Official Protocol Title:	Observer blinded, randomised study to investigate safety, tolerability and long-term immunogenicity of different dose regimens and formulations of MV-CHIK in healthy volunteers
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Protocol MV-CHIK-205

EudraCT 2018-000211-25

Observer blinded, randomised study to investigate safety, tolerability and long-term immunogenicity of different dose regimens and formulations of MV-CHIK in healthy volunteers

Sponsor	Themis Bioscience GmbH Muthgasse 11 1190 Vienna, Austria Tel: PPD
Contract Research Organisation Study Site	Celerion 22-24 Lisburn Road Belfast BT9 6AD Tel: PPD
Study Phase	Clinical Phase II
Protocol Version	Version 1.4
Version Date	08-August-2018

Protocol Approval

SPONSOR

09-Aug-2018
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Investigator's Approval

I confirm that I have read the protocol and that I agree to conduct this study in accordance with the protocol, International Conference on Harmonisation and GCP guidelines and with the applicable local regulatory requirements. Moreover, the site will keep all information obtained from the participation in this study confidential unless otherwise agreed in writing.

INVESTIGATOR

Date	Signature
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Address	Name

1. STUDY SYNOPSIS

Title of the Study	Observer blinded, randomised study to investigate safety, tolerability and long-term immunogenicity of different dose regimens and formulations of MV-CHIK in healthy volunteers
Study Centres	Celerion, 22-24 Lisburn Road, Belfast
Study Period	Study start, FSI Jun-2018
Study Phase	Clinical phase II
Objectives	– To investigate immunogenicity and safety of different MV-CHIK dose regimes, 28 days after one or two vaccinations – To investigate the immunogenicity and safety of a novel liquid vaccine formulation – To compare immunogenicity, safety and tolerability between different doses and formulations of MV-CHIK during a treatment period of 56 days – To evaluate the long-term immunogenicity of MV-CHIK up to one year after the first vaccination – To evaluate cellular responses induced by one or two immunisations – To collect human sera for efficacy evaluation, by passive transfer of anti-Chikungunya antibodies to virus susceptible non-human primates
Study Design	Observer blinded, randomised immunogenicity and safety phase II trial in 60 healthy volunteer female and male subjects. After completion of screening procedures, subjects will be randomised to one of five treatment groups (A, B, C, D or E): A: 12 subjects will receive two low dose treatments with MV-CHIK $5x10^4$ ($\pm$0.5 log) $TCID_{50}$ /dose on day 0 and 28 (lyophilised formulation) B: 12 subjects will receive two low dose treatments with MV-CHIK $1x10^5$ ($\pm$0.5 log) $TCID_{50}$ /dose on day 0 and 28 (liquid frozen formulation) C: 12 subjects will receive two low dose treatments with MV-CHIK $1x10^5$ ($\pm$0.5 log) $TCID_{50}$ /dose on day 0 and 28 (liquid SPS® formulation) D: 12 subjects will receive two high dose treatments with MV-CHIK $1x10^6$ ($\pm$0.5 log) $TCID_{50}$ /dose on day 0 and 28 (liquid frozen formulation) E: 12 subjects will receive one high dose treatment with MV-CHIK $1x10^6$ ($\pm$0.5 log) $TCID_{50}$ /dose on day 0 (liquid frozen formulation) and placebo on day 28 After the screening visit, subjects will perform visits on study days 0, 14, 28, 42 and 56 for immunogenicity sample collection and safety follow up and additionally return to site on day 182 (6 months) and 365 (12 months) for long-term immunogenicity serum sampling. Serum samples for passive transfer will be collected on days 0, 28, 56, 365.

Number of Subjects	60 healthy subjects
Main Inclusion Criteria	Healthy female or male volunteers, 18 - 55 years old at screening.
Investigational Medicinal Product	MV-CHIK, a live-attenuated recombinant measles vaccine expressing Chikungunya virus antigens, in concentrations of: $5x10^4$ (±0.5 log) $TCID_{50}$ / dose – lyophilised - low dose $1x10^5$ (±0.5 log) $TCID_{50}$ / dose – liquid frozen - low dose $1x10^5$ (±0.5 log) $TCID_{50}$ / dose – liquid SPS® - low dose $1x10^6$ (±0.5 log) $TCID_{50}$ / dose – liquid frozen - high dose Presented in three different formulations: - Lyophilised formulation: powder for suspension in water for injection - Liquid frozen formulation: suspension for injection - Liquid SPS® formulation: suspension in a proprietary "Stabilising and Protecting Solution" for injection MV-CHIK vaccine administered by intra muscular (i.m.) injection.
Placebo	Physiological saline solution (0.9% NaCl), administered by i.m. injection
Duration of the Study	Study duration per subject will be 12 months, comprising of 8 weeks treatment period Visit 1 - Visit 5 (study day 0 until day 56) and 44 weeks follow up period, including Visit 6 + Visit 7 (post day 56 until day 365). Study subjects are free to participate in other clinical studies after completion of the treatment period on day 56. The end of study will be defined as date of the last visit of the last subject.
Criteria for Evaluation	**Primary end-point** – Immunogenicity 28 days after the last MV-CHIK vaccination confirmed by the presence of functional antibodies as determined by the plaque reduction neutralisation test ($PRNT_{50}$) **Secondary end-points:** – Rate of solicited and unsolicited adverse events during the treatment period up to study day 56 – Serious adverse events during the treatment period until study day 56 – Immunogenicity on days 0, 28 (for group A-D), 0, 56 (for group E) and on day 182 and 365 (for all) confirmed by the presence of functional antibodies as determined by the plaque reduction neutralisation test ($PRNT_{50}$). – Measurement of anti-Chikungunya antibodies on days 0, 28, 56, 182 and 365 determined by enzyme linked immunosorbent assay (ELISA) – Measurement of anti-Measles antibodies on days 0, 28, 56 determined by enzyme linked immunosorbent assay (ELISA) – Induction of Chikungunya specific immune responses on days 0, 14, 28, 42 and 56 as determined by T-cell assay. – Safety laboratory parameters (haematology, serum chemistry, coagulation, urinalysis)

Statistical Methods	Data Analysis: The immunogenicity analysis will compare the anti-Chikungunya $PRNT_{50}$ antibody geometric mean titre (GMT) 28 days after the last MV-CHIK vaccination in the Per Protocol (PP) analysis population between the treatment groups. GMTs and GMT ratios will be estimated by applying an analysis of variance including the factor treatment group. This will be done using log_{10} transformed data and taking the anti-log of the resulting point estimates for the least squares means, least squares means differences and the corresponding 2-sided 95% CIs. P-values will also be provided to compare GMTs between the treatment groups adjusted for multiple comparisons according to Tukey-Kramer. Likewise, seroconversion rates will be compared between groups. Seroconversion will be defined as anti-Chikungunya $PRNT_{50}$ titres ≥10. Measles- and Chikungunya- ELISA titres will be analysed as described above. In addition, the long-term stability of anti-Chikungunya antibodies determined by $PRNT_{50}$ and ELISA, 6 months and one year after the first immunisation, will be analysed compared between the five treatment groups. The analysis of safety will be performed by means of descriptive measures in the Safety Population. All subjects entered into the study who receive at least one vaccination will be included in the safety analysis. For solicited local, solicited systemic and unsolicited AEs, the number and percentage of subjects with AEs will be summarised for each treatment group, overall, by system organ class /preferred term, AE grade, and relatedness. Solicited and unsolicited AEs (local and systemic) will be analysed descriptively. Laboratory values and vital signs as well as changes in laboratory values from baseline will be analysed descriptively by time point and treatment group. Interim Analysis: A preliminary analysis including safety and immunogenicity data will be performed after all subjects completed Visit 5 (day 56) and therefore terminated the treatment period of the study. The purpose of this analysis is to provide highly valuable data for further Chikungunya vaccine development including the efficacy evaluation, by passive transfer. Results from this interim analysis will not have any impact on the study design or the subjects during the follow up period of the study. The final analysis will be conducted once the last subject has completed the study.

2. LIST OF ABBREVIATIONS

Ab	Antibody
AE	Adverse Event
AESI	Adverse Event of special interest
ALT	Alanine Aminotransferase (SGPT)
aPTT	Abbreviated Partial Thrombin Time
AST	Aspartate Aminotransferase (SGOT)
CA	Competent Authority
CCID50	Cell Culture Infectious Dose 50%
CHIKV	Chikungunya Virus
CRO	Clinical Research Organisation
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
(e)CRF	(electronic) Case Report Form
EC	Ethics Committee
ELISA	Enzyme Linked Immunosorbent Assay
ER	Emergency Room
FDA	(United States) Food and Drug Administration
FSI	First Subject In
GCP	Good Clinical Practice
GMO	Genetically Modified Organism
GMT	Geometric Mean Titre
HAS	Human Serum Albumin
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSD	Honest Significant Difference
IEC	Independent Ethics Committee
i.m.	Intra Muscular
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
ITT	Intent-To-Treat
MAE	Medically-attended Adverse Event
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-To-Treat
Mo	Month
MV	Measles Virus
PBMC	Peripheral Blood Mononuclear Cell
PP	Per-Protocol
$PRNT_{50}$	Plaque Reduction Neutralisation Test 50%
PT	Prothrombin Time
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SPS®	Stabilising and Protecting Solutions
SUSAR	Suspected Unexpected Serious Adverse Reaction
$TCID_{50}$	Tissue Culture Infective Dose 50%
WHO	World Health Organisation

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4. BACKGROUND INFORMATION AND RATIONAL

4.1 Investigational Drug

The MV-CHIK vaccine includes a backbone of measles virus (Schwarz Vaccine strain), which has been developed at the Institute Pasteur (Combredet C. 2003). Chikungunya Virus structural proteins have been inserted into the MV genome (MV-CHIK) and are expressed as the vaccine antigens. The backbone has already been tested in previous trials of MV-HIV construct (Lorin C. 2004; Stebbings R. 2012; ClinicalTrials.gov Identifier: NCT01320176). A detailed description of the vaccine construct and mechanism of action can be found in the current version of the Investigators' Brochure.

4.2 Disease Background

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 [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 characterised 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 polyarthralgia. 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 oedematous polyarthritis. Chronic

disease is defined by symptoms that persist for more than 3 months [CDC/PAHO Chikungunya virus, 2011].

The frequency of persons reporting persistent symptoms varies substantially by study and the time that had elapsed between symptom onset and follow-up. Studies from South Africa note that 12%−18% of patients will have persistent symptoms at 18 months and up to 2 to 3 years later. More recent studies in India revealed that the proportion of patients with persistent symptoms at 9-12 months was 22% and additional 22% reported symptoms for more than one year (Paul BJ, 2011).

Data from La Réunion have found that as many as 60% of patients complained of persistent symptoms 36 months after disease onset. In almost one half of these patients the joint pain had negative impact on their everyday life and on their ability to work (Schilte C, 2013).

During 2005-2006 several European countries reported imported cases from travellers returning from the Indian Ocean region including France (808 imported cases). A detailed follow up of 47 patients that returned to Marseilles. 38 patients remained symptomatic after the tenth day with severe joint pain and tenosynovitis with a dramatically limited ability to ambulate and carry out activities in daily life (Simon F, 2007).

4.3 Preclinical Studies

Immunogenicity of MV-CHIK and protective effects against infection have previously been demonstrated in a series of preclinical studies in mice and cynomolgous monkeys. These experiments are described in detail in the current version of the Investigators' Brochure.

4.3.1 Preclinical Immunogenicity Studies

Preclinical evaluation of the MV-CHIK vaccine was demonstrated in the measles vaccine virus susceptible mouse strain CD46-IFNAR. The animals are transgenic for the human CD46 receptor, which allows measles vaccine entry into host cells. In addition, the animals are deficient in the Type 1 Interferon receptor (IFNAR) to facilitate virus replication.

Single or two vaccinations of the mice resulted in high levels of chikungunya virus neutralising antibodies. All immunised mice were protected from lethal challenge with chikungunya virus, even after a single immunisation, demonstrating a strong protective capacity in this experimental model. Passive transfer of immune sera to highly susceptible 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 neutralisation against homologous and heterologous circulating strains of chikungunya virus. The vaccine characterisation and proof of concept is described by Brandler, et al [Brandler 2013].

Immunogenicity was also demonstrated in immunocompetent animals (non-human primates). Cynomolgus macaques were immunised twice with MV-CHIK at 2 doses. Neutralising antibodies, as determined by plaque reduction neutralisation test ($PRNT_{50}$), were induced by one or 2 immunisations. In addition, all vaccinated animals were protected against viremia and disease symptoms after challenge with the homologous Chikungunya virus wild type strain (For details please refer to the current Investigators Brochure).

4.3.2 Preclinical Toxicity Studies

4.4 Clinical Studies

MV-CHIK has been evaluated in one completed phase I study (MV-CHIK-101, EudraCT 2013-001084-23). Currently there are 3 ongoing clinical trials with MV-CHIK. One additional phase I clinical trial conducted in the USA under an IND (NTC03028441) and two further phase II trials, conducted in Europe (MV-CHIK-202 in Austria and Germany, EudraCT 2015-004037-26/ NTC02861586) and in the USA (MV-CHIK-204 in Puerto Rico, NTC0310111).

Safety data from all 3 ongoing studies will be shared in real time with the sponsor and communicated to all clinical trial sites in order to ensure that any safety signals that emerge are promptly addressed.

4.4.1 Phase I Study

MV-CHIK-101:

A phase I study has been conducted in Austria at the Medical University, Vienna, Department of Clinical Pharmacology (EudraCT: 2013-001084-23):

Observer blinded, block-randomised, active and placebo-controlled, dose escalation trial to evaluate the optimal dose of MV-CHIK, a new vaccine against Chikungunya virus, in regard to immunogenicity, safety and tolerability in healthy volunteers.

The purpose of this first-in-man study was to evaluate the optimal dose considering immunogenicity, safety and tolerability of the MV-CHIK vaccine. We further analysed the immunogenicity, safety and tolerability of MV-CHIK during the vaccination period up to 28 days after the last vaccination.

Each subject received an i.m. injection of one of the doses of MV-CHIK on day 0 and either an i.m. injection with MV-CHIK on day 28 and an i.m. injection with placebo control on day 90 or the other way around (placebo control on day 28 and MV-CHIK on day 90).

Doses (volumes administered):

Cohort #1: 0.05 mL; $1{,}5x10^4$ ($\pm$ $0{,}5x10^4$) $TCID_{50}$

Cohort #2: 0.25 mL; $7{,}5x10^4$ ($\pm$ $2{,}7x10^4$) $TCID_{50}$

Cohort #3: 1 mL; $3{,}0x10^5$ ($\pm$ $1{,}1x10^5$) $TCID_{50}$

A control group received Priorix®, an approved Measles, Mumps, Rubella vaccine that contains the parental measles vaccine Schwarz strain. Sterile saline has been injected as placebo treatment. The phase I clinical batch was present at a ready to use liquid formulation at a single concentration. In contrast, the proposed phase II clinical trial IMP will be presented as lyophilised product.

Out of 44 healthy male or female volunteers screened 42 subjects (98% (41/42) Caucasians, 55% (23/42) female, mean age 30.5 ± 7.3 years) have been enrolled into the study from November 2013 to June 2014.

Immunogenicity Analysis:

The vaccine immunogenicity was determined by the presence and quantity of functional Chikungunya virus neutralising antibodies in the sera of vaccinated subjects. The neutralisation titres were determined by 50% plaque reduction neutralisation test ($PRNT_{50}$) and total antibody levels were determined by hemagglutination inhibition assays (HIA).

The primary end-point of the study, which was immunogenicity on day 28 after the first vaccination, showed that a single MV-CHIK immunisation elicits functional antibodies in all treatment cohorts. The medium and high dose groups induced similar levels of neutralising antibodies, which were significantly higher than the low dose group and the Priorix® control groups. A second immunisation at one or three months interval boosted the titres in all treatment groups clearly, figure 1). The low dose group showed relatively low titres of both functional and total antibody levels. A second immunisation did boost the titres clearly, however, this dose level is not suitable for a single shot vaccine. The medium dose group elicited significantly higher titres after one or two immunisations. The highest functional and total anti-CHIKV titres were elicited in the highest dose group. The functional antibodies were persistent over the study period. Very similar results were obtained by HAI. Total antibody levels have been induced by a single immunisation and boosted by a second dose.

The Measles antibody titres as measured by ELISA were boosted in all treatment groups (MV-CHIK and Priorix®) after the first vaccination. No clear trend between the individual groups was visible but an increase in Measles virus dose increased the geometric mean titre (GMT). The measles ELISA titres that were induced after the first immunisation were close to the detection limit of the assay. Therefore, a not additive effect was visible after a second immunisation.

The subjects that entered the study were not pre-selected for a specifically high or low Measles antibody titre. The subjects were randomly allocated to the individual treatment cohorts. To analyse the effect of pre-existing anti-measles immunity on the immunogenicity of the MV-CHIK vaccine, we investigated if a baseline anti-measles titre would cause a difference between the $PRNT_{50}$ titres on day 28 after the first immunisation of all subjects in MV-CHIK dose groups (independently of their dose group). This analysis showed that subjects that had a low, medium or high measles titre at baseline were equally prone for the immunogenicity of the MV-CHIK vaccine. Thus, the pre-existing immunity to the vaccine vector did not interfere with the vaccine immunogenicity.

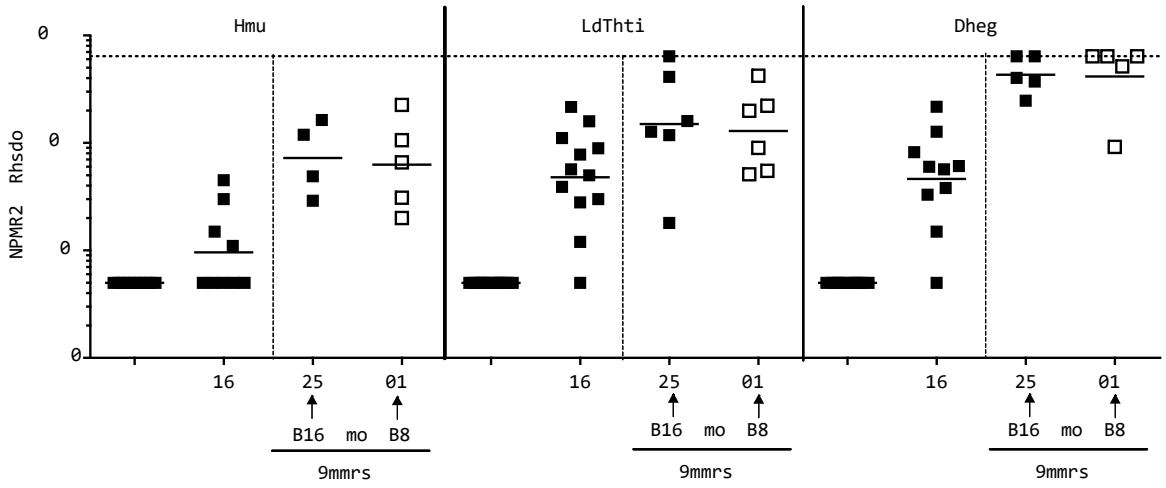


Figure 1: Induction of neutralising antibodies after two immunisations.
Closed squares show the results of subjects that were boosted on Day 28 after the first immunisation. Open squares depict PRNT levels of subjects that were boosted on day 90 after the first immunisation.

Safety Analysis:

Vital sign checks including measurement of blood pressure, pulse rate and axillary body temperature as well as physical examination were performed at all visits. Additionally, the injection site was inspected and evaluated for local side effects by the investigator at each visit and before and 6 hours after each vaccination. Grading of local pain, tenderness, redness, swelling, itching and induration was performed according to the respective FDA guidance for industry and the guidance of the Brighton Collaboration. Accordingly, local side effects were graded as mild (grade 1), moderate (grade 2), severe (grade 3) and potentially life-threatening (grade 4). All participants were provided with a template that allows estimation of local reactions (supplementary information).

Pain, itching and induration were graded as mild if they did not interfere with daily activity, as moderate if they did compromise activity or necessitated repeated use of non-narcotic pain reliever/anti-inflammatory and pain relieving ointment (itching and induration) and as severe if they prevented daily activity. Grade 4 pain/itching included the need for Emergency room (ER)

visit or hospitalisation. Swelling and erythema below a diameter of 5 cm were rated as mild. Swelling/erythema exceeding 10 cm in diameter were considered to be severe and necrosis indicated grade 4 swelling/erythema (supplementary information).

