety or GCP finding to the Investigator, Sponsor and SME and direct the site to contact the IRB immediately and the DoD Research Monitor if required. Regular inspection of the eCRFs will be conducted by the CRA in order to assess subject enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the eCRFs, verification of eCRF data against original source documents, and occurrence of AEs. A full description of the responsibilities of the CRA, which will include reviewing the Investigational Site File on a routine basis, will be documented in the Monitoring Plan.

15.3.2 Unblinded Study Monitor

An unblinded CRA will also be assigned to verify appropriate accountability and storage of the IVP and ensure site pharmacy staff understand and conduct their IVP preparation and administration procedures according to protocol.

15.4 Sponsor and Government Audits

The purpose of an audit is to assess whether ethical, regulatory and quality requirements are met. The Sponsor may conduct an audit at the investigative site at any time. In addition, representatives from the Project Oversight Agency (WRAIR) may conduct inspections at the investigative site in order to assess contractor and subcontractor performance. The HRPO also conducts site visits as part of its responsibility for compliance oversight. These visits may include but are not limited to, inspection of the IVP supply, required documents, the informed consent process, and comparison of CRFs with source documents.

Government regulatory authorities may also inspect the Investigative site during or after the study. The Investigator or designee should contact the Sponsor/CRO and SME/HRPO immediately if this occurs. He/she must cooperate fully with regulatory authorities or other audits conducted in a reasonable manner.

15.5 Investigative Site Responsibilities

All study records including progress notes must be available for audit. The Investigator agrees to participate with audits conducted in a reasonable manner. The Investigator must provide monitors/auditors with full access to all source and study documents and clinical study facilities and equipment (vaccine storage and reconstitution areas, freezers, centrifuges, calibration logs, monitors, and laboratory equipment). Adequate time and space for monitoring visits should be made available by the Investigator.

The site must complete the eCRFs in a timely manner and on an ongoing basis to allow regular review by the CRAs, who will verify entries made in the eCRF. Whenever a subject name is revealed on a document that is to be collected for the Sponsor the name must be blacked out (for paper source) or encrypted (for electronic source) permanently by the site personnel, leaving the initials visible, and annotated with the subject code as identification.

15.6 Data Safety Monitoring Board

Following vaccination of at least 20 volunteers in Step 1, an independent DSMB will be convened to review safety data and provide recommendations to the Sponsor. The DSMB will review unblinded cumulative study data to evaluate safety, performance parameters, study conduct, and the scientific validity and integrity of the trial. Data from MV-CHIK 204 will also be made available to the DSMB by the Sponsor. The DSMB will specifically address the question of whether or not it is safe to proceed to Step 2 of the study in which previously exposed volunteers ages 51 to 65 will receive this vaccine. The DSMB will provide recommendations only; final decisions regarding study conduct rest with the Sponsor. Members of the DSMB serve in an individual capacity, the DSMB has no protocol enforcement authority. Refer to the DSMB Charter for detailed information on the DSMB.

16 Data Management

Clinical data (including AEs, concomitant medications and clinical laboratory data) will be entered into a 21 CFR Part 11-compliant validated computerized EDC system. Clinical data will be entered directly from the source documents at the investigational site. The EDC system includes password protection and internal quality checks, such as automatic edit checks, to identify data that appear inconsistent, incomplete, or inaccurate.

16.1 Electronic Case Report Forms

An eCRF will be used to store and transmit subject information. The file structure and format for the eCRF will be provided by the DF/net and should be handled in accordance with the instructions provided. The eCRF must be reviewed and electronically signed and dated by the Investigator.

Access to the eCRF will be strictly password protected and limited to authorized personnel directly participating in the study. Data should be entered into the eCRF completely by examining personnel or the study coordinator. The eCRF must be completed as soon as possible after any subject evaluation or communication. If data is to be changed due to erroneous input or other reason, an electronic audit trail will track these changes. The eCRFs and computers that store them must be accessible to the CRAs and to any regulatory auditor.

16.2 Data Collection

During each study visit, the Investigator will maintain progress notes in the subject's study record to document all significant observations. At a minimum, these notes will contain:

- The date of the visit and the corresponding day or visit in the study schedule (eg, screening, Day 0, Day 28, and so forth).
- General condition including subjective complaints, any significant medical findings, discussion of any documentation in the diaries, the severity, frequency, duration, and resolution of any reported AEs, and the Investigator's assessment as to whether or not the reported AE is IVP-related.
- Changes in concomitant medications or dosages.
- Documentation of the procedures performed.
- The signature or initials of all Investigators making an entry in the medical record via the progress notes.

In addition, any contact with the subject via telephone or other means that provides significant clinical information will also be documented in the study record (progress notes). Information from the study records (progress notes) and other source documents will be promptly entered in the appropriate section of the eCRF.

Changes to information in the study record (progress notes) and other source documents will be initialed and dated on the day the change is made by the Investigator or designee. Changes to information in an electronic record will be handled per DF/Net SOPs. If the reason for the change is not apparent, a brief explanation for the change will be written adjacent to the change.

16.3 Clinical Database Management

An electronic CRF and electronic data capture (EDC) will be used for this study, and a Data Management Plan will be prepared. The EDC system will be hosted by DF/Net. Note that the EDC system will not contain any of the subjects' personally identifying information: only de-identified (coded) data will be entered. DF-Net will not have access to any of the subjects' personally identifying information.

Previous and concomitant medications will be coded using version Global B3 September 1, 2018 WHO Drug Reference Dictionary. The same WHO Drug version will be used throughout the study even though a new release is expected by March 2019. Coexistent

diseases and AEs will be coded using version 21.1 of MedDRA (Medical Dictionary for Regulatory Activities) as documented in the Data Management Plan. The same version of MedDRA will be used throughout the study even though a new release is expected by March 2019.

Data cleaning will be an ongoing process throughout the trial. Specific reviews and processes used to clean the data are described in the Data Management Plan. External data sources and reconciliation processes are documented in the Data Management Plan. Vendor contact information and information regarding data formats and transfer frequency is provided in the Data Transfer Specifications. External data will be reconciled with CDMS data in accordance with DF/Net SOP 3.13 version 6 “Data Transfer from External Sources”. When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time can only be made by written agreement between the Investigator and DF/Net.

16.4 Source Document Maintenance

Source documents contain the results of original observations and activities of a clinical investigation. Source documents include, but are not limited to, study records (progress notes), computer printouts, screening logs and recorded data from automated instruments. Diaries provided to subjects will be used as a memory aid to inform Investigator documentation in the progress notes, but will not themselves be considered source documents.

All source documents from this study will be maintained by the Investigator and made available for inspection by authorized persons. The original signed informed consent for each subject shall be filed with records kept by the Investigator and a copy shall be given to the subject.

16.5 Record Maintenance

Records will be retained in accordance with the current ICH Guidelines. All essential study documents including records of subjects, source documents, eCRFs and IVP inventory will be maintained in the eTMF. US FDA regulations (21 CFR 312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of IVP, including eCRFs, consent forms, laboratory test results, and medical source documents, be retained by the PI for 2 years after marketing application approval. If no application is filed, DoD regulations (32 CFR 219.115[b]) require these records to be kept 3 years after

the investigation is discontinued and the US FDA and the applicable national and local Health Authorities are notified. The Sponsor or their representative will notify the PI of these events.

If an application is filed and approved, essential documents should be retained until at least 2 years after the last approval and until there are no pending or contemplated marketing applications in an ICH region, or until at least 2 years have elapsed since the formal discontinuation of clinical development of MV-CHIK. However, essential documents may be retained for a longer period if required by the applicable regulatory requirements or by agreement with the Sponsor. The Sponsor is responsible for informing the Investigator when these documents need no longer be retained.

The Investigator will not dispose of any records relevant to this study without written permission from the Sponsor, and will provide the Sponsor the opportunity to collect such records. The Investigator shall take responsibility for maintaining adequate and accurate hard copy source documents of all observations and data generated during this study. Such documentation is subject to inspection by the Sponsor, its representatives, and regulatory authorities.

If an Investigator moves, withdraws from an investigation, or retires, the responsibility for maintaining the records may be transferred to another person who will accept responsibility. Notice of transfer must be made to and agreed by the Sponsor.

