Statistical Analysis Plan

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1.0 Approvals

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2.0 Purpose

The statistical analysis plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under Protocol MV-CHIK-204.

3.0 Scope

This plan is a living document that will be created during the trial start-up and will be finalized prior to database lock. The SAP will require sign off from the Project Manager(s) and the lead Statistician.

The Statistical Analysis Plan outlines the following:

- Study objectives
- Study design
- Variables analyzed and analysis sets
- Applicable study definitions
- Statistical methods regarding important protocol deviations, study drug exposure, efficacy analysis, concomitant medications, adverse events handling, laboratory data and physical examinations

4.0 Introduction

This statistical analysis plan (SAP) describes the statistical methods to be used during the reporting and analyses of data collected under Themis Bioscience GmbH protocol MV-CHIK-204 entitled "*Phase 2 Study of a Live Attenuated Measles Virus-Vectored Chikungunya Vaccine in a Previously Epidemic Area*."

This SAP should be read in conjunction with the study protocol and case report form (CRF). This version of the plan has been developed using the protocol amendment 02, version 3.0, dated 15Dec2017 and CRF dated 07Feb2018. Any further changes to the protocol or CRF may necessitate updates to the SAP.

4.1 Changes from Protocol

There are no changes from the protocol at the time of the development of this SAP.

5.0 Study Objectives

Objectives	Endpoints
Primary Objective: To determine the safety of MV-CHIK administered in 2 doses separated by 28 days in previously exposed versus unexposed individuals.	• 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
Secondary 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.	• 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.
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.	• Measles virus genome equivalents per milliliter of serum

6.0 Study Design

6.1 Overview

This is a prospective randomized double-blind interventional clinical study to evaluate the safety and immunogenicity of 2 doses of an investigational live attenuated recombinant measles virus-vectored chikungunya vaccine (MV-CHIK), delivered in 2 vaccinations, 28 days apart compared with one dose of an active measles, mumps, and rubella (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.

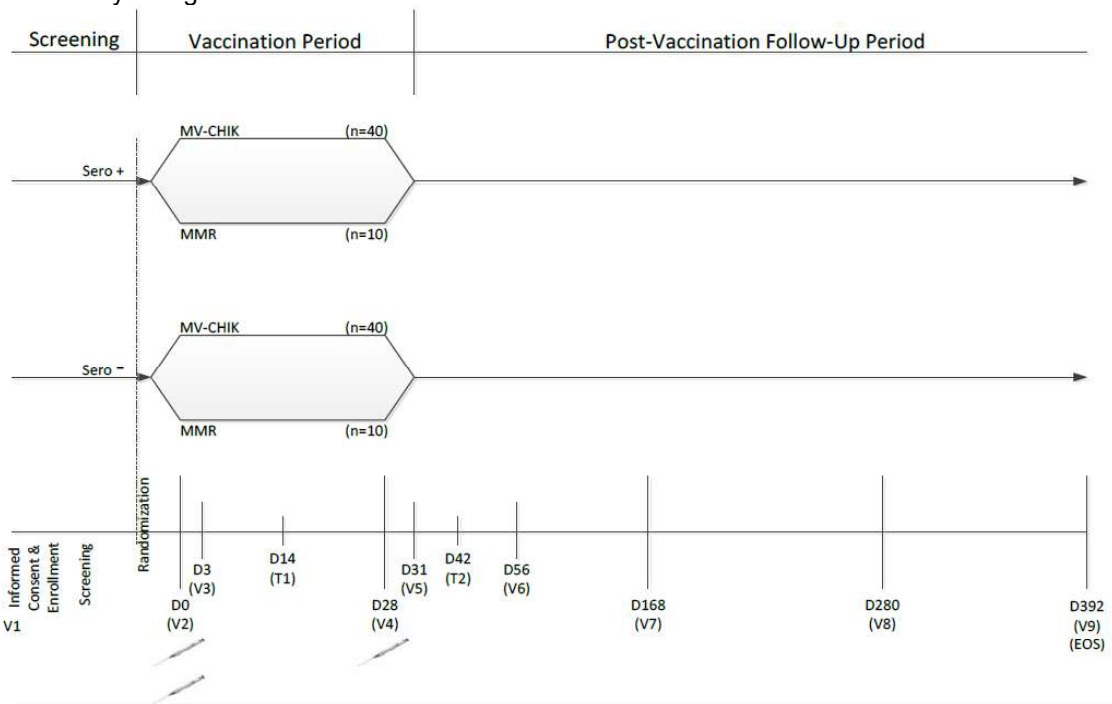
The study will randomize 50 subjects in the seropositive cohort to either MV-CHIK (40 subjects) or MMR (10 subjects) and 50 subjects in the seronegative cohort to either MV-CHIK (40 subjects) or MMR (10 subjects). One dose level (5×10^5 ±0.5 log[1] $TCID_{50}$) of MV-CHIK will be studied for this Phase 2 design. Forty subjects in each cohort will be vaccinated with MV-CHIK intramuscularly (deltoid) on study Day 0 and Day 28 (a subcutaneous dummy injection will be administered in the opposite arm on Day 0). Ten subjects in each cohort will be vaccinated with MMR subcutaneously on study Day 0 (an intramuscular

