STUDY PROTOCOL

PHASE 2 STUDY OF A LIVE ATTENUATED MEASLES VIRUS-VECTORED CHIKUNGUNYA VACCINE IN A PREVIOUSLY EPIDEMIC AREA

Investigational Vaccine Product: MV-CHIK

This will be a prospective randomized double-blind interventional clinical study investigating one dose level of the investigational vaccine product in subjects previously exposed and unexposed to chikungunya.

Protocol Number: MV-CHIK-204 **IND Number**: 17343

Study Phase: 2 **NCT Identifier:** NCT03101111

Version: 3.0
Amendment Number: 02
Date: 15 Dec 2017

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Silver Spring, Maryland
20910 United States
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This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) as set forth in the International Council for Harmonisation (ICH) guidelines on GCP (ICH E6), and applicable local regulatory requirements.

CONFIDENTIAL

1. SIGNATURES

Representatives of Sponsor, Funding Agency, and Clinical Research Organization

I have read and agree to the MV-CHIK-204 Protocol Amendment 02, Version 3.0, entitled "Phase 2 Study of a Live Attenuated Measles Virus-Vectored Chikungunya Vaccine in a Previously Epidemic Area." I am aware of my responsibilities under the guidelines of GCP, local regulations (as applicable), and the study protocol. I agree to conduct the study according to these responsibilities.

Sponsor Representative - Themis Bioscience GmbH:

Raimund M. Vielnascher	Clinical Study Manager
Print Name	Title
[signature]	20-Dec-2017
Signature	Date

Funding Agency's Representative – Walter Reed Army Research Institute:

Paul B. Keiser	Colonel, US Army
Print Name	Title
[signature]	16 Dec 2017
Signature	Date

Clinical Research Organization Representative - PRA Health Sciences:

Lynlee Burton	Director Project Delivery
Print Name	Title
[signature]	15 Dec 2017
Signature	Date

Investigator

I have read and agree to the MV-CHIK-204 Protocol Amendment 02, Version 3.0, entitled "Phase 2 Study of a Live Attenuated Measles Virus-Vectored Chikungunya Vaccine in a Previously Epidemic Area." I am aware of my responsibilities under the guidelines of GCP, local regulations (as applicable), and the study protocol. I agree to conduct the study according to these responsibilities and to appropriately direct and assist the staff under my control, who will be involved in the study.

Clinical Site: _____

Site Number: _____

Site Principal Investigator:

_____	_____
Print Name	Title
_____	_____
Signature	Date

2. PROTOCOL SUMMARY

TITLE: Phase 2 Study of a Live Attenuated Measles Virus-Vectored Chikungunya Vaccine in a Previously Epidemic Area

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 an active measles, mumps, and rubella (MMR) comparator delivered in one vaccination. Subjects will be consented adults 21-50 years of age. After providing informed consent, individuals will be screened for eligibility and then openly cohorted based on baseline serostatus to chikungunya virus. They will then be randomized in a double-blind fashion to receive either MV-CHIK or the licensed MMR vaccine in a 4:1 ratio.

OBJECTIVES:
Primary: To determine the safety of MV-CHIK administered in 2 doses separated by 28 days in previously exposed versus unexposed individuals.
Secondary: To determine the immunogenicity of MV-CHIK administered in 2 doses separated by 28 days in previously exposed versus unexposed individuals, by a neutralization assay.
Exploratory: To quantify measles viremia from both the investigational and the comparator vaccine and relate this to baseline measles antibody titers and the serologic response to chikungunya virus.

ENDPOINTS:
Primary: Incidence of solicited and unsolicited adverse events and incidence of grade 2 and higher solicited and unsolicited adverse events including clinically significant abnormal safety laboratory results, vital signs, and physical examination findings in previously exposed versus unexposed individuals.
Secondary: Immunogenicity on Days 0, 28, 56, 168, 280, and at the end of the study measured as geometric mean titer (GMT) of neutralizing antibodies to chikungunya in previously exposed versus unexposed individuals.
Exploratory: Measles virus genome equivalents per milliliter of serum.

POPULATION: The study population will be aged ≥21 to ≤50 years from a chikungunya virus endemic or previously epidemic area. Chikungunya exposure status will be confirmed prior to randomization. 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 will not be excluded. Subjects with acute chikungunya infection will be excluded but may be re-screened after resolution of their symptoms.

PHASE OF DEVELOPMENT: This Phase 2 study of MV-CHIK at one dose level 5×10^5 50% tissue culture infectious dose ($TCID_{50}$) will be the first study in an endemic or previously epidemic area. The study will be conducted in Puerto Rico.

INTERVENTION(S):
MV-CHIK, Dose and Mode of Administration: MV-CHIK is a recombinant live attenuated Schwarz strain measles-vectored vaccine expressing chikungunya virus surface proteins. It is manufactured as a lyophilized powder in a glass vial to which has been added D-sorbitol, sucrose, sodium phosphate, sodium chloride, hydrolyzed gelatin, and recombinant human serum albumin (HSA). The dose of MV-CHIK will be 5×10^5 $TCID_{50}$ per dose and will be given by intramuscular (IM) injection.
Comparator Vaccine, Dose and Mode of Administration: M-M-R® II (measles, mumps, and rubella virus vaccine live, [Merck and Co., Inc. 2015 product information]) is a sterile lyophilized preparation of the following:

(1) ATTENUVAX® (Measles Virus Vaccine Live), a measles virus strain derived from Enders' attenuated Edmonston strain and propagated in chick embryo cell culture; (2) MUMPSVAX® (Mumps Virus Vaccine Live), the Jeryl Lynn™ (B level) strain of mumps virus propagated in chick embryo cell culture; (3) MERUVAX® II (Rubella Virus Vaccine Live), the Wistar RA 27/3 strain of live attenuated rubella virus propagated in WI-38 human diploid lung fibroblasts.

M-M-R® II is reconstituted and each 0.5-mL dose contains not less than 1,000 $TCID_{50}$ of measles virus; 12,500 $TCID_{50}$ of mumps virus; and 1,000 $TCID_{50}$ of rubella virus. Each dose of the vaccine is calculated to contain

sorbitol (14.5 mg), sodium phosphate, sucrose (1.9 mg), sodium chloride, hydrolyzed gelatin (14.5 mg), recombinant human albumin (≤0.3 mg), fetal bovine serum (<1 ppm), other buffer and media ingredients, and approximately 25 mcg of neomycin. The product contains no preservative. The MMR vaccine will be given subcutaneously (SC). **Masking of Intervention:** For this Phase 2 study, one MV-CHIK dose level of 5×10^5 $TCID_{50}$ will be studied with MMR as the comparator. MV-CHIK will be studied in 40 seropositive subjects and 40 seronegative subjects, and as comparators, there will be 20 subjects (10 from the seropositive group and 10 from the seronegative group) randomized to receive MMR. Subjects randomized to MV-CHIK will receive the intervention IM (deltoid) on study Day 0 and Day 28 (SC dummy injection of sterile saline will be administered in the opposite arm on Day 0). Subjects randomized to MMR will receive intervention SC on study Day 0 (an IM dummy injection of sterile saline will be administered in the opposite arm on Day 0 and again on Day 28). Dummy injections are added to the design to maintain the blind.
STUDY PERIOD: The estimated date of last subject completed will be 24 months after study start. The recruitment period is defined as the date of Institutional Review Board (IRB) approval to the date of the initial randomization of the last subject. The maximum number of subjects to be randomized is 100. Subjects will be followed for 13 months after receiving their first vaccination. The estimated date of study completion is 24 months after study start.
DURATION: Subjects will be followed for safety and immunogenicity for one year after completing the series at the investigational site(s). Subjects will complete all study visits within approximately 14 months.
EVALUATIONS: **Safety Variables:** Spontaneous and solicited adverse events (AEs), serious adverse events (SAEs), and adverse events of special interest (AESIs). **Immunogenicity Variable:** Neutralization titers (chikungunya). **Exploratory Variable:** Measles genome equivalents per milliliter.

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4. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Text
AE	adverse event
AESI	adverse event of special interest
BMI	body mass index
BSL	biosafety level
CBER	Center for Biologics Evaluation and Research
CD	cluster of differentiation
CDMS	Clinical Data Management System
CFR	Case Fatality Ratio
CFR	Code of Federal Regulations
CIRO	Clinical Investigations Regulatory Office
CMI	Conceptual MindWorks Incorporated
CRA	clinical research associate
CRF	case report form
CRO	Contract Research Organization
CSDG	Clinical System Design Guide
eTMF	electronic Trial Master File
DMID	Division of Microbiology and Infectious Diseases
DoD	Department of Defense
eCRF	electronic case report form
EDC	electronic data capture
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GmbH	Gesellschaft mit beschränkter Haftung (limited liability company)
GMP	Good Manufacturing Practice
GMT	geometric mean titer
HBsAg	hepatitis B surface antigen
HCG	human chorionic gonadotrophin
HCV	hepatitis C virus
HEENT	head, eyes, ears, nose, and throat
HHS	Health and Human Services (US Department of)

Abbreviation	Text
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HRPO	Human Research Protection Office
HSA	Human Serum Albumin
ICF	informed consent form
ICH	International Council for Harmonisation
IFA	Immunofluorescence assay
IFNAR	interferon alpha/beta receptor knockout
IgG	immunoglobulin G
IM	intramuscular(ly)
IND	investigational new drug
IRB	Institutional Review Board
ITT	intent-to-treat
IVP	investigational vaccine product
IWRS	Interactive Web Response System
MedDRA	Medical Dictionary for Regulatory Activities
MMR	Measles, Mumps, and Rubella (vaccine)
MNt	microneutralization (assay)
MRMC	Medical Research and Materiel Command
MV-CHIK	measles-vectored chikungunya vaccine (the name of the experimental vaccine)
NIH	National Institutes of Health
NLM	National Library of Medicine
OHRP	Office for Human Research Protections
ORP	Office of Research Protections
PAHO	Pan American Health Organization
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PI	Principal Investigator
PP	per protocol
PRA	PRA Health Sciences
PRNT	plaque reduction neutralization test
QA	quality assurance
RNA	ribonucleic acid

Abbreviation	Text
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SLE	systemic lupus erythematosus
SOC	system organ class
SOP	Standard Operating Procedure
SUSAR	suspected unexpected serious adverse reaction
$TCID_{50}$	(median) tissue culture infective dose 50% (the amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated)
UPIRTSO	unanticipated problem involving risk to subjects or others
US/USA	United States/United Sates of America
USAMRMC	United States Army Medical Research and Materiel Command
VLP	virus-like particle
WBC	white blood cell
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

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), for example abstinence, combined or progestogen oral contraceptives, injectable progestogen, implants of levonorgestrel, oestrogenic 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.

Abbreviation	Text
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.

5. ETHICS

5.1 Ethics Committee

This study will be conducted in compliance with Institutional Review Board (IRB) and International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines - including Title 21 Part 56 of the United States of America (USA) Code of Federal Regulations (CFR) relating to IRBs and GCP as described in the United States Food and Drug Administration (FDA) CFR (21 CFR § 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.

5.1.1 Institutional Review Board

Before initiating this trial, the Investigator must have written and dated approval from the IRB for the study protocol, written 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 comply with GCP requirements and must identify the documents and versions reviewed.

5.1.2 The U.S. Army Human Research Protection Office

All United States Army Medical Research and Material Command (USAMRMC)-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 Sponsor is responsible for HRPO submission and must ensure that documentation is completed. See Section 16.5 for trial conduct requirements.