Data property rights are as specified in the trial contracts/agreements.

16.6 Confidentiality

All information obtained during the conduct of the study with respect to the subject's state of health will be regarded as confidential. The Investigator must ensure that each subject's anonymity is maintained. On CRFs and other documents submitted to the SME, Sponsor or the CRO, subjects must not be identified by name. Instead, subjects will only be known by the unique subject number allocated to them in order to ensure confidentiality on all study documentation. Subjects will retain this unique number throughout the study. The Investigator will keep a separate paper log of these codes in the Investigator Site File.

In order to comply with government regulatory guidelines and to ensure subject safety, it may be necessary for the Sponsor or representative, the DoD, the CRO, the IRB, or the FDA to review subjects' medical records as they relate to this study. Only the subject's unique number on the CRFs will identify him/her, but their full names may be made known to a pharmaceutical regulatory authority or other authorized government or health care officials, if necessary, and to personnel designated by the Sponsor.

Documents that are not for submission to the Sponsor or the CRO (eg, consent forms) will be maintained by the Investigator in strict confidence, except to the extent necessary to allow checking by study monitors, and auditing by the Sponsor, the Project Oversight Agency, or regulatory authorities. No documents identifying subjects by name will leave the investigative site, and subject identity will remain confidential in all reports or publications related to the study.

For any request to disclose the subject's identity, an agreement among the subject, Investigator, and the Sponsor or designee will be obtained in writing.

17 Administrative Procedures

17.1 Regulatory Approval

Themis Bioscience GmbH or their appointed agents will be responsible for ensuring that appropriate regulatory authority approvals are obtained, in accordance with FDA and DoD requirements. No subject may enter the study until this approval has been obtained. A copy of the FDA IND application approval will be provided to the Investigator and to the IRB.

17.2 Protocol Adherence

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, Standard Operating Procedures (SOPs) or Study Specific Procedures (SSPs). The noncompliance may be either on the part of the subject, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with ICH E6 (R2):

- Compliance with Protocol, Section 4.5,
- Quality Assurance and Quality Control, Section 5.1,

- Noncompliance, Sections 5.20.

The protocol must be read thoroughly, and the instructions must be followed. However, exceptions will be made in emergency situations when the protection, safety, and well-being of the subject requires immediate intervention based on the judgment of the Investigator or a responsible appropriately trained and credentialed professional(s) designated by the Investigator as a Subinvestigator.

Important protocol deviations are a subset of protocol deviations that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a subject's rights, safety, or well-being. In the event of an important protocol deviation due to an emergency, accident, or error, the Investigator or designee must contact the Sponsor, CRO and SME at the earliest possible time by telephone or email. This allows for an early joint decision to be made as to whether and to what extent the involved subject(s) should continue in the study.

It is the responsibility of the site to use continuous vigilance to identify and report protocol deviations to the CRA within 5 working days of identification of the protocol deviation or within 5 working days of the scheduled protocol-required activity.

17.3 Protocol Amendments

In accordance with ICH E6 (R2) Guideline for GCP, the Investigator should not implement any deviation from or changes to the protocol without agreement by the Sponsor and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to study subjects, or when the change(s) involve(s) only logistical or administrative aspects of the study (e.g., change in contact information or correction of typographical errors).

Substantive changes to the protocol must be handled as a protocol amendment. Any potential amendment must be approved by the Sponsor. A written amendment must be submitted to the appropriate regulatory authorities and to the IRB. The Investigator must await IRB approval of protocol amendments before implementing the changes, except where necessary to eliminate apparent immediate hazard to subjects. In these cases, the IRB must be notified within 5 days of the change.

All amendments to the protocol must be approved in writing by both the appropriate regulatory authorities and the IRB, except for administrative amendments, which require notification but not written approval. Once approved, the protocol amendment will be distributed to all recipients of the original protocol, with instructions to append the amendment to the protocol.

If, in the opinion of the IRB, Investigator, and/or Sponsor, the protocol amendment alters the study design or procedures and/or increases the potential risks to the subjects, the currently approved written ICF will require modification. The modified ICF must also be reviewed and approved by the Sponsor, appropriate regulatory authorities, and the IRB. In such cases, repeat informed consent must be obtained from subjects still enrolled in the study before participation continues.

17.4 Specific Requirements of the Human Research Protection Office

The Human Research Protection Office (HRPO) of the US Army Medical Research and Materiel Command (MRMC) must provide written approval before the study can begin. The HRPO may stop or suspend the use of the IVP in DoD-supported studies at any time. Accordingly, HRPO approval is contingent upon the following reporting requirements:

17.4.1 Unanticipated Problems Involving Risks to Self or Others

All unanticipated problems involving risk to subjects or others must be promptly reported by telephone PPD by email PPD PPD or by facsimile PPD to the HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report can be sent to the US Army Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000. The IRB and the HRPO will, in coordination with the Sponsor, ensure upward reporting of the unanticipated problems involving risk to subjects or others to the appropriate regulatory offices.

17.4.2 Subject Withdrawal of Consent Due to an Adverse Event

AE-related withdrawals of consent for either the second vaccine dose or for study participation in general need not be reported to the HRPO. Notification of the Sponsor, the CRA, the SME and the IRB should be done in accordance with Section 9.4. Note that reporting to HRPO of AEs, including SAEs, is not required.

17.4.3 Protocol Modifications

Substantive modifications to the research protocol and any modifications that could potentially increase risk to subjects must be submitted to the HRPO for approval prior to implementation. The HRPO defines a substantive modification as a change in PI, change or addition of an institution, elimination or alteration of the consent process, change to the study population that has regulatory implications (such as adding children, adding active duty population), significant change in study design (ie, that would prompt additional scientific review), or a change that could potentially increase risks to subjects.

17.4.4 Change of IRB

Any changes of the IRB used to review and approve the research will be promptly reported to the HRPO.

17.4.5 Suspension or Termination of Research

Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, Investigator, Sponsor, DoD Research Monitor or regulatory authorities will be promptly reported to the HRPO.

17.4.6 Continuing IRB Review

A copy of continuing review approvals by the IRB must be submitted to the HRPO as soon as possible after receipt. For greater than minimal risk research, a copy of the continuing review report approved by the IRB must also be provided. Please note that the HRPO also conducts random audits at the time of continuing review. Additional information and documentation may be requested at that time.

17.4.7 Pending Inspection

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

17.4.8 Final Study Report

The final study report and supporting documents, including any acknowledgement documentation, must be submitted to the IRB and HRPO when available.

17.5 Investigative Site File Management

The Investigator is responsible for assuring that the Investigational Site File is maintained. The Investigational Site File will contain, but will not be limited to, the information listed below:

1. Investigator's Brochure,
2. Current, signed version of the protocol and any previous versions of the protocol,
3. Protocol amendment(s),
4. Current ICF (blank) and any previous versions of the ICF,
5. Curricula Vitae of the PI, Subinvestigator(s), and photocopies of their respective professional license(s) where required by law; original US FDA Form 1572 signed by the PI at each site. The names of any Subinvestigators should appear on this form. The Investigator must also complete all regulatory documentation as required ICH GCP and by local or national regulations,
6. Documentation of IRB approval of the protocol, the ICF, any protocol amendments, and any ICF revisions,
7. All correspondence between the Investigator, IRB, and the Sponsor/CRO relating to study conduct,
8. Lab certification(s),
9. Monitoring log,
10. IVP shipment invoices,
11. Signature list of all staff completing the eCRF pages,
12. Site Delegation Log, which will include delegation of tasks or functions (including eCRF entry, IVP accountability, and other tasks) from PI to qualified staff and signatures of these staff members acknowledging their role(s),
13. Current Safety Report Form, Pregnancy Report Form (blank), and corresponding completion guidelines and any previous versions of the aforementioned forms and guidelines.

17.6 Contract Requirements

The Investigator and the Geneva Foundation will sign a Clinical Trial Agreement prior to the start of the study outlining overall Sponsor and Investigator responsibilities in relation

to the study. The contract should describe whether costs for pharmacy, laboratory, and other protocol-required services are being paid directly or indirectly. Financial Disclosure Statements will need to be completed, as required by 21 CFR part 54.