[1] The vaccine dosages are liable to the manufacturing dependent window of ±0.5 log steps. For ease of reading, this manufacturing dependent window will not be given in the other parts of this document.

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).

Figure 1. Study Design Schematic



Abbreviations: D, day; EOS, End of Study; MMR, measles, mumps, and rubella vaccine, MV-CHIK, measles-vectored chikungunya vaccine product; 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 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 in order 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 in order to maintain the blind.

6.2 Sample Size Considerations

A formal sample size calculation was not conducted. The sample size was determined based on prior experience in evaluating the safety and immunogenicity of vaccines and is typical for early phase clinical studies. In 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.

6.3 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)

The randomization will be completed by an Interactive Web Response System (IWRS). 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. Overall, a maximum of 100 subjects (50 seropositive and 50 seronegative for chikungunya) will be randomized to receive MV-CHIK or MMR vaccine.

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

6.4 Blinding

Dummy injections of sterile saline were 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 intramuscular (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 subcutaneous (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.

6.5 Schedule of Events

The Schedule of Events (Table 1) 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.

Table 1. 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 and 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										

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]				
Pregnancy test [g]	X	X			X				X		
Neutralizing antibody to chikungunya		X			X			X	X	X	X
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; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus.
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 leucocytes detected. Acute phase reactants, C-reactive protein and ferritin, will be measured to aid in characterizing, but not defining, AE's.
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.

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.
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 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.

7.0 Study Variables and Covariates

7.1 Primary Variables

7.1.1 Safety

7.1.1.1 Adverse Events

An adverse event is defined as 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 or physical examination 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. 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).

The following variables will be collected for each AE:

- Adverse event (verbatim)
- The date and time when the AE started and stopped
- Outcome (Recovered/resolved; Recovered/resolving; Not recovered/not resolved; Recovered/resolved with sequelae; Fatal; Unknown)
- Severity (Mild; Moderate; Severe)
- Action taken with Study Vaccine (Vaccine withdrawn; Vaccine delay; No action)
- Relationship to Study Vaccine (Unrelated; Related)
- Whether the AE is serious or not, and if yes, which category (AE resulted in death; AE is life-threatening; AE resulted in persistent or significant disability or incapacity; AE resulted in initial or prolonged hospitalization; AE is associated with a congenital anomaly or birth defect; AE is a medically important event not covered by other criteria).

The grading scales that will be used for local reactions to injectable product, systemic reactions to injectable product, systemic illnesses, and vital signs are detailed in Protocol Appendix 1: Toxicity Grading Scales. The investigator will assign the grade and document this severity in the eCRF.