Furthermore, participants were requested to keep a study diary in order to record all local and systemic symptoms and any adverse event occurring during the first two weeks after vaccination starting with the first entry 6 hours (±1) after vaccination. The subjects themselves recorded the assessments once daily at the same time each day. Completeness and accuracy of self-reported symptoms and side effects were verified at each visit by a study-related physician. Diaries were reviewed together with the particular participant at each visit and participants were interviewed for any adverse events. Diaries had to be signed by both the particular participant and the reviewing physician at the end of each vaccination period to confirm and ensure completeness and reliability of self-reporting. The investigator collected the diaries at each visit and new ones were dispensed after vaccination.

Solicited and non-solicited adverse events (AEs) were reported separately. Solicited AEs included pyrexia, flu like illness, headache and injection-site- related side effects (erythema, swelling, induration and pain).

In general, MV-CHIK vaccine had an acceptable tolerability profile. All adverse events observed were treatment emergent AEs and were recovered without any sequelae before or at study end.

The most frequently solicited AEs were headache (57.1%), injection site pain (50.0%), influenza like illness (45.2%), vaccination site pain (40.5%), fatigue (38.1%), nausea (31.0%), myalgia (26.2%) and arthralgia (23.8%). The most frequently related solicited AEs were injection site pain (50.0%), vaccination site pain (40.5%), headache (28.6%) and fatigue (23.8%).

Unsolicited AEs were AEs with none of the preferred terms of solicited AEs. 28 subjects (66.7%) had a total of 73 unsolicited AEs. Two subjects (4.8%) had two severe unsolicited AEs, five subjects (11.9%) had seven related unsolicited AEs and 19 subjects (45.2%) reported 33 unsolicited AEs where an action was taken. Two subjects (4.8%) reported two serious unsolicited AEs. The most frequently unsolicited AEs were nasopharyngitis (26.2%) and oropharyngeal pain (11.9%).

Special attention was directed to any occurrence of joint pain during the MV-CHIK study due to significant joint pathology caused by CHIKV. The subjects were asked to report any symptoms of joint pain in their subject diary and they were asked for any symptoms during each visit. Overall, 23.8% (10/42) of the subjects reported any signs of joint pain or arthralgia during the treatment period, 11.9% (5/42) of which were rated as related to the study treatment. In all 5 cases the joint pain was described as transient musculoskeletal pain, which was accompanied by flu like symptoms. Pain in the limbs associated with MV-CHIK injection was largely graded as mild (82%), was self-limiting and did not engender any withdrawals from the study. The rate of musculoskeletal pain decreased from 12% (n=5; 67% mild) at the first visit to 2% (n=1; 100% mild) at visit 5. No signs of inflammation were observed.

Severe and serious AEs occurred only for subjects treated with MV-CHIK. All serious AEs reported were non-local and unsolicited. Overall, seven severe AEs occurred (in six participants), of which five were solicited and related to vaccination including headache, local erythema, local induration, local pain and pyrexia.

The number of overall AEs and related AEs increased with the MV-CHIK dose. In addition, local reactions to MV-CHIK vaccination increased dose-dependently and were deemed related to the high inoculation volume (1mL) together with the formulation's salt buffer content.

The evaluation of safety laboratory parameters (haematology, serum chemistry, urinalysis) did not indicate any safety issues. There were no clinically relevant abnormalities observed in haematology, clinical chemistry, coagulation or urinalysis laboratory parameters.

The Clinical Study Report has been finalised in Q4/2014. The study results were published in Ramsauer et. al. Lancet Infect Dis 2015.

MV-CHIK-203:

This clinical phase I study (protocol 15-0038) sponsored by the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Disease (NIAID), and National Institute of Health (NIH), currently conducted in Iowa (USA), intends to investigate safety, immunogenicity and schedule of MV-CHIK in 180 subjects age 18-45. 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 currently ongoing, and no preliminary results are available.

4.4.2 Phase II Studies

Currently there are two clinical phase II studies with MV-CHIK going on. One in Europe (MV-CHIK-202; EudraCT: 2015-004037-26) and one in Puerto Rico (MV-CHIK-204; NCT03101111).

MV-CHIK-202:

This phase II 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 in order to assess the effect of recently boosted immunity to Measles on Chikungunya immunogenicity.

A database snapshot was performed in August 2017, providing preliminary interim safety and immunogenicity data up to study day 56.

Safety Analysis:

Until the database snapshot, overall 624 adverse events (AEs) were observed in 263 treated subjects, whereas 456 AEs [73.1%] were solicited by the means of a subjects' diaries and 168 unsolicited [26.9%], reported spontaneously. The intensity was mostly assessed as mild in 468 cases [75.0%] or moderate in 145 cases [23.2%] and 11 cases [1.8%] were classified as severe, disregarding relatedness.

Solicited AEs occurred more often in MV-CHIK group (91 subjects [72.7%]) compared to the control group (17 subjects [34.0%]) but unsolicited AEs were seen more frequently in the control group (17 subjects [34.0%] and 69 subjects [32.4%] in the MV-CHIK group respectively).

The most common solicited, systemic adverse event after immunisation was headache, which was reported by 42 subjects [19.7%] of the MV-CHIK group and by 10 subjects [20.0%] of the

control group. The severity of headache was mostly reported as mild (52 cases [65.0%]) or moderate (23 [28.8%]), 2 subjects reported severe headache.

After headache the most common solicited, systemic adverse events were observed in the following number of subjects: fatigue (33 [14.4%] MV-CHIK; 3 [8.8%] control group), flu like symptoms (16 [7.9%] MV-CHIK; 1 [2.9%] control group), nausea (14 [6.1%] MV-CHIK; 3 [8.8%] control group) and myalgia (14 [6.1%] MV-CHIK; 3 [8.8%] control group). Taken together, the frequency of solicited systemic AEs was equally distributed between MV-CHIK and control group.

Arthralgia, regarded as adverse event of special interest, was solicited and observed in 9 subjects [3.9%] treated with MV-CHIK and in 2 subjects [5.9%] of the control group.

The general local tolerability was assessed one hour after each vaccination by the investigator and additionally recorded in a diary by the subjects for 7 days upon treatment.

The most common local side effects were tenderness (57 subjects [26.8%] MV-CHIK; 4 subjects [8.0%] control group), pain (38 [17.4%] MV-CHIK; 4 [8.0%] control group), induration (19 [8.9%] MV-CHIK; 0 [0.0%] control group) and erythema (16 [7.5%] MV-CHIK; 1 [2.0%] control group) with higher reporting rates after MV-CHIK administration.

In addition, no clinical relevant abnormalities were detected in haematology or clinical chemistry laboratory results in either treatment group and no case of anaphylactic reaction occurred in the study population up to visit 3.

One serious adverse event was reported for a 49-year old male subject, who underwent surgery for umbilical hernia 120 days after his last vaccination. As this subject already had a medical history of umbilical hernia, the event was assessed as not related to MV-CHIK or control vaccine.

Overall the interims analysis of safety and tolerability data up to study visit 3, showed that the MV-CHIK vaccine is well tolerated and safe.

Immunogenicity Analysis:

Preliminary $PRNT_{50}$ conducted after completion of the primary endpoint, 28 days after one or two immunisations (day 56) clearly demonstrated that immunisation with one or two MV-CHIK doses (at either dose level) induced high levels of neutralising antibodies and a seroconversion rate of up to 95% in the prime/boost groups. The immunisation with a single MV-CHIK dose was less potent, however, higher titres and seroconversion rates were achieved in the high dose groups.

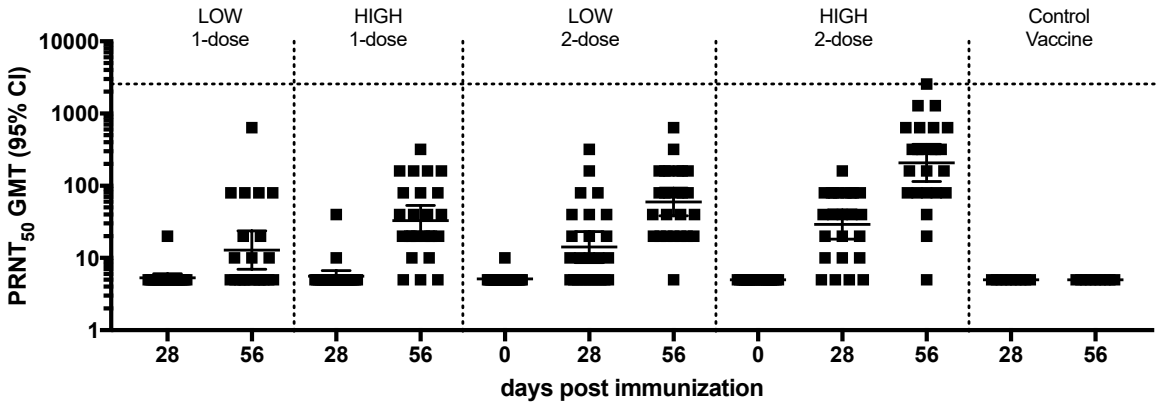


Figure 2: MV-CHIK 202 preliminary data.
Neutralising antibodies as determined by $PRNT_{50}$ after one or two immunisations.

MV-CHIK-204:

This clinical phase II study (NCT03101111) initiated in May-2017 and currently conducted in Puerto Rico, a previously epidemic area, evaluates the safety and immunogenicity of MV-CHIK in 100 subjects. The main objective is to determine the safety of MV-CHIK administered in 2 doses (5×10^4 and 5×10^5 $TCID_{50}$) separated by 28 days in previously exposed versus unexposed individuals. The subjects will be randomised in a double-blind fashion to receive either MV-CHIK or the licensed active measles, mumps, and rubella vaccine (Priorix®) in a 4:1 ratio. Additionally, immunogenicity of MV-CHIK will be investigated in previously exposed versus unexposed individuals by a neutralisation assay.

4.5 Study Rationale

The current phase II trial is designed to investigate the immunogenicity, safety and tolerability of MV-CHIK as well as the long-term durability of anti-Chikungunya antibody response after administration of different dose levels of MV-CHIK in three different formulations.

The concentrations of the evaluated doses are $5x10^4$ (± 0.5 log) $TCID_{50}$/dose, $1x10^5$ (± 0.5 log) $TCID_{50}$/dose and $1x10^6$ (± 0.5 log) $TCID_{50}$/dose and the compared formulations are lyophilised, liquid frozen and liquid SPS® (Stabilising and Protecting Solution), respectively.

The induction of functional anti-Chikungunya antibodies by MV-CHIK will be investigated in healthy adults aged 18 to 55 years, by means of immunoassays like ELISA and T-cell assay and $PRNT_{50}$. The timepoint day 28 and day 56 after immunisation were found to be suitable for immunogenicity analyses to reveal valuable information on future treatment recommendations (single shot treatment vs. double immunisation).

A randomised, observer-blinded study design was chosen to allow an objective recording and assessment of all adverse events.

The MV-CHIK doses used in previous clinical trials (phase I and phase II) was calculated between $1,5x10^4$ (± 0.5 log) - $5x10^5$ (± 0.5 log) $TCID_{50}$/dose. This dose was efficient to induce a seroconversion rate of up to 96% in the study population. To further investigate the capacity of the vaccine to induce sufficiently high neutralising titres and seroconversion rates after a single immunisation, an increased dose $1x10^6$ (± 0.5 log) $TCID_{50}$/dose will be administered once or twice.

The different vaccination schedules, two MV-CHIK vaccinations on days 0 and 28 or one MV-CHIK vaccination on day 0 and placebo on day 28, allow to compare the immunogenicity of these dosing regimens. A single shot or two vaccinations within a short period of time are desirable to allow for a quick immunisation before travelling to areas with active transmission of the Chikungunya virus.

In addition to the previously tested liquid frozen and lyophilised formulations, Themis has an ongoing project to improve the product stability in a variety of potential formulations. Recently, a liquid formulation (stored at 2-8°C) showed promising stability data. Clearly, a liquid formulation will substantially decrease the costs of good and will facilitate the use of the vaccine throughout the world. Thus, a group of subjects will receive the SPS formulation and safety and immunogenicity will be analysed.

The induction of virus specific T cells is a critical step in the generation of a functional immune response. For this purpose, the rate of Chikungunya virus specific T cells will be analysed in vitro.

Additionally, the study design will allow for the first time to gather data on the long-term stability of specific antibodies, by testing serum samples 6 months and 1 year after the first immunisation with MV-CHIK.

Up to now the level of protective anti-Chikungunya-Ab titre could only be estimated. Therefore, it is of special interest to evaluable and proof Ab titres that are able to prevent CHIKV infections. The design of the study will allow to collect human serum samples, to verify efficacy, by passive transfer of antibodies to non-human primates in future investigations.

This study was designed according to the Note for Guidance on Clinical Evaluation of New Vaccines (CHMP/VWP/164653/2005), where applicable, and will be conducted in compliance with the protocol, Good Clinical Practice (GCP) as set forth in the International Council on Harmonisation (ICH) guidelines on GCP (ICH E6), and applicable local regulatory requirements.

4.6 Benefit/Risk Assessment

General

Potential risks that are frequently associated with vaccination are the occurrence of local reactions such as oedema, 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. Additionally, needle picks for blood sampling may cause discomforts and local reactions.

MV-CHIK Related

As described above, adverse events (AEs) previously reported with MV-CHIK include injection site pain, headache, fatigue, and joint pain or arthralgia. Refer to the current version of the Investigators' 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 immunised individuals. The MV-CHIK vaccine is based on live measles virus and contains no live Chikungunya virus. Therefore, 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).

Potential Benefit

If the clinical development of MV-CHIK is successful, the potential benefit of MV-CHIK vaccination would be life-long protection against Chikungunya infection helping people, who are at risk of acquiring a possibly life-threatening infection with CHIKV.

A boost in measles immunity may be an additional benefit.

Conclusion

The benefit risk ratio is considered favourable.

5. TRIAL OBJECTIVES AND MEASUREMENTS

5.1 Primary Objective

- To investigate immunogenicity and safety of MV-CHIK in different dose regiments, 28 days after one or two vaccinations

The immunogenicity will be determined by means of $PRNT_{50}$ 28 days after a single shot treatment at Visit 3 (study day 28) and 28 days after two treatments with MV-CHIK at Visit 5 (study day 56). Local and systemic AEs will be recorded and assessed.

5.2 Secondary Objectives

- To compare immunogenicity, safety and tolerability between different doses and formulations of MV-CHIK during the treatment period up to day 56

- To investigate the immunogenicity and safety of a novel liquid vaccine formulation

To cover this objective immunogenicity will be determined by means of $PRNT_{50}$ at the following timepoints during the treatment period: Visit 1 (day 0), Visit 3 (day 28) and Visit 5 (day 56). Solicited and unsolicited AEs will be recorded at each visit during the treatment period.

- To evaluate cellular responses induced by one or two immunisations

The cellular response will be investigated by T cell analysis of PBMCs isolated at Visit 1(day 0), Visit 2 (day 14), Visit 3 (day 28), Visit 4 (day 42) and Visit 5 (day 56)

- To evaluate the long-term immunogenicity of MV-CHIK up to one year after the first MV-CHIK treatment.

The long-term immunogenicity will be observed by means of $PRNT_{50}$ detecting anti-Chikungunya antibodies 6 months and one year after the first treatment with MV-CHIK (at Visit 6 and Visit 7).

- To collect human sera for future efficacy evaluation, by passive transfer of anti-Chikungunya antibodies to virus susceptible non-human primates

On study day 0 (Visit 1), day 28 (Visit 3), day 56 (Visit 5) and day 365 (Visit 7) blood samples will be collected for isolation of anti-Chikungunya antibody containing human serum.

6. TRIAL DESIGN

6.1 Overall Study Design

This is a prospective, interventional, observer-blinded, block-randomised, phase II trial, comparing different dose levels of MV-CHIK in three different formulations (lyophilised, liquid frozen and liquid SPS®) to evaluate immunogenicity, safety and tolerability of this novel Chikungunya vaccine. Placebo (physiological saline solution) will be applied to blind two different treatment schedules, evaluating single shot versus double shot treatment.

The study will be conducted at a single study site, Celerion in Belfast (United Kingdom) and is registered online at http://www.ClinicalTrials.gov under EudraCT number 2018-000211-25.

After the screening procedures, 60 healthy male and female volunteers aged 18-55 years will be randomly assigned to one of five treatment groups (A, B, C, D or E). Subjects will be observed for safety and immunogenicity for 56 days during the treatment period, starting with the first MV-CHIK vaccination at Visit 1 (day 0) up to Visit 5 (day 56). After this treatment period subjects will return to site at Visit 6 (after 6 months) and at Visit 7 (after 1 year) for collection of serum to follow up the durability of specific antibodies against Chikungunya.

The investigator and site personnel assessing AEs, all subjects, as well as the sponsor's representatives involved in the monitoring and conduct of the study will be blinded to which vaccine was administered. Only the site personnel performing randomisation, preparation and administration of IMP will be unblinded.

6.2 Study Endpoints

6.2.1 Primary Endpoint

- Immunogenicity 28 days after the last MV-CHIK vaccination confirmed by the presence of functional antibodies as determined by the plaque reduction neutralisation test ($PRNT_{50}$)

6.2.2 Secondary Endpoints

- Rate of solicited and unsolicited adverse events during the treatment period until day 56
- Serious adverse events during the treatment period until study day 56
- Immunogenicity on days 0, 28 (group A, B, C, and D), days 0, 56 (group E) and long-term immunogenicity on day 182 (6 Mo) and 365 (12 Mo), confirmed by the presence of functional antibodies as determined by the plaque reduction neutralisation test ($PRNT_{50}$).
- Measurement of anti-Chikungunya antibodies on days 0, 28, 56, 182 and 365 determined by enzyme linked immunosorbent assay (ELISA)
- Measurement of anti-Measles antibodies on days 0, 28, 56 determined by enzyme linked immunosorbent assay (ELISA)
- Induction of Chikungunya specific immune responses on days 0, 14, 28, 42 and 56 determined by T-cell assay.