5.2 Ethical Conduct of the Study

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

5.3 Subject Information and 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 prior to the subject entering the study and before initiation of any study-related procedure (to include screening and administration of the investigational vaccine product).

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.

6. STUDY ADMINISTRATIVE STRUCTURE

The study governance and role of the study team is described. No steering committee or data monitoring committee will be used.

Sponsor
Themis Bioscience GmbH
Muthgasse 11/2
1190 Vienna
Austria

- Function: Vaccine development and manufacturing, fulfillment of Sponsor responsibilities as described in 21 CFR 312.50.

Walter Reed Army Institute of Research - Viral Diseases Branch
503 Robert Grant Avenue, Room 3A12
Silver Spring, Maryland

- Function: Funder's representative, study design and oversight, exploratory analyses of measles viremia; future studies of humoral and cellular immune responses to MV-CHIK and Measles, Mumps, and Rubella (MMR) vaccine.

The Human Research Protection Office (HRPO)
1101 Wootton Parkway, Suite 200
Rockville, Maryland 20852

- Function: Human subject's protection review and approval, ensuring adherence to ethical standards in DoD-supported research in accordance with DoD Instruction 3216.02.

Clinical Research Organization (CRO)
PRA Health Sciences
4130 Parklake Avenue, Suite 400
Raleigh, North Carolina 27612
United States
Tel: +1 (919) 786-8200

- Function: Medical Monitoring, Clinical Trial Management, Data Management, Serious Adverse Event Reporting, Statistical Analysis, and serving as the Sponsor's agent to the FDA.

PRA Medical Monitor (see PRA Health Sciences Medical Monitoring Plan)
Medical Monitor: Susie Sargent, MD
Medical Director
PRA Health Sciences
4130 Parklake Avenue, Suite 400
Raleigh, North Carolina 27612
United States
Tel: +1 (434) 951-4082 or +1 (866) 326-5053
Email: MVCHIK@prahs.com (Study-Specific Medical Support Center)

- Function: Serve as the central point of contact for scientific, medical, and protocol questions from the investigator(s) and site(s).

Research Monitor
Hermes Garcia, MD, MPH
P.O. Box 457
Gurabo, Puerto Rico, 00778-0457

- The Research Monitor will be a physician independent of the Sponsor and investigative site.

Serious Adverse Event Reporting
PRA Drug (and Vaccine) Safety Center
Email: CHOSafety@prahs.com (complete the Safety Report Form)
Fax: 1-888-772-6919 or 1-434-951-3482
Telephone: 1-800-772-2215 or 1-434-951-3489

Almac Clinical Services, Ltd.
Almac Clinical Services (Regional Site for Trial)
4204 Technology Drive
Durham, North Carolina 27704
Tel: +1 (919) 479-8850

- Function: clinical study supply management (vaccine storage and labeling)

BioClinica, Inc.
800 Adams Avenue
Audubon, Pennsylvania 19403
Tel: +1 (484) 928-6000

- Function: The interactive web response system (IWRS) will be used for screening and randomization

Conceptual MindWorks, Inc. (CMI)
9830 Colonnade Blvd.
San Antonio, Texas
Tel: +1 (210) 737-077

- Function: Independent third-party quality assurance (QA) of the data.

Clinical Laboratories:

Eurofins Central Laboratory, LLC
2430 New Holland Pike
Lancaster, Pennsylvania 17601
Tel: +1 (866) 633-4638

- Function: Supply lab kits for blood collection

Safety Laboratory and Baseline Serology Testing:

- The investigative site will subcontract with a commercial laboratory for safety laboratory tests and baseline serology (chikungunya virus serostatus).

Immunogenicity Laboratory Testing:

- Immunogenicity samples will be sent for testing to the central laboratory selected by the Sponsor to test for neutralizing antibodies to the chikungunya vaccine post-injection.

7. INTRODUCTION

7.1 Disease Review

Chikungunya virus, a mosquito-borne pathogen that causes chikungunya fever, has been spreading dramatically throughout tropical areas worldwide [Enserik 2008; Rezza 2007; Wang 2008]. Recent large outbreaks have been reported in parts of Asia (mainly southeast) and Latin America. Due to global travel and wide distribution of suitable mosquito vector species, the risk for spreading to temperate regions has increased [Jain 2008] with local transmission reported in Europe (Italy in 2007, France in 2010 and 2014) and the continental United States (Florida in 2014, Texas in 2015). Puerto Rico experienced a dramatic outbreak in 2014 with over 35,000 suspected and confirmed cases reported. Incidence has since dropped off significantly with only 177 confirmed cases in 2016 and fewer than 10 confirmed cases so far in 2017 [Pan American Health Organization (PAHO) Website].

Chikungunya virus is transmitted to humans primarily by the mosquitoes *Aedes aegypti* [Tsetsarkin 2007] and *Aedes albopictus*, which are common in many tropical and non-tropical urban areas [Vazeille 2007]. Transmission of the virus occurs predominantly in an urban cycle whereby the mosquito spreads the disease from human to human following an epidemiological pattern similar to dengue [Jain 2008]. Vertical transmission of chikungunya virus has also been described from mothers who were viremic at the time of delivery [Gerardin 2008].

Most individuals present with symptomatic disease after an incubation period of 2 to 12 days, although not all individuals infected with the virus will develop symptoms. However, individuals acutely infected with chikungunya virus, whether clinically apparent or asymptomatic, can contribute to the transmission of the disease if active vectors are present.

Acute disease is most often characterized by the acute onset of high fever (>102°F/39°C). The fever typically lasts from several days up to 2 weeks. Other signs and symptoms may include headache, diffuse back pain, myalgias, nausea, vomiting, polyarthritis, rash, and conjunctivitis. After the onset of fever, the majority of infected persons develop severe, often debilitating polyarthralgias. Joint symptoms are usually symmetric and occur most commonly in wrists, elbows, fingers, knees, and ankles but also more proximal joints. The lower extremity arthralgias can be severely disabling, resulting in a slow, broad based, halting gait that can persist for months. The patients may suffer from severe pain, tenderness, swelling, and stiffness, and they cannot perform normal tasks or go to work. Acute symptoms of chikungunya fever typically resolve within 7 to 10 days. Following the acute phase, a number of patients continue to experience prolonged symptoms, lasting several weeks to months, including fatigue, incapacitating joint pain, and tenosynovitis or edematous polyarthritis. Chronic disease is defined by symptoms that persist for more than 3 months [CDC/PAHO Chikungunya virus, 2011].

During the La Réunion epidemic, a number of unusual clinical presentations were observed, including hepatitis, autoimmune neurologic pathologies (Guillain-Barré), cardiologic

manifestations, and death. The case fatality ratio (CFR) during the 2006 La Réunion epidemic has been estimated to be 1:1000 (0.1%), with most deaths occurring in neonates, adults with underlying medical conditions and older people (>65 years) [Staples 2009]. Of greater public health significance is the risk of chronic disease and disability, which occurs in up to 50% of those infected [LaBeaud 2011]. This is of particular concern to the military, not only because of the potential impact on operations but because of the severe burden it can impose on military and veteran healthcare systems [Marimoutou 2012].

7.2 Investigational Vaccine Review

The MV-CHIK vaccine is a recombinant, live attenuated virus vaccine in which chikungunya structural genes have been inserted into a measles vaccine strain backbone [Brandler 2013]. Replication and virus-like particle (VLP) production occur after immunization. The advantage of the VLP antigen 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. The advantages of using a measles vector is that the live virus presumably elicits enduring cellular and humoral immune responses, and the methods for producing and up-scaling the vaccine are well established. See the Investigator Brochure for full discussion.

MV-CHIK will be presented as a lyophilized powder in a glass vial. The following excipients are added: D-sorbitol, sucrose, sodium phosphate, sodium chloride, hydrolyzed gelatin, recombinant human serum albumin (HAS). No adjuvants are added.

7.3 Preclinical Studies

7.3.1 Immunogenicity Studies

Preclinical evaluation of the MV-CHIK vaccine is described by Brandler et al [Brandler 2013].

In CD46-IFNAR mice, which express the human measles virus receptor, CD46, and are deficient in the interferon alpha/beta receptor and therefore susceptible to measles virus infection, the 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, demonstrating a strong protective capacity in this experimental model. Passive transfer of immune sera to highly susceptible interferon alpha/beta receptor knockout (IFNAR) mice conferred protection from lethal chikungunya virus challenge. Importantly, pre-existing immunity to measles virus did not impair the protective capacity of MV-CHIK. The vaccine also conferred protection against homologous and heterologous circulating strains of chikungunya virus.

Immunogenicity was also demonstrated in immunocompetent animals (non-human primates). Cynomolgus macaques were immunized twice with MV-CHIK at 2 doses. Neutralizing antibodies, as determined by a plaque reduction neutralization test (PRNT), were induced by one or 2 immunizations.

7.3.2 Preclinical Toxicity

Shedding Profile

The MV-CHIK vaccine infects host cells, leading to transcription and secretion of chikungunya VLPs. A biodistribution and shedding study for MV-CHIK was performed and is outlined in the Investigator's Brochure, Section 4.2.1. The shedding of the recombinant measles virus will be analyzed in more detail as part of a Phase 2 clinical trial being conducted in Germany and Austria.

A non-human primate (cynomolgus monkey) biodistribution and shedding study will be conducted prior to Phase 3. However, based on the data generated with a measles-vectored human immunodeficiency virus (HIV) vaccine candidate [Lorin 2012] and additional data generated on recombinant measles vaccines used in cancer therapy [Msaouel 2013], the potential to be shed into the environment is presumably very low.

Shedding of measles virus genomes was not observed in these studies.

The biodistribution and shedding assessment of the measles-vectored HIV vaccine, MV-1 F4, revealed measles ribonucleic acid (RNA) primarily in the draining (iliac) lymph nodes of virtually all vaccinated animals and occasionally in a few other tissues (mandibular lymph node, tongue, popliteal lymph node and cervical lymph node). This was also seen with the unvectored parent Schwarz strain. All shedding levels were found to be at or below limit of detection ($\leq$ 100 genome equivalents per reaction). Thus, no recombinant virus was released into the environment.

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.

7.4 Clinical Studies

MV-CHIK has been evaluated in one completed study. In addition, including this study, there are 3 ongoing clinical trials of MV-CHIK. Safety data from all 3 ongoing studies will be shared in real time with the Sponsor and communicated to the clinical trial sites to ensure that any safety signals that emerge are promptly addressed.

7.4.1 Study MV-CHIK-101

One Phase 1 study has been completed: MV-CHIK-101 [Ramsauer 2015]. This first-in-human trial was conducted in 42 healthy adults (aged 30.5±7.3 years) in Austria.

The vaccine induced neutralizing anti-chikungunya virus antibodies even in the presence of pre-existing anti-vector immunity in all treatment cohorts. The medium- and high-dose

groups induced similar levels of neutralizing antibodies, which were significantly higher than the low-dose group 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. The measles antibody titers were boosted in all dose groups after the first immunization. Overall, MV-CHIK induced a robust anti-chikungunya virus immune response. No immunological correlate of protection was established, and the protective titer is still unknown. However, the highest dose group induced the most persistent titer.

MV-CHIK had an acceptable safety and tolerability profile. 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 accompanied by flu-like symptoms and was self-limited. The most frequent related AEs were injection site pain (18/36 [5.0%]), headache (12/36 [33.3%]), and fatigue (10/36 [27.8%]).

