Protocol # MV-CHIK-202/ EudraCT 2015-004037-26

Double blinded, randomized, Priorix®- and placebo-controlled trial to evaluate the optimal dose of MV-CHIK vaccine (against Chikungunya virus) in regard to immunogenicity, safety and tolerability in healthy volunteers

Sponsor:	Themis Bioscience GmbH, Vienna, Austria

Development phase:	Phase 2
Version and date:	Amended Protocol # MV-CHIK-202, Version 1.6, 19 Apr 2017 Incorporating Amendment 1, Version 1.0, 26 Apr 2016 and Amendment 3, Version 1.0, 19 Apr 2017

PROTOCOL 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 Harmonization and GCP guidelines and with the applicable local regulatory requirements.

SPONSOR

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PROTOCOL 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 Harmonization 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
Address	Name

STUDY SYNOPSIS

TITLE OF STUDY:

Double blinded, randomized, Priorix®- and placebo-controlled trial to evaluate the optimal dose of MV-CHIK vaccine (against Chikungunya virus) in regard to immunogenicity, safety and tolerability in healthy volunteers

STUDY CENTRE(S):

- Department of Clinical Pharmacology, Medical University of Vienna (Austria)
- Hansa Sanatorium GmbH of Graz (Austria)
- Institute for specific Prophylaxis and Tropical Medicine, Vienna (Austria)
- Berlin Centre for Travel & Tropical Medicine, Berlin (Germany)
- Department of Tropical Medicine and Infectious Diseases, Rostock University Medical Center, Rostock (Germany)
- University Medical Centre Hamburg-Eppendorf, Bernhard Nocht Centre for Clinical Trials (BNCCT), Hamburg (Germany)

STUDY PERIOD:	**PHASE OF DEVELOPMENT:**
Start, August 2016	Phase 2

OBJECTIVES:

- To investigate the immunogenicity and safety of MV-CHIK 28 days after primary immunization regimen, consisting of one or two vaccinations
- To investigate the immunogenicity, safety and tolerability of MV-CHIK booster dose 24 weeks after primary immunization
- To investigate the immunogenicity, safety and tolerability of MV-CHIK during the treatment period up to 28 days after the last treatment
- To identify dose and schedule of MV-CHIK to forward to Phase 3 clinical development

STUDY DESIGN:

Double blinded, randomized, Priorix® and placebo-controlled dose finding, multi center phase 2 trial in 320 healthy volunteer subjects. (Priorix® is preferred as control vaccine, however in case of unavailability or shortness of supply also MMR-Vax-Pro® or an equal measles vaccine may be used as control-vaccine)

The subjects will be randomized to one of six treatment groups (A, B, C, D, M1 or M2).

Group A: MV-CHIK low dose/Priorix® on study day 0 and 28, placebo on day 196

Group B: placebo on study day 0 and MV-CHIK low dose/Priorix® on day 28 and boosting dose on day 196

Group C: MV-CHIK high dose/Priorix® on study day 0 and 28, placebo on day 196

Group D: placebo on study day 0 and MV-CHIK high dose/Priorix® on day 28 and boosting dose on day 196

Group M1: Priorix® on study day -28, MV-CHIK high dose on day 0 and 28 and placebo on day 168 and 196

Group M2: Priorix® on study day -28, placebo on day 0 and 28 and MV-CHIK high dose on day 168 and 196

All subjects of treatment group A, B, C and D will receive three i.m. injections (on study day 0, 28 and 196). Subjects of group A and B will receive MV-CHIK low dose or control vaccine Priorix® and subjects of group C and D will be treated with MV-CHIK high dose or the control-vaccine Priorix®. All subjects of group A, B, C and D additionally will be randomized to one of two treatment sequences: group A and C will receive MV-CHIK or control-vaccine on study day 0 and 28, followed by placebo (saline) on day 196, and group B and D receive placebo on study day 0 and MV-CHIK or Priorix® on day 28, followed by an additional vaccination of the same product on day 196 (boosting vaccination).

All subjects of the measles booster group M1 and M2 will receive five i.m. injections on study day -28, 0, 28, 168 and 196. The first vaccination will be Priorix® (or equivalent measles vaccine) on study day -28. Group M1 will receive MV-CHIK high dose vaccinations on day 0 and day 28 and placebo on day 168 and 196. Group M2 will receive placebo on day 0 and 28 and MV-CHIK high dose on day 168 and on day 196.

NUMBER OF SUBJECTS (PLANNED AND ANALYSED):

320 subjects

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION:

Healthy male or female volunteer subjects, 18 to 55 years old

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION:

MV-CHIK, a recombinant measles virus vaccine expressing Chikungunya virus antigens; powder for suspension for injection; concentration of low dose $5x10^4$ (± 0.5 log) $TCID_{50}$ per 0.3 mL or high dose $5x10^5$ (± 0.5 log) $TCID_{50}$ per 0.3 mL administered by i.m. injection.

CONTROL TREATMENTS, DOSE AND MODE OF ADMINISTRATION:

Control-vaccine: Priorix® or MMR-Vax-Pro® or equivalent measles vaccine, powder and solvent for suspension for injection of measles, mumps, and rubella vaccine (live). Administration by i.m injection of 0.5 mL after reconstitution in physiological saline solution 0.9%.

Placebo control: physiological saline solution 0.9%. Administration by i.m injection.

DURATION OF STUDY:

The estimated study duration per subject will be 33-37 weeks (~8 months). Women of childbearing potential will be followed up for additional 2 months to confirm no pregnancy

occurred within 3 months after the last vaccination. The overall study duration will take 3 years from study initiation until reporting.