- Safety laboratory parameters (haematology, serum chemistry, coagulation, urinalysis)

6.3 Treatment Groups

After the screening procedure, 60 subjects eligible for the study will be randomised to one of the following 5 treatment groups:

Group A:

two vaccinations with MV-CHIK lyophilised formulation, low dose, on day 0 and day 28

Group B:

two vaccinations with MV-CHIK liquid frozen formulation, low dose, on day 0 and day 28

Group C:

two vaccinations with MV-CHIK liquid SPS® formulation, low dose, on day 0 and day 28

Group D:

two vaccinations with MV-CHIK liquid frozen formulation, high dose, on day 0 and day 28

Group E:

one vaccination with MV-CHIK liquid frozen formulation, high dose on day 0 and placebo on day 28

The concentration of different MV-CHIK dose levels per treatment group are:

Group A: low dose: $5x10^4$ ±0.5 log $TCID_{50}$ /dose

Group B+C: low dose: $1x10^5$ ±0.5 log $TCID_{50}$ /dose

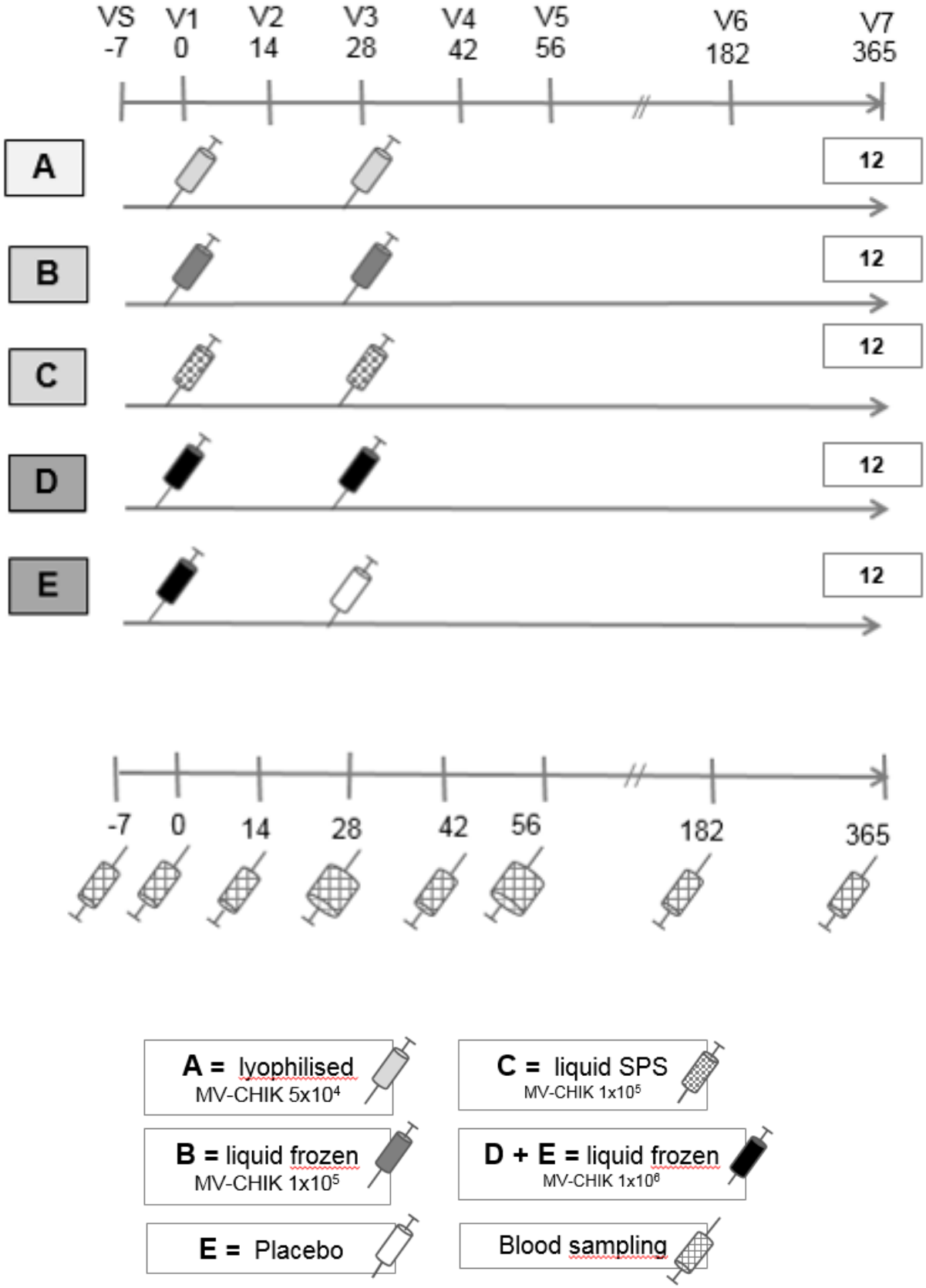
Group D+E: high dose: $1x10^6$ ±0.5 log $TCID_{50}$ /dose

The actual vaccine dosages are dependent on the manufacturing process, specifying and allowing a window of ±0.5 log. The five different treatment groups, group sizes and vaccination schedules are summarised in table 1.

Table 1: Treatment Groups and Vaccination Schedule

Treatment Group	N	Vaccine	Formulation	Vaccine on day	Placebo on day
A	12	MV-CHIK $5x10^4$ (±0.5 log) $TCID_{50}$	lyophilised	0 + 28	
B	12	MV-CHIK $1x10^5$ (±0.5 log) $TCID_{50}$	liquid frozen	0 + 28	
C	12	MV-CHIK $1x10^5$ (±0.5 log) $TCID_{50}$	liquid SPS®	0 + 28	
D	12	MV-CHIK $1x10^6$ (±0.5 log) $TCID_{50}$	liquid frozen	0 + 28	
E	12	MV-CHIK $1x10^6$ (±0.5 log) $TCID_{50}$	liquid frozen	0	28

6.4 Study Flowchart



6.5 Study Procedures

Table 2: Study Procedures

	Screening	Treatment Period					Follow up Period		
Visit	VS	V1	V2	V3	V4	V5	V6	V7	ET
Study Day	-28 to -1	0	14 ±1	28 ±5	42 ±5	56 ±5	182 ±14	365 ±14	(7)
Informed Consent	x								
Inclusion/Exclusion Criteria	x	x							
Medical History	x	x							
Vaccination History	x								
Vital Signs (1)	x	x		x		x			x
Physical Examination (2)	x	x		x		x			x
Adverse Events (3)		x	x	x	x	x			x
Concomitant Medication (4)	x	x	x	x	x	x			x
Clinical Laboratory (5)	x			x		x			x
Pregnancy Test	x	x		x		x			x
Study Treatment (8)		x		x					
Dispense subject diary		x		x					
Collect subject diary			x		x				x
Local Tolerability (6)		x	x	x	x	x			x
Immunogenicity ($PRNT_{50}$)		x		x		x	x	x	x
T-cell Immunity		x	x	x	x	x			x
CHIKV-antibodies (ELISA)		x		x		x	x	x	x
Measles-antibodies (ELISA)		x		x		x			x
Serum collection for passive transfer		x		x		x		x	x

(1) Assessment of vital signs will include systolic and diastolic blood pressure, pulse rate and body temperature

(2) A full physical examination will be performed at screening and a system based physical examination will be done at Visit 1, 3 and 5, if necessary according to findings in a symptom-directed physical examination

(3) Conditions/symptoms noted prior to first IMP administration will be reported as medical history and not as AEs, At Visit 6 and 7 AEs will not be recorded as subjects are allowed to participate in other clinical studies during the follow-up period.

(4) Medications administered within 30 days prior to screening until end of treatment period will be documented as concomitant medication.

(5) Clinical Laboratory parameters include:
- Virology: HB s-Ag, anti-HB c-Ab, anti-HC-Ab, HIV-Ab (only once at screening)
- Haematology: haemoglobin, haematocrit, erythrocyte count, differential and total white blood count, platelets
- Chemistry: creatinine, potassium, sodium, calcium, AST, ALT, alkaline phosphatase, bilirubin
- Coagulation parameter: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen
- Urinalysis: pH-value, glucose, protein, bilirubin, urobilinogen, blood, leucocytes esterase, nitrite, ketone and specific gravity. In case of abnormal findings, a microscopy will be done for confirmation.

Please refer to section 8.11.1

(6) Local tolerability to be investigated 2 hours after each vaccination and additionally at the subsequent visits and prior to IMP administration.

(7) If the early termination occurs within the treatment period (at or before Visit 5), subjects shall perform all procedures as defined for Visit 5. If the early termination occurs within the follow up period (after Visit 5), subjects shall perform all procedures defined for Visit 7

(8) Administration of IMP will be performed according to allocated treatment group, after randomisation at Visit 1

7. PARTICIPANT IDENTIFICATION

7.1 Trial Participants

Healthy male and female volunteers will be screened for inclusion and exclusion criteria and randomised to one of 5 treatment groups in case of eligibility.

60 subjects will be enrolled if all of the following inclusion criteria and none of the following exclusion criteria apply:

7.2 Inclusion Criteria

1. Signed informed consent obtained before any trial-related activities
2. Healthy men or women aged 18 to 55 years on the day of consenting
3. Ability to comprehend the full nature and purpose of the study, including possible risks and side effects; ability to cooperate with the investigator and to comply with the requirements of the entire study
4. All female subjects must have a negative serum pregnancy test at screening
5. Willingness not to become pregnant or to father a child during the entire study period by practicing reliable methods of contraception as specified in protocol section 8.11.4
6. Availability during the duration of the trial

7.3 Exclusion Criteria

The subject may not be enrolled in the study if any of the following applies:

1. Participation in another clinical study (including exposure to an investigational medicinal product ore device) within one month before the screening visit or planned concurrent participation in another clinical study before completion of the treatment period (day 56)
2. History of immunodeficiency, known human immunodeficiency virus (HIV) infection or current hepatitis B/C infection
3. History of drug addiction including alcohol dependence within the last 2 years
4. Inability or unwillingness to avoid intake of more than around 20g alcohol per day during 48 hours after each vaccination (equals roughly 0.5 L beer or 0.25 L of wine)
5. Vaccination within 4 weeks prior to first vaccination or planning to receive any non-study vaccine until end of treatment period (day 56)
6. Prior receipt of any Chikungunya vaccine
7. History of moderate or severe arthritis or arthralgia within the past 3 months prior to Screening Visit
8. Recent infection within 1 week prior to Screening Visit (possibility of deferral)

9. Blood donations including plasma donations, 90 days prior to Screening Visit and anticipated blood, plasma, tissue, sperm or organ donation, throughout the study until end of treatment period (day 56)
10. Clinically relevant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine, inflammatory, autoimmune or neurological diseases or clinically relevant abnormal laboratory values, that in the opinion of the investigator may interfere with the aim of the study
11. History of neoplastic disease (excluding non-melanoma skin cancer that was successfully treated) within the past 5 years or a history of any haematological malignancy
12. Behavioural, cognitive, or psychiatric condition that in the opinion of the investigator affects the ability of the participant to understand and cooperate with the study protocol
13. History of severe adverse reactions to vaccine administration, including anaphylaxis and related symptoms, such as urticaria, respiratory difficulty, angioedema and abdominal pain to vaccines, or history of allergic reaction likely to be exacerbated by any component of the vaccine
14. History of anaphylaxis to drugs or other allergic reactions, which the investigator considers compromising the safety of the volunteer
15. Use of medication during 2 weeks before the first vaccination and throughout the study, which the investigator considers affecting the validity of the study, except hormonal contraception or hormonal replacement therapy in female subjects. (Prior to taking any medication within 72 h before study vaccination, the subject should consult the investigator)
16. Use of immunosuppressive drugs like corticosteroids (excluding topical preparations) within 30 days prior to the first vaccination or anticipated use before completion of the treatment period (day 56)
17. Receipt of blood products or immunoglobulins within 120 days prior to the Screening Visit or anticipated receipt of any blood product or immunoglobulin before completion of the treatment period (day 56)
18. Pregnancy (positive pregnancy test at screening or during the treatment period) or lactation at screening, or planning to become pregnant during the treatment period
19. Unreliable contraception methods (for details please refer to protocol section 8.11.4)
20. Persons in a direct relationship with the sponsor, an investigator or other study team members. Direct dependent relationships include close relatives (i.e. children, parents, partner/spouse, siblings) as well as employees of the study site or the sponsor

8. TRIAL PROCEDURES

8.1 Informed Consent

Before any trial specific procedures are performed subjects will be informed about the exact nature of the trial, what it will involve for the participant, all procedures as determined by the clinical protocol, the known side effects and any risks involved in taking part. It will be clearly stated that the subject is free to withdraw from the trial at any time for any reason without prejudice to future care, without affecting their legal rights and with no obligation to give the reason for withdrawal.

The participant will be allowed as much time as needed to consider the information, and the opportunity to question the investigator or other independent parties to decide whether they will participate in the trial.

Written informed consent will then be obtained by means of participant's dated signature and dated signature of the qualified and experienced person who presented and obtained the informed consent. A copy of the signed informed consent will be provided to the subject and the original signed form will be retained at the site.

8.2 Randomisation

At Visit 1 (day 0) 60 eligible subjects will be randomly assigned to one of five treatment groups (A, B, C, D or E) by means of randomisation envelopes provided by data management.

Each subject will be assigned a unique three-digit randomisation number, stated on the envelope in ascending order. Only trained members of the unblinded study team will be authorised to open one envelope per subject, containing the information about the allocated treatment group.

Upon opening, the randomisation envelope shall be signed with date and time of randomisation and the number of the randomised subject shall be written down on it.

All 60 subjects will be equally distributed between the 5 groups using a randomisation ratio of 1 : 1 : 1 : 1 : 1 = A : B : C : D : E

A subject will be considered enrolled once the first IMP administration has been performed according to randomisation.

8.3 Blinding and Code-breaking

As this study will be conducted in an observer-blinded manner, unblinded study team members responsible for randomisation, preparation and administration of IMP, will otherwise not be involved in the conduct of the study.

All subjects, the investigator and site personnel performing study related assessments, as well as the sponsor's representatives involved in the monitoring and conduct of the study, will be blinded to treatment assignment.

Besides one set of randomisation envelopes, the site will receive an additional set of emergency envelopes stored in the ISF for unblinding purposes. As the knowledge of the subject's treatment might become mandatory (e.g. in case of emergency) the investigator has the possibility to open the emergency envelope identified with the subject's randomisation number and unblind the subject.

Please note that this code-breaking should only be done by the investigator if the knowledge of the subject's treatment influences the decision on further procedures. If unblinding would not make any difference for further treatment, the study team should remain blinded.

8.4 Screening Procedures

8.4.1 Screening Visit, (VS, 1-28 days prior to Visit 1)

After subjects have signed the informed consent form the screening visit will be performed within four weeks prior to Visit 1.

During this visit, all subjects will be checked for inclusion and exclusion criteria, undergo a full physical examination and evaluation of vital signs (systolic and diastolic blood pressure, pulse rate and body temperature). Medical history, vaccination history (covering the last three years prior to screening), prior/concomitant medication within 30 days prior to screening and demographic data will be recorded.

Blood and urine samples will be collected for clinical laboratory assays (HIV, hepatitis B and C, haematology, coagulation parameters, clinical chemistry and urinalysis). HIV tests with available results performed up to 30 days before screening are acceptable. For details on laboratory sampling and testing, see section 8.11.1.

A serum β-HCG pregnancy test will be performed in all women.

All subjects eligible for the clinical study will be asked to return to the study site within 28 days after the screening visit for Visit 1.

8.5 Treatment Period

8.5.1 Visit 1 (day 0)

Subjects found to be eligible will return to site for Visit 1 within 28 days after the screening visit. Inclusion and exclusion criteria will be checked again and (in case of changes since the screening visit) the medical history will be updated. Symptom-directed physical examination, system-based assessment (only if necessary according to findings in symptom-directed physical exam) and vital signs (systolic and diastolic blood pressure, pulse and body temperature) will be recorded. In addition, the subjects will be asked regarding changes in

concomitant medications since the last visit. A serum pregnancy test will be performed in all women.

Blood samples for baseline immunogenicity assessments (measles and Chikungunya antibody ELISA, $PRNT_{50}$) and isolation of serum for passive transfer will be drawn from an appropriate forearm or cubital vein. For T-cell assays blood samples will be collected and peripheral blood mononuclear cells (PBMCs) will be prepared. For details on laboratory sampling and testing, see section 8.11.

Subjects will be randomised to treatment group A, B, C, D or E and receive their first MV-CHIK vaccination.

The subjects' diaries will be explained and handed over. Subjects will be asked to report their body temperature and any adverse event in these diaries for 7 days, starting 6 hours after the injection.

After observation of the subjects for two hours, local reactions and adverse events (if any occurred) will be recorded.

Blood pressure and pulse rate will be checked, and participants will be discharged only if the investigating physician considers it to be safe for them to leave the department.

The subjects are invited to return to study site for Visit 2 on study day 14 (± 1 day).

8.5.2 Visit 2 (day 14 ±1 day)

Subjects will be requested to return to site for Visit 2, 13 to 15 days after the first vaccination.

The investigator or designee will assess local tolerability (of previous vaccination site) and ask about any new concomitant medications or AEs occurred since the last visit. The subject's diary will be collected and checked for completeness.

A blood sample will be collected and PBMCs will be prepared for T cell analysis.

The subjects will be asked to return to study site for Visit 3 on study day 28 (± 5 days).

8.5.3 Visit 3 (day 28 ±5 days)

Subjects will be invited to return to site for Visit 3, 23 to 33 days after Visit 1.

The investigator or designee will examine local tolerability (of previous vaccination site) and ask about any new concomitant medications or AEs occurred since the last visit.

In addition, symptom-directed physical examination, system-based assessment (only if necessary according to findings in symptom-directed physical exam) and vital signs (systolic and diastolic blood pressure, pulse rate and body temperature) will be recorded.

A serum pregnancy test will be performed in all women.

Blood samples will be taken for clinical chemistry, haematology and coagulation parameters and a urine sample will be collected for urinalysis.

Additional blood samples for immunogenicity assessments (measles and Chikungunya ELISA, $PRNT_{50}$) and isolation of serum for passive transfer will be drawn from an appropriate vein. For T-cell assays blood samples will be collected and PBMCs will be prepared. For details on laboratory sampling and testing, see section 8.11.

Subjects will receive their second vaccination of MV-CHIK or placebo, according to their randomised treatment group.

The subjects' diaries will be explained and handed over. Subjects will be asked to report their body temperature and any adverse event in these diaries for 7 days, starting 6 hours after the injection.

After observation of the subjects for two hours, local reactions and adverse events (if any occurred) will be recorded.

Blood pressure and pulse rate will be measured, and participants will be discharged only if the investigating physician considers it to be safe for them to leave the department.

The subjects are invited to return to study site for Visit 4 on study day 42 (± 5 days).

8.5.4 Visit 4 (day 42 ±5 days)

Subjects will be asked to return for Visit 4, 37 to 47 days after Visit 1.

The investigator or designee will assess local tolerability (of previous vaccination site) and ask about any new concomitant medications or AEs occurred since the last visit. The subject's diary will be collected and checked for completeness.

A blood sample will be collected and PBMCs will be prepared for T cell analysis.

The subjects are invited to return to study site for Visit 5 on study day 56 (± 5 day).

8.5.5 Visit 5 (day 56 ±5 days)

Subjects will be asked to return to site for Visit 5, 51 to 61 days after Visit 1.

The investigator or designee will examine and document local tolerability (of the previous vaccination site) and ask about any new concomitant medications or AEs occurred since the last visit.

Symptom-directed physical examination, system-based assessment (only if necessary according to findings in symptom-directed physical exam) and vital signs (systolic and diastolic blood pressure, pulse rate and body temperature) will be recorded. A serum pregnancy test will be performed in all women.

Blood samples will be taken for clinical chemistry, haematology, coagulation parameters and urine samples will be collected for urinalysis.

Additionally, blood samples for immunogenicity assessments (measles and Chikungunya ELISA, $PRNT_{50}$) and isolation of serum for passive transfer will be drawn from an appropriate

vein. For T-cell assays blood samples will be collected and PBMCs will be prepared. For details on laboratory sampling and testing, see section 8.11.

Upon Visit 5, the treatment period of this study will be completed. Subjects will be allowed to participate in other clinical trials after all procedures of Visit 5 will have been performed.

Blood pressure and pulse rate will be measured before participants will be discharged, and subjects are invited to return to study site for follow up Visit 6 on study day 182 (± 14 days).

8.6 Follow up Period

8.6.1 Visit 6 (day 182 ±14 days)

Subjects will be asked to return to site for follow up Visit 6, 168 to 196 days (6 months) after Visit 1.

Blood samples will be collected for immunogenicity assessments (Chikungunya antibody ELISA and $PRNT_{50}$). For details on laboratory sampling and testing, see section 8.11.

The subjects are invited to return to study site for the final Visit 7 on study day 365 (± 14 days).

8.6.2 Visit 7 (day 365 ±14 days), termination visit

Subjects will be asked to return for the final Visit 7, 351 to 379 days (1 year) after Visit 1.

Blood samples will be collected for immunogenicity assessments (Chikungunya antibody ELISA and $PRNT_{50}$) and for isolation of serum for passive transfer. For details on laboratory sampling and testing, see section 8.11.

8.7 Early Termination Visit (ET)

Subjects who are withdrawn or wish to discontinue the study prematurely for any reason shall be asked to attend an early termination visit.

If the early termination occurs within the treatment period (at or before Visit 5), subjects shall perform all procedures as defined for Visit 5. If the early termination occurs within the follow up period (after Visit 5), subjects shall perform all procedures defined for Visit 7.

8.8 Unscheduled Visits

Unscheduled visits can be conducted whenever deemed necessary. The extent of the examination performed at an unscheduled visit is at the discretion of the investigator. All procedures completed during an unscheduled visit should be documented in the source data and should also be entered to the electronic case report form (eCRF).

8.9 Discontinuation/Withdrawal of Subjects

Subjects may withdraw from participation in the study at any time. If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision should be recorded in the electronic case report forms (eCRFs) although it is recognised that the subject may refuse to provide a reason.

Generally, any enrolled/vaccinated subject may withdraw or be withdrawn from the study for the following reasons:

- The subject withdraws consent
- The subject fails to comply with the requirements of the protocol
- At the discretion of the investigator
- For an adverse event, which at the discretion of the investigator requires discontinuation of the subject or results in inability to continue to comply with trial procedures
- if the administration of a drug, which is not permitted by the study protocol, becomes necessary (this should be discussed and agreed between the investigator and the sponsor)
- violation of exclusion criteria 1, 4, 5, 7, 12, 15, 16, 17, 18, 19 or 20 during the study (this should be discussed and agreed between the investigator and the sponsor)

All subjects who withdraw or are withdrawn prematurely from the study will undergo an early termination visit (see section 8.7).