Seven severe AEs occurred in 6 subjects who received MV-CHIK. One case of severe injection site induration and one case of severe erythema of the injection site 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 9 volunteers who received the highest dose, all reported significant pain, and 3 experienced vasovagal syncope. After decreasing the volume of injection by splitting it into 2 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 will be administered in a lower volume (0.3 mL).

Three cases of severe headache after vaccination were reported; one was rated as probably related, and the other 2 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 who had pre-existing alcohol dependence and depression attempted suicide and met the seriousness criterion “hospitalization” (severe and not related), which led to an early termination. One other subject experienced a serious adverse event (SAE), a meniscus injury (moderate severity and not related).

All AEs were recovered without any sequelae by study end.

7.4.2 Study MV-CHIK-202

The MV-CHIK-202 Phase 2 study of MV-CHIK is being conducted at four sites across Austria and Germany. It is comparing 2 doses (5×10^4 and 5×10^5 $TCID_{50}$) of MV-CHIK administered at two different intervals (1 month and 6 months). Each of these four cohorts will have 70 consented subjects of which 60 will receive MV-CHIK and 10 will receive a licensed MMR vaccine as a comparator. In addition, two cohorts of 20 consented subjects

each will receive MMR either one month or 6 months before MV-CHIK to assess the effect of recently boosted immunity to measles on chikungunya immunogenicity.
This study has enrolled about half of the planned total of 320 subjects. Thus far, no significant safety issues have been reported. Immunogenicity data is not yet available.

7.4.3 Division of Microbiology and Infectious Diseases Protocol 15-0038

Protocol 15-0038, a clinical trial of MV-CHIK, is sponsored by the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Disease (NIAID), and National Institutes of Health (NIH). This study is comparing 2 doses of MV-CHIK (5×10^4 and 5×10^5 $TCID_{50}$) given at three different intervals (1 month, 3 months, and 6 months). Each of these six cohorts will contain 30 consented subjects of which 25 will receive MV-CHIK and 5 will receive saline placebo.

This study is just starting, and no preliminary results are available.

7.5 Potential Benefit

The potential benefit of MV-CHIK vaccination for those who have not previously been infected would be life-long protection against chikungunya infection if the vaccine is proven to be efficacious. A boost in measles immunity may be an additional benefit.

7.6 Potential Risks

General

Potential risks that are frequently associated with vaccination are the occurrence of local reactions such as edema, induration and erythema, transient local pain or tenderness at the injection site as well as mild to moderate headache, myalgia, flu-like symptoms, or fatigue. Vaccines have been known to induce allergic and anaphylactic reactions apart from reactions at the vaccination site and systemic flu-like reactions.

MV-CHIK Related

As described above, AEs previously reported with MV-CHIK include injection site pain, headache, fatigue, and joint pain or arthralgia. Refer to the current version of the Investigator's Brochure for more detail.

Potential for Severe Adverse Reaction

In the past, a live virus vaccine against chikungunya virus, which was based on an attenuated chikungunya-strain was associated with mild, transient arthralgia in immunized individuals. The MV-CHIK vaccine is based on a live measles virus and contains no live chikungunya virus. Thus, induction of chikungunya-like symptoms is not expected. However, the subjects will be monitored for occurrence of arthralgia, an adverse event of special interest (AESI). See Section 11.6 for testing of acute phase reactants to assess the inflammatory nature of any arthralgias that are reported.

7.7 Benefit:Risk Summary

The potential for serious adverse reaction is minimal and the benefit: risk ratio is considered favorable.

7.8 Clinical Study Rationale

The rationale for this study is to collect data on vaccine performance in a recently epidemic area to demonstrate safety and immunogenicity in previously exposed individuals. Such persons will inevitably receive the vaccine when field efficacy studies are conducted, and the vaccine is ultimately licensed for use in areas of ongoing transmission. Because some clinical manifestations of chikungunya may be immunologically mediated, there is a chance that a vaccine containing chikungunya antigens may re-activate an immunopathologic response.

8. STUDY OBJECTIVES AND STUDY ENDPOINTS

The study objectives are as follows:

8.1 Primary Study Objective

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

8.2 Secondary Study Objective

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

8.3 Exploratory Objective

- To quantify measles viremia from both the investigational and the comparator vaccine and relate it to baseline measles antibody titers and the serologic response to chikungunya virus.

This study will measure the following endpoints:

8.4 Primary Endpoint

- Incidence of solicited and unsolicited AEs and incidence of grade 2 and higher solicited and unsolicited AEs including clinically significant abnormal safety laboratory results, vital signs, and physical examination findings in previously exposed versus unexposed individuals.

8.5 Secondary Endpoint

- Immunogenicity on Days 0, 28, 56, 168, 280, and at the end of the study measured as geometric mean titer (GMT) of neutralizing antibodies to chikungunya.

8.6 Exploratory Endpoint

- Measles virus genome equivalents per milliliter of serum.

9. INVESTIGATIONAL PLAN

9.1 Overall Study Design and Plan

This will be a prospective randomized double-blind interventional clinical study to evaluate the safety and immunogenicity of 2 doses of MV-CHIK, a live attenuated recombinant measles virus-vectored chikungunya vaccine, delivered 28 days apart compared with one dose of an active MMR comparator in adults 21-50 years of age.

Consented study subjects will be screened for baseline seropositivity to chikungunya virus, with or without a clinical history of chikungunya infection and cohorted openly based on serostatus. They will then be randomized to receive either MV-CHIK (the experimental vaccine) or the licensed MMR (the comparator) in a blinded fashion in a 4:1 ratio.

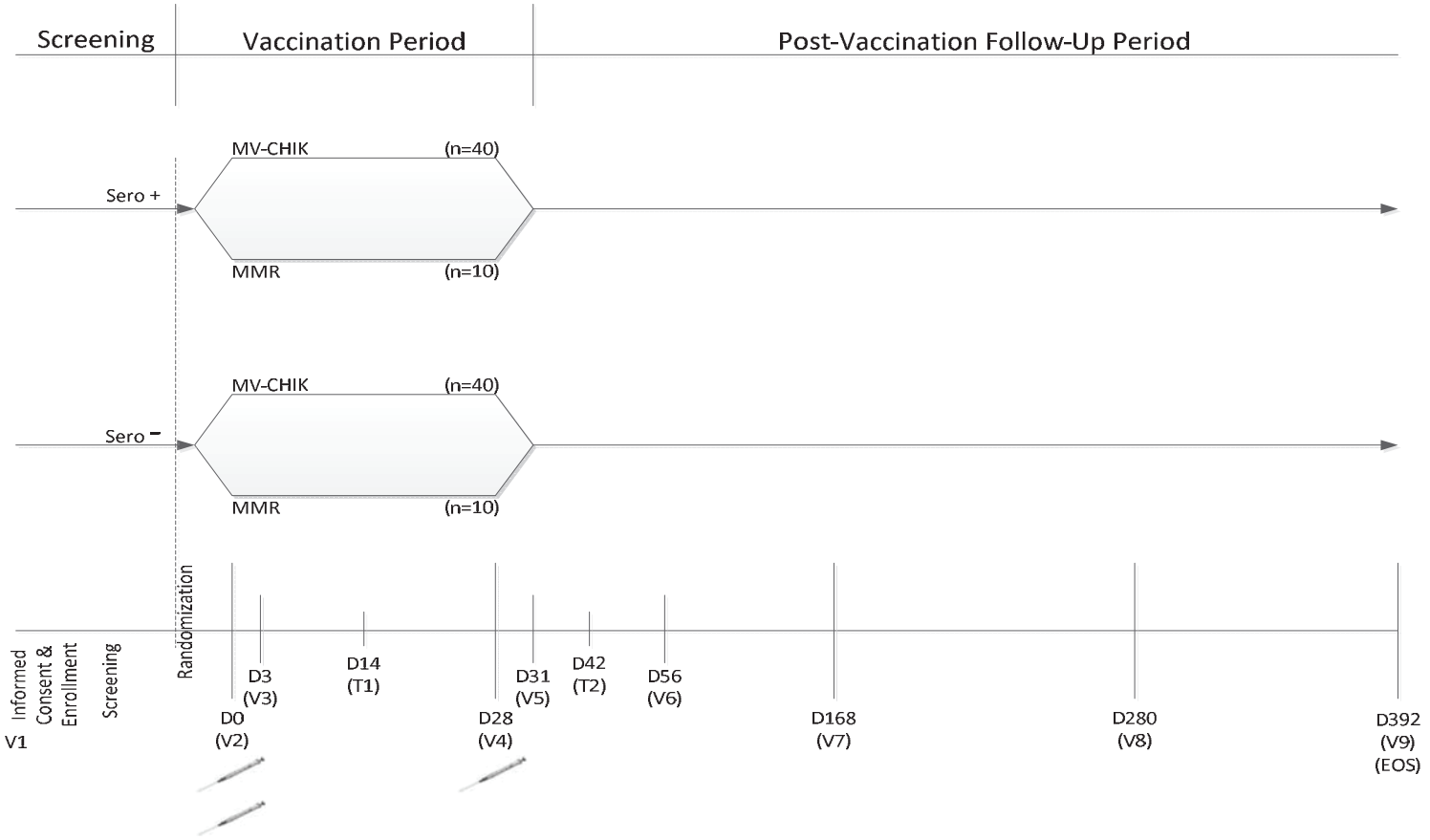
This investigational chikungunya vaccine uses Themis's proprietary Themaxyn® platform. It uses the Schwarz measles vaccine strain as a vector into which the gene for the structural polyprotein of chikungunya virus (La Réunion strain 06-49) has been inserted. The recombinant virus infects host cells, producing and secreting VLP that present the structural antigens directly to macrophages and dendritic cells, eliciting a corresponding immune response. Since this is a replicating vector vaccine, the antigens continue to be expressed after immunization.

The plan is to randomize 50 subjects in the seropositive cohort to either MV-CHIK (40 subjects) or MMR (10 subjects) and 50 subjects in the seronegative cohort to either MV-CHIK (40 subjects) or MMR (10 subjects). One dose level (5×10^5 $TCID_{50}$) of MV-CHIK will be studied for this Phase 2 design. Forty subjects in each cohort will be vaccinated with MV-CHIK IM (deltoid) on study Day 0 and Day 28 (an SC dummy injection will be administered in the opposite arm on Day 0). Ten subjects in each cohort will be vaccinated with MMR SC on study Day 0 (an IM dummy injection will be administered in the opposite arm on Day 0 and again on Day 28). Dummy injections are added to the design for the purpose of maintaining double-blind status. Subjects will be followed for safety and immunogenicity for one year after completing the series at the investigational site(s). The Study Design Schematic is displayed in Figure 1.

The study will be conducted in Puerto Rico, and individuals will be screened until 100 subjects are vaccinated (estimation: 300 to 500 subjects assuming 23.5% seroprevalence for chikungunya exposure based on blood donations in the San Juan area of Puerto Rico) [Simmons 2016].

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

Figure 1. Study Design Schematic



Abbreviations: D, day; EOS, End of Study; MMR, measles, mumps, and rubella vaccine, MV-CHIK, measles-vectored chikungunya vaccine; Sero, serostatus; V, visit; T, telephone call.