17.7 Insurance, Indemnity and Compensation

Themis Bioscience GmbH maintains an appropriate liability insurance policy. Deviations from the study protocol, such as the administration of a dose other than that scheduled in the study protocol or another route of administration, are not permitted and will not be covered by the subject insurance scheme.

17.8 Clinical Study Report

A final clinical study report will be prepared according to the ICH E3 guideline on Structure and Contents of Clinical Study Reports. A final clinical study report will be prepared regardless of whether the study is completed or prematurely terminated.

17.9 Publication Policy

After completion of the study, the Investigator may prepare a joint publication with the Sponsor and Project Oversight Agency. The Investigator must not submit any part of the data from this protocol for publication without the prior consent of the Sponsor and Project Oversight Agency.

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19 Appendices

19.1 Appendix 1: Adverse Event grading scales

19.1.1 Appendix Table A: Local Reaction to Injection

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain/Tenderness	Does not interfere with activity	Interferes with activity or repeated use of non-narcotic pain reliever	Prevents daily activity or any use of narcotic pain reliever	Emergency room (ER) visit or hospitalization
Erythema/Redness	2.5 - 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling	≤ 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis
Itching	Does not interfere with activity	Interferes with activity or repeated use of non-narcotic pain reliever	Prevents daily activity or repeated use of anti-inflammatory, pain-relieving ointment	Emergency room (ER) visit or hospitalization

19.1.2 Appendix Table B: Solicited Systemic Adverse Events

Systemic (General) Reaction	**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	ER visit or hospitalization for hypotensive shock
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Joint Pain*	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Fever (°C) (°F)	 38.0-38.4 100.4-101.1	 38.5-38.9 101.2-102.0	 39.0-40.0 102.1-104.0	 >40.0 >104.0

*Non-traumatic joint pain or stiffness that persists for more than 24 hours, or is associated with objective findings of effusion or soft tissue swelling, will be considered an AESI. See the protocol Section 13.5 and the Investigator Site File for this study for additional reporting requirements.

19.1.3 Appendix Table C: Unsolicited Adverse Events

Systemic Adverse Event (for solicited AEs occurring at other than solicited times, see Table B)	**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	ER visit or hospitalization

19.1.4 Appendix Table D: Vital Signs

Vital Signs*	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute**	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation
Fever (°C) (°F)	 38.0-38.4 100.4-101.1	 38.5-38.9 101.2-102.0	 39.0-40.0 102.1-104.0	 >40.0 >104.0

* Subjects should be at rest for all vital sign measurements.

** Use clinical judgment to characterize bradycardia among some healthy populations, for example, conditioned athletes.

19.1.5 Appendix Table E: Laboratory Results

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – < LLN***	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	> ULN*** – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	> ULN – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – < LLN	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – < LLN	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 > ULN – 110 > ULN – 125	 111 – 125 126 – 200	 > 125 > 200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen mg/dL	> ULN– 26	27 – 31	> 31	Requires dialysis
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
Hemoglobin (Female) - gm/dL	11.0 – < LLN	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) decrease from baseline - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 to < LLN	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) decrease from baseline – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm3	> ULN – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm3	2,500 – < LLN	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm3	750 – < LLN	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm3	1,500 – < LLN	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm3	> ULN – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm3	125,000 – < LLN	100,000 – 124,000	25,000 – 99,000	< 25,000

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

*** LLN=lower limit of the normal range; ULN=upper limit of the normal range.

19.2 Appendix 2: SF-36 Quality of Life Survey

19.2.1 SF-36 Quality of Life Survey in English

SF-36 Quality of Life Survey

Choose one option for each questionnaire item.

1. In general, would you say your health is:

- ○ Excellent
- ○ Very good
- ○ Good
- ○ Fair
- ○ Poor

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2. **Compared to one year ago**, how would you rate your health in general **now**?

- ○ Much better now than one year ago
- ○ Somewhat better now than one year ago
- ○ About the same
- ○ Somewhat worse now than one year ago
- ○ Much worse now than one year ago

The following items are about activities you might do during a typical day. Does **your health now limit you** in these activities? If so, how much?

3. **Vigorous activities**, such as running, lifting heavy objects, participating in strenuous sports?

- ○ Yes, limited a lot
- ○ Yes, limited a little
- ○ No, not limited at all

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4. **Moderate activities**, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

5. Lifting or carrying groceries?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

6. Climbing **several** flights of stairs?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

7. Climbing **one** flight of stairs?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

8. Bending, kneeling, or stooping?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

9. Walking **more than a mile**?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

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10. Walking **several blocks**?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

11. Walking **one block** (about 100 meters)?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

12. Bathing or dressing yourself?

- Yes, limited a lot
- Yes, limited a little
- No, not limited at all

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

13. Cut down the **amount of time** you spent on work or other activities?

- Yes
- No

14. **Accomplished less** than you would like?

- Yes
- No

15. Were limited in the **kind** of work or other activities?

- Yes
- No

---

16. Had **difficulty** performing the work or other activities (for example, it took extra effort)?

- Yes
- No

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

17. Cut down the amount of time you spent on work or other activities?

- Yes
- No

18. **Accomplished less** than you would like?

- Yes
- No

19. Didn't do work or other activities as **carefully** as usual?

- Yes
- No

---

20. During the **past 4 weeks**, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- Not at all
- Slightly
- Moderately
- Quite a bit
- Extremely

---

21. How much **bodily** pain have you had during the **past 4 weeks**?

- None
- Very mild
- Mild
- Moderate
- Severe
- Very severe

---

22. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

- Not at all
- A little bit
- Moderately
- Quite a bit
- Extremely

These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

How much of the time during the **past 4 weeks**...

23. Did you feel full of pep?

- All of the time
- Most of the time
- A good bit of the time
- Some of the time
- A little of the time
- None of the time

24. Have you been a very nervous person?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

25. Have you felt so down in the dumps that nothing could cheer you up?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

26. Have you felt calm and peaceful?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

27. Did you have a lot of energy?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

---

28. Have you felt downhearted and blue?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

29. Did you feel worn out?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

30. Have you been a happy person?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

31. Did you feel tired?

- ○ All of the time
- ○ Most of the time
- ○ A good bit of the time
- ○ Some of the time
- ○ A little of the time
- ○ None of the time

---

32. During the **past 4 weeks**, how much of the time has **your physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

How TRUE or FALSE is **each** of the following statements for you.

33. I seem to get sick a little easier than other people.

- Definitely true
- Mostly true
- Don't know
- Mostly false
- Definitely false

34. I am as healthy as anybody I know.

- Definitely true
- Mostly true
- Don't know
- Mostly false
- Definitely false

35. I expect my health to get worse.

- Definitely true
- Mostly true
- Don't know
- Mostly false
- Definitely false

36. My health is excellent.

- Definitely true
- Mostly true
- Don't know
- Mostly false
- Definitely false

19.2.2 SF-36 Quality of Life Survey in Spanish

Encuesta de Calidad de Vida SF-36

Marque una sola respuesta:

1. En general, usted diría que su salud es:

- ○ Excelente
- ○ Muy buena
- ○ Buena
- ○ Regular
- ○ Mala

---

2. **En comparación con hace un año**, ¿cómo diría que es su salud **actualmente**?

- ○ Mucho mejor ahora que hace un año
- ○ Algo mejor ahora que hace un año
- ○ Más o menos igual que hace un año
- ○ Algo peor ahora que hace un año
- ○ Mucho peor ahora que hace un año

Las siguientes preguntas se refieren a actividades o cosas que usted podría hacer en un día normal. **Su salud actual**, ¿**le limita** en estas actividades? Si es así, ¿cuánto?

3. ¿**Actividades intensas**, tales como correr, levantar objetos pesados, o participar en deportes agotadores?