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 investigational product will be considered and investigated.

All solicited local (injection site) reactions will be considered causally related to vaccination.

7.1.1.2 Serious Adverse Events

A serious AE (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.

Any SAE which occurs to any subject after entering into intervention in this study through the last study follow-up visit on Day 392 (±10 days) will be reported by the Investigator to the PRA Drug Safety Center regardless of whether or not the SAE is considered related to the IVP. SAEs that occur after the last study follow-up visit and which are deemed to be related to the IVP will also be reported. All subjects with SAEs will be followed up for outcome.

7.1.1.3 Subject Diaries

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.

7.1.1.4 Adverse Events 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 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 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 be obtained and other etiologic causes ruled out.

7.2 Secondary Variable

7.2.1 Immunogenicity: Neutralization titer (chikungunya)

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 plaque reduction neutralization test (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.

7.3 Exploratory Variable

7.3.1 Measles Viremia: Measles virus genome equivalents

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. 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.

Results of this and other exploratory analyses are not included in this analysis plan.

7.4 Other Variables

7.4.1 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 (Days 0, 3, 28, 31, 56, 168, 280, and 392) 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.

7.4.2 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.

7.4.3 Pregnancy Test

Pregnancy will be determined by evaluation of β- human chorionic gonadotrophin (HCG) in urine for all women of childbearing potential. 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, they will continue to be followed and the outcome of the pregnancy will be documented.

7.4.4 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.

7.4.5 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 study Schedule of Events:

Complete Blood Count (Screening and Days 0, 3, 28, 31, and 56): white blood cell count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), hemoglobin, hematocrit, platelet count.

Basic Metabolic Panel (Days 0, 3, 28, and 31): This blood test assesses the following analytes: glucose, sodium, potassium, carbon dioxide, chloride, urea nitrogen, and creatinine.

Comprehensive Metabolic Panel (Screening and Day 56): 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 (Screening; fresh urine clean catch specimen): protein, glucose, hemoglobin, erythrocytes, and leucocytes.

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

7.4.6 Serology

Chikungunya Baseline Serostatus: The local commercial laboratory will test subjects for chikungunya antibodies at screening. The screening value will serve as the baseline value in this study. The IgG antibody test will be performed via the enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (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.

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.

7.4.7 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. 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.

7.4.8 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. 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, 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.

8.0 Definitions

Variable	Definition
Age	Age will be based on time in years from date of birth to signing of informed consent. Age (years) = (date of informed consent – date of birth) / 365.25
Baseline	Baseline is defined as the last assessment prior to the first vaccination of either study drug at Day 0.
Change from baseline	Change from baseline will be defined as the post-baseline value minus the baseline value, where applicable (on a subject level). Change from baseline will only be calculated for subjects who have both baseline and at least one post-baseline value for any parameter.
Time in study	Time in study will be defined as the number of days from the date of informed consent to the date of study completion. Time in study (days) = date of study completion – date of informed consent
Duration of follow-up	Duration of follow-up will be defined as the number of days from the Day 28 vaccination to the date of study completion. Subjects who discontinue prior to the 2nd vaccination dose will not have a value for this variable. Duration of follow-up (days) = date of study completion – date of Day 28 vaccination
Days since last vaccine	If the AE start date is prior to Vaccine dose 2 (Day 28), then calculate as: (AE start date) – (1st vaccine date) If the AE start date is after Vaccine dose 2 (Day 28), then calculate as: (AE start date) – (2nd vaccine date)
Temperature	Temperature that is recorded as Fahrenheit (F) will be converted to Celsius (C) for the TFLs. C=(F – 32)/1.8
Fever	From the Subject Diary card, temperatures reported by the subject will be evaluated for fever based on the guidelines in Protocol Appendix Table C: Mild fever: 38.0-38.4C Moderate fever: 38.5-38.9C Severe fever: 39.0-40.0C Potentially life-threatening fever: >40.0C
Start/Stop Date	For partial dates collected related to AEs or prior/concomitant medications, the following imputation will be performed. Start Date: - If only 'day' is missing, and the month and year are not the same as the month of first dose, then impute day with '01'. Otherwise, if the month and year are the same as the first dose date, use the first dose date. - If 'day' and 'month' are missing, and 'year' is not missing, then impute month and day with month and day of the first dose date (assuming same 'year'). - If 'day' and 'month' are missing and 'year' is not missing and is not the same year as first dose date, then impute with '01' for both 'day' and 'month'. If the start date is completely missing, it will be set to the first dose date.