Note 1: Subjects will be tested at screening for chikungunya serostatus, and results will be provided to the IWRS (Interactive Web Response System) prior to randomization. Note 2: Vaccination days are shown with the syringe icons (Day 0 and Day 28). On Day 0 the MV -CHIK group and the MMR group will each receive one vaccination and 1 dummy injection to maintain the blind. On Day 28, only the MV-CHIK group will receive a second vaccination; the MMR group will receive a dummy injection to maintain the blind.

9.2 Discussion of Study Design

This study is a randomized, double-blind prospective interventional study with the MV-CHIK as the experimental vaccine and MMR as a comparator. The 1-year follow-up vaccination period will allow assessment of the durability of the immune response.

The MMR vaccine is used as a comparator to assess the proportion of AEs that is attributable to live attenuated virus vaccination versus exposure or re-exposure to chikungunya antigens. In addition, future studies of the immune response can explore how the cellular immune response to measles in the vaccine compares with that to measles vaccine virus without the chikungunya structural polyprotein gene. The baseline measles titer will be used to determine whether pre-existing immunity to the vaccine vector interferes with the vaccine immunogenicity.

The study sample size will provide a preliminary assessment of safety; however, the immunogenicity evaluation is not powered for statistical comparisons, and this secondary endpoint will be descriptive in nature.

The Investigator and site personnel assessing AEs, as well as the Sponsor and their representatives involved in the monitoring and conduct of the study, and the subjects will all be blinded to which vaccine was administered.

9.3 Study Duration

The recruitment period will be defined as from the date of IRB approval to the date on which the last subject is randomized. Recruitment will stop when 100 subjects have been vaccinated.

The maximum number of subjects to be randomized is 100. Subjects will be followed for 13 months after completing the series.

The end of study (EOS) will be defined as when the last subject visit is conducted. The last subject visit is either Visit 9 (EOS) of the last ongoing subject vaccinated or the last unscheduled follow-up visit of the last subject. The study will then be stopped.

The estimated date of study completion is 24 months after study start.

9.4 Study Population

The study population will be from a chikungunya virus endemic or previously epidemic area. Chikungunya exposure status will be confirmed prior to enrollment. 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 will not be excluded. Subjects with acute chikungunya infection will be excluded but may be re-screened after resolution of their symptoms. Subjects cannot be randomized before all inclusion and no exclusion criteria,

including test results, are met. Subjects who fail to meet criteria because of an identifiable temporary condition or who cannot be randomized within the 30-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. Because rheumatologic symptoms of chikungunya infection persist longer in elderly patients, this study will be limited to individuals 50 years of age and younger.

9.4.1 Inclusion Criteria

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

1. Aged ≥21 to ≤50 years on the day of enrollment.
2. Able to provide informed consent.
3. Available and accessible for the duration of the trial.
4. Able and willing to comply with all requirements of the study.
5. For women of childbearing potential, willing to practice a reliable form of contraception as specified in the protocol (see Definition of Terms) until 5 months after the second and final vaccination in accordance with recommendations following MMR vaccination.
6. Medical history and physical examination findings are considered normal or not clinically significant in the opinion of the Investigator.
7. 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.
8. History of previous measles vaccination, either in childhood or as an adult if more than 3 months before participation in this study.

9.4.2 Exclusion Criteria

If any of the following apply, the subject **MUST NOT** enter the study:

1. Taking medication or other treatment for unresolved symptoms attributed to a previous chikungunya virus infection.
2. Prior receipt of any chikungunya or other alphavirus vaccine.
3. Recent infection, including suspected chikungunya (within 1 week prior to Screening Visit).
4. History of an allergic or anaphylactic reaction to any vaccine.
5. An allergic reaction other than allergic contact dermatitis to any component of either vaccine (i.e., neomycin, gelatin), or a current egg allergy. Volunteers with a

childhood history of egg allergy who are able to tolerate egg in their diet now will not be excluded on this basis.

6. History of an immunosuppressive disorder (such as HIV infection, common variable immunodeficiency), 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.
7. History of moderate or severe non-traumatic arthritis or arthralgia within 3 months of the Screening Visit.
8. Recent (within 30 days), current, or anticipated use of any immunosuppressive or immune modifying medication including corticosteroids (excluding nasal, ophthalmic, and other topical preparations).
9. Other vaccination or planned vaccination within 4 weeks of either study dose (seasonal influenza vaccine excepted).
10. Measles vaccination or booster within the last 3 months or planned during the clinical study.
11. Receipt or planned receipt of blood products including immunoglobulins within 120 days of the Screening Visit.
12. Pregnant or lactating or planning pregnancy during the trial.
13. Known alcohol or other substance abuse that in the opinion of the Investigator affects the ability or willingness of the participant to understand and comply with the study protocol.
14. 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 concurrent participation in another clinical study during the study period.
15. Relevant history of any medical condition that, in the opinion of the Investigator, may interfere with the safety of the subject (volunteer) or aims of the study.
16. 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.
17. Behavioral or psychiatric disease or cognitive impairment that in the opinion of the Investigator affects the ability or willingness of the participant to understand and comply with the study protocol.
18. Non-consent to storage of blood specimens for future research.
19. Persons in direct relationship with the Sponsor or its contract service provider, 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 University of

Puerto Rico not directly employed by the Clinical & Translational Research Center will not be excluded.

9.4.3 Withdrawal and Replacement of Subjects

9.4.3.1 Criteria for Subject Withdrawal from the Study

In accordance with the Declaration of Helsinki and other 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 at the institution.

Subjects may also be withdrawn from the study for any of the following reasons:

1. The Investigator decides that the subject should be withdrawn due to any unforeseen circumstance that may affect the safety of the subject or the integrity of the study.
2. The subject is unwilling to continue in the study.
3. Lack of compliance with protocol.
4. The Investigator, PRA Medical Monitor, Research Monitor, or Sponsor, for any reason, stops 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 10.12).

9.4.3.2 Criteria for Discontinuing Individual Subjects from Further Vaccination

The following 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 adverse reaction 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 is discontinued from further vaccination because of an adverse event, appropriate measures to treat the subject will be taken, and the Sponsor or the Sponsor designee will be notified immediately.

9.4.3.3 Evaluations at Withdrawal

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

1. Perform an EOS visit (see Section 10.11). This assessment will 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 visit, telephone contact, 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.

9.4.3.4 Replacement of Subjects

Subjects who are withdrawn after the first vaccination will not be replaced. This will result in a decreased number of subjects in the predefined sample size (see Section 13.2).

9.4.4 Premature Termination or Suspension of the Study

The study may be temporarily suspended or prematurely terminated at any time if there is sufficient reasonable cause, and if agreed to by both the Investigator and the Sponsor as being in the best interests of subjects, and justified on either medical or ethical grounds. In terminating the study, the Sponsor, the CRO, and the Investigator will ensure that adequate consideration is given to the protection of the subjects' interests. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the PI, IRB, and HRPO.

9.4.4.1 Study Stopping Rules

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

- Any subject experiences an SAE that is assessed as at least possibly related to the IVP

- Three or more subjects experience the same or similar grade 3 or greater adverse events or abnormal laboratory tests that are assessed as at least possibly related to the IVP

The type of safety review or criteria required for study resumption will be determined by the Sponsor at the time of study suspension or termination and will require approval by the IRB(s) and regulatory authority.

9.5 MK-CHIK

MV-CHIK is described in **Table 1**.

9.5.1 MV-CHIK

MV-CHIK is a recombinant live Schwarz strain measles-vectored vaccine expressing chikungunya virus structural proteins. It is manufactured as a lyophilized powder in a glass vial to which has been added D-sorbitol, sucrose, sodium phosphate, sodium chloride, hydrolyzed gelatin, and recombinant human serum albumin (HSA).

9.5.1.1 Dose and Mode of Administration

The dose of MV-CHIK will be 5×10^5 $TCID_{50}$ (50% tissue culture infectious dose) per dose. MV-CHIK will be given by IM injection.

9.5.2 MMR Comparator Vaccine

M-M-R® II (measles, mumps, and rubella virus vaccine live, [Merck and Co., Inc. 2015 product information]) is a sterile lyophilized preparation of the following:

- ATTENUVAX® (Measles Virus Vaccine Live), an attenuated strain measles virus, derived from Enders' attenuated Edmonston strain and propagated in chick embryo cell culture;
- MUMPSVAX® (Mumps Virus Vaccine Live), the Jeryl Lynn™ (B level) strain of mumps virus propagated in chick embryo cell culture;
- MERUVAX® II (Rubella Virus Vaccine Live), the Wistar RA 27/3 strain of live attenuated rubella virus propagated in WI-38 human diploid lung fibroblasts.

9.5.2.1 Dose and Mode of Administration

M-M-R® II is reconstituted and each 0.5-mL dose contains not less than 1,000 $TCID_{50}$ of measles virus; 12,500 $TCID_{50}$ of mumps virus; and 1,000 $TCID_{50}$ of rubella virus. The MMR vaccine will be given SC. The Investigator must ensure that this vaccine is used only in accordance with the protocol.

9.5.3 Randomization

Subjects will be openly cohorted based on baseline serostatus to chikungunya virus and then randomized in a double-blind fashion to receive either MV-CHIK or the licensed MMR vaccine in a 4:1 ratio as follows:

- MV-CHIK 5×10^5 $TCID_{50}$ 0.3-mL IM injection on Days 0 and 28 (40 seropositive and 40 seronegative)

OR

- M-M-R® II 0.5-mL SC injection on Day 0 (10 seropositive and 10 seronegative)

See Section 9.5.13 for assignment to study intervention.

Once the subject numbers of one serostatus reach a threshold of approximately 90% or 45 subjects, the Screening Visit may be modified to reduce unnecessary assessments or testing of subjects with the alternate serostatus.

Table 1: Identity of Vaccine Products

Vaccine name	MV-CHIK	M-M-R® II
Manufacturer	Themis Bioscience GmbH	Merck and Co., Inc.
Formulation	Reconstituted lyophilized powder (re-suspend with 0.4 mL sterile water for injection, [a] gently swirl, and withdraw 0.3 mL for injection).	Reconstituted lyophilized preparation with sterile water[b] supplied commercially.
Strength	5×10^5 $TCID_{50}$ per dose	Each 0.5-mL dose contains not less than: 1,000 $TCID_{50}$ of measles virus; 12,500 $TCID_{50}$ of mumps virus; 1,000 $TCID_{50}$ of rubella virus.
Route of administration	Intramuscular injection	Subcutaneous injection
Storage (powder)	-20°C	-50°C to + 8°C; protect from light
Storage (reconstituted)	Diluent must be stored at room temperature (20°C to 25°C or 68°F and 77°F); [excursions between 15°C and 30°C (59°F and 86°F) are permitted][a] The lyophilized drug product will be removed from the -20°C freezer and allowed to warm to room temperature for 5-10 min. The vaccine should be used as soon as possible after reconstitution but not later than 30 min after reconstitution when stored at room temperature or 60 min after reconstitution when stored refrigerated.	Store diluent in refrigerator [b] The vaccine should be used as soon as possible after reconstitution. Store reconstituted vaccine in the vaccine vial in a dark place at 2°C to 8°C and discard if not used within 8 hours.
Batch numbers	TCB 001 04 16	Provided by clinical supply organization

TCID, tissue culture infectious dose
a: MV-CHIK diluent (sterile water for injection) will be supplied by Almac separately from the MV-CHIK;
b: MMR diluent will be supplied with the commercially available supply shipped by Almac.