- ○ Sí, me limita mucho
- ○ Sí, me limita un poco
- ○ No, no me limita nada

4. ¿ **Actividades moderadas**, como mover una mesa, pasar la aspiradora, jugar a los bolos o jugar al golf?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

5. ¿Levantar o llevar comestibles?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

6. ¿Subir **varios** pisos por la escalera?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

7. ¿Subir **un solo** piso por la escalera?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

8. ¿Doblar, arrodillarse o agacharse?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

9. ¿Caminar **más de una milla**?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

10. ¿Caminar **varias cuadras**?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

11. ¿Caminar **una cuadra** (unos 100 metros)?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

12. ¿Bañarse o vestirse por sí mismo?

- Sí, me limita mucho
- Sí, me limita un poco
- No, no me limita nada

Durante **las últimas 4 semanas**, ¿ha tenido usted alguno de los siguientes problemas con su trabajo u otras actividades diarias regulares **como resultado de su salud física**?

13. ¿Tuvo que reducir **el tiempo** dedicado al trabajo o a sus otras actividades?

- Sí
- No

14.¿**Hizo menos** de lo que hubiera querido hacer?

- Sí
- No

15.¿Estuvo limitado en el **tipo** de trabajo o otras actividades?

- Sí
- No

16.¿Tuvo **dificultad** para hacer su trabajo o sus actividades (por ejemplo, le costó más esfuerzo)?

- Sí
- No

Durante **las últimas 4 semanas**, ¿ha tenido alguno de los siguientes problemas con su trabajo u otras actividades diarias regulares **como resultado de cualquier problema emocional** (como sentirse deprimido o ansioso)?

17.¿Tuvo que reducir **el tiempo** dedicado al trabajo o a otras actividades?

- Sí
- No

18.¿**Hizo menos** de lo que hubiera querido hacer?

- Sí
- No

19.¿No hizo su trabajo o otras actividades tan **cuidadosamente** como de costumbre?

- Sí
- No

---

20. Durante **las 4 últimas semanas**, ¿hasta qué punto su salud física o los problemas emocionales han interferido con sus actividades sociales habituales con familiares, amigos, vecinos o grupos?

- Nada
- Un poco
- Regular
- Bastante
- Mucho

---

21. ¿Cuánto dolor **de cuerpo** ha tenido durante **las 4 últimas semanas**?

- ○ No, ninguno
- ○ Sí, muy poco
- ○ Sí, un poco
- ○ Sí, moderado
- ○ Sí, mucho
- ○ Sí, muchísimo

---

22. Durante **las 4 últimas semanas**, ¿hasta qué punto **el dolor** le ha dificultado su trabajo habitual (incluido el trabajo fuera de casa y las tareas domésticas)?

- ○ Nada
- ○ Un poco
- ○ Regular
- ○ Bastante
- ○ Mucho

Las preguntas que siguen se refieren a cómo se ha sentido y cómo le han ido las cosas durante **las 4 últimas semanas**. En cada pregunta responda lo que se parezca más a cómo se ha sentido usted. Durante **las 4 últimas semanas**, ¿cuánto tiempo…

23. ¿Se sintió lleno de vitalidad?

- ○ Siempre
- ○ Casi siempre
- ○ Muchas veces
- ○ Algunas veces
- ○ Sólo alguna vez
- ○ Nunca

24. ¿Ha sido usted una persona muy nerviosa?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

25. ¿Se ha sentido tan bajo en ánimo que nada podía animarle?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

26. ¿Se ha sentido calmado y tranquilo?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

27. ¿Tuvo mucha energía?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

28. ¿Se sintió desanimado y triste?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

29. ¿Se sintió agotado?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

30. ¿Ha sido una persona feliz?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

31. ¿Se sintió cansado?

- Siempre
- Casi siempre
- Muchas veces
- Algunas veces
- Sólo alguna vez
- Nunca

32. Durante **las 4 últimas semanas**, ¿con qué frecuencia **su salud física o los problemas emocionales** han interferido con sus actividades sociales (como visitar a los amigos o familiares, etcétera)?

- Siempre
- Casi siempre
- Algunas veces
- Sólo alguna vez
- Nunca

Diga si le parece CIERTA o FALSA **cada una** de las siguientes frases.

33. Parece que me pongo enfermo más fácilmente que otras personas.

- Totalmente cierta
- Bastante cierta
- No lo sé
- Bastante falsa
- Totalmente falsa

34. Estoy tan saludable como cualquier persona que conozco.

- Totalmente cierta
- Bastante cierta
- No lo sé
- Bastante falsa
- Totalmente falsa

35. Creo que mi salud va a empeorar.

- Totalmente cierta
- Bastante cierta
- No lo sé
- Bastante falsa
- Totalmente falsa

36. Mi salud es excelente.

- Totalmente cierta
- Bastante cierta
- No lo sé
- Bastante falsa
- Totalmente falsa

19.2.3 Terms and Conditions for Using the SF-36

HEALTH CARE

Terms and Conditions for Using the 36-Item Short Form Survey (SF-36)

RAND hereby grants permission to use RAND 36-Item Short Form Health Survey in accordance with the following conditions, which shall be assumed by all to have been agreed to as a consequence of accepting and using this document:

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https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form/terms.html

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Final Audit Report 2021-01-05

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2021-01-05 - 9:18:20 AM GMT

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CLINICAL STATISTICAL ANALYSIS PLAN

DF/Net Research, Inc.

PHASE 2 STUDY OF A LIVE ATTENUATED MEASLES VIRUS-VECTORED CHIKUNGUNYA VACCINE IN PREVIOUSLY EXPOSED ADULTS

Client: Themis
Protocol: MV-CHIK-206 Version 2.1, 23DEC2020

SAP Version: 2
Date of Version: 20AUG2021

Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from Themis (or others, as applicable), unless it is necessary to obtain informed consent from potential study subjects.

SPONSOR APPROVAL

By signing below, I certify that I approve of the Statistical Analysis Plan for this project.

Printed Name and Title:

PPD , Themis Bioscience GmbH

Signature and Approval Date:

PPD 25-Aug-2021

DF/NET APPROVAL

By signing below, I certify that I approve of the Statistical Analysis Plan for this project. I also certify that the analyses will be performed in accordance with this Statistical Analysis Plan.

Printed Name and Title:

PPD DF/Net Research Inc.

Signature and Approval Date:

PPD 24-Aug-2021

Revision History

Version	Date	Author(s)	Organization(s)	Purpose
1.0	21MAR2019	PPD	DF/Net Research, Inc.	New document
2.0	20AUG2021	PPD	DF/Net Research, Inc.	Updates made to be consistent with the updated version 2.1 of the protocol

TABLE OF CONTENTS

1-INTRODUCTION

The purpose of this document is to describe the reporting and statistical analyses that will guide the preparation of the clinical portion of the final study report for Themis study MV-CHIK-206.

The study report will be prepared after all data in the database have been reviewed, all data queries have been resolved and the database has been locked.

All individual subject listings, summary tables, and statistical analyses described below will be provided in separate appendices to the study report. Unless other indicated, all listings and summary tables will be provided by treatment assignment, subject identifier and visit.

This SAP is written with consideration of the recommendations outlined in the International Conference on Harmonization (ICH) E9 Guideline entitled Guidance for Industry: Statistical Principles for Clinical Trials [ICH 1998] and the ICH E3 Guideline entitled Guidance for Industry: Structure and Content of Clinical Study Reports [ICH 1995].

2-STUDY OBJECTIVES

The main objective of this trial is to evaluate the safety and immunogenicity of an investigational live recombinant measles-vectored chikungunya vaccine (MV-CHIK) delivered in 2 vaccinations, 28 days apart compared with saline placebo.

2.1 Primary Objective

To determine the safety of MV-CHIK administered in 2 doses separated by 28 days in previously exposed individuals.

2.2 Secondary Objectives

To determine the immunogenicity by a neutralization assay of MV-CHIK administered in 2 doses separated by 28 days in previously exposed individuals.

2.3 Exploratory Objectives

To assess the relationship of Quality-of-Life scores and acute phase reactants with adverse events experienced post-vaccination.

3-STUDY ENDPOINTS

3.1 Primary Endpoint

- Solicited AEs (fever, fatigue, headache, malaise, myalgia, nausea/vomiting, joint pain or injection site itching, pain/tenderness, erythema/redness or induration/swelling)
- Unsolicited AEs including clinically significant abnormal safety laboratory results, vital signs, and physical examination findings
- Solicited and unsolicited AEs of grade 2 and higher.