CRITERIA FOR EVALUATION:

Primary end-point:

- Immunogenicity on day 56 confirmed by the presence of functional anti-chikungunya antibodies as determined by the plaque reduction neutralization test ($PRNT_{50}$)

Secondary end-points:

- Immunogenicity on days 0, 28, 196 and 224; additionally, for group M1 and M2 on day -28 and 168 as confirmed by the presence of functional anti-chikungunya antibodies as determined by the plaque reduction neutralization test ($PRNT_{50}$) and by ELISA.
- Measurement of anti-measles antibodies on days 0, 28 and 56; additionally, for group M1 and M2 on day -28 as determined by ELISA.
- Solicited local and systemic adverse events (AEs)
- Serious adverse events (SAEs)
- Rate of AEs during the 28 day post-vaccination period
- Safety laboratory parameters (hematology, serum chemistry, urinalysis)
- Shedding of live recombinant virus until day 196 (subset of subjects)
- Induction of a Chikungunya virus specific T cell response (subset of subjects)
- Pre-existing anti-vector immunity:
  - Immunogenicity of Chikungunya vaccine in the presence of recently boosted measles immunity (measles booster groups M1 and M2).
  - Relation of post-vaccination anti-chikungunya plaque reduction neutralization titers ($PRNT_{50}$) and baseline anti-measles ELISA titers (all groups).

STATISTICAL METHODS:

The primary immunogenicity analysis will compare the $PRNT_{50}$ antibody geometric mean titer (GMT) 28 days after one or two immunizations 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 vaccination group and adjusting for multiple comparisons according to Tukey-Kramer. This will be done using log-10 transformed data and taking the anti-log of the resulting point estimates the least squares means, least squares means differences and the corresponding 95% CIs.

All subjects entered into the study, who receive at least one vaccination, will be included in the safety analysis. For solicited local and solicited systemic AEs, the number and percentage of subjects with AEs will be summarized for the 7 day diary period, by AE grade (mild, moderate, severe), by group and by dose. Relatedness will not be assessed for solicited AEs.

For unsolicited AEs, the number and percentage of subjects with treatment emergent adverse events (AEs) and serious adverse events (SAEs) will be presented for each treatment group overall and by system organ class/preferred term, and by AE grade, and relatedness. For unsolicited AEs, all subjects will be included in the safety analysis based on the product received ("as treated") for the time period summarized. Changes in laboratory values will be analyzed descriptively by grade and by group from study entry until time point of assessment.

Data Analysis:
A preliminary analysis including safety and immunogenicity data will be performed after all subjects completed Visit 3 (day 56). The goal of this analysis is to provide highly valuable data of the novel Chikungunya vaccine for further development. Results from this analysis will have no impact on the study design and treatment of the subjects in this study. A final analysis will be conducted once the last subject has completed the study.

Data Safety Monitoring Board:

An independent DSMB will be installed to periodically review accruing safety information, and if necessary, to determine whether study or individual subject stopping rules have been met. The DSMB will periodically review listings and summary tabulations of SAEs, deaths, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination. After about 50% of subjects of treatment groups A, B, C and D completed Visit 3 (day 56), the DSMB will review safety and immunogenicity in order to evaluate the risk-benefit of the MV-CHIK administration and make recommendations to the sponsor regarding the further study conduct and/or protocol modifications, if needed. A written DSMB charter will be developed.

BENEFIT/RISK ASSESSMENT:

The measles virus vector is well known. Possible risks that are frequently associated with vaccination are the occurrence of local reactions e.g. edema, induration and erythema, transient local pain or tenderness at the injection site as well as mild to moderate headache, myalgia, arthralgia, flu-like symptoms or fatigue.

As any other vaccine, MV-CHIK might induce allergic and anaphylactic reactions, apart from the described local reactions at the vaccination site and systemic flu-like reactions. In rare cases the injection can lead to a vasovagal reaction immediately after injection of the vaccine. The needle pricks for blood sampling may also cause local reactions such as edema. The subjects cannot expect direct benefit from study participation, except for potential boosting of measles immunity and increasing immunity to CHIKV.

LIST OF ABBREVIATIONS

Ab	Antibody
Ag	Antigen
AE	Adverse event
AESI	AEs of Special Interest
ALT	Alanine aminotransferase (SGPT)
aPTT	Abbreviated partial thrombin time
AST	Aspartate aminotransferase (SGOT)
$CCID_{50}$	Cell culture infectious dose 50%
CHIKV	Chikungunya Virus
cm	Centimeter(s)
CRO	Contract research organization
dL	Deciliter(s)
DSMB	Data safety monitoring board
e.g.	For example
EC	Ethics committee
eCRF	Electronical case report form
EudraCT	European clinical trials database
ER	Emergency room
FDA	Food and drug administration
GCP	Good clinical practice
GMT	Geometric mean titer
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus
i.e.	This is
i.m.	Intramuscularly
ICH	International conference on harmonization
IMP	Investigational medicinal product
IRB	Institutional review board
M1	Measles booster treatment group 1
M2	Measles booster treatment group 2
MedDRA	Medical dictionary for regulatory activities
mg	Milligram(s)
mL	Milliliter(s)
MV	Measles Virus
NA	Not assessable
NCR	Not clinically relevant
No.	Number(s)
PBMC	Peripheral blood mononuclear cells
PP	Per protocol
$PRNT_{50}$	Plaque reduction neutralization test 50%
SAE	Serious adverse event
SAR	Suspected adverse reaction
SCR	Seroconversion rate
SOP	Standard operating procedures
SUSAR	Suspected unexpected serious adverse reaction
$TCID_{50}$	Tissue culture infecting dose 50%
WHO	World health organization

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1 TREATMENT GROUPS AND STUDY PLAN

1.1 Treatment groups

MV-CHIK vaccine:

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

Group C + D: high dose $5x10^5$ (± 0.5 log) $TCID_{50}$/ dose

Group M1 + M2: high dose $5x10^5$ (± 0.5 log) $TCID_{50}$/ dose

Control vaccine:

Priorix® or MMR-Vax-Pro® or equivalent measles, mumps rubella vaccine

Placebo injection:

Physiological saline solution 0.9%.