8.9.1 Safety Stopping Rules

Stopping rules are established at subject level and entire study level.

<u>Individual Subjects Stopping Rules</u>

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:

- Subjects who develop serious adverse reaction (SAR) at the discretion of the investigator
- Subjects who develop a potentially life-threatening (grade 4) solicited systemic adverse reaction that occurs without alternative etiology within 7 days after the study vaccination
- Subjects who develop a medical condition for which continued participation, in the opinion of the investigator, would pose a risk to the subject or would be likely to confound interpretation of the results
- Subjects who become pregnant during the treatment phase of the study
- Subjects who experience anaphylaxis within 24 hours or generalised urticaria within 72 hours after administration of the study vaccine

If any of these occur, appropriate measures to treat the subject will be taken and the sponsor or the sponsor designee will be notified immediately.

Entire Study Stopping Rules

The study will be stopped (no new enrolments and no additional vaccine administered) pending a safety review by the investigator and the sponsor, if any of the following occurs:

- One or more subjects experience a serious adverse reaction (SAR), based on the current reference safety information (Investigator's Brochure)
- One or more subjects' deaths assessed by the investigator, safety manager or sponsor as related to investigational product
- One or more subjects develop laryngospasm, bronchospasm or anaphylaxis within 24 hours of vaccine administration
- Three or more subjects develop injection-site ulceration, abscess or necrosis
- Three or more subjects experience generalised urticaria within 72 hours after administration of study product

Upon completion of this review, the sponsor will determine if study enrolment or treatment should be discontinued, or if study enrolment and treatment may continue according to the protocol.

Irrespective of the stopping rules 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.

Whenever the study is stopped the competent authorities and ethical committees will be informed.

In case the entire study has to be discontinued prematurely, all concerned subjects should be asked to perform an early termination visit.

8.9.2 Replacement of Subjects

Subjects who are discontinued before randomisation will be regarded as screening failures and screening will be continued until 60 subjects will be randomised.

Subjects who withdraw or are withdrawn after randomisation will not be replaced. This may result in a decreased number of subjects in the predefined sample size.

8.10 End of Study

The end of study will be defined as the date of the last visit of the last subject. The study will then be stopped. For premature termination or suspension of the study, refer to section 8.9.1

8.11 Handling of Samples

The procedures of sample collection, preparation and storage will be described in detail in a study specific Lab-Manual. Blood sampling kits and labels will be supplied by the CRO supply vendor. Timepoints and blood volumes are summarised in Table 3.

8.11.1 Samples for Safety Laboratory Parameters

Safety laboratory parameters will be analysed by local laboratories according to applicable laboratory SOP

The following parameters will be measured to evaluate inclusion and exclusion criteria only once at screening visit (SV).

- Virology: Hepatitis B surface-Ag, anti-Hepatitis B core-Ab, anti-Hepatitis C-Ab, HIV Ab. HIV tests that were performed up to 30 days before screening and where results are available are acceptable and do not require a new test.

The following laboratory parameters will be assessed for subject's safety at screening, Visit 3, Visit 5 and additionally at Early Termination (if applicable) as depicted in Table 2.

- Haematology: haemoglobin, haematocrit, erythrocyte count, differential and total white blood count, platelets
- Chemistry: creatinine, potassium, sodium, calcium, AST, ALT, alkaline phosphatase, bilirubin
- Coagulation parameter: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen
- Urinalysis: a standard urine test dipstick for determining pH-value, glucose, protein, bilirubin, urobilinogen, blood, leucocytes esterase, nitrite, ketone and specific gravity will be used. In case of abnormal findings, a microscopy will be done for confirmation.
- Laboratory values deviating from the normal ranges defined by the local laboratory will be evaluated by the investigator. Clinically relevant deviations have to be reported as adverse events and graded according to the FDA Guidance for Industry (Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, Sept. 2007)

8.11.2 Immunogenicity Samples

Plaque reduction neutralisation assay ($PRNT_{50}$)

Humoral systemic immune response will be determined by anti-Chikungunya neutralisation assay. The 50% plaque reduction neutralisation titre ($PRNT_{50}$) will be measured to identify immunogenicity in sera of all subjects.

At Visits 1, 3, 5, 6 and 7 a volume of 8-10 ml blood will be collected, to isolate serum and freeze two aliquots (0.5-1.2 ml /vial) at -80°C, for determination of anti-Chikungunya antibodies by $PRNT_{50}$.

Enzyme linked immunosorbent assay (ELISA)

In addition, humoral systemic immune response mediated by serum IgG antibodies against Chikungunya and against Measles will be determined by IgG ELISA.

At Visits 1, 3, 5, 6 and 7 a volume of 8-10 ml blood will be collected, to isolate serum and freeze two aliquots (3-4 ml /round bottom cryovial) at -80°C, for determination of anti-Chikungunya antibodies by ELISA.

Anti-Measles antibodies will be determined by ELISA out of the same samples collected at Visit 1, 3 and 5. Therefor no additional blood sapling will be required for this assay.

T cell Analysis

Cellular immunogenicity will be determined by evaluation of T cell immune response. Therefore, PBMCs will be isolated from fresh blood within 8 hours after blood draw, aliquoted in 3-5 cryovials (~0.8 ml /vial) and frozen at -80°C for further analysis. The T cells will be re-stimulated in vitro with Chikungunya virus structural protein peptides to determine the number of Chikungunya virus specific T cells after one or two immunisations.

At Visits 1, 2, 3, 4 and 5 a volume of 24-30 ml blood will be collected for isolation of peripheral blood mononuclear cells (PBMCs).

8.11.3 Serum Samples for Passive Transfer

At Visit 1, a volume of 50 ml and at Visit 3, 5 and 7, a volume of 200 ml blood will be collected for isolation of human serum and frozen at -80°C for future efficacy evaluation of the MV-CHIK vaccine. Sera of different anti-Chikungunya antibody titres will be transferred to non-human primates to investigate protective potential against Chikungunya virus challenge.

Table 3: Blood Sample Collection

Test	Type of Vials	Volume ml	Visits	Total Volume ml
Virology (HIV, HBV, HCV)	serum	* 0	VS	* 0
β-HCG Pregnancy Test	serum	*0, 1x 3-5	VS, V1, V3, V5	3-5
Haematology	EDTA	3 - 5	VS, V3, V5	9-15
Coagulation	citrate tubes	3 - 5	VS, V3, V5	9-15
Clinical Chemistry	serum	8 - 10	VS, V3, V5	24-30
$PRNT_{50}$	serum	8 - 10	V1, V3, V5, V6, V7	40-50
ELISA CHIKV	serum	8 - 10	V1, V3, V5, V6, V7	40-50
ELISA Measles	serum	** 0	V1, V3, V5	** 0
PBMCs for T-cell Analysis	citrate tubes	24-30	V1, V2, V3, V4, V5	120-150
Passive Transfer	serum	50 + 3x 200	V1, V3, V5, V7	650
				895-965

* 0 ml: Virology testing at screening and pregnancy testing at screening, V3 and V5 will be performed from the same sample as Clinical Chemistry, no additional blood draw will be necessary

** 0 ml: ELISA for determination of Measles antibodies will be performed from the same sample as the ELISA for CHIK-Ab, no additional blood draw will be necessary

Total blood volume collected during the entire study duration of one year will be 895-965 ml

8.11.4 Pregnancy Test – Contraception

Pregnancy will be determined by evaluation of β-HCG in serum at screening, Visit 1, 3 and 5 (or at early termination) for all women.

A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Only females with a negative result will be allowed to receive study vaccinations.

Subjects with a positive pregnancy test will be excluded from further treatments and the Investigator will inform the CRO immediately by completing a pregnancy report from. Please see section 10.6.

After the last IMP use (Day 56) all subjects will be follow up until Day 365.

Contraception

Birth control methods which may be considered as highly effective are those that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

_ combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:

- Oral
- Intravaginal
- transdermal

_ progestogen-only hormonal contraception associated with inhibition of ovulation:

- oral
- injectable
- implantable

_ intrauterine device (IUD)
_ intrauterine hormone-releasing system (IUS)
_ bilateral tubal occlusion
_ vasectomised partner
_ sexual abstinence

Acceptable birth control methods which may not be considered as highly effective are those that result in a failure rate of more than 1% per year include:

_ progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
_ male or female condom with or without spermicide
_ cap, diaphragm or sponge with spermicide

Birth control methods which are considered unacceptable include:

Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM) are not acceptable methods of contraception. Female condom and male condom should not be used together

8.11.5 Labelling, Storage and Transport of Samples

Please refer to the study specific Lab-Manual for detailed information about labelling, storage and transport of study serum sample vials.

Labelling of Samples

Each sample tube will be clearly and unequivocally identified by a label resistant to the storage temperature which contains at least the following information:

study code, subject number, visit code, test allocation (e.g. ELISA, $PRNT_{50}$)

Samples Storage

Aliquots of samples will be stored at -80°C in a temperature monitored freezer. The temperature will be controlled either by a connected central monitoring system or a min/max thermometer, which will be controlled and documented each working day.

Transport of Samples

Study samples packed in sufficient dry ice will be shipped by an authorised courier from the study site to the sponsor, a dedicated analytical laboratory or a sample storage facility. Pick up of serum samples will be organised together with the site personnel at the end of the study or during the study as necessary.

9. INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

9.1 Investigational Medicinal Product Description

9.1.1 MV-CHIK vaccine – test product

The MV-CHIK vaccine candidate is a recombinant live attenuated viral vectored vaccine, based on the backbone of the measles Schwarz virus strain for prophylaxis of Chikungunya infection. The heterologous antigens are derived from the Chikungunya virus.

Nucleotide sequences encoding Chikungunya structural proteins were inserted into the Measles virus Schwarz strain to produce the candidate vaccine MV-CHIK expressing Chikungunya virus antigens.

The MV-CHIK vaccine is provided by the sponsor in three different formulations in glass vials:

(1) as lyophilised powder for suspension in water for injection:

- MV-CHIK $5x10^4$ (± 0.5 log) $TCID_{50}$/ dose

(2) as liquid frozen suspension for injection in two concentrations:

- MV-CHIK $1x10^5$ (±0.5 log) $TCID_{50}$ / dose
- MV-CHIK $1x10^6$ (±0.5 log) $TCID_{50}$ / dose

(3) suspension in stabilising and protecting solution (SPS®) for injection:

- MV-CHIK $1x10^5$ (±0.5 log) $TCID_{50}$ / dose

(1) The lyophilised product will be reconstituted in 0.5 ml/ dose injection grade water and 0.4ml/ dose will be administered i.m. Following excipients are present: D-sorbitol, sucrose, sodium phosphate, sodium chloride, hydrolysed gelatine and recombinant human serum albumin (HAS). No adjuvants are added. The solvent for suspension will be provided by the study sites.

(2) The frozen formulation will be thawed at room temperature for about 30 min before 0.4 ml will be drawn up into a syringe for i.m. administration.

(3) The solution stabilised in protective SPS® formulation will be kept outside the fridge for about 10 min to adapt to room temperature before i.m. administration of 0.4 ml

For a detailed description of the vaccine construct and mechanism of action please refer to the current version of the MV-CHIK Investigators' Brochure.

For detailed description of receipt, storage, preparation, accountability and destruction of IMP please refer to the study specific IMP-Manual.

9.1.2 Placebo

A sterile physiological saline solution will be used as placebo to ensure blinding of the treatment schedule in case of single vaccination regimen (treatment group E). The

physiological saline solution (0.9% NaCl) is a clear colourless solution ready to use, stored at room temperature and provided by the study site.

9.2 Packaging and Labelling

Individual cardboard boxes will contain single 2 ml amber glass vials, with rubber stopper and aluminium seal, containing either lyophilised powder, frozen liquid or cooled SPS® liquid. The labels on vials and boxes will be in English language.

The ready to use IMP syringe will be labelled fulfilling all requirements of EudraLex Volume 4, GMP Annex 13, 2010.

For further details of IMP labelling, please refer to the study specific IMP-Manual.

9.3 Dose Regimen

All 60 eligible subjects will receive their first treatment with MV-CHIK at Visit 1 (day 0) and 4 weeks later their second treatment with MV-CHIK or placebo at Visit 3 (day 28 ±5)

Visit	VS	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
Study Day		0	14 ±1	28 ±5	42 ±5	56 ±5	182 ±14	365 ±14
Study Treatment		❶		❷				

The 5 treatments groups (A, B, C, D and E) differ in the concentration and formulation of MV-CHIK as well as in single or double shot treatment regimen.

Group A: Lyophilised formulation
2x low dose MV-CHIK $5x10^4$ (±0.5 log) $TCID_{50}$/dose day 0 and day 28

Group B: Liquid frozen formulation
2x low dose MV-CHIK $1x10^5$ (±0.5 log) $TCID_{50}$/dose day 0 and day 28

Group C: Liquid SPS® formulation
2x low dose MV-CHIK $1x10^5$ (±0.5 log) $TCID_{50}$/dose day 0 and day 28

Group D: Liquid frozen formulation
2x high dose MV-CHIK $1x10^6$ (±0.5 log) $TCID_{50}$/dose day 0 and day 28

Group E: Liquid frozen formulation
1x high dose MV-CHIK $1x10^6$ (±0.5 log) $TCID_{50}$/dose day 0
1x placebo day 28

Table 4: Dose Regimen

Treatment Group	N	Vaccine	Formulation	Vaccine on day	Placebo on day
A	12	MV-CHIK $5x10^4$ /0.4 ml	lyophilised	0 + 28	
B	12	MV-CHIK $1x10^5$ /0.4 ml	liquid frozen	0 + 28	
C	12	MV-CHIK $1x10^5$ /0.4 ml	liquid SPS®	0 + 28	
D	12	MV-CHIK $1x10^6$ /0.4 ml	liquid frozen	0 + 28	
E	12	MV-CHIK $1x10^6$ /0.4 ml	liquid frozen	0	28

9.4 Storage and Transport Conditions

The IMP has to be stored under continuously temperature-controlled condition in a lockable room, or lockable freezer with limited access.

The minimum and maximum temperature will be recorded daily in the temperature logs throughout the study, except on weekends and holidays if min/max thermometer will be used.

In case the freezer/fridge is connected to an alarm system and corresponding copies of the readout can be filed, a min/max temperature does not need to be recorded daily. It is acceptable to use a site-specific temperature log, which covers all the required information.

MV-CHIK has to be kept in the outer package to be protected from light.

The lyophilised formulation of MV-CHIK will be stored and transported at -20°C and shall be administered within 30 min after preparation, the ready to use formulation can be stored at 2-8°C for up to 1 hour.

The liquid frozen formulation of MV-CHIK will be stored and transported at -80°C and shall be administered within 30 min after preparation, the ready to use formulation can be stored at 2-8°C for up to 1 hour.

The liquid SPS® formulation of MV-CHIK will be stored and transported at 2°- 8°C and shall be administered within 30 min after preparation, the ready to use formulation can be stored at 2-8°C for up to 1 hour.

The 0.9% saline solution and sterile water diluent will be stored according to instruction leaflet. It is under the site's responsibility to ensure the storage conditions according to the summary product characteristics.

After administration, all used containers properly labelled with subject ID and appropriate visit identifier (e.g. visit number, date of administration) will be stored at site until checked by the unblinded monitor.

9.5 IMP Preparation and Distribution

MV-CHIK and placebo will be prepared and administered by unblinded, authorised and qualified staff members (investigator, pharmacist or nurse) otherwise not involved in the conduct of the trial after randomisation. Preparation of MV-CHIK for injection will be performed in a dedicated lab appropriate for handling of GMOs applying biosafety level 2 (BSL2)

standards although MV-CHIK is assessed as a biosafety level 1 product. BSL2 is applied because there is a negative system biological safety level 2 cabinet available within a containment room, which usually is used for preparing IMPs.

MV-CHIK and placebo will be exclusively used for the present clinical trial and will only be administered to subjects enrolled in the study.

For detailed description of receipt, storage, preparation, accountability and destruction of IMP, please refer to the study specific IMP-Manual.

9.6 GMO Handling

MV-CHIK vaccine is a genetically modified organism requiring biosafety level 1 standards. Nevertheless, preparation and disposal of IMP will be performed in a dedicated lab according to applicable hygiene standards of biosafety level 2. Needles and syringes that have been in contact with MV-CHIK, as well as all other potentially contaminated materials, will be collected in dedicated containers and will be destroyed in a safe manner.

This study will be conducted under regulations for contained use of a GMO.

9.7 Route and Method of Administration of IMP

All subjects will receive intra muscular (i.m.) injections of MV-CHIK vaccine or placebo in the deltoid region of the non-dominant arm (preferred) or the dominant arm. In case of ongoing local AEs from previous vaccinations at the respective injection site, vaccination in the contra-lateral arm should be performed.

9.8 Treatment Compliance

The study medication will be prepared at site and administered during the study Visit 1 and 3. Each treatment will be documented in the subject's charts, the eCRF, an IMP Preparation Log and an IMP Inventory Log. Subjects must receive all assigned vaccinations to be considered compliant.

9.9 Study Drug Accountability

IMP accountability will be performed throughout the entire study, starting with the initial receipt of medication.

The authorised, unblinded study staff members will confirm the number and condition of received vials, by signing and dating an IMP receipt form.

Upon receipt an IMP inventory log will be kept current by the site, detailing the batch numbers, dates and quantity of IMP obtained, used for administration on a per subject basis and

destroyed or returned to the sponsor. This documentation will only be available to an unblinded monitor verifying drug accountability during the study.

At the end of the study, all unused and used MV-CHIK vials (marked with subject ID and visit number) will be returned to the sponsor or destroyed by the investigator in a confidential manner, after final reconciliation and confirmation of correctness of drug accountability.

9.10 Prior and Concomitant Therapy

9.10.1 Permitted Prior and Concomitant Therapy

Any prior vaccination within the last three years prior screening and any medication within 30 days prior screening has to be documented.

Any medication taken during the study up to Visit 5 (study day 56) has to be reported to the investigator and will be documented.

Additionally, any treatment that will be considered necessary for the subject's welfare may be given at the discretion of the Investigator. All 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 preferred, if possible.

All concomitant medications will be coded using the WHO Drug Dictionary.

9.10.2 Forbidden Prior and Concomitant Therapy

Treatments with non-study licensed vaccines within 4 weeks prior to first study vaccination until the end of the treatment period (day 56) are not allowed.

Use of immunosuppressive drugs like corticosteroids (excluding topical preparations) within 30 days prior to the first vaccination or anticipated use before completion of the treatment period (day 56) is not allowed.

Receipt of blood products or immunoglobulins within 120 days prior to the Screening Visit or anticipated receipt of any blood product or immunoglobulin before completion of the treatment period (day 56) is not allowed.

Subjects have to be asked about concomitant medication and vaccinations at each visit. Any concomitant medication or vaccination has to be documented.

10. SAFETY REPORTING

10.1 Definitions

10.1.1 Adverse Event (AE)

Any untoward medical occurrence in a participant to whom an investigational medicinal product (IMP) has been administered, not necessarily caused by or related to that product.

An AE can therefore be any unfavourable or unintended sign, abnormal laboratory finding, symptom or disease temporally associated with the use of an IMP whether or not considered related to the IMP.

10.1.2 Adverse Reaction (AR)

An untoward and unintended response in a participant to an investigational medicinal product which is at least possibly related to any dose administered to that participant.

All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.

10.1.3 Serious Adverse Event (SAE)

A serious adverse event is any untoward medical occurrence that:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- consists of a congenital anomaly or birth defect

Other ‘important medical events’ may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences.

The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy will be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of “serious”.

NOTE: to avoid confusion or misunderstanding of the difference between the terms “serious” and “severe”, the following note of clarification is provided: “Severe” is used to describe the intensity of a specific adverse event, while “seriousness” is used for defining regulatory reporting obligations as supplied above.

10.1.4 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A serious adverse reaction, the nature, severity or outcome of which is not consistent with the applicable product information.