3.2 Secondary Endpoints

- Fold-increase from Day 0 in geometric mean titer (GMT) of neutralizing antibodies to chikungunya on Days 28, 56, and at the end of the study.

3.3 Exploratory Endpoints

- Change in serum C-reactive protein levels from pre-vaccination to three days postvaccination in recipients of MV-CHIK versus placebo.
- Changes in plasma fibrinogen from pre-vaccination to seven days post-vaccination in recipients of MV-CHIK versus placebo.
- Changes from baseline in Quality-of-Life survey (SF36) scores at four weeks post-vaccination and at the end of the study in recipients of MV-CHIK versus placebo.

4-DEFINITIONS

The following definitions apply to the summary tables and statistical analyses planned for the study report:

Screened population: All subjects who signed informed consent.

Intent-to-treat (ITT) population: Subjects who receive at least one dose of vaccine and will be the population for the safety analyses.

Per-Protocol (PP) population: A subset of the ITT population that includes subjects who received both doses of IVP, have at least one post-vaccination immunogenicity assessment, and do not experience a protocol deviation that would affect their evaluation for immunogenicity. The protocol deviations that affect evaluation for immunogenicity will be determined based on a blinded data review prior to database lock.

Baseline value: Endpoint value at Day 0

Visit Day: Planned visit based on schedule of events, each visit day has a range of allowed days from previous visit.

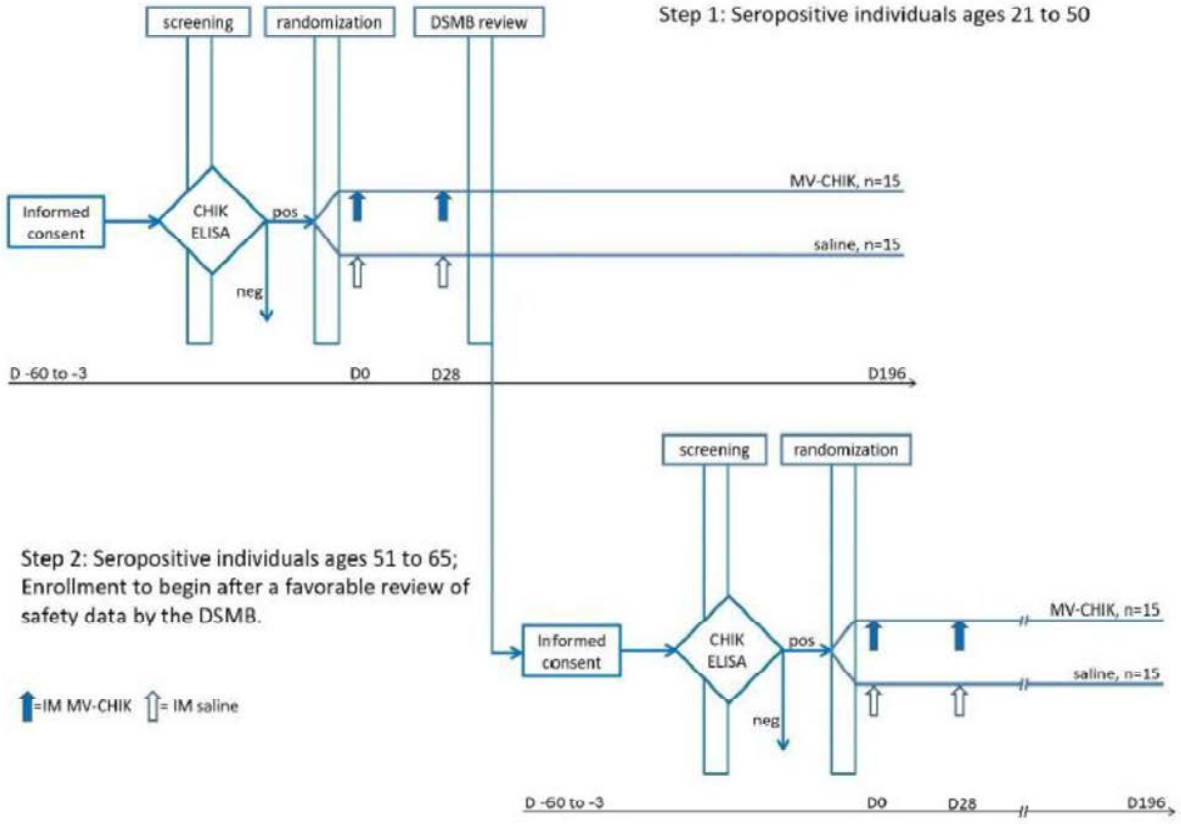
Study Day: Number of days from Visit Day 0, with day on Day 0 equal to 1 and the day before Day 0 equal to -1.

Protocol Deviation: Any noncompliance with the clinical trials protocol, Good Clinical Practice (GCP), or SOP requirements. Noncompliance may be either on the part of the subject, the investigator, or the study site staff.

5-STUDY DESIGN

This will be a prospective randomized double-blind interventional clinical trial to evaluate the safety and immunogenicity of MV-CHIK in two intramuscular injections 28 days apart versus placebo. Consented study subjects will be screened for baseline seropositivity to chikungunya virus, with or without a clinical history of chikungunya infection, and then randomized to receive either MV-CHIK (the experimental vaccine) or saline (the placebo) in a blinded fashion in a 1:1 ratio. One dose level (5×10^5 $TCID_{50}$) of MV-CHIK will be studied. Subjects will be followed for safety and immunogenicity for 24 weeks after completing the series. Because rheumatologic symptoms of chikungunya infection are more likely, more severe and more persistent in older patients, this study will initially be limited to adults aged 21 to 50 years of age (Step 1). After at least 20 volunteers in Step 1 have been vaccinated, a DSMB will review the safety data and, with their recommendation, the study will proceed to Step 2 and enroll subjects aged 51 to 65 years.

The study schematic is:



6-STUDY POPULATION

The study will be conducted in Puerto Rico where a chikungunya epidemic occurred in 2014 but where transmission is now negligible. Individuals will be screened until 30 subjects are vaccinated for each Step. Subjects still under treatment for symptoms attributed to a previous chikungunya virus infection will be excluded from the study. Subjects who attribute only mild and subjective symptoms such as fatigue to previous chikungunya infection may be eligible at investigator discretion; subjects with acute chikungunya infection will be excluded but may re-screen no sooner than 3 months after symptoms (other than mild subjective symptoms not requiring treatment) have resolved. This study will measure, but not control for, baseline Quality-of-Life using the SF36 questionnaire. The seroprevalence of chikungunya in San Juan is approximately 23.5% [Simmons 2016]. Up to 500 subjects may be screened for each Step but it is hoped that existing databases of seropositive study volunteers will allow enrollment to be completed much more efficiently.

Subjects will not be randomized unless all inclusion and no exclusion criteria, including lab test results, are met. Subjects who fail to meet criteria because of an identifiable temporary condition or who cannot be randomized within the 60-day screening window may repeat the screening evaluation up to two times after the initial screening (total of three) at the discretion of the Investigator.

6.1 Inclusion Criteria

1. Previous infection with chikungunya as verified by a serum immunoassay.
2. Age appropriate for the Step being conducted:
    a. ≥21 to ≤50 years on the day of enrollment for Step 1.
    b. ≥51 to ≤65 years on the day of enrollment for Step 2.
3. Able to provide informed consent.
4. Available and accessible for the duration of the trial.
5. Able and willing to comply with all requirements of the study.
6. For women of childbearing potential, willing to practice adequate contraception (see Definition of Terms) for the duration of the study. This is similar to recommendations following MMR vaccination [McLean 2013] but for a longer duration (six months versus one month) due to the higher concentration of measles virus and the lack of reproductive or developmental toxicology data on MV-CHIK.
7. Medical history and physical examination findings are considered normal or not clinically significant in the opinion of the Investigator, which includes resolution of any arthralgias that may have occurred during prior chikungunya infection, as well as the absence of synovitis.
8. Laboratory values are considered normal or not clinically significant in the opinion of the Investigator. If laboratory screening tests are out of the normal reference range and of potential clinical significance, the test(s) may be repeated up to 2 times (a total of 3 per screening evaluation) at the discretion of the Investigator, and the repeat values and their potential clinical significance will be used to determine eligibility.
9. History of immunity to measles. For persons born after 1957, this will be established by a history of compliance with vaccination policies that included measles vaccination or known vaccination as an adult at least one month before they are randomized. Volunteers

born before 1957 will be presumed to have immunity to measles based on natural exposure in accordance with CDC guidelines [McLean 2013].