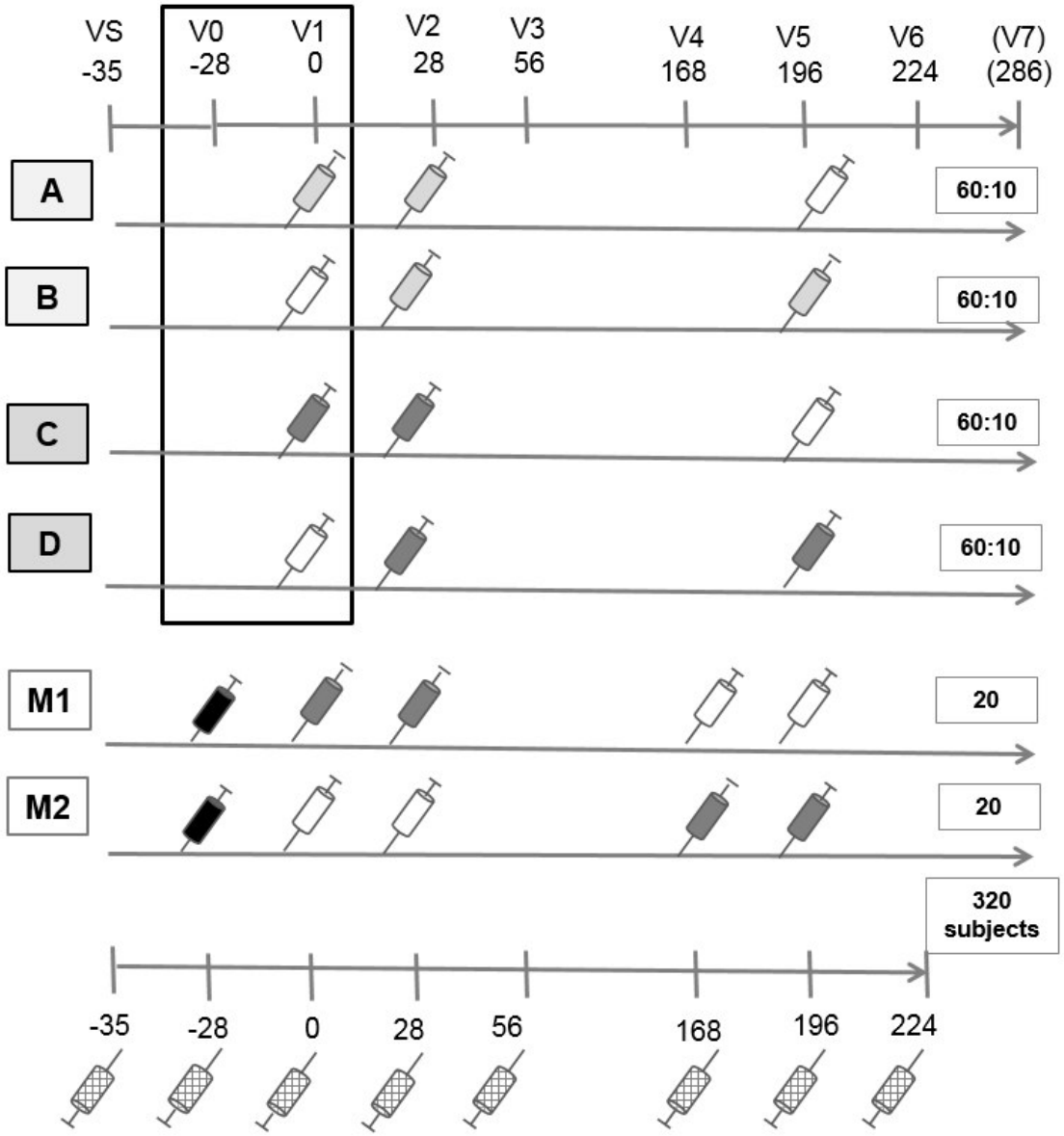
Table 1 Treatment groups and vaccination schedule

Group	Vaccine	Number of Subjects	Vaccine on day	Placebo on day
A	MV-CHIK low dose or	60	0 + 28	196
	control vaccine	10		
B	MV-CHIK low dose or	60	28 + 196	0
	control vaccine	10		
C	MV-CHIK high dose or	60	0 + 28	196
	control vaccine	10		
D	MV-CHIK high dose or	60	28 + 196	0
	control vaccine	10		
M1	control vaccine and	20	-28	168 + 196
	MV-CHIK high dose		0 + 28	
M2	control vaccine and	20	-28	0 + 28
	MV-CHIK high dose		168 + 196	
	Total Number of Subjects	320		

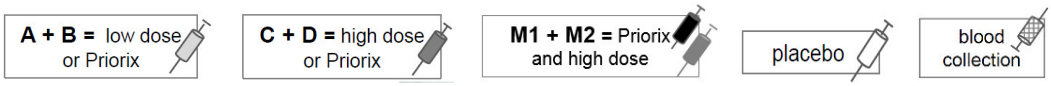
1.2 Clinical Study Plan

Subjects of group A and C will receive MV-CHIK or control-vaccine on day 0 and 28 and placebo (saline) on day 196. Subjects of group B and D will receive placebo (saline) on day 0, MV-CHIK or control-vaccine on day 28, followed by a booster of the same vaccine on day 196. Subjects randomized to measles booster group M1 or M2 will receive the control vaccine (Priorix® MMR-Vax-Pro® or equivalent measles vaccine) as first vaccination on day -28. Group M1 will then receive two MV-CHIK high dose vaccinations on day 0 and 28, followed by two placebo injections on day 168 and 196. Group M2 will receive two placebo injections on day 0 and 28, followed by two MV-CHIK high dose vaccinations on day 168 and 196.