9.5.4 MV-CHIK Formulation

MV-CHIK was manufactured under responsibility of Themis Bioscience and will be supplied as powder for reconstitution. MV-CHIK was manufactured, quality control checked and released in accordance with good manufacturing practices (GMP) at the facility of Batavia Biosciences (Bioscience Park Leiden, Zernikedreef 16, 2333 CL

Leiden, The Netherlands). The drug substance was shipped to Biofabri, SL (A Relva s/n, Polígono II, 36410 O Porriño (Pontevedra), Spain) and manufacturing, quality control, and release was performed in accordance with GMP. The following excipients are added to ensure stability: D-sorbitol, sucrose, sodium phosphate, sodium chloride, hydrolyzed gelatin, recombinant HSA. The excipients are frequently used in standard vaccine formulations that are approved for use in humans. The hydrolyzed gelatin used is from porcine origin and has been used in approved vaccines including M-M-R® II (Merck). The use of material of porcine origin will be mentioned in the ICFs. 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 vial of lyophilized product will be removed from the -20°C freezer and allowed to stand at room temperature for 5-10 min and then will be reconstituted with a total of 400 µL injection grade sterile water (not provided) by injecting into the vials and then 300 µL will be removed with a syringe and injected intramuscularly in the deltoid.

9.5.5 Comparator Vaccine

M-M-R® II is manufactured by Merck and Co., Inc., and is commercially available. The package insert is available at: http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/Approved Products /UCM123789.pdf

M-M-R® II is reconstituted, and each 0.5-mL dose contains not less than 1,000 $TCID_{50}$ of measles virus; 12,500 $TCID_{50}$ of mumps virus; and 1,000 $TCID_{50}$ of rubella virus.

Each dose of the vaccine is calculated to contain sorbitol (14.5 mg), sodium phosphate, sucrose (1.9 mg), sodium chloride, hydrolyzed gelatin (14.5 mg), recombinant human albumin (≤0.3 mg), fetal bovine serum (<1 ppm), other buffer and media ingredients, and approximately 25 mcg of neomycin. The product contains no preservative.

Before reconstitution, the lyophilized vaccine will be stored at 36°F to 46°F (2°C to 8°C). The vaccine will be used as soon as possible after reconstitution. The reconstituted vaccine in the vaccine vial will be stored in a dark place at 36°F to 46°F (2°C to 8°C) and discarded if not used within 8 hours.

9.5.6 MV-CHIK Labeling and Packaging

Both MV-CHIK and the comparator vaccine will be supplied as powder in a single-use vial. The labeling of MV-CHIK will include, at least, the following information:

- Product manufacturer name
- Sponsor name
- Vaccine name
- Study identifier
- Dosage form

- Concentration of vaccine
- Lot number
- Route of administration
- Storage conditions
- Expiration date
- Caution: New Vaccine Limited by Federal Law To Investigational Use
- Directions for use: See protocol

Packaging and labeling of investigational vaccine products (IVPs) for clinical trial use will be performed by Almac Clinical Services. The MV-CHIK vaccine will be shipped as hazardous and labeled as live attenuated measles vaccine expressing foreign antigens on dry ice. The biosafety level (BSL) / Risk Group will be 2.

The MMR vaccine will be supplied in the standard commercial label.

9.5.7 Blinding of Study Vaccines

Dummy injections of sterile saline are added to the design for the purpose of maintaining double-blind status due to the inability to mask the mode of administration.

Subjects randomized to the MV-CHIK group will be vaccinated by IM (deltoid) injection on study Day 0 and Day 28. To maintain the blind, a subcutaneous (SC) dummy injection will be administered in the opposite arm on Day 0, but not on Day 28.

Subjects randomized to the MMR group will be vaccinated by SC (upper arm) injection on study Day 0 only. To maintain the blind, on Day 0 an intramuscular dummy injection will be administered in the opposite arm. On Day 28, the MMR group will receive a dummy injection intramuscularly in order to maintain the blind.

This is a randomized, double-blind, controlled study with limited access to the randomization code. The vaccines will be injected by an unblinded pharmacist or designee who will have minimal interaction with the study subject. The Investigators will not be able to discern the treatments by the appearance of the syringes, and the intervention each subject will receive will not be disclosed to the Investigator, other study site staff, subject, Sponsor, or CRO. The intervention codes will be held according to the IWRS. Refer to the Investigator Site File for further instructions on the IWRS.

For details of the procedure for unblinding of individual subjects see Section 9.5.14.

9.5.8 MV-CHIK Accountability

MV-CHIK material should not be used for purposes other than as defined in this protocol.

9.5.9 MV-CHIK Storage

Lyophilized MV-CHIK will be stored frozen at -20°C and should be used as soon as possible but not later than 30 min after reconstitution when stored at room temperature or 60 min when stored refrigerated.

The comparator vaccine (M-M-R® II) must be stored between -58°F and +46°F (-50°C to +8°C) and, for this study, will be stored between 36°F and 46°F (2°C to 8°C) (see Protocol Section 9.5.5). Dry ice may subject M-M-R® II to temperatures colder than -58°F (-50°C) and should therefore not be used. The vaccine will be protected from light at all times, since such exposure may inactivate the virus. The diluent may be stored in the refrigerator with the lyophilized vaccine or separately at room temperature, but will not be frozen.

9.5.10 MV-CHIK Accountability

All supplies of MV-CHIK and comparator (MMR vaccine) will be accounted for in accordance with GCP. An individual study MV-CHIK 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 comparator) supplies received during the study. These records should include the amounts and dates supplies were received, administered to the subject, or returned to Almac. If errors or damages in the vaccine supply shipments occur, the Investigator should contact the PRA Health Sciences Clinical Trial Management personnel immediately. Copies of the investigational vaccine accountability records will be provided by each Investigator for inclusion in the Trial Master File after database lock. The Clinical Research Associate (CRA) will periodically check the supplies of investigational vaccine held by the Investigator or pharmacist to verify accountability of all investigational vaccine 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, then investigational vaccine and all containers will be returned to Almac for destruction, and documentation will be returned to the Sponsor. The Sponsor or CRO designee will verify that a final report of vaccine product accountability is prepared and maintained in the Investigator's file and the electronic Trial Master File (eTMF).

9.5.11 Prior and Concomitant Therapy

Any treatment that will be considered necessary for the subject's welfare may be given at the discretion of the Investigator. Administration of concomitant medications must be reported in the appropriate section of the eCRF along with dosage information, dates of administration, and reasons for use. Generic names for concomitant medication should be used, if possible. The total daily dose should be filled in whenever possible.

9.5.12 MV-CHIK Compliance

MV-CHIK should be dispensed by the unblinded pharmacist or designee. An up-to-date treatment inventory/dispensing record must be maintained (see Section 9.5.8). Subjects must receive all assigned vaccinations to be considered compliant.

9.5.13 Assignment to Intervention

Subjects will be assigned to the intervention using the IWRS. Accessing a password protected web site, the site personnel will submit subject data and request for randomization code. The IWRS will provide the code to the designated unblinded study pharmacist who will dispense the assigned vaccine to an unblinded vaccinator. A dummy injection (sterile saline for injection) will be administered in the opposite deltoid muscle (for those who received the assigned vaccine SC) or overlying subcutaneous tissue (for those who received the assigned vaccine IM). The subject and all study personnel involved in assessing AEs, including the Investigator and study coordinator, will be blinded to the vaccine assignment.

9.5.14 Unblinding Procedures

During the study, individual subjects' IVP assignments may be unblinded for safety reasons that constitute a medical emergency or expedited safety reporting to the regulatory authority.

Treatment allocation will only be known by the responsible pharmacy and vaccination personnel. An unblinded CRA will be available to assist the site as needed. The Sponsor, the CRO, and the site Principal Investigator (PI) responsible for subject safety and welfare will be unblinded only if necessary for decisions associated with volunteer safety.

The procedure for breaking the blind will be included in site facing documents, and is documented internally via the PRA Health Sciences Work Instruction (PRA 060 W11 B) for 'Unblinding Procedures for Safety Reasons' as outlined in the Medical Management Plan. The PRA Medical Monitor will discuss the safety issue with the Sponsor prior to requesting the unblinding of a subject's IVP assignment. Upon approval, the PI will be granted permission to obtain the subject IVP assignment directly via the IWRS.

Group unblinding during the study will not occur as there is no plan for interim analysis or data monitoring committee.

9.6 Efficacy and Safety Variables

Safety Variables: Spontaneous and solicited AEs, SAEs, AESIs;
Immunogenicity Variable: Neutralization titer (chikungunya);
Exploratory Variable: Measles viremia expressed as genome equivalents per milliliter.

10. STUDY EVALUATIONS BY VISIT

The Schedule of Events (**Table 2**) provides the procedures/assessments to be performed at each scheduled visit for the screening, vaccination, and post-vaccination/follow-up periods. Data to be collected during the telephone contacts are included. Prior to conducting any procedures, the subject will provide informed consent (See Section 5.3).

Table 2: Schedule of Events

Procedure	Screening	Vaccination Period				Post-Vaccination Follow-up Period					
	Day -30 to Day-7	Day 0	Day 3 (±1 day)	Day 14 (±3 days)	Day 28 (±3 days)	Day 31 (3 [±] 1 days post dose 2)	Day 42 (14 [±3] days post dose 2)	Day 56 (28 [±3] days post dose 2)	Day 168 (± 7 days)	Day 280 (± 7 days)	Day 392 (± 10 days) EOS visit
Visit	1	2	3		4	5		6	7	8	9
Telephone				1			2 [a]				
Informed consent	X										
Clinical Assessments											
Medical & medication history	X										
Medication history	X										
Complete physical examination	X										
Brief physical examination		X	X		X	X		X	X	X	X
Vital signs [b]	X	X	X		X	X		X	X	X	X
Dispense diary [c]		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 [d]											
Serology: chikungunya [e], measles, HBsAg, anti-HCV, anti-HIV ½	X										
Complete blood count [f]	X	X	X		X	X		X			
Comprehensive metabolic panel[e]	X							X			
Basic metabolic panel [f]		X	X		X	X					
Urinalysis [f]	X										
Pregnancy test [g]	X	X			X				X		
Neutralizing antibody to chikungunya		X			X			X	X	X	X