	Stop Date: - If only 'day' is missing, impute day with last day of the month. - If 'day' and 'month' are missing, and 'year' is not missing, then impute month with '12' and day with '31' (or date of study discontinuation/completion if earlier than 12-31 and year is the same as the year of discontinuation). - If the stop date is completely missing, it will be set to the date of study discontinuation/completion. A stop date will not be applied to ongoing AEs.

9.0 Analysis Sets

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 population. The per protocol population will be used for secondary analyses of immunogenicity.

9.1 Intent-to-Treat

The intent-to-treat (ITT) population will include all subjects who are randomized in the study, and received at least one dose of IVP. The ITT population will be the population for the safety analyses. Subjects will be reported based on actual treatment received.

9.2 Per Protocol

The per protocol (PP) population is a subset of the ITT population that includes subjects who receive 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. Refer to Section 12.2 for the process surrounding Important Protocol Deviations.

10.0 Interim Analyses

Interim analyses are not planned for this study.

11.0 Data Review

11.1 Data Handling and Transfer

Please refer to the Data Management Plan for details concerning data handling and transfer.

11.2 Data Screening

Beyond the data screening built into the PRA Data Management Plan, the PRA programming of analysis datasets, tables, figures, and listings (TFL) provides additional data screening. Presumed data issues will be output into SAS logs identified by the word "Problem" and extracted from the logs by a SAS macro and sent to Data Management.

Review of a pre-freeze TFL run on clean subjects and a post-freeze TFL run on the frozen database allow for further data screening prior to lock. The post-freeze TFL will be discussed with the sponsor in a data review meeting to identify any final data issues and seek corrections prior to database lock. The PRA statistician and the sponsor must approve database lock.

12.0 Statistical Methods

All analyses will use SAS version 9.4 or higher. Results will be reported by treatment group (MV-CHIK or MMR), and further cohorted based on baseline serostatus to chikungunya virus. Sites will be pooled together for all analyses.

Unless otherwise noted, categorical variables will be summarized using counts and percentages. Percentages will be rounded to one decimal place, except 100% will be displayed without any decimal places and percentages will not be displayed for zero counts.

Continuous variables will be summarized using the number of observations (n), mean, standard deviation (SD), median, minimum and maximum. The minimum and maximum values will be displayed to the same level of precision as the raw data, the mean and median to a further decimal place and the SD to two additional decimal places.

Confidence intervals will be two-sided and use the exact binomial method at a 95% confidence level.

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.

12.1 Subject Disposition

The number of screened subjects, subjects randomized, subjects who received at least one dose of IVP, and subjects who were included in the Per Protocol population will be summarized. Reasons for screen failure will be collected, and exclusions from an analysis population will be listed.

Reasons for discontinuation from vaccine and from study will be tabulated.

The number and percentage of subjects at each study visit will also be presented, and descriptive statistics will be used to summarize the time in study and the duration of follow-up in days.

12.2 Important Protocol Deviations

Per PRA processes, protocol deviation data will be entered into our Clinical Trials Management System (CTMS). The study team and the sponsor will conduct on-going reviews of the protocol deviation data from CTMS and the resulting set of evaluable subjects throughout the study, adjusting the protocol deviation criteria as seems appropriate. The evaluable subjects set will be finalized at the post-freeze data review meeting (or earlier), prior to database lock. Important protocol deviations for ITT subjects will be summarized in a table, and all protocol deviations will be listed.