Figure 1 Treatment Schedule



V_S	V0	V1	V2	V3	V4	V5	V6	(V7)
-35	-28	0	28	56	168	198	224	286
screening / enrolment	randomi zation							
	M1 + M2 1st shot	M1 + M2 2nd shot	M1 + M2 3rd shot		M1+ M2 4th shot	M1 + M2 5th shot		
	A+B+C+D V0 + V1 together on the same day 1st shot		A+B+C+ D 2nd shot			A+B+C+ D 3rd shot		



1.3 Visit procedures

Table 2. Study Procedures

	Screening Visit*	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Early
Study Day	Day -35*	Day -28	Day 0	Day 28	Day 56	Day 168	Day 196	Day 224	Day 286	Termination
Time windows / treatment groups	M1+M2: -35 to -29 A-D: -7 to -1	M1+M2: ±5 days **A-D -28 is study day 0	M1+M2 day 0 = V0+V1 at once on d 0	±5 days all groups	± 5 days all groups	±10 days only M1+M2	± 10 days all groups	± 10 days all groups	± 10 days all groups	
Informed consent	X									
Inclusion/exclusion criteria (1)	X	X	X (14)	X	X	X (14)	X			
HIV, hepatitis B/C, [blood: 8-10 mL]	X									
Demographic data	X									
Physical examination	X									
Vaccination history	X									
Hematology blood sample [3-5 mL] (6)	X				X			X		X
Coagulation parameter [blood: 3-5 mL] (7)	X				X			X		X
Clinical chemistry serum [8-10 mL] (8)	X				X			X		X
Urinalysis (10)	X				X			X		X
Medical history	X	X (2)								
Concomitant medications	X	X	X (14)	X	X	X (14)	X	X		X
Assessment of arthralgia	X	X	X (14)	X	X	X (14)	X	X		X
Symptom-directed physical exam (3)		X	X (14)	X	X	X (14)	X	X		X
Vital signs (4)	X	X	X (14)	X	X	X (14)	X	X		X
Urine pregnancy test (9)	X	X	X (14)	X	X	X (14)	X	X	(15)	X
Randomization		M1+M2 X	A B C D							
Study treatment: vaccination		X (14)	X	X		X (14)	X			
Immunogenicity 1: serum [blood: 2x 8-10 mL]		X (14)	X	X	X	X (14)	X	X		X
Immunogenicity 2 (5.1): [blood: 16-20 mL]		X (14)	X	X	X	X (14)	X	X		X
Measles antibody titer [no additional blood sample]		X (14)	X	X	X					X
T- cell analysis (5.2): [blood: 24-30 mL]			X	X	X	X (14)	X	X		X
Measles virus shedding (13): [blood: 2-3 mL, urine and saliva]			X (13)	X (13)			X (13)			
Local tolerability (12)		X (14)	X	X	X	X (14)	X	X		X
Dispense subject diary (11)		X (14)	X	X		X (14)	X			
Collect subject diary (11)			X (14)	X	X		X (14)	X		X
Adverse events		X (14,2)	X (2)	X	X	X (14)	X	X		X

* Screening visit within 7 days before randomization

(1) After randomization only exclusion criteria, which could influence the subject's eligibility throughout the study will be checked. The following exclusion criteria are concerned: 1, 7, 8, 10, 19, 20, 21, 22 and 23

(2) Symptoms noted prior to randomization are not considered as adverse events but will be recorded as medical history

(3) Including system-based assessment if necessary according to symptom-directed physical examination. (section 11.2.2)

(4) Systolic and diastolic blood pressure, body temperature and pulse

(5.1) Sites outside of Vienna: serum samples (blood 2x 8-10 mL) to be collected for additional immunogenicity analyses (cross neutralization and passive transfer)

(5.2) Vienna sites only: PBMCs will be isolated from fresh blood (blood 24-30 mL and once at V3 48-60 mL). (section 8.3.2)

(6) Hemoglobin, hematocrit, erythrocyte count, differential white blood count, platelets (EDTA blood: 3-5 mL)

(7) Prothrombin time, aPTT, fibrinogen (blood: 3-5mL)

(8) Creatinine, sodium, potassium, calcium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, bilirubin (blood 8-10 mL),

(9) Pregnancy test will be performed for women of childbearing potential. (section 8.3.6)

(10) Glucose, protein, pH, erythrocytes, leucocytes, nitrite, ketones, urobilinogen, bilirubin and specific gravity

(11) The subjects will assess local tolerability and systemic reactions themselves over a period of 7 consecutive days after each injection. (section 11.2.5)

(12) Investigation of injection site reaction 1 hour after vaccination and additionally before each subsequent vaccination. (section 11.2.4)

(13) Shedding: only in AKH Vienna, only in a subset of group A-D, on day 0, 28, 196 and additional shedding sampling visits on day 7, 10 and 14 (±1 day each) after the first vaccination on day 0. (section 8.3.1)