6.2 Exclusion Criteria

1. Taking medication or other treatment for unresolved symptoms attributed to a previous chikungunya virus infection.
2. Prior receipt of any investigational chikungunya or other alphavirus vaccine. To date, no alphavirus vaccines have been commercially available in the United States.
3. Recent infection:
    a. self-limited upper respiratory infections until afebrile without medication for >1 week;
    b. chikungunya unless/until asymptomatic (other than mild subjective symptoms not requiring treatment) for >3 months;
    c. non-recurrent upper respiratory or urinary tract infections successfully treated with antibiotics, until asymptomatic for 1 month after full antibiotic course has been completed.
4. History of an acute allergic or anaphylactic reaction to any vaccine.
5. History of an immunosuppressive disorder (such as HIV infection, Common Variable Immune Deficiency), chronic infection (such as chronic hepatitis B or C), autoimmune disease (such as rheumatoid arthritis, systemic lupus erythematosus (SLE), autoimmune thyroid disease), or any medical condition that, in the opinion of the Investigator, could lead to an atypical immune response to the vaccine.
6. History of moderate or severe non-traumatic arthritis or arthralgia within 3 months of the Screening Visit.
7. Recent (within 30 days), current or anticipated use of any immunosuppressive or immune modifying medication including corticosteroids (excluding nasal, ophthalmic, and other topical preparations).
8. Other vaccination or planned vaccination within 4 weeks of either study dose (within 2 weeks for seasonal influenza vaccine).
9. Receipt or planned receipt of blood products including immunoglobulins within 120 days of the Screening Visit.
10. Pregnant or lactating or planning pregnancy during the trial.
11. Known alcohol or other substance abuse that in the opinion of the Investigator affects the ability or willingness of the subject to understand and comply with the study protocol.
12. Participation in another clinical study within the past 30 days in which the subject was exposed to an investigational product (pharmaceutical product or placebo or device) or planned participation in another interventional clinical study while participating in this study.
13. Relevant history of any medical condition that, in the opinion of the Investigator, may interfere with the safety of the subject or aims of the study.
14. History of neoplastic disease (excluding successfully treated non-melanoma skin cancer or cervical intraepithelial neoplasia) within the past 5 years or a history of any hematological malignancy.
15. Behavioral or psychiatric disease or cognitive impairment that in the opinion of the Investigator affects the ability or willingness of the subject to understand and comply with the study protocol.

16. Non-consent to storage of blood specimens for future research.
17. Persons in direct relationship with the Sponsor or its contracted service providers, the CRO or its subcontractors, the Investigator, or study site staff. Direct relationship includes first degree relatives or dependents (children, spouse/partner, siblings or parents), as well as employees (site or Sponsor). Employees of the San Juan City Hospital not directly employed by the Research Unit will not be excluded.

6.3 Withdrawal of Study Subject

A subject has the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the physician or the institution.

Subjects may also be withdrawn from the study if the Investigator determines that the subject should be withdrawn due to any unforeseen circumstance that may affect the safety of the subject, staff or the integrity of the study.

6.4 Randomization and Blinding

Initially, thirty (30) Step 1 subjects will be randomized at Visit 1, Day 0 using a Clinical Data Management System (CDMS). Subsequent to DSMB review, another thirty (30) Step 2 subjects will be enrolled and randomized at their Visit 1, Day 0. Visit 1 occurs after Screening (Visit S, Day -60 to -3) and includes enrollment, randomization and vaccine administration. At Visit 1 subjects will be randomized after enrollment and before vaccine administration. Subjects in each Step will be assigned to one of two groups. Block randomization of appropriate size will accommodate balanced enrollment in ratio of 1:1 into each of the two treatment groups for the planned 30 Step 1 and 30 Step 2 subjects.

Step	N	Treatment Assignment	Study Days of Administration
1	15	MV-CHIK 5×10^5 $TCID_{50}$, 0.4mL IM injection	0, 28
	15	Saline, 0.4mL IM injection	0, 28
2	15	MV-CHIK 5×10^5 $TCID_{50}$, 0.4mL IM injection	0. 28
	15	Saline, 0.4mL IM injection	0, 28

The list of randomized treatment assignments will be prepared by statisticians at DF/Net Research. The blinded randomization number will be provided through the randomization module in the data management system. The designated unblinded site personnel will be provided with the unblinded treatment key, which links the randomization number to the actual treatment assignment and should be securely stored.

Instructions for use of the data management system for randomization will be included in the Study Specific Procedures (SSP) Manual. The SSP will also detail the manual backup randomization procedures in the event that the site temporarily loses access to the internet or the randomization module is unavailable. All documentation of the randomization procedure and output will be maintained by DF/Net until the end of the study.

Vaccine administration will be double-blind, with the Investigator, study coordinator, all study personnel involved in assessing AEs and the subject being unaware of the treatment assignment. To achieve this, the thawed MV-CHIK vaccine and saline will be given the same wait time after procurement to allow both temperature equalization and non-discrepant wait times between treatments. Furthermore, only designated site personnel (pharmacist, etc.), unblinded CRAs and the unblinded biostatistician (at DF/Net) will have access to the treatment assignments.

Unblinding will occur after the last subject's last visit (Visit 10) and database lock, though the safety data may be unblinded upon Sponsor or DSMB request.

6.5 Schedule of Events

	Screening	Vaccination Period				Post-Vaccination Follow-up Period					
	Day -60 to Day-3	Day 0	Day 3 (-1,+2 days)	Day 7 (+5 days)	Day 28 (-3,+7 days)	Day 31 (3 [-1,+2] days post dose 2)	Day 35 (7 [+5] days post dose 2)	Day 56 (±7 days)	Day 84 (± 7 days)	Day 140 (±14 days)	Day 196 (±14 days) EOS visit
Visit	**S**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Informed consent	X										
Randomization		X									
Clinical Assessments											
Medical & medication history	X										
SF36 Questionnaire		X			X			X			X
Physical examination	X										
Directed physical examination		X	X	X	X	X	X	X	X	X	X
Vital signs [a]	X	X	X	X	X	X	X	X	X	X	X
Dispense diary [b]		X			X						
Collect and/or review diaries			X	X	X	X	X	X			
Review concomitant medications		X	X	X	X	X	X	X	X	X	X
Interim history/adverse events		X	X	X	X	X	X	X	X	X	X
Review inclusion/exclusion criteria	X	X			X						
Laboratory Assessments [c]											
Serology: chikungunya [d], HBsAg, anti-HCV, anti-HIV-1 and -2	X										
Complete blood count [e]	X	X		X	X		X				
Complete metabolic panel [e]	X							X			
Basic metabolic panel [e]		X	X		X	X					
Urinalysis (clean catch)	X										
Pregnancy test [f]	X	X			X						X
Neutralizing antibody to chikungunya		X			X			X			X
Sera for future immunogenicity studies [g]		X	X	X	X	X	X	X	X	X	X
C-reactive protein		X	X		X	X		X			

	Screening	Vaccination Period				Post-Vaccination Follow-up Period					
	Day -60 to Day-3	Day 0	Day 3 (-1,+2 days)	Day 7 (+5 days)	Day 28 (-3,+7 days)	Day 31 (3 [-1,+2] days post dose 2)	Day 35 (7 [+5] days post dose 2)	Day 56 (±7 days)	Day 84 (± 7 days)	Day 140 (±14 days)	Day 196 (±14 days) EOS visit
Visit	S	1	2	3	4	5	6	7	8	9	10
Fibrinogen		X		X	X		X				
Ferritin	X										
Vaccination											
Blinded vaccination		X			X						

Abbreviations: AE, adverse event; EOS, end of study; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus.
Note: See Section 9.4.3 for Early Termination Visit procedures/assessments.
a: To include body temperature, pulse rate, systolic and diastolic blood pressure.
b: The paper subject diary will assess solicited local injection site reactions (erythema/redness, induration/swelling, itching, and pain/tenderness) after each injection for up to 7 days. Systemic signs and symptoms (fever, fatigue, headache, malaise, myalgia, nausea/vomiting, and joint pain) will also be solicited for 7 days. The diary will include a section for recording unsolicited AEs and concomitant medications.
c: The cumulative total of blood drawn in this study is approximately 300 mL per subject.
d: Determine chikungunya exposure status using an enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin G (IgG).
e: See Section 12.5 for individual tests performed.
f:Urine pregnancy testing will be done on women of childbearing potential at Screening, on vaccination days and at the EOS visit.
g: On Day 0, an 80-mL blood sample will be collected for sera; on other collection days, 10 mL will be collected for sera. These samples will be shipped to the Viral Diseases Branch of WRAIR for storage pending future testing and analysis. Refer to the Investigator Site File for sample preparation, handling, and shipping instructions.