Table 2: Schedule of Events

Procedure	Screening	Vaccination Period				Post-Vaccination Follow-up Period					
	Day -30 to Day-7	Day 0	Day 3 (±1 day)	Day 14 (±3 days)	Day 28 (±3 days)	Day 31 (3 [±] 1 days post dose 2)	Day 42 (14 [±3] days post dose 2)	Day 56 (28 [±3] days post dose 2)	Day 168 (± 7 days)	Day 280 (± 7 days)	Day 392 (± 10 days) EOS visit
Visit	1	2	3		4	5		6	7	8	9
Telephone				1			2 [a]				
Sera for measles viremia and/or future immunogenicity studies [h]		X	X		X	X		X	X	X	X
Cells/plasma for future immunogenicity studies [i]		X			X			X	X		X
C-reactive protein		X	X		X	X		X			
Ferritin	X	X			X			X			
Vaccination											
Injection in blinded fashion [j]		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 10.12 for Early Termination Visit procedures/assessments.
Note: See Section 11.5.4 for human leukocyte antigen (HLA) testing for exploratory immunology analysis.
a: The telephone follow-up is not required unless or until after the subject receives dose 2.
b: To include body temperature, pulse rate, systolic and diastolic blood pressure.
c: 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, diarrhea, nausea/vomiting, and joint pain) will be solicited from Day 0 through Day 28. The diary will also include a section for recording unsolicited AEs and concomitant medications.
d: The cumulative total of blood drawn in this study is approximately 375 mL per subject.
e: Confirm chikungunya exposure serostatus using enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) to detect immunoglobulin G (IgG).
f: The complete blood count will include: hemoglobin, hematocrit, white blood cell count and differential, platelets. The comprehensive metabolic panel will include: glucose, calcium, sodium, potassium, carbon dioxide, chloride, urea nitrogen, creatinine, albumin, total protein, and ALP (alkaline phosphatase), ALT (alanine amino transferase), AST (aspartate aminotransferase), bilirubin. The basic metabolic panel will include: glucose, sodium, potassium, carbon dioxide, chloride, urea nitrogen, and creatinine. Screening urinalysis will consider the clinical significance of any glucose, protein, hemoglobin, erythrocytes or leukocytes detected. Acute phase reactants, C-reactive protein and ferritin, will be measured to aid in characterizing, but not defining, AEs.
g: Urine pregnancy testing will be done on women of childbearing potential at Screening and at the subsequent scheduled time points.
h: On sera collection days, a 5-mL blood sample will be collected for neutralization antibody testing. In addition, a 10-mL blood sample will be collected and shipped to the Viral Diseases Branch of the Walter Reed Army Institute of Research (WRAIR) for measles viremia testing and future exploratory analyses. A backup 5-mL tube of sera will be retained at the study site and shipped to Themis at the end of the study. Refer to the Investigator Site File for sample preparation, handling, and shipping instructions.
i: On Day 0, a 60-mL blood sample will be collected for cells, and on other cell collection days, 40 mL will be collected for cells. Cells to be shipped to the Viral Diseases Branch of WRAIR for testing and analysis. Refer to the Investigator Site File for sample preparation, handling, and shipping instructions.
j: On Day 0, the subject will be randomized receive in a blinded fashion either MV-CHIK IM and dummy injection SC, or MMR SC and dummy injection IM depending on the

Table 2: Schedule of Events

Procedure	Screening	Vaccination Period				Post-Vaccination Follow-up Period					
	Day -30 to Day-7	Day 0	Day 3 (±1 day)	Day 14 (±3 days)	Day 28 (±3 days)	Day 31 (3 [±] 1 days post dose 2)	Day 42 (14 [±3] days post dose 2)	Day 56 (28 [±3] days post dose 2)	Day 168 (± 7 days)	Day 280 (± 7 days)	Day 392 (± 10 days) EOS visit
Visit	1	2	3		4	5		6	7	8	9
Telephone				1			2 [a]				

vaccine arm the subject is randomized to. On study Day 28, the subject will receive in a blinded fashion either MV-CHIK IM, or dummy injection IM depending on the study arm (deltoid) randomized. The dummy injection will be sterile saline for injection. See Section 9.5 and Pharmacy Manual for details and administration instructions.

10.1 Screening Visit 1: Day -30 to -7

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

- Medical history
- Complete physical examination and vital signs
- Medication(s) history
- 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)
- Measles serology to determine subject's baseline titer using the IgG test
- Hepatitis B surface antigen (HBsAg), anti-hepatitis C virus (anti-HCV), anti-HIV-1/2 serology
- Complete blood count
- Comprehensive Metabolic Panel
- Urinalysis
- Ferritin
- Pregnancy test (urine, women of child bearing potential only)

10.2 Randomization Visit 2: Day 0

The following assessments/procedures will be performed:

- Pregnancy test (urine, women of childbearing potential only)
- Review of inclusion/exclusion criteria
- Brief physical examination and vital signs
- Interim history
- Concomitant medication review

- Baseline immunogenicity test samples
  - Neutralizing antibody to chikungunya
  - Sera for measles viremia and future immunogenicity analyses
  - Cells/plasma
- Complete blood count
- Basic metabolic panel
- Acute phase reactants (C-reactive protein and ferritin)
- Randomization (via IWRS) to either the MV-CHIK group or the MMR group
- Vaccine administration in a blinded fashion (see Section 9.5.7)
  - MV-CHIK group (receives IM injection of vaccine and 1 SC dummy injection)
  - MMR group (receives SC injection of vaccine and 1 IM dummy injection)
- Monitor for at least 30 minutes post-vaccination
- Dispense diary

10.3 Visit 3: Day 3 (±1 day)

The following assessments/procedures will be performed:

- Brief physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Review diary
- Complete blood count
- Basic metabolic panel
- Sera for measles viremia and future immunogenicity analyses

- Acute phase reactant blood sample (C-reactive protein only)

10.4 Telephone 1: Day 14 (± 3 days)

The following assessments will be evaluated:

- Interim history/adverse events
- Concomitant medication review
- Review diary

10.5 Visit 4: Day 28 (± 3 days)

The following assessments/procedures will be performed:

- Brief physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Collect/review the diary
- Pregnancy test (urine, women of childbearing potential only)
- Review of inclusion/exclusion criteria
- Immunogenicity Test Samples
  - Neutralizing antibody to chikungunya
  - Sera for measles viremia and future immunogenicity analyses
  - Cells/plasma
- Complete blood count
- Basic metabolic panel
- Acute phase reactants (C-reactive protein and ferritin)
- Measles viremia by quantitative polymerase chain reaction (PCR)
- Vaccine administration will be in a blinded fashion (see Section 9.5.7)

- MV-CHIK group (receives IM injection of vaccine)
- MMR group (receives IM dummy injection)

- Monitor for at least 30 minutes post-vaccination
- Dispense second diary

10.6 Visit 5: Day 31 (3 [±1] 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:

- Brief physical examination and vital signs
- Interim history
- Concomitant medication review
- Review the diary
- Complete blood count
- Basic metabolic panel
- Sera for measles viremia and future immunogenicity analyses
- Acute phase reactant (C-reactive protein only)

10.7 Telephone 2: Day 42 (14 [± 3] days post dose 2)

The telephone follow-up is not required unless or until after the subject receives dose 2.

The following assessments will be evaluated:

- Interim history/adverse events
- Concomitant medication review
- Review the diary

10.8 Visit 6: Day 56 (28 [± 3] days post 2nd dose)

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

The following assessments/procedures will be performed:

- Brief physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review
- Review and collect the diary
- Immunogenicity Test Samples
  - Neutralizing antibody to chikungunya
  - Sera for measles viremia and future immunogenicity analyses
  - Cells/plasma
- Complete blood count
- Comprehensive metabolic panel
- Acute phase reactants (C-reactive protein and ferritin)

10.9 Visit 7: Day 168 (± 7 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
  - Cells/plasma

- Pregnancy test (urine, women of childbearing potential only)

10.10 Visit 8: Day 280 (± 7 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

10.11 Visit 9: Day 392 (± 10 days) EOS visit

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
  - Cells/plasma

10.12 Early Termination Visit

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

The following assessments/procedures will be performed:

- Brief physical examination and vital signs
- Interim history/adverse events
- Concomitant medication review

- Pregnancy test (urine, women of childbearing potential only), if termination is prior to Day 168
- Collect and review the diary if termination is prior to Day 56
- Immunogenicity Test Samples
    - Neutralizing antibody to chikungunya
    - Sera for future immunogenicity analyses
    - Cells/plasma
- Complete blood count
- Comprehensive metabolic panel

11. METHODS OF ASSESSMENT

11.1 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 brief 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.

11.2 Vital Signs

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

Systolic and diastolic blood pressure will be assessed by sphygmomanometer measurement after the subject has been in a supine/sitting position for 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 for safety reasons.

11.3 Pregnancy Test

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 passes since 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.

11.4 Concomitant Medications

At each scheduled visit and telephone contact subjects will be asked by the Investigator if he/she has taken any medication since the last visit. All concomitant prescription and non-prescription medication taken by the subject will be recorded in the corresponding eCRF page.

11.5 Laboratory Testing

Venous blood samples will be taken for hematology and chemistry testing by a trained phlebotomist or qualified designee. The following parameters will be determined according to the schedule indicated in **Table 2**:

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

Basic Metabolic Panel: This blood test assesses the following analytes: glucose, sodium, potassium, carbon dioxide, chloride, urea nitrogen, and creatinine.

Comprehensive Metabolic Panel: This blood test assesses the BMP analytes: as well as calcium, albumin, total protein, ALP (alkaline phosphatase), ALT (alanine amino transferase), AST (aspartate aminotransferase), and bilirubin.

Urinalysis (fresh urine clean catch specimen): protein, glucose, hemoglobin, erythrocytes, and leukocytes.

The urinalysis done at the Screening Visit can be repeated twice if necessary to ensure there is no clinically significant underlying medical condition. No other urine samples will be collected.

Further details of the procedures to be followed for sample collection, storage, and shipment will be documented in the Investigator Site File.

Additional and repeat laboratory safety testing may be performed at the discretion of the Investigator.

11.5.1 Baseline Serology Testing

Baseline serology testing will be conducted during the screening process. Blood sampling kits (containing tubes, needles and labels) will be supplied by the CRO supply vendor. Baseline serology testing will be performed by a local commercial laboratory selected by the investigative site.

11.5.1.2 Chikungunya Baseline Serostatus

The local commercial laboratory will test subjects for chikungunya antibodies at baseline. The IgG antibody test will be performed via the EIA or ELISA to determine the subject's pre-vaccination chikungunya serostatus. Serology results that range from equivocal to positive

will be reflexed to the immunofluorescence assay (IFA) method for confirmation. Subjects who are found to be seropositive at screening and who require re-screening do not need to have their chikungunya serology repeated.

Seropositivity will be defined as the presence of baseline chikungunya virus antibodies at a titer of 1:20 (reference range: <1:10) (Source: Quest Diagnostics).

The serostatus results will be entered into the IWRS for randomization procedures by the site personnel.

11.5.1.3 Measles Baseline Serostatus

A blood sample for the IgG antibody test via immunoassay will be sent for analysis to the local laboratory. The baseline measles serostatus result will not be a factor in cohorting to a chikungunya serostatus cohort or randomization to a vaccine study group. The baseline measles titer will support the exploratory analysis of the effect of pre-existing anti-measles immunity on measles viremia and the immunogenicity of the MV-CHIK vaccine. Undetectable measles antibody at screening will not be used to exclude individuals who give a history of having received MMR previously.

11.5.1.4 HBsAg, anti-HCV, anti-HIV 1/2

Baseline screening for HBsAg, anti-HCV, anti-HIV-1/2 will require one blood sample to be sent to the local laboratory for analysis.

11.5.2 Immunogenicity Testing

Subjects will be tested for vaccine-induced neutralizing anti-chikungunya virus antibodies in order to determine seroconversion following vaccination. Immunogenicity samples will be sent for testing (Days 0, 28, 56, 168, 280, and 392) to a central laboratory selected by the Sponsor. The assay to be used for functional chikungunya virus antibody (anti-CHIK antibody) seroconversion testing will be the PRNT or the microneutralization (MNt) assay. Seroconversion for chikungunya using neutralization testing will be defined as a 4-fold or greater increase. A value below a 4-fold increase will be considered non-seroconversion.

11.5.2.1 Sera

On sera collection days a 5-mL blood sample will be collected for neutralization antibody testing. In addition, a 10-mL blood sample will be collected for the Viral Diseases Branch of the Walter Reed Army Institute of Research (WRAIR) for quantification of measles viremia and future exploratory analysis.