(14) Only measles booster group M1 and M2

(15) women of childbearing potential will be followed up by a phone call, to ensure no pregnancy occurred 3 months after the last vaccination

** Group A, B, C, and D will perform Visit 0 and Visit 1 procedures on one day, group M1 and M2 will perform V0 and V1 on two days (28 days apart)

2 INTRODUCTION

2.1 Disease Background

Chikungunya virus (CHIKV), a mosquito-borne pathogen that causes Chikungunya fever (CHIKF), has been spreading throughout Asia, Africa, and parts of Europe in recent times. (Enserik 2008; Rezza 2007; Wang 2008) Formerly indigenous to tropical Africa, recent large outbreaks have been reported in parts of South East Asia and due to global travel, the risk of spreading CHIKV in non-endemic regions, such as Europe and the United States, has increased. (Jain 2008)

CHIKV transmits to humans primarily by *Aedes aegypti* (Tsetsarkin 2007) and *Ae.albopictus,* which are common in many non-tropic urban areas. (Vazeille 2007). Transmission of the virus can occur in an urban cycle whereby the mosquito spreads the disease from an infected human to an uninfected human, following an epidemiological pattern similar to dengue fever (Jain 2008). Even vertical transmission of CHIKV from mother to fetus, causing congenital illness and fetal death, seems possible. (Ramful 2007; Gerardin 2008).

Most individuals will present with symptomatic disease after an incubation period of three to seven days (range, 2-12 days). Not all individuals infected with the virus will develop symptoms, approximately 3%-25% of persons with antibodies to Chikungunya virus have asymptomatic infections. 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 mostly characterized by the acute onset of high fever (>102°F/ 39°C). The fevers typically last from several days up to two weeks. Other signs and symptoms may include headache, diffuse back pain, myalgias, nausea, vomiting, polyarthritis, rash, and conjunctivitis. After the onset of fever, the majority of infected persons develop severe, often debilitating polyarthralgias. Joint symptoms are usually symmetric and occur most commonly in wrists, elbows, fingers, knees, and ankles but also more proximal joints. The lower extremity arthralgias can be severely disabling resulting in slow, broad based, halting gait, which can persist for months. The patients suffer from severe pain, tenderness, swelling and stiffness and they cannot perform normal tasks or go to work.

During the La Reunion Epidemic a number of unusual clinical presentations were observed, including hepatitis, autoimmune neurologic pathologies (Guillain-Barré), cardiologic manifestations and death. The case fatality ratio (CFR) during the 2006 La Reunion epidemic has been estimated to be 1:1000 (0,1%), with most deaths occurring in neonates, adults with underlying medical conditions and older people (>65 years) (Staples JE, 2009). During an

epidemic in Mauritius (2005-2006) and India (2006) a CFR of 4.5% and 4.9% was reported. The difference in fatality rates can be explained by difference in pathogenicity of the viral strains causing the epidemic, but also by the difference in medical care of infected patients (Renault P, 2008). Calculated estimates from annualized averages over combined epidemic and interepidemic periods suggest 33 to 26K deaths annually attributed to Chikungunya fever. Up to 46K patients per year can develop a chronic infection (LaBeaud AD, 2011).

Acute symptoms of Chikungunya fever typically resolve within 7 to 10 days. Following the acute phase, a number of patients develop prolonged symptoms lasting several weeks to months including fatigue, incapacitating joint pain, and tenosynovitis or edemous polyarthritis of their digits. Chronic disease is defined by symptoms that persist for more than three 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 1 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 travelers 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).

2.2 Investigational Drug

The 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 investigators' brochure.

2.3 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 more detail in the investigators' brochure.

2.4 Phase 1 Study

A Phase 1 study has been conducted in Austria at the Medical University, Vienna, Department of Clinical Pharmacology (EudraCT: 2013-001084-23):

Observer blinded, block-randomized, 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 analyzed 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 round (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 1 clinical batch was present at a ready to use liquid formulation at a single concentration. In contrast, the proposed Phase 2 clinical trial IMP will be presented as lyophilized 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 neutralizing antibodies in the sera of vaccinated subjects. The neutralization titers were determined by 50% plaque reduction neutralization test (PRNT50) 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 immunization elicits functional antibodies in all treatment cohorts. The medium and high dose groups induced similar levels of neutralizing antibodies, which were significantly higher than the low dose group and the Priorix control groups. A second immunization at one or three months interval boosted the titers in all treatment groups clearly, figure **2**). The low dose group showed relatively low titers of both functional and total antibody levels. A second immunization did boost the titers clearly, however, this dose level is not suitable for a single shot vaccine. The medium dose group elicited significantly higher titers after one or two immunizations. The highest functional and total anti-CHIKV titers 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 immunization, and boosted by a second dose.

The Measles antibody titers 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 titer (GMT). The measles ELISA titers that were induced after the first immunization were close to the detection limit of the assay. Therefore, a not additive effect was visible after a second immunization.

The subjects that entered the study were not pre-selected for a specifically high or low Measles antibody titer. The subjects were randomly allocated to the individual treatment cohorts. To analyze the effect of pre-existing anti-measles immunity on the immunogenicity of the MV-CHIK vaccine, we investigated if a baseline anti-measles titer would cause a difference between the $PRNT_{50}$ titers on day 28 after the first immunization 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 titer 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.