10.1.5 Causality

The relationship of each AE to the IMP must be determined by a medically qualified person according to the following definitions:

Definitely: Temporal relationship to the administration of the study drug and course following a known reaction pattern

Probably: Good reasons and sufficient documentation to assume a causal relationship

Possibly: A causal relationship is conceivable and cannot be dismissed

Unlikely: The event is most likely related to an etiology other than the trial treatment

Not Related: No temporal relationship to the administration of the drug or other factors have caused the event

10.1.6 Severity

AEs must be graded by a medically qualified person as being mild, moderate, severe or life-threatening and their approximate duration given. Definitions of severity are as follows:

Mild: an AE that requires minimal or no treatment and does not interfere with daily activities;

Moderate: an AE that is sufficiently discomforting to interfere with normal activities;

Severe: an AE that is incapacitating or prevents normal activities and may require systemic drug therapy or other treatment.

Potentially life threatening: an AE that requires immediate intervention to prevent death.

"life-threatening" refers to an AE in which the subject was at risk of death at the time of the event, it does not refer to an AE which hypothetically might have caused death if it were more severe.

Please see Appendix 1 for stencil to be used for grading local injection site reactions and Table 5 and 6 for grading of systemic and local reactions.

10.1.7 Expectedness

Expectedness will be determined considering the current Investigators' Brochure.

Expected: an AE that is listed in the current Investigator's Brochure of MV-CHIK.

Unexpected: an AE that is not listed in the current Investigator's Brochure or differs due to greater severity or greater specificity.

All SAEs assessed as unexpected and suspected to be related to the IMP qualify for a SUSAR (suspected unexpected serious adverse reaction) and require expedited reporting.

10.1.8 Outcome

Recovered: A subject has recovered from an AE, when all signs or symptoms returned to normal.

Stabilised: An AE is stabilised when, according to the investigator, the subject is in a clinically stable condition. This term should only be used for chronic conditions and for a given subject only when he/she has completed the study.

Recovered with sequelae: As a result of the SAE, the subject is suffering from persistent or significant disability/incapacity (e.g. became blind, deaf, paralysed). Any AE recovered with sequelae should be rated as an SAE since an SAE criterion is fulfilled.

Not Recovered: An AE currently ongoing.

Ongoing at final examination: An AE ongoing at the subject's last visit.

Died: An AE that caused death.

10.2 Recording of Adverse Events

All AEs occurring during the treatment period starting after the first vaccination at Visit 1 until termination of Visit 5 (day 56) observed by the investigator or reported by the subject (verbally or in the subject diary), will be recorded, whether or not related to the study treatment.

AEs will be requested at each visit during the treatment period up to Visit 5 (day 56) and additionally solicited by a subject's diary for 7 days after each vaccination. (see section 11.7)

The subjects will be asked generally (e.g. "How are you?") as well as specifically (e.g. "Have you experienced any problems since the last visit?").

The investigator must record all adverse events in the eCRF. One single adverse event page must be used per adverse event, from start to resolution, stating the following information:
AE description, date of onset and end date, severity, relatedness, seriousness and action taken. Please consider definitions of section 10.1. Follow-up information should be provided as necessary.

If possible, the investigator should record the diagnosis. If a diagnosis is not available, the investigator should record each sign and symptom as individual adverse events. However, as soon as a diagnosis becomes available, all signs and symptoms should be summarised and replaced by the underlying adverse event term (e.g. common cold instead of separate AEs for headache, rhinorrhoea, arthralgia, fever etc.)

The AE should be documented stating the highest severity (i.e. if an AE goes from mild severity to moderate severity or vice versa, the whole episode of the AE should be reported with the moderate severity). Please consider Table 5 and 6 for grading local injection site reactions and systemic reactions.

For causality reporting purposes, the categories "definitely", "probably" and "possibly" qualify an event as "suspected" adverse reaction. AEs with missing causality assessment will be regarded as possibly related unless further specified.

Any medical condition that is present at screening will be considered as medical history and not reported as an AE. However, if the subject's condition deteriorates at any time during the study, it will be recorded as an AE.

It will be left to the investigator's clinical judgment to decide whether an AE requires the subject's removal from the study. In case of discontinuation, the subject will undergo an early termination visit.

10.3 Reporting of Serious Adverse Events

The investigator should report all SAEs occurring during the treatment period (whether or not related to the IMP) within 24 hours of the site study team becoming aware of the event, to the Assign Safety Desk by completing an SAE Report Form.

The completed SAE Report Form should be submitted to the Assign Safety Desk either by fax or as pdf per e-mail:

Fax: PPD

Email: PPD

For urgent questions, please call the 24-hours Safety Hotline: PPD

Under certain circumstances, the first notification can be done by phone, nevertheless, a written Serious Adverse Event Report Form should be submitted for confirmation to Assign Safety Desk.

After the initial SAE report the investigator will follow up proactively each subject and provide further information like copies of hospital case reports, laboratory reports or autopsy reports, to the Assign Safety Desk within 24 h after receipt.

Assign Safety Desk 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 reported in an expedited manner to the regulatory authorities as required per local regulations within the required time frame.

The original copy of the SAE Report Form and the fax confirmation sheet must be kept with the documentation at the study site. Each SAE has also to be documented in the eCRF as a serious adverse event in the corresponding AE section.

10.4 Follow-up of Adverse Events

During and following a subject's participation in a clinical trial, the investigator will ensure that adequate medical care is provided to the subject for any adverse events, including clinically significant laboratory values related to the trial.

All non-serious and serious adverse events will be followed until the subject has recovered, stabilised, recovered with sequelae or died until end of the study.

Follow-up information about a previously reported SAE must also be reported within 24 hours of the investigator receiving it. The initial SAE report form should be used, stating that this is a follow-up report to the previously reported SAE and giving the number and date of the follow-up report. The information provided should describe whether the event has resolved or continues, if and how it was treated, and whether the subject continued or discontinued study participation, if not already stated in the initial report.

10.5 Reporting of SUSARs

A serious adverse reaction that is not expected according to the current IB and were the causality is assessed as "definitely", "probably" or "possibly", qualifies as "suspected" unexpected serious adverse reaction.

On behalf of the sponsor, Assign Safety Desk will be responsible to fulfil the requirements of expedited reporting of all SUSARs (suspected unexpected serious adverse reactions) to the Competent Authorities, Ethics Committees and principal investigators concerned according to national and European law and regulations.

For fatal and life-threatening SUSARs, this will be done no later than 7 calendar days after the sponsor or delegate is first aware of the reaction. Any additional relevant information to the initial report that was not available within the 7 calendar days will be reported within 8 additional calendar days. All other SUSARs will be reported within 15 calendar days.

Treatment codes will be unblinded for specific participants.

Principal investigators will be informed of all SUSARs related to the relevant IMP for all studies conducted with the same IMP, whether or not the event occurred in the current trial.

10.6 Reporting of Pregnancies

Women should not become pregnant during the treatment period of the study. If a subject becomes pregnant within the treatment period up to day 56, she should immediately inform the investigator about the pregnancy.

The investigator will complete a corresponding Pregnancy Report Form and send the form within 24 hours after becoming aware to Assign Safety Desk via fax or as pdf per e-mail:

Fax: PPD

Email: PPD

The first notification can be done via phone 24-hours Safety Hotline: PPD

Nevertheless, a written Pregnancy Report Form must be submitted for confirmation. The subject should attend follow-up visits as planned. In addition, subjects who become pregnant within the treatment period up to day 56 (and who received at least one dose) will be followed until the pregnancy outcome, even if this occurs after the study is completed.

Pregnancy itself is not regarded as an AE unless there is a suspicion that the IMP may have interfered with the effectiveness of a contraceptive medication. However, the outcome of all pregnancies that begin before day 56 (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) will be followed up and documented even if the subject was discontinued from the study.

A pregnancy outcome of a congenital abnormality/birth defect would qualify as an SAE and will be reported to Assign Safety Desk for processing to the regulatory authority. A pregnancy outcome of an elective abortion without evidence of complications would not be processed as an AE.

10.7 Development Safety Update Reports

Assign Data Management and Biostatistics GmbH will submit a DSUR once a year throughout the clinical trial to the competent authority (MHRA), ethics committee, HRA (where required) and Host NHS trust according to applicable regulations and requirements.

11. ASSESSMENT OF SAFETY

Adverse event rates compared between 5 different treatment groups will be used to evaluate the secondary study objective. Please also refer to Table 2, summarising study procedures including safety assessments.

11.1 Safety Laboratory Parameter Assessments

Haematology, blood chemistry, coagulation parameters and urinalysis will be assessed for subject's safety at screening, Visit 3, Visit 5 and additionally at Early Termination (if applicable). Please refer to section 8.11.1

11.2 Symptom-directed Physical Examination

A full physical exam will be performed at screening and a system based physical examination will be done, if necessary according to findings in a symptom-directed physical examination at Visit 1, 3 and 5. This means if a symptom is reported by the subject, a system-based assessment will be performed, if needed, for a detailed check of the affected body system.

Any symptom reported after first vaccination, including worsening of pre-existing conditions (i.e. medical history/concomitant diseases), will be recorded as AE.

11.3 System-based Physical Examination

All subjects will undergo a system-based physical examination, including but not limited to assessment of general appearance and skin, head/eyes/ears/nose/throat, respiratory system, cardiovascular system, abdominal and gastrointestinal system, musculoskeletal system, neurological system and lymph nodes.

This system-based physical examination will only be performed if necessary according to findings of the symptom-directed physical exam at Visit 1, 3 and 5.

11.4 Vital Signs and Body Temperature

Systolic and diastolic blood pressure and pulse rate as well as body temperature will be recorded at screening and Visit 1, 3 and 5 with the subject at rest in a sitting position.

11.5 Post Vaccination Reactogenicity Assessments

All subjects enrolled in the study and who receive at least one study treatment (MV-CHIK or placebo) will be followed up 28 days after each study treatment. Solicited and unsolicited AEs will be recorded at each visit until the end of the treatment phase at Visit 5.

11.6 Local and Systemic Tolerability

The local tolerability (i.e. injection site reactions) will be inspected and evaluated by the investigator two hours after each IMP administration.

Additionally, local tolerability (i.e. inspection of the previous injection site) will be assessed at the following visits and prior to application of the subsequent vaccination at Visit 3 (day 28). Furthermore, local tolerability will be evaluated by the subjects in the subject diaries for 7 days starting 6 hours after each treatment.

The grading of local reaction will be performed by means of a stencil (provided to the subject as shown in Appendix 1) and per "Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials", modified, reflecting the guidance of the Brighton Collaboration (Table 5).

Findings in the local tolerability examination by the investigator as well as local tolerability findings recorded in the subject diary will be covered as adverse events in the eCRF.

11.7 Subject Diary

The subjects will be instructed to complete a diary for 7 days after each vaccination to document local and systemic tolerability as well as body temperature.

Solicited local and solicited systemic AEs will be assessed by the subjects themselves by checking for presence of the listed symptoms and measuring the size of the affected area where appropriate (a template will be provided for local reaction grading, as shown in Appendix 1). The diary will also provide space for recording unsolicited AEs and concomitant medication.

Recording should be done approximately at the same time each day, starting on the day of vaccination. The first entry should be made 6 hours $\pm$1h after vaccination at Visit 1 and Visit 3.

The subjects' diaries will be collected and verified for completeness by the investigator at Visit 2 and Visit 4. Any AE recorded in the subject diaries will be entered in the eCRF by the investigator or authorised delegates. The investigator will re-evaluate the severity of the reported local and systemic AEs according to the below Tables 5 and 6.

Diary solicited systemic AEs include: nausea, vomiting, headache and fatigue, also to be documented in the eCRF.

In addition, body temperature will be recorded daily for 7 days and fever will be graded by the investigator according to the FDA Guidance for Industry. For details see below Table 6.

Table 5: Grading of Local Reactions

Local reaction to injectable product	Mild (grade 1)	Moderate (grade 2)	Severe (grade 3)	Potentially life threatening (grade 4)
Pain	Does not interfere with activity	Interfere with activity or repeated use of non-narcotic pain reliever	Prevents daily activity or repeated use of non-narcotic pain reliever	Emergency room (ER) visit or hospitalisation
Tenderness	Mild pain to touch	Pain with movement	Significant pain at rest	ER visit or hospitalisation
Erythema/ Redness[1]	≤ 5 cm	5.1–10 cm	> 10 cm	Necrosis or exfoliate dermatitis
Swelling[2]	≤ 5 cm and does not interfere with activity	5.1–10 cm or interfere 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-inflammation and pain-relieving ointment	Emergency room (ER) visit or hospitalisation
Induration[1]	≤ 1 cm Does not interfere with activity	> 1 to < 3 cm Interferes with activity or repeated use of non-narcotic pain reliever	≥ 3 cm Prevents daily activity or repeated use of anti-inflammation and pain-relieving ointment	Not applicable

[1] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
[2] Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Table 6: Grading of Systemic Reactions

Systemic Reaction (General)	Mild (grade 1)	Moderate (grade 2)	Severe (grade 3)	Potentially life threatening (grade 4)
Nausea/ vomiting	No interference with activity or 1-2 episodes/ 24 hours	Some interference with activity or >2 episodes/ 24 hours	Prevents daily activity, requires outpatient IV hydration	Emergency room (ER) visit or hospitalisation for hypotensive shock
Diarrhoea	2-3 loose stools or < 400 gms/ 24hours	4-5 stools or < 400-800 gms/ 24hours	6 or more watery stools or >800 gms/ 24 hours or requires outpatient IV hydration	ER visit or hospitalisation
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 hospitalisation
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalisation
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalisation
Illness or clinical AE (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 hospitalisation
Fever (°C)	38.0 - 38.4	38.5 - 38.9	39.0 - 40.0	>40.0

12. STATISTICS

The data will be analysed by Assign Data Management and Biostatistics GmbH. A Statistical Analysis Plan (SAP) will be finalised before database snapshot/closure providing a detailed description on the statistical methods and evaluation of study results.

An interim analysis will be performed after all subjects completed the treatment period and the final analysis will be conducted after the last subject completed the last visit.

12.1 Interim Analysis

A preliminary analysis including safety and immunogenicity data will be performed after all subjects completed Visit 5 (day 56) and therefore terminated the treatment period of the study. The purpose of this analysis is to provide highly valuable data for further Chikungunya vaccine development including the efficacy evaluation, by passive transfer of anti-Chikungunya antibodies to virus susceptible non-human primates.

Results from this interim analysis will not have any impact on the study design or the subjects during the follow up period of the study.

12.2 Methods of Statistical Immunogenicity Analysis

The immunogenicity analysis will compare the anti-Chikungunya $PRNT_{50}$ antibody geometric mean titre (GMT) 28 days after the last MV-CHIK vaccination in the Per Protocol (PP) analysis population between the treatment groups. GMTs and GMT ratios will be estimated by applying an analysis of variance including the factor treatment group. This will be done using $\log_{10}$ transformed data and taking the anti-log of the resulting point estimates for the least squares means, least squares means differences and the corresponding 2-sided 95% CIs.

P-values will also be provided to compare GMTs between the treatment groups adjusted for multiple comparisons according to Tukey-Kramer. Likewise, seroconversion rates will be compared between groups.

Seroconversion will be defined as anti-Chikungunya $PRNT_{50}$ titres ≥10.

Measles- and Chikungunya- ELISA titres will be analysed as described above.

In addition, the long-term stability of anti-Chikungunya antibodies determined by $PRNT_{50}$ and ELISA, 6 months and one year after the first immunisation, will be analysed compared between the five treatment groups.

12.3 Methods of Statistical Safety Analysis

The analysis of safety will be performed by means of descriptive measures in the Safety Population. Solicited and unsolicited AEs (local and systemic) will be analysed descriptively.

AEs and concomitant diseases will be coded using the MedDRA coding dictionary.

Solicited AEs (reactogenicity):

Solicited local and systemic AEs gathered by the subjects' diaries will be summarised for the 7-day period, by AE grading (mild, moderate, severe) and by treatment group. The number and percentage of subjects with AEs will be presented. Fisher exact tests will be provided for a comparison of AE rates between treatment groups in summary tables.

Unsolicited AEs:

Separately, the number and percentage of subjects with unsolicited/spontaneous adverse events (AEs), medically-attended AEs (MAEs) and serious adverse events (SAEs) will be presented for each treatment group overall, and by system organ class/preferred term, by AE grade, and relatedness. Fisher exact tests will be provided for a comparison of AE rates between treatment groups.

Laboratory values and vital signs as well as changes in laboratory values from baseline will be analysed descriptively by time point and treatment group.

12.4 Determination and Justification of Sample Size

A formal sample size calculation was not conducted. The sample size of 60 subjects has been determined based on prior experience in evaluating the safety and immunogenicity of vaccines and is typical for early phase clinical studies. Sample size for this study was determined on grounds of feasibility and common practice in similar trials.

12.5 Analysis Populations

12.5.1 Safety Population

All safety analyses will be based on the safety population, defined as subjects who entered into the study and received at least one IMP administration. All analysis based on the Safety Population will be carried out using the actual treatment received.

12.5.2 Modified Intent-to-Treat (mITT) Population

The secondary immunogenicity analyses will be based on the modified ITT population. The modified intent-to-treat (mITT) analysis population is defined to include all subjects randomised who receive at least one IMP administration. Subjects will be analysed according to the treatment group they had been allocated to, rather than by the actual treatment they received.

12.5.3 Per-Protocol (PP) Population

As a primary analysis, immunogenicity will be assessed on the per-protocol (PP) population. The PP population includes subjects without protocol deviations that could impact immune response. Examples that may lead to exclusion from the PP population are provided here (further criteria may be defined in the SAP):

- Immunosuppressive drugs: Use of corticosteroids (excluding topical preparations) or immunosuppressive drugs within 30 days prior to first IMP administration, or anticipated use during the trial.
- Subjects with any confirmed immunosuppressive or immunodeficient condition, including human immunodeficiency virus (HIV), hepatitis A, B or C infection or a family history of congenital or hereditary immunodeficiency
- Subjects who received the wrong or no IMP

These criteria for potential protocol violation are identified at the time of planning the study. However, during the course of the trial unforeseen events may occur or new scientific knowledge may become available, therefore final decisions on whether any protocol violation could impact immune response and thus lead to exclusion from the PP population will be made by the sponsor on a case by case basis in a blinded manner (and prior to study unblinding). Sample testing issues may also lead to exclusion from the PP population for particular time points.

12.6 Statistical Analysis Plan

A Statistical Analysis Plan (SAP) will be written and finalised 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.

12.7 Missing Data

All attempts will be made to prevent missing data and generally, missing will not be imputed, and analysis will be limited to observed values. However, for missing data in AE evaluation (e.g. missing information about severity or causality) a worst-case approach will be applied.

13. QUALITY CONTROL AND QUALITY ASSURANCE

The trial will be conducted in accordance with the current approved protocol, ICH GCP guidelines, relevant regulations and standard operating procedures.

13.1 Periodic Monitoring

A designated Clinical Research Associate (CRA) will monitor study progress by scheduling and performing on-site study visits throughout the study including site initiation visit, several site monitoring visits and site close out visit. At regular intervals throughout the study monitoring visits will be performed to verify completeness, accuracy and consistency of data in the eCRFs, protocol adherence, adherence to GCP and the applicable regulatory requirements. Frequency and scope of source data verification will be determined before study start and detailed in the Monitoring Plan.
Therefore, the monitor must have access to all source records needed to verify the entries on the eCRFs. The investigator will cooperate with the monitor to ensure that any discrepancies identified are resolved.
The periodic monitoring will be performed by Celerion, 22-24 Lisburn Road, Belfast BT9 6AD
Tel: PPD

13.2 Audit and Inspection

Upon request, the investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the sponsor or to regulatory inspectors.

The investigator or designee should contact the sponsor/CRO immediately upon announcement of an audit by the regulatory authority. He/she further agrees to fully cooperate with regulatory authorities and participate with audits conducted at a convenient time in a reasonable manner.

The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, to assess whether ethics, regulatory and quality requirements are met and to verify that all data relevant for the assessment of safety and immunogenicity of the investigational product have appropriately been reported to the sponsor.

14. ETHICS

14.1 Ethical Conduct of the Study

This study will be conducted in compliance with relevant regulatory requirements, ICH GCP guidelines of the Declaration of Helsinki for biomedical research involving human subjects and the EU directive 2005/28/EC.

14.2 Independent Ethics Committees (IEC)

Prior to initiation of the study, the protocol, subject informed consent form, any other written information to be provided to the subject, any proposed advertising material, investigator's brochure (IB), information about payments and compensation available to subjects if not mentioned in the subject information, investigator's current CV and other documentation evidencing qualifications, and other documents as required by the independent ethics committee should be submitted.