7-SAMPLE SIZE

7.1 Determination of Sample Size

60 subjects are intended to be enrolled in the study. A formal sample size calculation was not conducted for this study. The sample size was determined based on prior experience in evaluating the safety and immunogenicity of vaccines and is typical for early phase clinical studies. In previous studies MV-CHIK was associated with joint pain in about 4% of previously unexposed recipients. The largest study to date is MV-CHIK 202 in which 9/229 reported joint pain (95% confidence interval: 1.8 to 7.3%). A higher risk of joint pain in previously exposed individuals would be suggested if 4/15 individuals who receive MV-CHIK in either Step 1 or 2 of the current study develop joint pain (95% confidence interval: 7.8% to 55.1%) while the placebo group develops none.

It is not possible to define a statistical threshold of safety that will rule out any increased risk of this vaccine in previously exposed individuals or guarantee market acceptance of this or any vaccine for an infection associated with joint pain, as demonstrated by the experience with LYMErix. This study is not expected to provide a final answer on whether or not to continue development of this vaccine candidate, but rather contribute to the incremental gathering of information on vaccine safety. In this regard, the details of individual cases including the time course and severity of their symptoms and the associated changes in their acute phase reactants and quality of life scores, will have to be examined individually. A qualitative description of these events to include their assessed causality, severity, duration, and association with evidence of inflammation and immunopathology will provide critical data to inform the consent of participants in field trials conducted in areas of on-going transmission.

8-STATISTICAL CONSIDERATIONS

SAS® version 9.3 or higher will be used for statistical analyses and the production of tables, listings, and figures (TLFs).

Categorical variables will be summarized using the number frequency and percentage by Step, treatment and overall of subjects falling into each category. Continuous variables will be summarized using mean, standard error or standard deviation, median, minimum, maximum, and number of subjects with observations by Step of subjects falling into each category. Continuous variables will be summarized using mean, standard error or standard deviation, median, minimum, maximum, and number of subjects with observations treatment and overall.

8.1 Demographic and Other Baseline Characteristics

Demographics will be summarized by age (in years, at time of signing informed consent), gender and ethnicity. Baseline characteristics and medical history will also be summarized by relevant characteristics and classifications.

8.2 Study Objective Analyses

8.2.1 Primary Endpoints

All verbatim terms for any reported AEs will be coded to the appropriate system organ class and preferred term according to the current version of MedDRA and graded by the Investigator for severity as per the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adults Enrolled in Vaccine Clinical Trials; 2007. The number and percentage of subjects experiencing an AE (all, serious, and vaccine-related) will be reported.

All AEs will be summarized by IVP received according to the MedDRA system organ class (SOC) and preferred term. An additional summary will be provided for the incidence of all AEs by severity. Summaries will be presented for SAEs, AEs of grade 2 (moderate) and higher, IVP-related AEs, AESIs, and AEs where an action was taken. All AE data will be listed by subject, and a separate listing will include all SAEs, including any deaths on study.

Adverse events will also be summarized separately for solicited and unsolicited events. Specific local injection site reactions (erythema/redness, induration/swelling, itching, and pain/tenderness) will be solicited using the subject diary from 0 to 7 days after each injection and Investigator interviews at follow-up visits. Specific systemic AEs (fever, fatigue, headache, malaise, myalgia, nausea/vomiting and joint pain) will be solicited using the subject diary from 0 to 7 days and Investigator interviews at follow-up visits. Unsolicited AEs will be all of those excluding the preferred terms of solicited AEs. Similar summaries will be presented for solicited and unsolicited AEs as noted above.

8.2.2 Secondary Endpoints

Analyses of immunogenicity will be conducted in the PP population according to IVP received. Chikungunya virus neutralizing antibody titer values which are less than the assay cut-off of 10 ED50 titer will be replaced with half the assay cut-off (i.e. 5 ED50 titer). The analysis of immunogenicity will be measured as GMTs of neutralizing antibodies on Days 0, 28, 56, and at EOS. The GMT will be calculated using a mixed effects model with IVP (MV-CHIK versus placebo), visit and study Step (age group) and treatment visit interaction as fixed factors and subject as a random effect. The analysis of variance will be fit using log10 transformed data. The estimates for

the least squares means and corresponding 95% confidence intervals (CIs) will be back-transformed by taking the anti-log to obtain the GMTs and CIs.

The following will be summarized quantitatively:

- Fold-increase from Day 0 in geometric mean titer (GMT) of neutralizing antibodies to chikungunya on Days 28, 56, and at the end of the study.

8.2.3 Exploratory Endpoints

The following will be summarized quantitatively:

- Change in serum C-reactive protein levels from pre-vaccination to three days postvaccination in recipients of MV-CHIK versus placebo.
- Changes in plasma fibrinogen from pre-vaccination to seven days post-vaccination in recipients of MV-CHIK versus placebo.
- Changes from baseline in Quality-of-Life survey (SF36) scores at four weeks postvaccination and at the end of the study in recipients of MV-CHIK versus placebo.

8.3 Subject Disposition

The following will be summarized categorically:

- Subjects in each of the populations.
- Subjects randomized and who received at least one dose of IVP.
- Subjects with protocol deviations and reason for deviation.
- Subjects who discontinued the study early along with the reasons for discontinuation.
- Screen failures and reasons for screen failure.
- Subjects at each study visit, and the number of subjects with important protocol deviations.

8.4 Vaccine Exposure

The number and percentage of subjects who receive only one of the IVP doses and the number and percentage of subjects who receive both of the IVP doses will be summarized.

8.5 Concomitant Medications

Concomitant medications will be coded by WHODrug. The number and percentage of subjects who received each category of medication will be summarized by IVP administered.

8.6 Safety Laboratory Tests

Clinical laboratory test results and changes from baseline will be summarized by time point. Clinically significant abnormal laboratory results will also be captured as AEs. Abnormal results of CRP, fibrinogen or ferritin will not be captured as AEs.

8.7 Vital Signs and Physical Exam Findings

Vital signs results (including blood pressure, heart rate, and body temperature) and changes from baseline will be summarized by time point. Abnormal vital signs will be graded and findings from physical examinations will be assessed for clinical significance and included in the tabular summaries and by-subject AE listings.

8.8 Missing Values

The intervention is not expected to have any effect on missing data. As such, any missing data will be considered Missing Completely At Random (MCAR) and will be assumed to have no effect on the analysis and no imputations will be performed. All attempts will be made to prevent missing data. An observed cases approach will be applied for all endpoints.

9-STUDY DISCONTINUATION

Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, Investigator, Sponsor, DoD Research Monitor or regulatory authorities will be promptly reported to the HRPO.