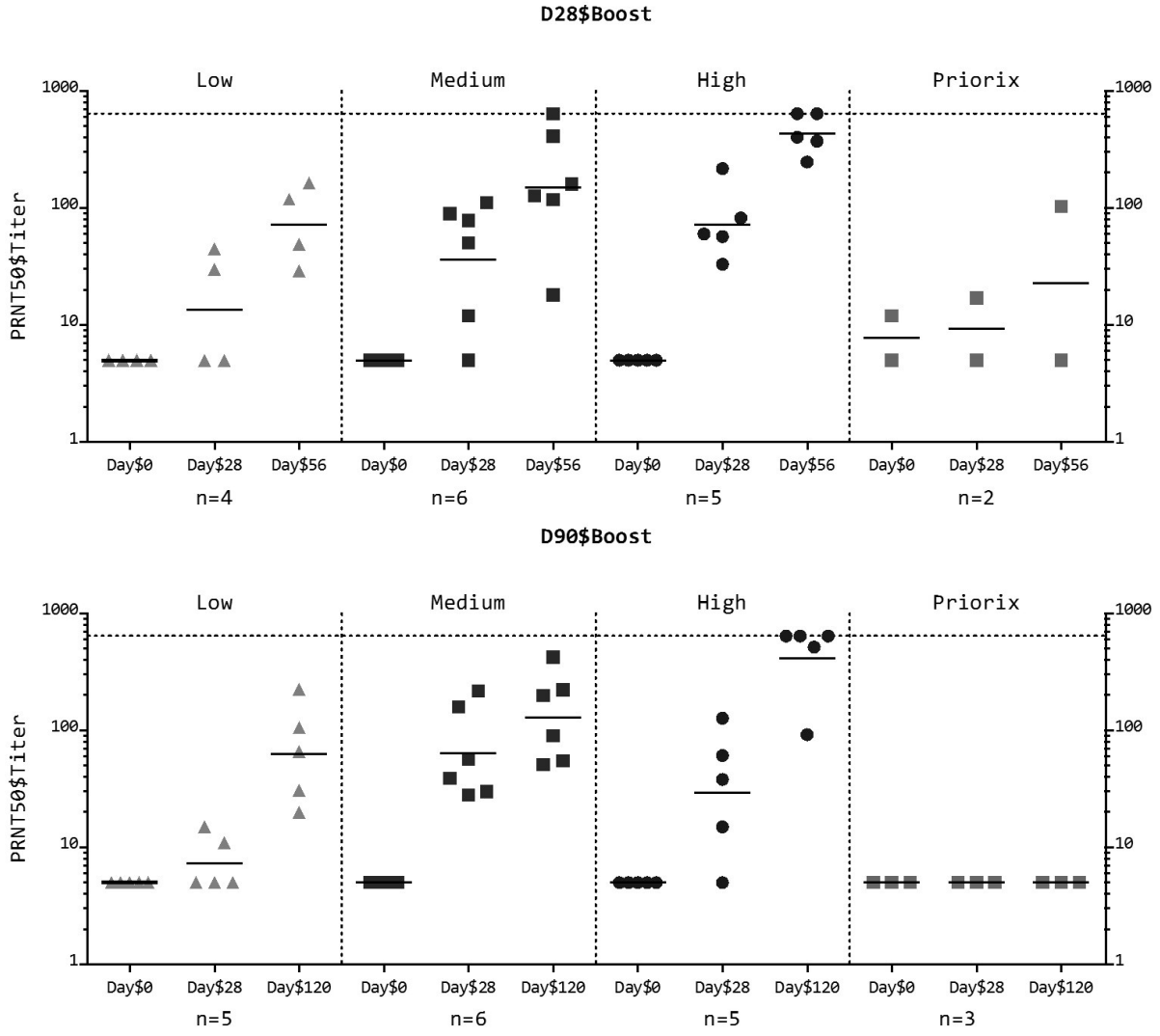


Figure 2: Induction of neutralizing antibodies after two immunizations – 1 month interval (Upper panel), 3 month interval (Lower panel). The data are depicted as 50% plaque reduction neutralization titers ($PRNT_{50}$) for the individual subjects and Geometric Mean titers (GMT) for the cohorts on day 0, 28 and 56 (upper panel) or day 120 (lower panel). The cohorts were divided in three dose groups: low ($1.5x10^4$ $TCID_{50}$/mL) medium ($7.5x10^4$ $TCID_{50}$/mL) and high ($3x10^5$ $TCID_{50}$/mL); the control group received Priorix, a commercially available Measles, mumps rubella vaccine. Titers <10 are referred as seronegative samples. For the purpose of display in the graph and for calculation of a GMT the negative values were depicted as 5.

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 hospitalization. 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 side pain (50.0%), influenza like illness (45.2%), vaccination side pain (40.5%), fatigue (38.1%), nausea (31.0%), myalgia (26.2%) and arthralgia (23.8%). The most frequently related solicited AEs were injection side 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 (hematology, serum chemistry, urinalysis) did not indicate any safety issues. There were no clinically relevant abnormalities observed in hematology, clinical chemistry, coagulation or urinalysis laboratory parameters.

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

2.5 Study rationale

This Phase 2 trial is designed to investigate the immunogenicity, safety and tolerability of MV-CHIK.

Two different doses of MV-CHIK at a concentration of $5x10^4$ (± 0.5 log) $TCID_{50}$/ dose and $5x10^5$ (± 0.5 log) $TCID_{50}$/ dose per 0.3 mL will be assessed to healthy adults for the induction of functional anti-chikungunya neutralizing antibodies. In addition, the direct effect of the pre-existing vector immunity will be addressed by the immunization of a small subset of subjects with Priorix® prior immunization with two doses of MV-CHIK.
This study was designed according to the Note for Guidance on Clinical Evaluation of New Vaccines (CHMP/VWP/164653/2005), where applicable.