12.3 Demographic and Baseline Characteristics

Demographics will be summarized for both the ITT and PP population, and will include age (in years, at time of signing informed consent), gender, race and ethnicity.

Baseline serology results for chikungunya and measles will be tabulated based on the number of ITT subjects with a sample collected, and the number and percentage of positive and negative results.

Medical history will also be summarized and coded using MedDRA v20.1.

12.4 Treatments

12.4.1 Compliance and Vaccine Exposure

For all ITT subjects, the number and percentage of subjects who receive only 1 vaccine dose and the number and percentage of subjects who receive both injections at Day 0 will be summarized. Additionally, the number and percentage of subjects receiving an injection at Day 28 will be tabulated. The summaries will also include, as applicable, vaccine route (intra-muscular (IM) or subcutaneous (SC)) and vaccine site (right or left). It is expected that each subject will receive an IM as well as an SC injection on Day 0, and all subjects will receive an IM injection on Day 28.

12.4.2 Prior and Concomitant Medications

Medications received during the study, categorized by generic term according to WHODRUG (B3 format), will be summarized for the ITT population. The number and percentage of subjects receiving each category of medication will be summarized by vaccine received and prior exposure to chikungunya. Any medications taken after enrollment but stopped prior to the first dose of vaccine on Day 0 will be tabulated separately as a prior medication. Concomitant medications are those ongoing at Day 0 or with a start date during the vaccination or post-vaccination follow-up portion of the study. Any partial start or stop dates will be imputed as described in Section 8.0.

12.5 Efficacy Analyses

12.5.1 Immunogenicity: Neutralization titer (chikungunya)

The primary immunogenicity analysis will be conducted in the PP population according to vaccine received. A secondary immunogenicity analysis will also be reproduced for the ITT population. This additional summary would allow inclusion of subjects with deviations that may have excluded them from the PP population (e.g. visit window violations).

The analysis of immunogenicity will be measured as geometric mean titers (GMT) of neutralizing antibodies on Days 0, 28, 56, 168, 280, and at EOS (Day 392 ± 10 days).

Antibody titer values below the lower limit of quantification (LLoQ=10) for the PRNT or MNt will imputed to half of the LLoQ. Values above the upper limit of quantification (ULoQ=1280) are imputed to the ULoQ.

The GMT will be calculated using a mixed-model repeated measures (MMRM) analysis, with the log-transformed value of the titer as the dependent variable. The independent variables in the model include fixed effects for treatment (MV-CHIK versus MMR), visit (Days 0, 28, 56, 168, 280, and EOS), serostatus (Seropositive versus Seronegative), and treatment-by-visit-by-serostatus interaction. An unstructured covariance matrix will be used to model the covariance amongst the repeated measures. However, if the model does not converge an alternative covariance matrix will be used which is appropriate for the repeated measurements (e.g., first-order autoregressive). Since this is a repeated measures analysis with potential missing data, the Kenward-Roger approximation will be used for calculating the denominator degrees of freedom and adjusting standard errors.

The SAS code to be used for the repeated measures model is:

```
proc mixed data=<<dataset1>>;
       class usubjid treatment serostatus visit;
       model log-transformed titer = treatment visit serostatus
                                     visit*treatment*serostatus / solution ddfm=kr;
       repeated visit/type = un subject = usubjid;
       lsmeans visit*treatment*serostatus / alpha=0.05 cl;
       ods output LSMEANS=<<dataset2>>;
run;
```

The estimates for the repeated measures model adjusted means (LS-means) and corresponding 95% confidence intervals (CIs) will be back-transformed by taking the anti-log to obtain the GMTs and CIs.