A backup tube of sera (5 mL) will be collected for Themis and shipped to WRAIR separately from those tubes collected at the same time that are destined for WRAIR. Procedures for aliquoting, freezing and shipping these samples will be provided in detail in the Investigator Site File. Samples will be logged at the site and a laboratory binder will be maintained to maintain accountability.

11.5.2.2 Cells

On Day 0, a 60-mL blood sample will be collected, and on other cell collection days 40 mL will be collected. The samples will be processed on site in accordance with a standard operating procedure. Isolated peripheral blood mononuclear cells (PBMCs) will be frozen in a stepwise fashion and then kept in liquid nitrogen until and during shipment to WRAIR. Plasma isolated during the process will be frozen at –70° C and shipped to WRAIR on dry ice. Samples will be logged at the site, and a laboratory binder will be used to accomplish this.

Note: Human leukocyte antigen (HLA) testing may be performed on these specimens in the future if deemed useful to analyzing and interpreting the subject's cellular immune response. Subjects will provide consent for possible HLA typing as part of providing consent to participate in the study.

11.5.3 Measles Viremia

Sera for measles viremia testing will be collected at 5 visits during the study: Day 0, 3, 28, 31, and 56. The purpose of the testing is to assess the magnitude of viremia that results from MV-CHIK and from the MMR. Testing will be performed on the serum sample sent to WRAIR using a quantitative PCR.

11.5.4 Future Use of Stored Blood Samples

Subjects will be required to agree to the future use of blood specimens and consent will be obtained.

At the end of the study, the sites will ship any remaining plasma, sera, and cells to the Viral Diseases Branch of WRAIR for exploratory research possibly including HLA testing. The blood samples will be shipped and retained via approved storage techniques, such as coding of samples. For samples stored at WRAIR, protection of subject confidentiality during any future research with the stored specimens will be guaranteed through the following process: the samples will be labeled 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. 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.

11.6 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 components [Javelle, 2015]. Because of this possibility, safety studies of vaccines that contain chikungunya virus components in previously exposed subjects are necessary before large scale efficacy studies or vaccination campaigns can be conducted in endemic regions. 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. Fears of vaccine-induced arthritis resulted in a drop in demand for the Lyme vaccine and its subsequent withdrawal from the market despite the fact that overwhelming evidence has shown this risk to have been negligible [Poland, 2011]. To add clarity and objective data to the assessment of arthralgia reported in this trial, and to help determine if joint symptoms that study participants report in this trial are due to an immunological or inflammatory mechanism, acute phase reactants will be included in the laboratory tests assessed routinely to monitor participant safety.

11.6.1 C-reactive Protein

C-reactive protein (CRP) is a blood biomarker signifying an inflammatory response. 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 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.

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 played 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. The Laboratory Manual provides more detailed information.

11.6.2 Ferritin

Ferritin is a blood cell protein containing iron that increases in inflammatory conditions. Ferritin has also been shown to correlate with viremia as well as chronic arthralgia in chikungunya infection [Anfasa, 2017]. Because so little is known about how ferritin responds to vaccination, abnormal laboratory values will not be considered AEs. However, levels that

are increased or more persistent compared to those of other study subjects may be considered by the investigators as evidence that arthralgia(s) or other inflammatory symptoms are related to vaccination in a manner analogous to natural infection.

Because serum ferritin levels do not change as rapidly as CRP, serum ferritin levels will be obtained less frequently. Blood samples will be obtained by a phlebotomist at screening (as an indicator of iron status and possible risk of developing anemia during the trial) and on Days 0, 28, and 56 and at additional time points in individual study subjects at the discretion of the site investigators. Refer to the Investigator Site File for detailed information.

12. SAFETY MEASUREMENTS AND VARIABLES

12.1 Adverse Events

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

Adverse events will be monitored throughout the entire study. The investigator will 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 392 (±10 days).

If known, the name of the illness should be recorded, in preference to the listing of individual signs or symptoms.

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 generally 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 (Appendix Table A), systemic reactions to injectable product (Appendix Table B), chikungunya-like systemic illness (Appendix Table C), and vital signs (Appendix Table D). The Investigator will assign the grade and document in the eCRF.

Relationship 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, based on natural history of the underlying diseases, 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 investigational vaccine product?"

NO : The AE is not causally related to administration of the study vaccine(s). There are other, more likely causes and administration of the study vaccine(s) is not suspected to have contributed to the AE.

YES : There is at least a reasonable possibility that the study vaccine(s) may be related to the AE.

12.2 Serious Adverse Events

An SAE is any untoward medical occurrence or effect that fulfills the following criteria:

- results in death;
- is life threatening;
- requires hospitalization or prolongation of existing inpatient 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, for example, require medical intervention to prevent one of the preceding outcomes.

Events associated with hospitalization for the following will not be considered as an SAE:

a) Evaluation or treatment of a pre-existing and non-exacerbating condition as long as the condition(s) associated with the hospitalization:

- was 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

b) Treatment that is elective or planned of a pre-existing and non-exacerbating condition.

12.3 Reporting of Serious Adverse Events

Reporting requirements for SAEs will be managed on behalf of the Sponsor by PRA Health Sciences. 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 392 (±10 days) must be reported by the

Investigator to the CRO 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 CRO,
- All subjects with SAEs must be followed up for outcome.

All SAEs must be reported by the investigative site to PRA Health Sciences within 24 hours of learning about the event. This can be done by sending a completed Safety Report Form via fax or email to the PRA Drug Safety Center. 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 PRA Drug Safety Center (includes vaccine reporting):

PRA Drug Safety Center (includes vaccine reporting)
Email: CHOSafety@prahs.com
Fax: 1-888-772-6919 or 1-434-951-3482
Telephone: 1-800-772-2215 or 1-434-951-3489

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 PRA safety team within 5 days of receipt of the alert report.

PRA 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. Reports of Suspected Unexpected Serious Adverse Reactions (SUSARs) will be expedited to the regulatory authorities as required per local regulations as an Investigational New Drug (IND) Safety Report within the required time frame.

The PRA safety team may be required to collect further information for a final evaluation of the SAE case and for submitting an expedited follow-up report to the regulatory authorities.

PRA will be responsible for IND safety reporting to the regulatory authorities of reportable SAEs as required. Correspondence with the IRB relating to the reporting of SAEs will be retained in the eTMF.

After the initial SAE report, the Investigator is required to follow up proactively each subject and provide further information to PRA on the subject's condition. The investigative site is responsible for reporting SAEs to the Research Monitor and the IRB as required and maintaining all IRB correspondences on file at the site.

See Section 16.5 for HRPO reporting requirements.

12.4 Assessment of Subjects with Adverse Events

Each subject must be carefully assessed for AEs by the Investigator or designee. This includes review of subjective symptoms reported on the diary or at follow-up visits, objective physical findings, and laboratory results. Assessments must be made of the seriousness, severity (intensity), and relationship to the IVP. During the study all AE/SAEs should be followed up 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.

12.4.1 Adverse Event of Special Interest

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 (within 24 hours after identification) by the Investigator to the PRA Medical Monitor and Sponsor. Such an event might warrant further investigation, including unblinding 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 effusion or soft tissue swelling. To characterize these symptoms, additional serologic, immunologic, and radiographic data may need to be obtained and other etiologic causes ruled out. The basis for selection of persistent joint symptoms as an AESI is discussed in Section 7.1 and Section 7.4. If the Investigator has any question about a case of persistent joint symptoms being an AESI, the PRA Medical Monitor should be contacted.

An AESI should be reported by the investigative site to the PRA Drug Safety Center within 24 hours of learning about the event by completing the Safety Report Form and sending via fax or email. The documentation and processing of AESIs is further detailed in the Investigator Site Files.

12.5 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.

An exception is made for CRP and ferritin 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. 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.

12.6 Abnormal Physical Examination 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.

12.7 Additional Safety Assessments

12.7.1 Reactogenicity

The severity of local injection site reactions (Appendix Table A), systemic reaction to injectable product (Appendix Table B), chikungunya-like systemic illness (Appendix Table C), and vital signs (Appendix Table D) will be assessed at follow-up visits and graded according to the modified FDA/CBER Guidance for Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007) (see **Appendix 1** for grading scales). The Investigator will assign the grade and document in the eCRF.

The reactogenicity evaluation will consider data entered into the subject diaries. The subject diaries will not be considered source documents, but rather memory aids.

12.7.2 Subject Diary

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: Appendix Table A: Local Reactions to Injectable Product).
- Systemic symptoms to be recorded are fatigue, headache, malaise, diarrhea, nausea/vomiting and joint pain. (See Appendix 1, Toxicity Grading Scale, Appendix Table B, Systemic Reactions to Injectable Product).
- The diary will also include a section for recording temperature and entering unsolicited AEs and concomitant medications.

Diary Day 8 – 28 entries:

- Systemic symptoms to be recorded are fatigue, headache, malaise, diarrhea, nausea/vomiting and joint pain. (See Appendix 1, Toxicity Grading Scale, Appendix Table B for the Toxicity Grading Scale: Systemic Reactions to Injectable Product).
- This diary will also include a section for recording temperature if the patient feels feverish and entering unsolicited AEs and concomitant medications.

Throughout the duration of the study, SAEs and AESIs will be reported. See Appendix 1, Appendix Table C for the Toxicity Grading Scale: Systemic Illness.

12.8 Treatment of a Deviation in Dose Administration of MV-CHIK

No experience with a deviation in dose administration (overdosing or underdosing) with MV-CHIK in humans has been recorded. Subjects who receive less than a full dose will not be re-vaccinated. No specific treatment for overdosing is known. Treatment given to a subject in case of overdosing should be symptomatic and supportive. Any deviation in dose administration, with or without associated AEs, must be reported to PRA. Deviations will be recorded in the eCRF. All reports of deviations must be filed in the eTMF. Any AEs associated with the deviation should be reported on relevant AE/SAE sections in the eCRF.

12.9 Procedures in Case of Pregnancy

Pregnancy in itself is not regarded as an AE unless there is a suspicion that the IVP may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies that begin before Day 168 (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even if the subject was discontinued from the study. A pregnancy outcome of a congenital abnormality/birth defect or of a spontaneous miscarriage would qualify as an SAE and be reported to PRA Health Sciences for processing to the regulatory authority. A pregnancy outcome of an elective abortion without evidence of complications would not be processed as an AE.

13. STATISTICAL ANALYSIS

Data management, analysis, and reporting will be performed by the CRO, PRA Health Sciences.

13.1 Data Collection, Coding of Terms, and Database Lock

An electronic CRF and electronic data capture (EDC) will be used for the current study, and a Data Management Plan will be prepared by the CRO, PRA Health Sciences.

Previous and concomitant medications will be coded using the latest available WHO Drug Reference Dictionary. Coexistent diseases and AEs will be coded using the current version of MedDRA (Medical Dictionary for Regulatory Activities) as documented in the Data Management Plan.

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 Themis Bioscience and the PRA Health Sciences project team.

13.2 Sample Size Estimation

A formal sample size calculation was not conducted. The sample size has been determined based on prior experience in evaluating the safety and immunogenicity of vaccines and is typical for early phase clinical studies. In the European Phase 2 study, MV-CHIK 202, arthralgia was observed in 9 out of 229 subjects that received MV-CHIK (3.9%, 95% confidence interval = 1.4 - 6.5%) and in 2 out of 34 subjects that were treated with the MMR control vaccine (5.9%). Assuming a similar proportion of baseline chikungunya seronegative subjects report arthralgia in this study, and that a lesser or equal proportion of MMR recipients report arthralgia, more than 6/40 (15%, 95% confidence interval = 7.0 - 30%) chikungunya seropositive subjects reporting arthralgia would be indicative of an increased risk of that AE in previously infected individuals.