2.6 Benefit/Risk Assessment

The measles virus vector is well known. Possible risks that are frequently associated with vaccination are the occurrence of local reactions e.g. edema, induration and erythema, transient local pain or tenderness at the injection site as well as mild to moderate headache, myalgia, flu-like symptoms or fatigue.
In the past, a live virus vaccine against Chikungunya virus, which was based on an attenuated Chikungunya strain caused mild, transient arthralgia in immunized patients. The MV-CHIK vaccine is based on the measles virus technology and NO LIVE ATTENUATED Chikungunya virus is used. Thus, induction of Chikungunya like symptoms is not expected. However, the subjects will be monitored for occurrence of arthralgia.

As any other vaccine, MV-CHIK might induce allergic and anaphylactic reactions, apart from the described local reactions at the vaccination site and systemic flu-like reactions. In rare cases the injection can lead to a vasovagal reaction immediately after injection of the vaccine. The needle pricks for blood sampling may also cause local reactions such as edema. The subjects cannot expect direct benefit from study participation, except for potential boosting of measles immunity and mounting immunity to CHIKV.

If the clinical development of MV-CHIK is successful, this vaccine might help people, who are at risk of acquiring a possible life-threatening infection with CHIKV.
Thus, the benefit risk ratio is considered positive.

3 STUDY OBJECTIVES

3.1 Primary Objective

- To investigate the immunogenicity and safety of MV-CHIK 28 days after primary immunization regimen, comprising one or two vaccinations

3.2 Secondary Objectives

- To investigate the immunogenicity, safety and tolerability of MV-CHIK booster dose 24 weeks after primary immunization
- To investigate the immunogenicity, safety and tolerability of MV-CHIK during the treatment period up to 28 days after the last treatment
- To identify dose and schedule of MV-CHIK to forward to Phase 3 clinical development

4 STUDY PLAN

4.1 Study Endpoints

4.1.1 Primary endpoint

- Immunogenicity on day 56 confirmed by the presence of functional anti-chikungunya antibodies as determined by the plaque reduction neutralization test ($PRNT_{50}$)

4.1.2 Secondary endpoints

- Immunogenicity on day 0, 28, 196 and 224; additionally, for group M1 and M2 on day -28 and 168 as confirmed by the presence of functional anti-chikungunya antibodies as determined by the plaque reduction neutralization test ($PRNT_{50}$) and by ELISA.
- Measurement of anti-measles antibodies on day 0, 28, and 56; additionally, for group M1 and M2 on day -28 as determined by ELISA.
- Solicited local and systemic adverse events (AEs)
- Serious adverse events (SAEs)
- Rate of AEs during the 28 day post-vaccination period
- Safety laboratory parameters (hematology, serum chemistry, urinalysis)
- Shedding of live recombinant virus until day 196 (subset of subjects)
- Induction of a Chikungunya virus specific T cell responses (subset of subjects)
- Pre-existing anti-vector immunity:
  - Immunogenicity of Chikungunya vaccine in the presence of recently boosted measles immunity (measles booster groups M1 and M2).
  - Relation of post-vaccination anti-chikungunya plaque reduction neutralization titers ($PRNT_{50}$) and baseline anti-measles ELISA titers (all treatment groups).

4.2 Safety

Systemic tolerability

- Vital signs (blood pressure, pulse rate, body temperature)
- Laboratory parameters (blood cell count, serum chemistry, coagulation, urinalysis)
- (Serious) adverse events

Local tolerability

- Local injection site reactions

5 INVESTIGATIONAL MEDICINAL PRODUCT

5.1 Investigational Medicinal Product Description

5.1.1 Test product – MV-CHIK

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 have been inserted into the measles virus Schwarz strain to produce the candidate vaccine MV-CHIK expressing Chikungunya virus antigens.

The study drug is provided as lyophilized powder for suspension for injection in two concentrations:

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

The lyophilized product will be reconstituted in 0.4 mL injection grade water and 0.3 mL/ dose will be administered. The solvent for suspension will be provided by the study sites.

5.1.2 Control treatments

Control-vaccine: Priorix® /Glaxo Smith Kline GSK will be used as a control-vaccine because MV is the backbone of MV-CHIK. In case of unavailability or shortness of supplies, also MMR-Vax-Pro /Sanofi Pasteur MSD or an equal measles vaccine approved in the EU and US may be used as control-vaccine. Priorix® is a sterile lyophilized mixed preparation containing the attenuated Schwarz measles virus strain, the RIT 4385 strain of mumps virus (derived from the Jeryl Lynn strain) and the Wistar RA 27/3 rubella virus strain. Each virus strain is separately obtained by propagation in either chick embryo tissue cultures (mumps and measles) or MRC5 human diploid cells (rubella). Priorix® is presented as a white to slightly pink pellet for reconstitution with sterile Water for Injection diluent. Each 0.5 mL dose of the reconstituted vaccine contains not less than $10^{3.0}$ $CCID_{50}$ (cell culture infectious dose 50%) of the Schwarz measles, not less than $10^{3.7}$ $CCID_{50}$ of the RIT 4385 mumps and not less than $10^{3.0}$ $CCID_{50}$ of the Wistar RA 27/3 rubella virus strains. The three virus strains are mixed prior to lyophilization. The lyophilized vaccine also contains lactose, neomycin sulfate, amino acids, as well as sorbitol and mannitol as stabilizers.

- MMR-Vax-Pro® is a powder and solvent for suspension for injection in pre-filled syringe.

Before reconstitution, the powder is a light yellow compact crystalline cake and the solvent is clear colorless Water for Injection.