10-SUGGESTED TABLES/LISTINGS/FIGURES

Type	Title	Population
Listing	L16.2.1.1- Patient Disposition: Screen Failures, Patients Enrolled and Eligibility	Screened
Listing	L16.2.1.2- Patient Disposition: Study Summary	ITT
Listing	L16.2.1.3- Patient Disposition: Visit Summary	ITT
Listing	L16.2.1.3- Patient Disposition: Patients Who Discontinued or Were Lost to Follow-Up	ITT
Listing	L16.2.2.1-Inclusion/Exclusion Criteria	Screen Failures
Listing	L16.2.2.2-Protocol Deviations	ITT
Listing	L16.2.3-Patients Excluded from the Per-Protocol Population	ITT
Listing	L16.2.4.1-Demographics	ITT
Listing	L16.2.4.2-Medical History	ITT
Listing	L16.2.5.1-Vaccine Exposure	ITT
Listing	L16.2.5.2-Concomitant Medications	ITT
Listing	L16.2.6.1-Chikungunya Serology	ITT
Listing	L16.2.6.2-Neutralizing Antibody to Chikungunya	ITT
Listing	L16.2.6.3-C-Reactive Protein	ITT
Listing	L16.2.6.4-Fibrinogen	ITT
Listing	L16.2.6.5-SF-36 Quality of Life Scores	ITT
Listing	L16.2.7.1-Adverse Events	ITT
Listing	L16.2.7.2-Serious Adverse Events	ITT
Listing	L16.2.7.3-Adverse Events of Grade 2 or Higher	ITT
Listing	L16.2.7.4-Study Injection-Related Adverse Events	ITT
Listing	L16.2.7.5-Adverse Events of Special Interest	ITT
Listing	L16.2.7.6-Adverse Events with an Action Taken	ITT
Listing	L16.2.7.7-Solicited Adverse Events	ITT
Listing	L16.2.7.8-Unsolicited Adverse Events	ITT
Listing	L16.2.8.1-Ferritin	ITT
Listing	L16.2.8.2-Serology Laboratory Results	ITT
Listing	L16.2.8.3-Urinanalysis Laboratory Results	ITT
Listing	L16.2.8.4-Pregnancy Test Laboratory Results	ITT
Listing	L16.2.8.5-Complete Blood Count Laboratory Results	ITT
Listing	L16.2.8.6-Metabolic Panel Laboratory Results	ITT
Listing	L16.2.9-Vital Signs	ITT
Listing	L16.2.10-Physical Examination Findings	ITT

Listing	L16.2.11-Pregnancy Report	ITT
Table	T14.1.1.1-Disposition Summary of Screen Failures	Screened
Table	T14.1.1.2-Disposition Summary	Screened
Table	T14.1.1.3-Protocol Deviation Summary	ITT
Table	T14.1.1.4-Visit Summary	ITT
Table	T14.1.2-Demographic Summary	ITT
Table	T14.2.1.1-Neutralizing Antibodies to Chikungunya Summary	PP
Table	T14.2.1.2-Fold-increase from Baseline in Neutralizing Antibodies to Chikungunya Summary	PP
Table	T14.2.1.3-Statistical Analysis of Neutralizing Antibodies to Chikungunya Summary	PP
Table	T14.2.2-Change in Serum C-reactive Protein Levels (mg/L) Summary	PP
Table	T14.2.3-Change in Plasma Fibrinogen (mg/dL) Summary	PP
Table	T14.2.4-Change in SF-36 Quality of Life Scores Summary	PP
Table	T14.3.1.1.1-Adverse Events Summary	ITT
Table	T14.3.1.1.2-Incidence of Adverse Events by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.1.3- Incidence of Serious Adverse Events by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.1.4- Incidence of Adverse Events of Grade 2 or Higher by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.1.5- Incidence of Adverse Events of Special Interest by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.1.6- Incidence of Adverse Events by System Organ Class and Preferred Term, by Severity	ITT
Table	T14.3.1.1.7- Incidence of Adverse Events by System Organ Class and Preferred Term, by Relationship to Study Injection	ITT
Table	T14.3.1.1.8- Incidence of Adverse Events with an Action Taken by System Organ Class and Preferred Term	ITT
Table	T14.3.1.2.1-Solicited Adverse Events Summary	ITT
Table	T14.3.1.2.2-Incidence of Solicited Adverse Events by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.2.3- Incidence of Solicited Serious Adverse Events by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.2.4- Incidence of Solicited Adverse Events of Grade 2 or Higher by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.2.5- Incidence of Solicited Adverse Events of Special Interest by System Organ Class and Preferred Term Summary	ITT

Table	T14.3.1.2.6- Incidence of Solicited Adverse Events by System Organ Class and Preferred Term, by Severity	ITT
Table	T14.3.1.2.7- Incidence of Solicited Adverse Events by System Organ Class and Preferred Term, by Relationship to Study Injection	ITT
Table	T14.3.1.2.8- Incidence of Solicited Adverse Events with an Action Taken by System Organ Class and Preferred Term	ITT
Table	T14.3.1.3.1-Unsolicited Adverse Events Summary	ITT
Table	T14.3.1.3.2-Incidence of Unsolicited Adverse Events by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.3.3- Incidence of Unsolicited Serious Adverse Events by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.3.4- Incidence of Unsolicited Adverse Events of Grade 2 or Higher by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.3.5- Incidence of Unsolicited Adverse Events of Special Interest by System Organ Class and Preferred Term Summary	ITT
Table	T14.3.1.3.6- Incidence of Unsolicited Adverse Events by System Organ Class and Preferred Term, by Severity	ITT
Table	T14.3.1.3.7- Incidence of Unsolicited Adverse Events by System Organ Class and Preferred Term, by Relationship to Study Injection	ITT
Table	T14.3.1.3.8- Incidence of Unsolicited Adverse Events with an Action Taken by System Organ Class and Preferred Term	ITT
Table	T14.3.4.1-Serology Laboratory Results at Screening Summary	ITT
Table	T14.3.4.2-Urinalysis Laboratory Results Summary at Screening	ITT
Table	T14.3.4.3-Pregnancy Test Laboratory Results Summary	ITT
Table	T14.3.4.4-Complete Blood Count Laboratory Results Summary	ITT
Table	T14.3.4.5-Metabolic Panel Laboratory Results Summary	ITT
Table	T14.3.5.1-Vaccine Exposure Summary	ITT
Table	T14.3.5.2-Incidence of Concomitant Medications by ATC Level 4 and Preferred Drug Name	ITT
Table	T14.3.5.3-Vital Signs Summary	ITT
Table	T14.3.5.4-Physical Examination Summary	ITT

APPENDIX I: ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse event
AESI	Adverse event of special interest
BMP	Basic metabolic panel
CBC	Complete blood count
CDC	US Centers for Disease Control and Prevention
CDMS	Clinical Data Management System
CI	Confidence Interval
CRA	Clinical Research Associate (study monitor)
CRO	Clinical Research Organization
CRP	C-reactive protein
DoD	Department of Defense
DSMB	Data Safety Monitoring Board
EOS	End-of-study
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GmbH	Gesellschaft mit beschränkter Haftung (German for Limited Liability Company)
GMT	Geometric Mean Titer
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (of the MRMC)
ICH	International Conference on Harmonization
ID	Identification
IRB	Institutional Review Board
ITT	Intention-to-treat
IVP	Investigational vaccine product (used to indicate either MV-CHIK or placebo)
MCAR	Missing Completely at Random
MedDRA	Medical Dictionary for Regulatory Activities
MMR	Measles, Mumps and Rubella vaccine
MRMC	US Army Medical Research and Materiel Command

MV-CHIK	Measles-vectored chikungunya vaccine
PP	Per-protocol
QoL	Quality-of-life
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SF36	Standardized quality-of-life questionnaire
SOC	System-organ-class
SOP	Standard Operating Procedure
SSP	Study Specific Procedure
TCID, $TCID_{50}$	Tissue culture infective dose; the subscript refers to the percent of cells in culture that are infected after inoculation.
US, USA	United States of America
WHO	World Health Organization
WHODrug	An internal classification of medicines

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Author: PPD | Creation Date: 24 Aug 2021, 06:37:08, PDT | Completion Date: 25 Aug 2021, 00:22:33, PDT

Document Details:

Name: Themis MV-CHIK-206 SAP V2.0

Type:

Document Ref: 3a1e17d944643e98ad475cfb8d110099ed0a1543f898a148714c4f239b6bc50d

Document Total Pages: 20

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Consent: eSignature Consent Accepted
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Invitation Sent	Invitation sent to PPD on 24 Aug 2021, 06:42:52, PDT
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