13.3 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.

13.4 Randomization

Randomization will be completed by an IWRS. A maximum of 100 subjects (50 seropositive and 50 seronegative for chikungunya) will be randomized to receive MV-CHIK or MMR vaccine in a 4:1 ratio.

Drop-outs after the first vaccination will not be replaced.

13.5 Analysis Populations

All of the analysis populations will be identified and finalized before the blind is broken for the study. The primary analyses of safety will use the intent-to-treat (ITT) population. The per protocol (PP) population will be used for secondary analyses of immunogenicity. The exploratory analysis of measles viremia will look at all subjects in whom these samples were collected.

13.5.1 Intent-to-treat Population

The ITT population will include all subjects randomized in the study and will be the population for the safety analyses.

13.5.2 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.

13.6 Statistical Methods

Categorical variables will be summarized using the number and percentage 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.

13.6.1 Missing Data

All attempts will be made to prevent missing data. An observed cases approach will be applied for all endpoints. No missing data will be imputed.

13.6.2 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.

13.6.3 Subject Disposition

The number of subjects randomized and who received at least one dose of IVP will be summarized. The number of subjects who discontinued the study early along with the reasons for discontinuation will be summarized. The number of screen failures and reasons for screen failure will also be presented. The number and percentage of subjects at each study visit, and the number of subjects with important protocol deviations (defined in Section 16.2) will also be presented.

13.6.4 Compliance and Vaccine Exposure

The number and percentage of subjects who receive only 1 of the IVP doses and the number and percentage of subjects who receive both of the IVP doses will be summarized.

13.6.5 Concomitant Medications

Concomitant medications will be coded by WHODRUG. The number and percentage of subjects receiving each category of medication will be summarized by IVP received and prior exposure group.

13.6.6 Safety

The primary analysis of safety will be conducted in the ITT population according to baseline serostatus cohort. Safety will be assessed by actual IVP received and by baseline chikungunya serostatus (seropositive or seronegative). Safety analysis will include analyses of AEs.

13.6.6.1 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 the MedDRA dictionary 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 intervention received and baseline chikungunya serostatus 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. Solicited local injection site reactions (erythema/redness, induration/swelling, itching, and pain/tenderness) will be entered into the subject diary from 0 to 7 days after each injection. Solicited systemic AEs (fever, fatigue, headache, malaise, diarrhea, nausea/vomiting and joint pain) will be entered into the subject diary from 0 – 28 days. 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.

13.6.6.2 Clinical Laboratory Testing

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.

13.6.6.3 Vital Signs and Physical Examination 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.

13.6.7 Immunogenicity

The analysis of immunogenicity will be measured as GMTs of neutralizing antibodies on Days 0, 28, 56, 168, 280, and at EOS. The GMT will be calculated using an analysis of variance with IVP (MV-CHIK versus MMR), visit and serostatus as fixed factors. The analysis of variance will be fit using $\log_{10}$ 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 seroconversion rate will be calculated as the proportion of the baseline seronegative group with detectable neutralizing antibody post-vaccination.

Analyses of immunogenicity will be conducted in the PP population according to vaccine received.

14. INVESTIGATIONAL SITE 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, an on-site Research Monitor, a PRA Medical Monitor, and blinded and unblinded PRA CRAs will be employed for the duration of the study.

14.1 Research Monitor and PRA Medical Monitor

The Research Monitor is a physician independent of the Sponsor responsible for serving as advocate for the medical safety of volunteers. 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.

The Research Monitor will be 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.

A PRA Medical Monitor will also be assigned and may review monitoring plans and will serve as the central point of contact for scientific, medical, and protocol questions from the site and investigator(s).

14.2 Clinical Research Associate

The CRA will monitor study progress by scheduling and performing on-site study visits throughout the study including a site initiation visit, several interim monitoring visits and a site close out visit. The CRA will also communicate with the site via phone, email and formal visit confirmation and follow-up letters. The CRA will be responsible for 100% source document verification of study data, including reviewing all UPIRTSOs associated with the protocol. The CRA may escalate any critical subject safety or GCP finding to the Investigator and Research Monitor and direct the site to contact the IRB immediately and then report to the PRA and Sponsor management teams as per PRA procedure. 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 Clinical Management Plan.

14.3 Investigative Site

The Investigator must provide the CRA 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.

14.4 Inspections and Auditing Procedures

The Sponsor may conduct an audit at the investigative site. In addition, the Contracting Officer's Representative from the funding agency (WRAIR) may conduct inspections at the investigative site in order to assess contractor performance. These visits may include but are not limited to, IVP supply, required documents, the informed consent process, and comparison of CRFs with source documents. All study records including progress notes must be available for audit. The Investigator agrees to participate with audits conducted at a convenient time in a reasonable manner.

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

The purpose of an audit is to assess whether ethics, regulatory and quality requirements are met.

15. DATA MANAGEMENT AND QUALITY OVERSIGHT

Clinical data (including AEs, concomitant medications and clinical laboratory data) will be entered into a 21 CFR Part 11-compliant validated web-based 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.

15.1 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 Sponsor or their representative 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 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.

15.2 Data Collection

During each study visit, the Investigator will maintain progress notes in the subject's study records 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 on 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 study vaccine-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 the PRA SOPs. If the reason for the change is not apparent, a brief explanation for the change will be written adjacent to the change.

15.3 Clinical Database Management and Quality Control

Data will be captured in the EDC system, DataLabs, which will be hosted by PRA. Data cleaning will be an ongoing process throughout the trial. Specific reviews and processes used to clean the data are documented in the Data Management Plan.

External data sources and reconciliation processes are documented in the Data Management Plan. All external data sources are listed in the System Set Up for Clinical System Design Guide (CSDG). Vendor contact information and information regarding data formats and transfer frequency is provided in the Data Transfer Specifications. Edit checks for reconciling external data and clinical data management systems (CDMS) data are outlined in the Edit Check Plan.

Quality Control reviews are built into data processing procedures to ensure that PRA produces analyzable, high quality data.

Additional quality controls that will be applied for the study are described in Sections 15.4 through Section 15.7.

15.4 Independent Data Quality Assurance

Conceptual MindWorks, Inc. (CMI), has been engaged to conduct independent third-party QA of the data. CMI will ensure data collection and cleaning is of the highest quality by ensuring compliance with the elements of GCP, ie, complete quality audit of data. CMI will work closely with the PRA data management team in coordinating activities and timelines for response and resolution of issues. The reliability of the trial data is the main objective of the independent QA.

15.5 Source Documents 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.

15.6 Record Maintenance

Records will be retained in accordance with the current ICH Guidelines on GCP. All essential study documents including records of subjects, source documents, eCRFs and study 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.

15.7 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 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 PRA supplied 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 as funding agency, the CRO personnel, the IRB, or the US 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 the PRA CRAs, the Sponsor, and auditing by funding and 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.

16. ADMINISTRATION PROCEDURES

16.1 Regulatory Approval

Themis Bioscience 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(s).

16.2 Protocol Adherence and Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP), or Manual of Procedures requirements (PRA/Sponsor/Vendor SOPs). 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:

- 4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, Section 5.1.1
- 5.20 Noncompliance, Sections 5.20.1 and 5.20.2.

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.

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 sub-investigator.

In the event of an important protocol deviation due to an emergency, accident, or error, the Investigator or designee must contact the PRA Medical Monitor through the Medical Support Center at the earliest possible time by telephone or email. This allows for an early joint decision to be made as to whether or not the involved subject(s) should continue in the study. The Investigator, the Sponsor, Research Monitor, and the PRA Medical Monitor will document this decision.

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.

16.3 Protocol Amendments

In accordance with ICH Topic 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 of a protocol amendment, except where necessary to

eliminate an immediate hazard(s) to study subjects, or when the change(s) involves only logistical or administrative aspects of the study (e.g., change in PRA Medical Monitor, change of telephone number).

Any change 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 IRBs. 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 judgment of the local IRB, the 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.

16.4 End of Study Definition

The end of study will be defined as when the last subject visit will be conducted. The last subject visit is either Visit 9 (EOS) of the last ongoing subject included in the double-blind vaccination period or the last unscheduled follow-up visit of the last subject. The study will then be stopped. For premature termination or suspension of the study, refer to Section 9.4.4.

16.5 Requirements of the U.S. Army Human Research Protection Office

The protocol will not be initiated until written notification of approval of the research project is issued by the HRPO.

The DoD is funding this study and their representatives are authorized to review subject's research records and private health information.

16.5.1 Study Termination Authorization

The USAMRMC Office of Research Protections, Human Research Protection Office (ORP, HRPO), may stop or suspend the use of the IVP at any time.

16.5.2 Unanticipated Problems Involving Risks to Subjects or Others

All unanticipated problems involving risk to subjects or others must be promptly reported by telephone (+1 301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.

mil), or by facsimile (301-619-7803) 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.

16.5.3 Reporting of Serious Adverse Events

Serious AEs related to participation in the study should be promptly reported by telephone, email, or fax to the IRB within 48 hours of awareness. A complete written report should follow the initial notification.

16.5.4 Subject Withdrawal of Consent Due to an Adverse Event

Any AE-related withdrawal of consent for either the second vaccine dose or for study participation in general must be reported immediately (within 24 hours of identification) by email or fax to the IRB. The Medical Monitor must also be notified.

16.5.5 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, would prompt additional scientific review), or a change that could potentially increase risks to subjects.

16.5.6 Reporting to the HRPO

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

Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the Sponsor, or regulatory agencies will be promptly reported to the HRPO.

A copy of the continuing review approval notification by the IRB of Record 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.

16.5.7 Pending Inspections/Issuance of Reports

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 USAMRMC ORP HRPO.

16.5.8 Final Study Report

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

16.6 Investigational 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, sub-investigator(s), and Research Monitor at the site 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 sub-investigators at each site should appear on this form. The Investigator must also complete all regulatory documentation as required the 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) Study vaccine 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

16.7 Contractual and Financial Details

The Investigator and the CRO will sign a clinical study 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.

16.8 Insurance, Indemnity and Compensation

Themis Bioscience undertakes to maintain an appropriate clinical study insurance policy. Deviations from the study protocol, such as the administration of a dose other than those scheduled in the study protocol or another route of administration, are not permitted and will not be covered by the statutory subject insurance scheme.

16.9 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.

16.10 Publication Policy

After completion of the study, the Investigator(s) may prepare a joint publication with the Sponsor and funding agency's representative. The Investigator(s) must undertake not to submit any part of the data from this protocol for publication without the prior consent of the Sponsor and funding agency.

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18. APPENDIX

18.1 Appendix 1: Toxicity Grading Scales

Appendix Table A: Local Reaction to Injectable Product

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

Appendix Table B: Systemic Reaction to Injectable Product

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
Diarrhea	2 – 3 loose stools or weight assessment per Investigator/24 hours	4 – 5 stools or weight assessment per Investigator /24 hours	6 or more watery stools or weight assessment per Investigator; or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia/Joint Pain*	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

*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 12.4.1 and the Investigator Site File for this study for additional reporting requirements.

Appendix Table C: Systemic Illness

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

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

* Subjects should be at rest for all vital sign measurements.
** When resting heart rate is between 60 – 100/minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.