Written approval/favourable opinion must be obtained from the IEC prior to commencement of the study. This approval should include a statement that these documents comply with GCP requirements and must identify the documents and versions reviewed.

During the trial, the investigator must promptly report the following to the IEC: amendments to the protocol, updates to IB, unexpected SAEs where a causal relationship cannot be ruled out, notes of administrative changes, deviations to the protocol implemented to eliminate immediate hazards to the trial subjects, new information that may affect adversely the safety of the subjects or the conduct of the trial, annually written summaries of the trial status, and other documents as required by the local IEC.

Amendments must not be implemented before approval/favourable opinion, unless necessary to eliminate immediate hazards to the subjects.

14.3 Regulatory Authority

The regulatory authority will receive the protocol, amendments, reports on SAEs, and related relevant safety information, including the final report according to EU Directive and national regulations.

Written approval/favourable opinion must be obtained from the regulatory authorities prior to commencement of the study and for all substantial amendments to the original documents before implementation.

15. DATA HANDLING AND RECORD KEEPING

15.1 Source Data and Records

Source documents are where data are first recorded and from which subjects' eCRF data are obtained. These include, but are not limited to, original charts (subjects' records), laboratory and pharmacy printouts, diaries, and data from automated instruments. On all trial-specific documents, other than the signed consent, the participant will be referred to by subject number, not by name.

At least the following data will be documented in the source records:

- Study identification, date of subject's study entry and termination
- Subject number
- Documentation of informed consent procedure
- Date of each study visit
- Medical history, demographic data
- Any examination findings, including local injection site reactions
- Adverse events
- Concomitant medication intake
- Early withdrawal date and withdrawal reason, if applicable
- Treatment dates
- Completed subject diaries

Source data entries must be made in accordance with GCP and local requirements.

The investigator will permit study-related monitoring, audits, IRB/IEC review and regulatory inspections, by providing direct access to source data. Source records should be preserved for the maximum period of time required by local regulations.

15.2 Access to Data

Direct access to source data and documents will be granted to authorised representatives from the sponsor, host institution and the competent authorities to permit trial-related monitoring, IRB/IEC review, audits and inspections.

15.3 Data Collection

During each study visit, the investigator will collect and maintain notes in the subject's study records to document all procedures, significant observations and assessments, which are regarded as source data. Additionally, diaries and laboratory result reports signed and dated by the investigator, have to be kept within the subject's records. Changes to information in the study record and other source documents will be initiated and dated on the day the change is made. All documents will be stored safely under confidential conditions.

15.4 Electronic Case Report Forms (eCRFs)

15.4.1 eCRF Entries

Information from the subjects' study records and other source documents will be entered into a 21 CFR Part 11-compliant validated electronic Case Report Form (eCRF) as soon as possible after each visit. The participants will be identified by a unique trial specific subject number. The name or any other identifying detail will NOT be included in the eCRF.

Entries and corrections in the eCRF may only be performed by the investigator or authorised study site staff via personal user account and password for access. This password must be kept confidential and must only be used by the person to whom it was assigned to ensure that each entry/change can be allocated to the person who performed the entry/change.

An automatic audit trail will log each data entry/change performed in the eCRF.

The eCRF system includes internal quality checks, to identify data that appear inconsistent, incomplete or inaccurate. Maintenance of the eCRF and the study database will be performed by the data management centre at Assign Data Management and Biostatistics GmbH.

15.4.2 Changes to eCRF Data

Necessary data changes of the eCRF data may be identified as follows:

Entries are checked by the eCRF during data entry or when the eCRF page has been submitted/saved. If the data does not fulfil particular quality criteria, a message will specify the type of problem and assist in its correction.

Monitors may ask for correction of data during monitoring (e.g., if the eCRF entry does not match the source data).

Computerised data-check programs and/or manual checks will identify clinical data discrepancies. Corresponding queries aiming at the resolution of these discrepancies will be created within the eCRF system and the study site will be informed about new issues to be resolved on-line.

All discrepancies will be resolved on-line directly by the investigator or by authorised staff.

As long as an eCRF page is not locked, required data changes can be conducted by the investigator or authorised site staff at any time.

15.4.3 eCRF Entry Validation

The principal investigator or the authorised delegate will thoroughly review the eCRF data and will finally certify the contents of the eCRF by electronically signing the eCRFs within the data capturing system directly. If a correction was made to the eCRF data after the investigator's approval, the certification must be repeated after the changes were performed.

15.5 Confidentiality of Subject's Data

The investigator will ensure that all subject's information obtained during the conduct of the study will be kept confidential maintaining subject's anonymity.

On eCRFs or any other documents submitted to the sponsor, subjects will only be identified by subject number and therefore remain confidential in all reports or publications related to the study. Documents revealing subject's identification, e.g. subject identification log and original informed consent forms, will be maintained by the investigator in strict confidence, except to the extent necessary to allow checking by CRAs, the Sponsor or competent authorities.

15.6 Record Maintenance, Archiving and Destruction

The investigator is responsible for compiling and keeping all essential documents in the Investigator Site File (ISF) during the study and for the maximum period of time in accordance with ICH GCP guidelines and local regulatory requirements.

The ISF has to be stored in a safe and secure location with restricted access to staff personnel only and will include all essential documents like the clinical protocol, competent authorities' approvals, original informed consent forms, source data, IMP dispensing and accountability logs, subjects' logs and all correspondence pertaining to the study.

The investigator will agree to archive the study documentation and will not dispose of any records relevant to this study without prior written permission from the sponsor. If an investigator moves, withdraws, or retires, the responsibility for maintaining the records may be transferred to another person upon sponsor's agreement.

The sponsor or their representative will notify the investigator when study documents need no longer be retained.

16. CHANGES IN THE CONDUCT OF THE STUDY

16.1 Protocol Amendments

Any proposed changes to the protocol must be covered in a written amendment to the protocol and submitted to the appropriate ethics committees or regulatory authorities. Amendments may only be implemented after approval of the appropriate ethics committees or regulatory authorities has been obtained except where necessary to eliminate apparent immediate hazard to subjects.

16.2 Study Termination

If the sponsor or the investigator decides to terminate the study before it is completed, they will notify each other in writing stating the reasons for early termination. In terminating the study, the sponsor and the investigator will ensure that adequate consideration is given to the protection of the subjects' interest. The investigator, sponsor or CRO will notify the relevant ethics committee and regulatory authority in writing in accordance with local requirements. Documentation will be submitted for filing in Central File and Investigator File.

17. DEVIATIONS FROM THE PROTOCOL

17.1 Relevant Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, manuals or local requirements. The noncompliance may be either on the part of the subject, the Investigator, or the study site staff. Consequently, corrective actions are to be developed and implemented promptly.

Important protocol deviations are 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.

Inclusion of subjects not satisfying the entry criteria will be subject to prior discussion with the sponsor and written approval from the sponsor. Subjects developing exclusion criteria during the study will be withdrawn, exceptions need prior discussion and written approval from the sponsor.

All protocol deviations will be listed in the study report and assessed as to their influence on the quality of the study analysis. No deviations from the protocol of any type will be made without complying with all the ethics committee's or regulatory authority's established procedures in accordance with applicable regulations.

17.2 Serious Breaches

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the sponsor becoming aware of the breach.

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial, or

- the scientific value of the trial".

If a serious breach is suspected the sponsor must be contacted within 1 working day. In collaboration with the principal investigator, the serious breach will be reviewed by the sponsor and, if appropriate, the sponsor will report it to the REC committee, Regulatory Authority and the NHS host organisation within 7 calendar days.

17.3 Premature Subject Withdrawal

Subjects have the right to withdraw from the study at any time for any reason, without the need to justify. The investigator also has the right to withdraw subjects in case of AEs, protocol violations or administrative reasons. Please refer to section 8.9 for details.

Since an excessive rate of withdrawal can render the study inconclusively, the unnecessary withdrawal of subjects must be avoided.

18. FINANCE AND INSURANCE

18.1 Contractual and Financial Details

The investigator, the sponsor and 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 will describe costs for pharmacy, laboratory and other protocol-required services.

Financial Disclosure Statements will be completed, as required by 21 CFR part 54.

18.2 Insurance

All subjects participating in this clinical study will be insured through Themis Biosciences GmbH. The Insurance will be contracted by the assigned CRO.

19. REPORTING AND PUBLICATION

19.1 Clinical Study Report

After all subjects terminated their study participation (either by completion of Visit 7 (day 365) or prematurely by early termination or are confirmed as lost to follow up), a clinical study report will be prepared by the sponsor or delegate in accordance with relevant guidelines.

19.1 Publication Policy

All results generated in this study will be considered to be strictly confidential. The investigators may not submit the results for publication or presentation without prior written permission of the sponsor. Authorship for any publication will be determined in mutual agreement.

20. REFERENCES

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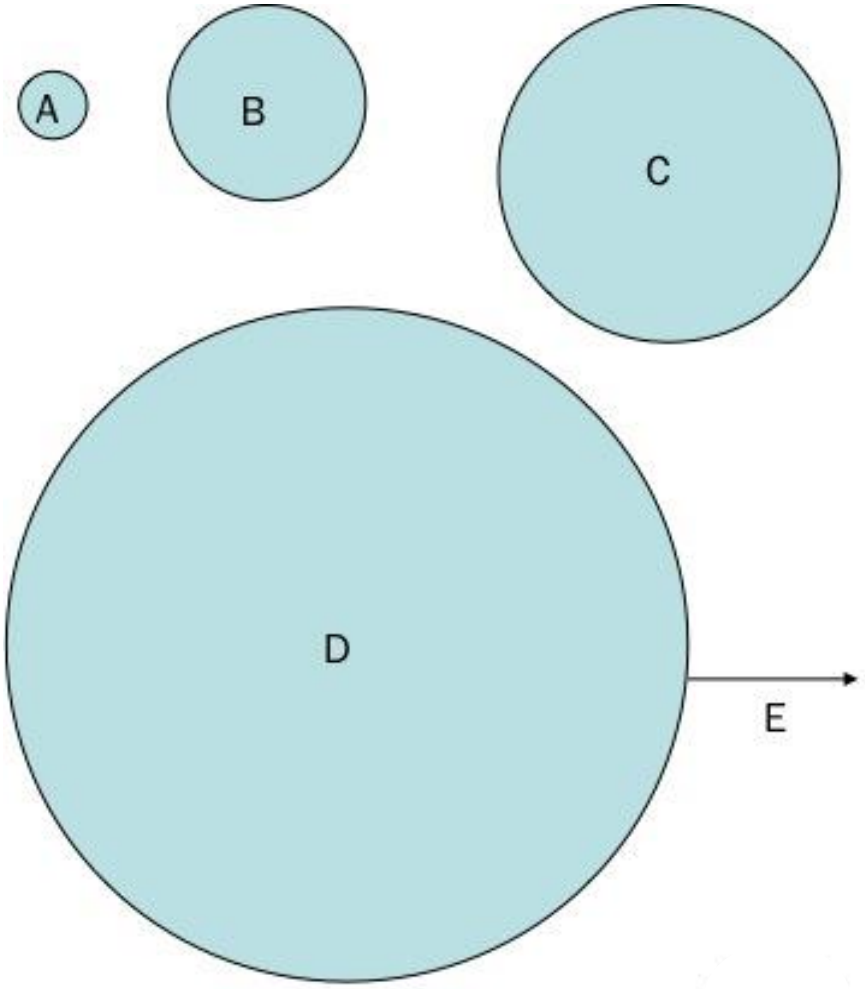
21. APPENDICES

Appendix 1: Template distributed to subjects to assess local reaction



Statistical Analysis Plan

Preliminary and Final Analysis

Observer blinded, randomised study to investigate safety, tolerability and long-term immunogenicity of different dose regimens and formulations of MV-CHIK in healthy volunteers

Protocol: MV-CHIK-205

Confidential

Sponsor: Themis Bioscience GmbH, Vienna, Austria

Table of Contents

ASSIGN DMB

Document	
Study	MV-CHIK-205
Document	Statistical Analysis Plan (SAP)
Version	FINAL 1.0
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Revision History

Version	Date	Reason for Revision
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Approval

Name	Role	Date	Signature
	Author		
PPD	Biostatistician	20-Feb-2019	PPD
	Assign DMB		
	Senior Biostatistician	20-Feb-2019	
	Themis Bioscience GmbH		
	Clinical Project Manager	20. Feb. 2019	

List of Abbreviations

AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
BDRM	Blind Data Review Meeting
CI	Confidence Interval
CSP	Clinical Study Protocol
CSR	Clinical Study Report
DRM	Data Review Meeting
eCRF	Electronic Case Report Form
ELISA	Enzyme Linked Immunosorbent Assay
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
HIV	Human Immunodeficiency Virus
IMP	Investigational Medicinal Product
IMP	Investigational Medical Product
LLOQ	Lower Limit of Quantification
mITT	Modified Intent-to-Treat
PP	Per-Protocol
$PRNT_{50}$	Plaque Reduction Neutralization Test 50 %
PT	Prothrombin Time
SOP	Standard Operating Procedure
SPS®	Stabilizing and Protecting Solutions
TLF	Tables Listings and Figures

1. OVERVIEW

1.1 Study Design

MV-CHIK-205 is a prospective, interventional, observer blinded, block-randomized phase II trial, comparing different dose levels of MV-CHIK in three different formulations ((lyophilized, liquid frozen and liquid Stabilizing and Protecting Solutions (SPS®)) to evaluate immunogenicity, safety and tolerability of this novel Chikungunya vaccine in 60 healthy volunteer female and male subjects. Placebo (physiological saline solution) will be applied to blind two different treatment schedules, evaluating single shot versus double shot treatment. The study will be conducted as a single study site.

After completion of screening procedures, subjects will be randomized to one of five treatment groups (A, B, C, D or E) and will receive injections according to the scheme outlined in the study flow chart (see Section 1.5).

Group A: 12 subjects will receive two low dose treatments with MV-CHIK $5x10^4$ (±0.5 log) TCID50 /dose on day 0 and 28 (lyophilized formulation).

Group B: 12 subjects will receive two low dose treatments with MV-CHIK $1x10^5$ (±0.5 log) TCID50 /dose on day 0 and 28 (liquid frozen formulation).

Group C: 12 subjects will receive two low dose treatments with MV-CHIK $1x10^5$ (±0.5 log) TCID50 /dose on day 0 and 28 (liquid SPS® formulation).

Group D: 12 subjects will receive two high dose treatments with MV-CHIK $1x10^6$ (±0.5 log) TCID50 /dose on day 0 and 28 (liquid frozen formulation).

Group E: 12 subjects will receive one high dose treatment with MV-CHIK $1x10^6$ (±0.5 log) TCID50 /dose on day 0 (liquid frozen formulation) and placebo on day 28.

Subjects will be observed for safety and immunogenicity for 56 days during the treatment period, starting with the first MV-CHIK vaccination at day 0 (Visit 1), day 14 (Visit 2), day 28 (Visit 3), day 42 (Visit 4) and day 56 (Visit 5). After this treatment period subjects will return to site at day 182 (Visit 6) and at day 365 (Visit 7) for collection of serum to follow up the durability of specific antibodies against Chikungunya. Serum samples for passive transfer will be collected on days 0, 28, 56 and 365.

Study duration per subject will be 12 months, comprising of 8 weeks treatment period from study day 0 (Visit 1) until day 56 (Visit 5) and 44 weeks follow up period, including Visit 6 and Visit 7 (post day 56 until day 365). Study subjects are free to participate in other clinical studies after completion of the treatment period on day 56. The end of study will be defined as date of the last visit of the last subject.

1.2 Study Objectives

1.2.1 Primary Objective

- To investigate immunogenicity and safety of MV-CHIK in different dose regiments, 28 days after one or two vaccinations

The immunogenicity will be determined by means of Plaque Reduction Neutralization Test 50 % ($PRNT_{50}$) 28 days after a single shot treatment at Visit 3 (study day 28) and 28 days after two treatments with MV-CHIK at Visit 5 (study day 56). Local and systemic AEs will be recorded and assessed.

1.2.2 Secondary Objectives

- To compare immunogenicity, safety and tolerability between different doses and formulations of MV-CHIK during the treatment period up to day 56
- To investigate the immunogenicity and safety of a novel liquid vaccine formulation
- To evaluate cellular responses induced by one or two immunizations
- To evaluate the long-term immunogenicity of MV-CHIK up to one year after the first MV-CHIK treatment
- To collect human sera for future efficacy evaluation, by passive transfer of anti-Chikungunya antibodies to virus susceptible non-human primates

1.3 Endpoints

1.3.1 Primary Endpoint

- Immunogenicity 28 days after the last MV-CHIK vaccination confirmed by the presence of functional antibodies as determined by the plaque reduction neutralization test ($PRNT_{50}$)

1.3.2 Secondary Endpoints

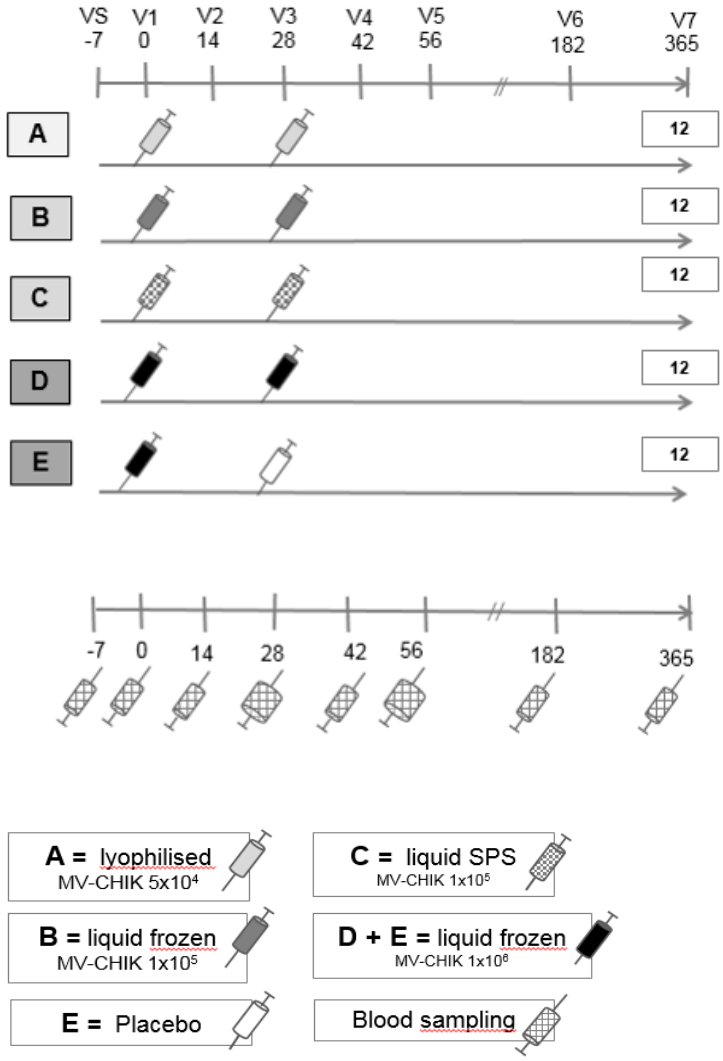
- Rate of solicited and unsolicited adverse events (AEs) during the treatment period until day 56
- Serious adverse events during the treatment period until study day 56
- Immunogenicity on days 0, 28 (group A, B, C, and D), days 0, 56 (group E) and long-term immunogenicity on day 182 (6 Month) and 365 (12 Month), confirmed by the presence of functional antibodies as determined by the plaque reduction neutralization test ($PRNT_{50}$).
- Measurement of anti-Chikungunya antibodies on days 0, 28, 56, 182 and 365 determined by enzyme linked immunosorbent assay (ELISA)
- Measurement of anti-Measles antibodies on days 0, 28, 56 determined by enzyme linked immunosorbent assay (ELISA)

- Induction of Chikungunya specific immune responses on days 0, 14, 28, 42 and 56 determined by T-cell assay.
- Safety laboratory parameters (hematology, serum chemistry, coagulation, urinalysis)

1.4 Sample Size Calculation

A formal sample size calculation was not conducted. The sample size of 60 subjects has been determined based on prior experience in evaluating the safety and immunogenicity of vaccines and is typical for early phase clinical studies. Sample size for this study was determined on grounds of feasibility and common practice in similar trials.