12.5.2 Seroconversion Rate

The primary seroconversion rate analysis will be conducted in the PP population, and will be repeated in the ITT population as a secondary analysis. The seroconversion rate will be calculated as the proportion of the baseline seronegative group with a titer greater than or equal to 1:20 post-vaccination, based on the number of subjects in the group with available data. The 95% confidence interval for the seroconversion rate will be calculated using the Clopper-Pearson method. Seroconversion for chikungunya using neutralization testing will be defined as a 4-fold or greater increase (including going from <10 to 20 and from 640 to >1280). A value below a 4-fold increase (as well as a decrease) will be considered non-seroconversion.

The rate of subjects with seropositive chikungunya at baseline with a 4-fold or greater post-baseline increase will also be analyzed in a similar manner as the above in the PP population, as well as the ITT population.

12.5.3 Exploratory Analysis: Measles virus genome equivalents

Results of this exploratory analysis will be reported separately, and are not included in this analysis plan.

12.6 Safety Analyses

Safety will be assessed in the ITT population by actual IVP received and by baseline chikungunya serostatus (seropositive or seronegative). Safety analysis will include analyses of AEs, including events entered into the AE case report form as a result of findings from laboratory, vital sign and physical examinations.

12.6.1 Adverse Events

12.6.1.1 Adverse Events Reported on the AE CRF Page

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 Medical Dictionary for Regulatory Activities (MedDRA v20.1) 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 with adjustments made to the ranges for grade 1 abnormalities based on the normal ranges reported by the laboratory.

The overall number and percentage of subjects experiencing an AE (all, serious, and related) throughout the duration of the study will be reported. For each AE category (ie. all, serious, and related), the number and percent of subjects with AEs of 'moderate' or 'severe' severity, the number of subjects with AEs of 'fatal' outcome, and the number with action requiring 'vaccine withdrawal' or 'vaccine delay' will be tabulated.

In addition, all AEs will be summarized by IVP received and baseline chikungunya serostatus according to the MedDRA system organ class (SOC) and preferred term. Note that counting will be by subject, not event, and subjects will be counted once within each body system or preferred term.

Similar summaries by system organ class and preferred term will be provided for:

- AEs with a grading of severe.
- Serious adverse events (SAEs)
- IVP-related AEs. Events without evaluation of relatedness to vaccine will be summarized as related.
- AEs leading to withdrawal of vaccine.

Any partial start or stop dates will be imputed as described in Section 8.0.

All AE data will be listed by subject, and a separate listing will include all SAEs, including any deaths on study.

12.6.1.2 Solicited Events Reported in the Subject Diary

Adverse events will also be summarized separately for solicited events collected in the subject diary.

Solicited local injection site reactions will include pain/tenderness, erythema/redness, induration/swelling, and itching. These symptoms will be analyzed for each of the two vaccination days (Day 0 and Day 28), based on events reported in the subject diary from 0 – 7 days after each injection. The number of days reporting the symptom up to seven days post-injection will be summarized using descriptive statistics, and the maximum severity reported up to seven days post-injection will be tabulated for each symptom except

for redness and swelling/hardness. Additionally, the largest diameter (cm) reported by the subjects for redness and swelling/hardness will be summarized descriptively.

Solicited systemic AEs will include malaise, nausea/vomiting, diarrhea, headache, fatigue, joint pain, and fever. The summary of these events will be tabulated similarly to the injection site reactions, for each of the two vaccination days (Day 0 and Day 28), based on events reported in the subject diary from 0 – 28 days after each injection. To determine cases of fever, all temperatures reported by the subject will be assessed and categorized into the following fever gradings: None (<38.0ºC), Mild (38.0-38.4ºC), Moderate (38.5-38.9ºC), Severe (39.0-40.0ºC), Potentially life-threatening (>40.0ºC).

12.6.1.3 Adverse Events of Special Interest

Adverse events of special interest (AESIs) are 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. The occurrence and 95% exact binomial confidence interval of subjects experiencing AESIs will be presented. Additionally, the total number of incidences of an AESI for a subject throughout the study will be summarized descriptively, and the maximum duration of reported AESIs will be tabulated based on the following categories: >24 – 48 hrs, >48 – 72hrs, >72 hrs. A review of coded adverse events terms will be performed in order to determine which preferred terms will be included in the assessment of AESIs (ex. Joint Pain, Stiffness, Arthralgia).