1.5 Flowchart



1.6 Schedule of Assessments

	Screening	Treatment Period					Follow up Period		
Visit	VS	V1	V2	V3	V4	V5	V6	V7	ET
Study Day	-28 to -1	0	14 ±1	28 ±5	42 ±5	56 ±5	182 ±14	365 ±14	(7)
Informed Consent	x								
Inclusion/Exclusion Criteria	x	x							
Medical History	x	x							
Vaccination History	x								
Vital Signs (1)	x	x		x		x			x
Physical Examination (2)	x	x		x		x			x
Adverse Events (3)		x	x	x	x	x			x
Concomitant Medication (4)	x	x	x	x	x	x			x
Clinical Laboratory (5)	x			x		x			x
Pregnancy Test	x	x		x		x			x
Study Treatment (8)		x		x					
Dispense subject diary		x		x					
Collect subject diary			x		x				x
Local Tolerability (6)		x	x	x	x	x			x
Immunogenicity ($PRNT_{50}$)		x		x		x	x	x	x
T-cell Immunity		x	x	x	x	x			x
CHIKV-antibodies (ELISA)		x		x		x	x	x	x
Measles-antibodies (ELISA)		x		x		x			x
Serum collection for passive transfer		x		x		x		x	x

(1) Assessment of vital signs will include systolic and diastolic blood pressure, pulse rate and body temperature
(2) A full physical examination will be performed at screening and a system based physical examination will be done at Visit 1, 3 and 5, if necessary according to findings in a symptom-directed physical examination
(3) Conditions/symptoms noted prior to first IMP administration will be reported as medical history and not as AEs, At Visit 6 and 7 AEs will not be recorded as subjects are allowed to participate in other clinical studies during the follow-up period.
(4) Medications administered within 30 days prior to screening until end of treatment period will be documented as concomitant medication.
(5) Clinical Laboratory parameters include:
- Virology: HB s-Ag, anti-HB c-Ab, anti-HC-Ab, HIV-Ab (only once at screening)
- Hematology: hemoglobin, hematocrit, erythrocyte count, differential and total white blood count, platelets
- Chemistry: creatinine, potassium, sodium, calcium, AST, ALT, alkaline phosphatase, bilirubin
- Coagulation parameter: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen
- Urinalysis: pH-value, glucose, protein, bilirubin, urobilinogen, blood, leucocytes esterase, nitrite, ketone and specific gravity. In case of abnormal findings, a microscopy will be done for confirmation.

Please refer to CSP section 8.11.1
(6) Local tolerability to be investigated 2 hours after each vaccination and additionally at the subsequent visits and prior to IMP administration.
(7) If the early termination occurs within the treatment period (at or before Visit 5), subjects shall perform all procedures as defined for Visit 5. If the early termination occurs within the follow up period (after Visit 5), subjects shall perform all procedures defined for Visit 7
(8) Administration of IMP will be performed according to allocated treatment group, after randomization at Visit 1

2. GENERAL CONSIDERATIONS

2.1 Conduct of Analysis

A preliminary analysis including safety and immunogenicity data will be performed after all subjects completed Visit 5 (day 56) and therefore terminated the treatment period of the study. The purpose of this analysis is to provide highly valuable data for further Chikungunya vaccine development including the efficacy evaluation, by passive transfer. Thus, immunogenicity analysis for $PRNT_{50}$ and ELISA results for chikungunya antibodies in the preliminary analysis will be performed for Visit 1 (day 0), Visit 3 (day 28), and Visit 5 (day 56). All safety analysis will be done in the preliminary analysis. Results from this preliminary analysis will not have any impact on the study design or the subjects during the follow up period of the study.

The final analysis will be conducted once the last subject has completed the study. All data of the study will already be available at the preliminary analysis except the immunogenicity data from Visit 6 and Visit 7, end of study data as well as other analyses requested from Visit 1 to Visit 5 for which no laboratory results are available at the time of the Day 56 analysis (T-cell analysis and ELISA results for measles). Thus only these data will be analyzed at the final analysis. If unexpected changes occur e.g. in the baseline or adverse event data, analyses may also be repeated at the final analysis.

2.2 Statistical Software and Quality Control

All statistical analyses will be performed using SAS® version 9.3 or higher. Tables, figures and data listings will be generated in Microsoft® Word® as well as PDF® format.

Quality control of SAS® programs will include a review of the whole process of result generation:

- Review of all analysis SAS® programs
- Review of SAS® log for errors, warnings and other notes that could indicate mistakes in the programs
- Review of all tables, listings and figures for completeness and correctness

2.3 Applicable Standard Operation Procedures

CCI

[redacted]

2.4 Blinding and Randomization

At Visit 1 (day 0) 60 eligible subjects will be randomly assigned to one of five treatment groups (A, B, C, D or E) by means of randomization envelopes provided by data management. All of these subjects will be equally distributed between the 5 groups using a randomization ratio of 1:1:1:1:1=A:B:C:D:E. A subject will be considered enrolled once the first IMP administration has been performed according to randomization.

As this study will be conducted in an observer-blinded manner, unblinded study team members responsible for randomization, preparation and administration of IMP, will otherwise not be involved in the conduct of the study. All subjects, the investigator and site personnel performing study related assessments, as well as the sponsor's representatives involved in the monitoring and conduct of the study, will be blinded to treatment assignment.

The primary endpoint as well as most of the secondary endpoints will be covered in the preliminary analysis after Day 56. The sponsor personnel and ADMB statisticians (blinded statistician) that perform and review the preliminary analysis will be unblinded at this point in time, but after the Blind Data Review Meeting (Section 2.9).

2.5 Descriptive Analyses

Descriptive analyses of continuous variables (summary statistics) will be described with the number of non-missing observations, arithmetic mean, standard deviation (±SD), median, quartiles (Q1 and Q3) and range (minimum and maximum).

Descriptive analysis of continuous immunogenicity variables (i.e. tables for the GMT and GMFI) will be described with the number of non-missing observations, geometric mean, confidence intervals for the geometric mean, standard deviation of logarithmic values, median, quartiles (Q1 and Q3) and range (minimum and maximum).

Categorical variables (frequency statistics) will be described with the number of non-missing observations and percentages (%). Percentages will be calculated within each stratum on the total number of non-missing observations, if not stated otherwise.

2.6 Inferential Analyses

The immunogenicity analysis will compare the functional anti-Chikungunya $PRNT_{50}$ antibody geometric mean titer (GMT) 28 days after the last MV-CHIK vaccination between the treatment groups. The significance level will be 0.05. All p-values will be two-sided and rounded to four decimal places.

GMTs and GMT ratios will be estimated by applying an analysis of variance (ANOVA) including the factor treatment group. This will be done using log10 transformed data and taking the anti-log of the resulting point estimates for the least squares means, least squares means differences and the corresponding 2-sided 95% Confidence Intervals (CI). P-values will also be provided to compare GMTs between treatment groups adjusted for multiple comparisons according to Tukey-Kramer.

Measles- and Chikungunya-ELISA titers as well as long-term stability of anti-Chikungunya antibodies determined by $PRNT_{50}$ and ELISA will be analyzed likewise.

Seroconversion rates will be compared between groups using a Fisher-Freeman-Halton test.

For the safety analysis Fisher exact tests will be provided for a comparison of AE rates between treatment groups.

2.7 Center and Country Effect

Not applicable since this is a single-center study.

2.8 Handling Missing Data

Generally, missing values of immunogenicity variables will not be imputed, and the analysis will be limited to observed values. For missing data in AE evaluation concerning seriousness, severity or causality a worst case approach will be applied. In case of missing assignment to solicited or unsolicited, this AE will neither be counted in tables for solicited AEs nor in tables for unsolicited AEs but in tables for all AEs.

2.9 Protocol Deviations

Protocol deviations will be collected only for the preliminary analysis. A listing of the potential protocol deviations will be prepared by Assign Data Management and Biostatistics GmbH using information from the eCRF documentation and monitoring reports. These protocol deviations will be classified into major or minor protocol deviations based on their possible impact on the study results in a Blind Data Review Meeting (BDRM) prior to the preliminary analysis attended by Assign Data Management GmbH and Themis Bioscience GmbH (sponsor).

Major protocol deviations will include but are not limited to the following:

- Subjects with any confirmed immunosuppressive or immunodeficient condition, including human immunodeficiency virus (HIV), hepatitis A, B or C infection or a family history of congenital or hereditary immunodeficiency
- Subjects who received the wrong or no IMP
- Subjects who received less than the protocol-defined number of vaccinations
- Subjects who received a wrong dose other than they were randomized to
- Subjects who entered the study even though they did not satisfy the entry criteria
- Subjects with substantial time window violations on vaccination visits
- Subjects who received an forbidden prior or concomitant medication
    - Immunosuppressive drugs: Use of corticosteroids (excluding topical preparations) or immunosuppressive drugs within 30 days prior to first IMP administration, or anticipated use during the trial
    - Treatments with non-study licensed vaccines within 4 weeks prior to first study vaccination until the end of the treatment period (day 56)
    - Receipt of blood products or immunoglobulins within 120 days prior to the Screening Visit or anticipated receipt of any blood product or immunoglobulin before completion of the treatment period (day 56)
- Further protocol deviations that could impact immune response that have not been described above

These criteria for potential protocol violation are identified at the time of planning the study. However, during the course of the trial unforeseen events may occur or new scientific knowledge may become available, therefore final decisions on whether any protocol violation could impact immune response and thus lead to exclusion from the per-protocol (PP) population will be made by the sponsor on a case by case basis in a blinded manner (and prior to study unblinding).

All protocol deviations will be listed in the analysis and described in the Clinical Study Report (CSR).

It is not planned to define a second PP population prior before database closure since it is not expected that protocol deviations after day 56 affect the primary immunogenicity endpoint. In case of unexpected changes or further protocol deviations documented after the BDRM, it may be discussed in a second DRM prior to database closure which analyses may be repeated for the final analysis.

2.10 Medical Coding

Adverse events and medical history will be coded using MedDRA in the most current version. Concomitant medications and vaccination history will be coded using WHO Drug Reference List and Anatomical Therapeutic Chemical (ATC) Classification System in the most current version.

2.11 Analysis Populations

2.11.1 Safety Population

The safety population is defined to include all subjects who entered into the study and received at least one IMP administration. All analysis based on the safety population will be carried out using the actual treatment received. Subjects with vaccination protocol deviations will be allocated to a treatment group after a case-by-case review in the Blind Data Review Meeting. If a subject received different doses at different visits, the minimum dose received will decide the treatment group.

2.11.2 Modified Intent-to-Treat (mITT) Population

The modified intent-to-treat (mITT) population is defined to include all randomized subjects who receive at least one IMP administration. Subjects will be analyzed according to the treatment group they had been allocated to, rather than by the actual treatment they received.

2.11.3 Per-Protocol (PP) Population

The per-protocol (PP) population is defined as mITT population including subjects with at least one major protocol deviation that might have an impact on the immune response, see Section 2.9. The PP population is the primary population for the immunogenicity analysis and will be defined in the BDRM that is conducted before preliminary analysis.

2.12 Subject-Level Data Listings

All treated subjects will be included in listings if not stated otherwise. Data listings will include the subject number as identifier (and parameter and/or visit if available) and will be sorted by subject number (and parameter and/or visit if available). Columns that indicate the treatment group will be shown in all listings.

2.13 Changes in the Conduct of the Study or Planned Analysis

All statistical analyses will be performed according to the CSP. In case of analyses deviate from SAP, all changes will be described and justified in the CSR.

3. OVERALL STUDY INFORMATION

The following information will be analyzed descriptively and corresponding details on the subject level will be provided in data listings:

- Subject overview
- Vaccination
- Visit attendance
- Protocol deviations
- Study status (incl. drop-outs and reason)
- Screening failures

Specifications on TLFs are provided in Section 7.

4. BASELINE EVALUATION

For the preliminary analysis baseline data (screening visit, VS) will be presented for the Safety and mITT population by treatment group and overall. For the final analysis all tables will be repeated for the PP population. In case populations do not differ, tabulations will not be repeated.

4.1 Data Points

The following information will be analyzed descriptively in tables and corresponding details on the subject level will be provided in data listings:

- Demographic information (age, sex, race)
- Physical examination (will be listed only)
- Vaccination history
- Virology (will be listed only)
- Medical history
- Prior and concomitant medications

Pregnancy test will be only listed in the safety section. Specifications on TLFs are provided in Section 7.

4.2 Derivations and Definitions

- Medications stopped clearly prior (<) to Day 0 (Visit 1) will be considered prior medications, all other medications are considered to be concomitant. Medications with a missing or incomplete stop date where it cannot clearly be decided if the stop date was before or after Day 0 will be considered concomitant.

5. IMMUNOGENICITY ANALYSIS

The immunogenicity analysis will be performed for the mITT and PP population. All tables will be presented by treatment group and overall. Data from the early termination visit or unscheduled visits will be listed only and will not be shown in summary tables by time point.

5.1 Data Points

The following information will be analyzed by visit descriptively in tables and corresponding details on the subject level will be provided in data listings:

- GMT of anti-Chikungunya $PRNT_{50}$ antibody (incl. ANOVA with fixed treatment group)
- Seroconversion rate for Anti-Chikungunya $PRNT_{50}$ antibody
- GMT of Chikungunya-ELISA antibodies (incl. ANOVA with fixed treatment group)
- Seroconversion rate for Chikungunya-ELISA antibodies
- GMT of Measles-ELISA antibodies
- T cell results (will be listed only)
- Sampling details for $PRNT_{50}$, ELISA CHIK, ELISA measles; T-cells and passive transfer (will be listed only)

Specifications on TLFs are provided in Section 7.

5.2 Derivations and Definitions

- For the preliminary analysis, only $PRNT_{50}$ will be analyzed included time points Visit 1 (day 0), Visit 3 (day 28) and Visit 5 (day 56). For the final analysis all immunogenicity parameters will be analyzed at all available time points except passive transfer.
- Analysis of passive transfer results are not described in this SAP.
- The primary analysis compares the $PRNT_{50}$ titer 28 days after last MV-CHIK vaccination between treatment groups. Therefore, titers from Day 56 in Groups A-D will be considered and the titer at Day 28 for Group E.
- Seroconversion rate for anti-Chikungunya $PRNT_{50}$ titers is defined as proportion of subjects achieving anti-Chikungunya $PRNT_{50}$ titers ≥ 10 at a certain time point.
- Seroconversion rate for Chikungunya-ELISA is defined as proportion of subjects achieving Chikungunya-ELISA titers ≥ 16 at a certain time point.
- The lower limit of quantification (LLOQ) are not known for immunogenicity parameters at SAP development and therefore the following general rules will apply:
    - In data listings, the original reported value “<LLOQ” will be displayed.

- For calculations the half of the true LLOQ value will be used for such values.

6. SAFETY ANALYSIS

All safety analyses will be performed for the safety population.

6.1 Extent of Exposure

6.1.1 Data Points

The following information will be analyzed descriptively in tables and corresponding details on the subject level will be provided in data listings:

- Treatment duration
- Study duration
- Study vaccine administration (will be listed only)

6.1.2 Derivations and Definitions

- Treatment duration [days] = date of last administered vaccine – date of first vaccination +1
- Study duration [days] = date of last attended visit (or date of study discontinuation, if applicable) – date of signed informed consent + 1

6.2 Adverse Events

6.2.1 Data Points

The following analyses will be provided for all Adverse Events:

- Adverse event overview for solicited and unsolicited events (e.g. any AE, any SAE, any related AE). This table will include a statistical test for comparison of treatment groups (see Section 2.6).
- Solicited Adverse Events by symptom
- Solicited Adverse Events by symptom and severity
- Unsolicited Adverse Events by SOC and PT, overall and for specific types of Adverse Events (e.g. SAEs, related AEs)
- Unsolicited Adverse Events by severity and for specific types of Adverse Events
- Unsolicited Adverse Events by causality and for specific types of Adverse Events

Specifications of TLFs are provided in Section 7.

6.2.2 Derivations and Definitions

- All solicited and unsolicited Adverse Events up to day 56 (Visit 5) will be analyzed for the preliminary analysis. AE reporting and documentation is completed at Day 56. Thus all AE in the database are considered for the adverse event endpoints. In case of unexpected data change, new reported AEs or follow-up information, affected tables and/or listings may be repeated for the final analysis. Otherwise,

adverse event tables are not repeated at the final analysis. This decision may be done in a DRM prior to database closure, see Section 2.9.

- Percentages for AE rates are based on N (treatment group totals).
- Medical Conditions/symptoms noted prior to first IMP administration will be reported as medical history and not as adverse events. In case of an incomplete start date where it cannot clearly be decided if the Adverse Event started before the first IMP the Adverse Event will be considered to be started before first IMP.
- An Adverse Events is considered to be related to study treatment if “Relationship to Study Treatment” is answered with “definitely”, “probably” or “possibly” or the causality is missing.
- Adverse Events where an action was taken are considered if “Action taken with Study Treatment” is answered with “Second IMP Administration Delayed” or “Second IMP not Administered”.
- Tables showing “severe” events will include events with grade 3 or 4 or missing grade.
- In tables showing subjects with AEs by maximum severity, subjects will be counted only in the highest grading category, but events will be counted in each reported grading category.
- In tables showing AEs by causality, subjects will be counted only in the strongest relationship category, but events will be counted in each category.

6.3 Laboratory Parameters

Data from the early termination visit or unscheduled visits will be listed only and will not be shown in summary tables by time point. All tables will be presented by treatment group and overall. Laboratory data from scheduled visits (VS, Visit 3 and Visit 5) will be tabulated by time point. Specifications on TLFs are provided in Section 7.

6.3.1 Data Points

- Hematology
  - Hemoglobin
  - Hematocrit
  - Erythrocytes
  - Leukocytes
  - Neutrophils
  - Lymphocytes
  - Monocytes
  - Basophils
  - Eosinophils
  - Neutrophils/Leukocytes
  - Lymphocytes/Leukocytes

  - Monocytes/Leukocytes
  - Basophiles/Leukocytes
  - Eosinophils/Leukocytes
  - Platelets
- Clinical Chemistry
  - Creatinine
  - Potassium
  - Sodium
  - Calcium
  - Aspartate Aminotransferase
  - Alanine Aminotransferase
  - Alkaline Phosphatase
  - Bilirubin
- Coagulation
  - Prothrombin Time
  - Activated Partial Thromboplastin Time
  - Fibrinogen
- Urinalysis
  - pH
  - Specific gravity
  - Glucose
  - Protein
  - Bilirubin
  - Urobilinogen
  - Erythrocytes
  - Leukocyte Esterase
  - Nitrite
- Urinalysis (Micro)
  - Ketones
  - Leukocytes
  - Erythrocytes
  - Casts
  - Bacteria

The following parameters will be analyzed descriptively by time point:

- Number of subjects with laboratory samples drawn for hematology, clinical chemistry, coagulation and urinalysis
- Absolute values (summary statistics for hematology, clinical chemistry and coagulation)

- Absolute change from baseline (Screening) (summary statistics for hematology, clinical chemistry and coagulation)
- Number of subjects with values above/below normal range (for hematology, clinical chemistry and coagulation)
- Urinalysis results (summary statistics for quantitative parameters, frequency statistics for qualitative parameters)

6.3.2 Derivations and Definitions

- No conversion of laboratory parameters will be performed. The parameters listed above will be tabulated and listed in the units reported by the study site.
- Absolute change from baseline will be calculated as difference from value of the current visit and baseline value (screening).