Similar summaries will be provided for the top 5 most occurring adverse events by preferred term.

12.6.2 Laboratory Data

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.

Results of laboratory data measurement will be reported in SI units, as defined in the table below:

Laboratory Group	Test	Standard unit
Complete Blood Count (Screening and Days 0, 3, 28, 31, and 56)	White Blood Cells	$x10^9/L$
	Hemoglobin	g/L
	Hematocrit	fraction of 1.0
	Platelets	$x10^9/L$
	Neutrophils Absolute	$x10^9/L$
	Lymphocytes Absolute	$x10^9/L$
	Monocytes Absolute	$x10^9/L$
	Eosinophils Absolute	$x10^9/L$
	Basophils Absolute	$x10^9/L$
Basic Metabolic Panel (Days 0, 3, 28, and 31)	Glucose	mmol/L
	Sodium	mmol/L
	Potassium	mmol/L
	Carbon dioxide	mmol/L

	Chloride	mmol/L
	Blood Urea Nitrogen	mmol/L
	Creatinine	umol/L
Comprehensive Metabolic Panel (Screening and Day 56):	<includes all BMP assessments above as well as the items listed below>	
	Protein Total	g/L
	Albumin	g/L
	Calcium	mmol/L
	ALP (alkaline phosphatase)	U/L
	ALT (alanine aminotransferase)	U/L
	AST (aspartate aminotransferase)	U/L
	Bilirubin Total	µmol/L
Ferritin (Screening and Days 0, 28, and 56)	Ferritin	pmol/L
C-Reactive Protein (Days 0, 3, 28, 31, and 56)	CRP	mg/L

In addition, urinalysis results for erythrocytes, leukocytes, protein, glucose, and blood will be listed.

12.6.3 Vital Signs

Summary tables will be presented on absolute values at Screening, and Days 0, 3, 28, 31, 56, 168, 280, 392, and change from baseline values by observation timepoint for the following continuous parameters:

- body temperature
- pulse rate
- systolic and diastolic blood pressure

Change from baseline for all timepoints will use the pre-vaccination value collected at Day 0 for the baseline assessment.

12.6.4 Physical Examinations

Findings from physical examinations will be assessed for clinical significance and included in the tabular summaries and by-subject AE listings.

13.0 Validation

PRA's goal is to ensure that each TFL delivery is submitted to the highest level of quality. Our quality control procedures will be documented separately.

14.0 References

FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007).

Merck and Co., Inc. M-M-R-II. Package Insert, 2015. Available at:

http://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM123789.pdf

Appendix 1 Glossary of Abbreviations

Glossary of Abbreviations:	
AE	Adverse event
AESI	Adverse event of special interest
CRF	Case Report Form
EIA	Enzyme immunoassay
ELISA	Enzyme-linked immunosorbent assay
FDA	Food and Drug Administration
GMT	Geometric mean titer
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
IgG	immunoglobulin G
ITT	Intention-to-treat
IM	Intramuscular
IVP	Investigational vaccine product
IWRS	Interactive Web Response System
LLoQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
MMR	Measles, Mumps, and Rubella (vaccine)
MNt	Microneutralization (assay)
MV-CHIK	Name of the investigational vaccine
PP	Per Protocol
PRNT	Plaque reduction neutralization test
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Subcutaneous
SOC	System Organ Class
$TCID_{50}$	(median) tissue culture infective dose 50% (the amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated)
ULoQ	Upper Limit of Quantification
WRAIR	Walter Reed Army Institute of Research

Appendix 2 Tables, Figures, Listings, and Supportive SAS Output Appendices

Please refer to the Table, Figure and Listing shells to support this SAP, provided in a separate document.