6.4 Other Safety Parameters

Data from the early termination visit or unscheduled visits will be listed only and will not be shown in summary tables by time point. All tables will be presented by treatment group and overall. Vital signs will be analyzed for Screening, Visit 3 and Visit 5:

- Diastolic blood pressure
- Systolic blood pressure
- Pulse rate
- Temperature

The following data will be listed only:

- Pregnancy test
- Local tolerability
- Local tolerability – post vaccination
- Symptom-directed physical examination
- System-based physical examination

7. LIST OF TABLES, DATA LISTINGS AND FIGURES

7.1 List of Tables

No. for IA	No. for FA	Legend	Content
Overall Subject Information			
1.1.1.1	2.1.1.1	Subject Overview (Safety Population)	• Randomized • Safety Population • mITT Population • PP Population
1.1.1.2	2.1.1.2	Subjects by Visit (Safety Population)	
1.1.1.3	n/a	Vaccinated Subjects and Reason for missed Vaccinations (Safety Population)	• Number of subjects that received first vaccination • Number of subjects that received second vaccination • Number of subjects that missed any vaccination • Reason for missing any vaccination
1.1.1.4	2.1.1.4	Protocol Deviations by Deviation Type (Safety Population)	• Any major PD • Any major PD by category • Any minor PD • Any minor PD by category
1.1.1.5	2.1.1.5	Study Status (Safety Population)	• Visit attendance status • Primary reason for early termination
1.1.1.6	n/a	Number of Screening Failures and Reasons	• Number of screening failures • Reason for screening failure
1.1.2.2-1.1.2.5	2.1.2.2-2.1.2.5	Repeat tables x.1.1.1-x.1.1.5 for mITT population (if not the same as Safety Population)	
1.1.3.2-1.1.3.5	n/a	Repeat tables x.1.1.1-x.1.15 for PP population	
Baseline Evaluation			
1.2.1.1	n/a	Summary Table of Demographic Information (Safety Population)	• Age • Gender • Race • Height [cm] • Weight [kg]
1.2.1.2	n/a	Vital Signs at Baseline (Safety Population)	• Systolic blood pressure [mmHg] • Diastolic blood pressure [mmHg] • Pulse rate [beats/min] • Temperature [C]
1.2.1.3	n/a	Medical History Entry by SOC and PT (Safety Population)	
1.2.1.4	n/a	Prior Medications by ATC Level 2 and 3 (Safety Population)	
1.2.1.5	n/a	Concomitant Medications and by ATC Level 2 and 3 (Safety Population)	
1.2.1.6	n/a	Vaccination History by ATC Level 2 and Level 3 (Safety Population)	

1.2.2.1-1.2.2.6	n/a	Repeat tables x.2.1.1-x.2.1.6 for mITT population (if not the same as Safety Population)	
1.2.3.1-1.2.3.6	n/a	Repeat tables 2.1.1-x2.1.6 for PP population (if not the same as Safety Population)	
Immunogenicity Results			
Primary Endpoint			
1.3.1.1	2.3.1.1	GMT of anti-Chikungunya $PRNT_{50}$ Antibody 28 days after last MV-CHIK vaccination (mITT Population)	
1.3.1.2	2.3.1.2	ANOVA with fixed factor treatment group for GMT of anti-Chikungunya $PRNT_{50}$ Antibody 28 days after last MV-CHIK vaccination (mITT Population)	
Anti-Chikungunya $PRNT_{50}$ Antibody			
1.3.1.3	2.3.1.3	GMT of anti-Chikungunya $PRNT_{50}$ Antibody by Visit (mITT Population)	• Preliminary Analysis: Visit 1, 3 and 5 • Final Analysis : All available time points
1.3.1.4	2.3.1.4	ANOVA for GMT of anti-Chikungunya $PRNT_{50}$ Antibody between treatment groups by Visit (mITT Population)	• Preliminary Analysis: Visit 1, 3 and 5 • Final Analysis : All available time points
1.3.1.5	2.3.1.5	Seroconversion rate for Anti-Chikungunya $PRNT_{50}$ Antibody by Visit (mITT Population)	• Preliminary Analysis: Visit 1, 3 and 5 • Final Analysis : All available time points
Chikungunya-ELISA Antibodies			
1.3.1.6	2.3.1.6	GMT of Chikungunya-ELISA Antibodies 28 days after last MV-CHIK vaccination (mITT Population)	
1.3.1.7	2.3.1.7	ANOVA for GMT of Chikungunya-ELISA Antibodies between treatment groups 28 days after last MV-CHIK vaccination (mITT Population)	
n/a	2.3.1.8	GMT of Chikungunya-ELISA Antibodies by Visit (mITT Population)	• All available time points
n/a	2.3.1.9	ANOVA for GMT of Chikungunya-ELISA Antibodies between treatment groups by Visit (mITT Population)	• All available time points
n/a	2.3.1.10	Seroconversion rate of Chikungunya-ELISA Antibodies by Visit (mITT Population)	• All available time points
Measles-ELISA antibodies			
n/a	2.3.1.11	GMT of Measles-ELISA Antibodies 28 days after last MV-CHIK vaccination (mITT Population)	• All available time points

1.3.1.1 – 1.3.1.7	1.3.2.1 – 1.3.2.11	Repeat tables for PP Population	
Safety Results			
Extent of Exposure			
1.4.1.	2.4.1	Extent of Exposure (Safety Population)	• Study duration • Treatment duration
Adverse Events			
1.4.2	n/a	Summary Table of solicited and unsolicited Adverse Events (Safety Population)	Number of subjects with: • Any AEs • Any related AEs • Any severe AEs • Any related severe AEs • Any SAEs • Any related SAEs • Any medically attended AEs • Any related medically attended AEs • Any AEs leading to study discontinuation • Any AEs leading to second vaccination not administered
1.4.3	n/a	Summary Table of solicited Adverse Events (Safety Population)	• Any solicited AEs • Any related solicited AEs • Any severe solicited AEs • Any related severe solicited AEs • Any solicited SAEs • Any related solicited SAEs • Any medically attended solicited AEs • Any related medically attended solicited AEs • Any solicited AEs leading to study discontinuation • Any solicited AEs leading to second vaccination not administered
1.4.4	n/a	Summary Table of solicited local Adverse Events (Safety Population)	• Any solicited local AEs • Any related solicited local AEs • Any severe solicited local AEs • Any related severe solicited local AEs • Any solicited local SAEs • Any related solicited local SAEs • Any medically attended solicited local AEs • Any related medically attended solicited local AEs • Any solicited local AEs leading to study discontinuation • Any solicited local AEs leading to second vaccination not administered
1.4.5	n/a	Summary Table of solicited systemic Adverse Events (Safety Population)	• Any solicited systemic AEs • Any related solicited AEs • Any severe solicited AEs

			• Any related severe solicited systemic AEs • Any solicited systemic SAEs • Any related solicited systemic SAEs • Any medically attended solicited systemic AEs • Any related medically attended solicited systemic AEs • Any solicited systemic AEs leading to study discontinuation • Any solicited systemic AEs leading to second vaccination not administered
1.4.6	n/a	Summary Table of unsolicited Adverse Events (Safety Population)	• Any unsolicited AEs • Any related unsolicited AEs • Any severe unsolicited AEs • Any serious unsolicited AEs • Any medically attended unsolicited AEs • Any related severe unsolicited AEs • Any related serious unsolicited AEs • Any related medically attended unsolicited AEs • Any unsolicited AEs leading to second vaccination not administered • Any unsolicited AEs caused study discontinuation
1.4.7	n/a	Subjects with at least one Adverse Event classified by Maximum Severity (Safety Population)	
Solicited Adverse Events			
1.4.8	n/a	Subjects with solicited local Adverse Events by Symptom (Safety Population)	
1.4.9	n/a	Subjects with solicited systemic Adverse Events by Symptom (Safety Population)	
1.4.10	n/a	Subjects with solicited Adverse Events classified by Maximum Severity (Safety Population)	
1.4.11	n/a	Subjects with solicited local Adverse Events by Symptom classified by Maximum Severity (Safety Population)	
1.4.12	n/a	Subjects with solicited systemic Adverse Events by Symptom classified by Maximum Severity (Safety Population)	
Unsolicited Adverse Events			
1.4.13	n/a	Subjects with unsolicited Adverse Events by SOC and PT (Safety Population)	
1.4.14	n/a	Subjects with related unsolicited	

		Adverse Events by SOC and PT (Safety Population)	
1.4.15	n/a	Subjects with severe unsolicited Adverse Events by SOC and PT (Safety Population)	
1.4.16	n/a	Subjects with serious unsolicited Adverse Events by SOC and PT (Safety Population)	
1.4.17	n/a	Subjects with medically attended unsolicited Adverse Events by SOC and PT (Safety Population)	
1.4.18	n/a	Subjects with related severe unsolicited Adverse Events by SOC and PT (Safety Population)	
1.4.19	n/a	Subjects with related serious unsolicited Adverse Events by SOC and PT (Safety Population)	
1.4.20	n/a	Subjects with related medically attended unsolicited Adverse Events by SOC and PT (Safety Population)	
1.4.21	n/a	Subjects with unsolicited Adverse Events leading to second Vaccination not administered (Safety Population)	
1.4.22	n/a	Subjects with unsolicited Adverse Events caused study discontinuation (Safety Population)	
1.4.23	n/a	Subjects with at least one unsolicited Adverse Event by Maximum Severity (Safety Population)	
1.4.24	n/a	Subjects with at least one medically attended unsolicited Adverse Event by Maximum Severity (Safety Population)	
1.4.25	n/a	Subjects with at least one unsolicited Adverse Event by Causality (Safety Population)	
1.4.26	n/a	Subjects with at least one medically attended unsolicited Adverse Event by Causality (Safety Population)	
1.4.27	n/a	Subjects with at least one serious unsolicited Adverse Event by Causality (Safety Population)	
Laboratory Parameters			
1.4.28	n/a	Absolute Values for Hematology Parameters by Parameter and Visit (Safety Population)	
1.4.29	n/a	Absolute Values for Clinical	

		Chemistry Parameters by Parameter and Visit (Safety Population)	
1.4.30	n/a	Absolute Values for Coagulation Parameters by Parameter and Visit (Safety Population)	
1.4.31	n/a	Absolute Changes from Baseline for Hematology Parameters by Parameter and Visit (Safety Population)	
1.4.32	n/a	Absolute Changes from Baseline for Clinical Chemistry Parameters by Parameter and Visit (Safety Population)	
1.4.33	n/a	Absolute Changes from Baseline for Coagulation Parameters by Parameter and Visit (Safety Population)	
1.4.34	n/a	Subjects with Hematology Parameters Outside Normal Range by Parameter and Visit (Safety Population)	
1.4.35	n/a	Subjects with Clinical Chemistry Parameters Outside Normal Range by Parameter and Visit (Safety Population)	
1.4.36	n/a	Subjects with Coagulation Parameters Outside Normal Range by Parameter and Visit (Safety Population)	
1.4.37	n/a	Urine Laboratory Results by Parameter and Visit (Safety Population)	
1.4.38	n/a	Absolute Changes from Baseline for pH and Specific Gravity by Visit (Safety Population)	
Other Safety Parameters			
1.4.38	n/a	Systolic Blood Pressure [mmHg] by Visit (Safety Population)	
1.4.39	n/a	Diastolic Blood Pressure [mmHg] by Visit (Safety Population)	
1.4.40	n/a	Pulse Rate [beats/min] by Visit (Safety Population)	
1.4.41	n/a	Body Temperature [°C] by Visit (Safety Population)	

7.2 List of Data Listings

No for IA	No for FA	Legend	Content
Overall Subject Information			
1.1	2.1	Analysis Population Details	Subject number, Treatment (Safety), Treatment (mITT), Safety Population, Reason not in Safety Population, mITT Population, Reason not in mITT Population, PP Population, Reason not in PP Population

1.2	2.2	Screening Failures with Reason	Subject number, Date of informed consent, Subject's withdrawal of consent, In/Ex criterion not satisfied, Other, Specification other reason
1.3	2.3	Violated Entry Criteria	Subject number, Visit, Criterion ID not met, Criterion description
1.4	2.4	Attended Visits with Dates (Safety Population)	Subject number, Treatment (Safety), Visit, Reason for unscheduled visit, Date, Reason out of time window
1.5	n/a	Protocol Deviations (Safety Population)	Subject number, Treatment (Safety), PD Category, Classification, Reason for classification, PD Description
1.6	2.6	Study Status (Safety Population)	Subject number, Treatment (Safety), Visit attendance status, Primary reason early termination/treatment discontinuation, Other reason specification, AE Term, Death date, Primary cause of death, Date of study discontinuation
Baseline Evaluation			
1.2.1	2.2.1	Demographic Information (Safety Population)	Subject number, Treatment (Safety), Date of birth, Age [years], Gender, Childbearing potential, Race, Specification other race
1.2.3	2.2.3	Full Physical Examination at Visit 0 (Safety Population)	Subject number, Treatment (Safety), Examination performed, Reason not performed, Exam Date
1.2.4	2.2.4	Medical History (Safety Population)	Subject number, Treatment (Safety), MH Term, Preferred Term (MedDRA [Version]), SOC (MedDRA [Version]), Started prior to study, Start date, End date, Ongoing, Ongoing at first IMP administration
1.2.6	2.2.6	Prior Medications (Safety Population)	Subject number, Treatment (Safety), Medication, ATC Level 2 (WHODD [Version]), ATC Level 3 (WHODD [Version]), Active Ingredients, Indication category, Indication, Dose, Dose Unit, Frequency, Route, Taken prior to study, Start date, End date, Ongoing, Ongoing at final examination
1.2.7	2.2.7	Concomitant Medications (Safety Population)	Subject number, Treatment (Safety), Medication, ATC Level 2 (WHODD [Version]), ATC Level 3 (WHODD [Version]), Active Ingredients, Indication category, Indication, Dose, Dose Unit, Frequency, Route, Taken prior to study, Start date, End date, Ongoing, Ongoing at final examination
1.2.5	2.2.5	Vaccination History (Safety Population)	Subject number, Treatment (Safety), Vaccination within 3 years prior screening, Date, Vaccination/Indication, ATC Level 2 (WHODD [Version]), ATC Level 3 (WHODD [Version]),
1.2.8	2.2.8	Virology	Subject number, Treatment (Safety), Virology test, Test performed, Result, Date
Immunogenicity Analysis			
1.3.1	2.3.1	Functional Antibodies Assessment ($PRNT_{50}$) (ITT Population)	Subject number, Country, Treatment group, Visit, Sample performed, Reason not performed, Sample ID, Date/Time of sample, Titer [IU/ml], Serconversion
1.3.2	2.3.2	Functional Antibodies Assessment (ELISA) (ITT Population)	Subject number, Country, Treatment group, Visit, Sample performed, Reason not performed, Sample ID, Date/Time of sample, Titer [IU/ml] , Serconversion
n/a	2.3.3	Measles Antibodies Assessment (ITT Population)	Subject number, Country, Treatment group, Visit, Sample performed, Reason not performed, Sample ID, Date/Time of sample, Titer [IU/ml]

n/a	2.3.4	T-cell details (ITT Population)	Subject number, Country, Treatment group, Visit, Sample performed, Reason not performed, Sample ID, Date/Time of sample, Titer [IU/ml]
n/a	2.3.5	Serum collection for passive transfer (ITT Population)	Subject number, Country, Treatment group, Visit, Sample performed, Reason not performed, Sample ID, Date/Time of sample
Safety Analysis			
Extent of Exposure			
1.4.1	2.4.1	Extent of Exposure (Safety Population)	Subject number, Treatment (Safety), Date first vaccination, Date second vaccination, Treatment duration [days], Date informed consent, Date of last attended visit, Study duration [days]
1.4.2	2.4.2	Study Medication Details (Safety Population)	Subject number, Treatment (Safety), Visit, IMP administered, Reason IMP not administered, Date/Time, Package label barcode
Adverse Events			
1.4.3	n/a	Adverse Events Part 1 (Safety Population)	Subject number, Treatment (Safety), Record ID, AE Term, MedDRA PT, MedDRA SOC, AE type, Start date/time, End date/time, Medically attended, Severity, Serious AE, SAE Criteria, Causality
1.4.4	n/a	Adverse Events Part 2 (Safety Population)	Subject number, Treatment (Safety), Record ID, AE Term, AE type ,Action taken with Study Treatment, Other Action taken, Outcome, Ongoing at final examination, Caused study discontinuation
1.4.5	n/a	Unsolicited Adverse Events Part 1 (Safety Population)	Subject number, Treatment (Safety), Record ID, AE Term, MedDRA PT, MedDRA SOC, Start date/time, End date/time, Medically attended, Severity, Serious AE, SAE Criteria, Causality
1.4.6	n/a	Unsolicited Adverse Events Part 2 (Safety Population)	Subject number, Treatment (Safety), Record ID, AE Term, Action taken with Study Treatment, Other Action taken, Outcome, Ongoing at final examination, Caused study discontinuation
1.4.7	n/a	Related Unsolicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.5
1.4.8	n/a	Related Unsolicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.6
1.4.9	n/a	Severe Unsolicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.5
1.4.10	n/a	Severe Unsolicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.6
1.4.11	n/a	Serious Unsolicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.5
1.4.12	n/a	Serious Unsolicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.6
1.4.13	n/a	Medically attended unsolicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.5
1.4.14	n/a	Medically attended unsolicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.6
1.4.15	n/a	Unsolicited Adverse Events caused Study Discontinuation Part 1 (Safety Population)	See Listing 1.4.5
1.4.16	n/a	Unsolicited Adverse Events caused Study Discontinuation Part 2 (Safety Population)	See Listing 1.4.6
1.4.17	n/a	Unsolicited Adverse Events leading to second Vaccination not administered	See Listing 1.4.5

		Part 1 (Safety Population)	
1.4.18	n/a	Unsolicited Adverse Events leading to second Vaccination not administered Part 2 (Safety Population)	See Listing 1.4.6
1.4.19	n/a	Solicited Adverse Events Part 1 (Safety Population)	Subject number, Treatment (Safety), Record ID, AE Term, Start date/time, End date/time, Medically attended, Severity, Serious AE, SAE Criteria, Causality
1.4.20	n/a	Solicited Adverse Events Part 2 (Safety Population)	Subject number, Treatment (Safety), Record ID, AE Term, Action taken with Study Treatment, Other Action taken, Outcome, Ongoing at final examination, Caused study discontinuation
1.4.21	n/a	Related Solicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.19
1.4.22	n/a	Related Solicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.20
1.4.23	n/a	Severe Solicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.19
1.4.24	n/a	Severe Solicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.20
1.4.25	n/a	Serious Solicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.19
1.4.26	n/a	Serious Solicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.20
1.4.27	n/a	Medically attended Solicited Adverse Events Part 1 (Safety Population)	See Listing 1.4.19
1.4.28	n/a	Medically attended Solicited Adverse Events Part 2 (Safety Population)	See Listing 1.4.20
1.4.29	n/a	Solicited Adverse Events caused Study Discontinuation Part 1 (Safety Population)	See Listing 1.4.19
1.4.30	n/a	Solicited Adverse Events caused Study Discontinuation Part 2 (Safety Population)	See Listing 1.4.20
1.4.31	n/a	Solicited Adverse Events leading to second Vaccination not administered Part 1 (Safety Population)	See Listing 1.4.19
1.4.32	n/a	Solicited Adverse Events leading to second Vaccination not administered Part 2 (Safety Population)	See Listing 1.4.20
Laboratory Parameters			
1.4.33	n/a	Hematology Values outside Normal Range (Safety Population)	Subject number, Treatment (Safety), Visit, Date/Time, Parameter, Value, Unit, Not done, Reason not done, Clinically significant, Likely cause, Change from baseline
1.4.34	n/a	Clinical Chemistry Values outside Normal Range (Safety Population)	Subject number, Treatment (Safety), Visit, Date/Time, Parameter, Value, Unit, Not done, Reason not done, Clinically significant, Likely cause, Change from baseline
1.4.35	n/a	Coagulation Values outside Normal Range (Safety Population)	Subject number, Treatment (Safety), Visit, Date/Time, Parameter, Value, Unit, Not done, Reason not done, Clinically significant, Likely cause, Change from baseline
1.4.36	n/a	Urinalysis Values outside Normal Range (Safety Population)	Subject number, Treatment (Safety), Visit, Date/Time, Parameter, Result, Not done, Reason not done, Clinically significant, Likely cause

Other Safety Parameters			
1.4.37	n/a	Vital Signs (Safety Population)	Subject number, Treatment (Safety), Visit, Vital signs collected, Reason not collected, Date, Test, Result, Unit, Clinically significant, not done
1.4.38	n/a	Symptom-directed Physical Exam (Safety Population)	Subject number, Treatment (Safety), Exam performed, Reason not performed, Exam date
1.4.39	n/a	System Based Physical Exam (Safety Population)	Subject number, Treatment (Safety), Exam performed, Exam date
1.4.40	n/a	Local Tolerability (Safety Population)	Subject number, Treatment (Safety), Assessment performed, Reason not performed, Exam Date/Time, Time recorded
1.4.41	n/a	Local Tolerability – Post Vaccination (Safety Population)	Subject number, Treatment (Safety), Assessment performed, Reason not performed, Exam Date/Time, Time recorded
1.4.42	n/a	Pregnancy test (Safety Population)	Subject number, Treatment (Safety), visit, Pregnancy test performed, Reason not performed, Date, Result