Each 0.5 mL dose of the reconstituted vaccine contains not less than $10^{3.0}$ $CCID_{50}$ (cell culture infectious dose 50%) of the Enders' Edmonston measles, not less than $12.5x\ 10^{3.0}$ $CCID_{50}$ of

Jeryl Lynn mumps virus strain and not less than $10^{3.0}$ $CCID_{50}$ of the Wistar RA 27/3 rubella virus. Each virus strain is separately obtained by propagation in either chick embryo tissue cultures (mumps and measles) or in WI-38 human diploid cells (rubella).
The lyophilized vaccine also contains lactose, neomycin, hydrolysed gelatin, as well as sorbitol and sucrose as stabilizers.
Placebo control: A sterile physiological saline solution of 0.9% will be used as placebo.

5.2 Dose regimen

The following concentrations and volumes will be administered according to the treatment schedule defined for the six treatment groups:
Group A + B: MV-CHIK low dose $5x10^4$ (± 0.5 log) $TCID_{50}$/ 0.3 mL and placebo 0.3 mL or control-vaccine (Priorix® or equal vaccine) 0.5 mL and placebo 0.5 mL
Group C + D: MV-CHIK high dose $5x10^5$ (± 0.5 log) $TCID_{50}$/ 0.3 mL and placebo 0.3 mL or control-vaccine (Priorix® or equal vaccine) 0.5 mL and placebo 0.5 mL
Group M1 + M2: control-vaccine (Priorix® or equal vaccine) 0.5 mL and MV-CHIK high dose $5x10^5$ (± 0.5 log) $TCID_{50}$/ 0.3 mL and placebo 0.3 mL.

Treatment schedule

Group	Vaccine	Number of Subjects	Vaccine on day	Placebo on day
A	MV-CHIK **$5x10^4$** (± 0.5 log) $TCID_{50}$ or	60	0 + 28	196
	control vaccine	10		
B	MV-CHIK **$5x10^4$** (± 0.5 log) $TCID_{50}$ or	60	28 + 196	0
	control vaccine	10		
C	MV-CHIK **$5x10^5$** (± 0.5 log) $TCID_{50}$ or	60	0 + 28	196
	control vaccine	10		
D	MV-CHIK **$5x10^5$** (± 0.5 log) $TCID_{50}$ or	60	28 + 196	0
	control vaccine	10		
M1	control vaccine and	20	-28	168 + 196
	MV-CHIK $5x10^5$ (± 0.5 log) $TCID_{50}$		0 + 28	
M2	control vaccine and	20	-28	0 + 28
	MV-CHIK $5x10^5$ (± 0.5 log) $TCID_{50}$		168 + 196	
	Total Number of Subjects	320		

5.3 Route and method of administration of study medication

All subjects will receive intra muscular (i.m.) injections of study medication (MV-CHIK, control vaccine or placebo) in the deltoid region (upper left arm or non-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.

5.4 Study medication preparation and distribution

The study medication (MV-CHIK, control-vaccine and placebo) will be prepared by unblinded, authorized staff members (nurse, investigator or pharmacist), otherwise not involved in the conduct of the trial after randomization. MV-CHIK, control-vaccine and placebo will be exclusively used for the present clinical trial and will only be administered to the subjects enrolled in the study. Details for instructions of study medication preparation will be provided in the IMP manual.

5.5 Packaging and labeling

Individual boxes will contain single vials with a lyophilized powder of the test product. The labels on vials will be in English and the labels on the boxes will be in German language. Each box will contain a leaflet with information in English and a translation in German and Spanish.

The vials will be labeled with:

Themis Bioscience GmbH
MV-CHIK-202, i.m.
Concentration: XXXXX
Batch No.:XXX
For clinical trial use only
Store frozen at -20°C
EudraCT: 2015-004037-26
Visit No.:
Subject ID:

Each box will be labeled with:

Bitte entnehmen Sie die Informationen zur sicheren Handhabung und Entsorgung des Produktes dem IMP-Manual!
Lagerung bei -20°
Re-test Datum: XXX
Visiten Nummer: ____
Probanden ID: _____
Prüfarzt: _____

MV-CHIK-202 Chikungunya Impfstoff
Genetisch veränderter Organismus
Pulver zur Herstellung einer Injektionslösung intramuskulär
1 Einzeldosisampulle pro Karton
Konzentration: XXX
Ch.-B.: XXX
EudraCT: 2015-004037-26
Nur zur klinischen Prüfung bestimmt!

Sponsor: Themis Bioscience GmbH
Muthgasse 11/2
1190 Wien
Tel.: +43 1 2367151

CRO: Assign Clinical Research GmbH
Hainburger Straße 33
1030 Wien
Tel.: +43 1 40338050

The prepared syringe ready for IMP administration will be labelled with the following information:

Sponsor: Themis Bioscience GmbH
Muthgasse 11/2, 1190 Wien
CRO: Assign Clinical Research GmbH
Hainburger Straße 33, 1030 Wien
Studie: MV-CHIK-202
MV-CHIK od. Priorix® od. Placebo
Probanden ID: _____
Visiten Nummer: _____
Injektionslösung zur intramuskulären
Verabreichung vor (Uhrzeit): _____

5.6 Storage and transport conditions

The MV-CHIK vaccine will be stored and transported at -20°C and shall be administered shortly after preparation. The lyophilized control-vaccine (Priorix® or MMR-Vax-Pro® or equal measles vaccine) shall be stored and transported refrigerated at 2°C-8°C and should be used immediately after reconstitution; however, in-use stability has been demonstrated for 8 hours when refrigerated at 2°C-8°C.

5.7 Used supplies

After administration, used containers properly labeled with subject ID and visit number (see above) will be stored on site until checked by the unblinded monitor.

5.8 Study drug accountability

After receipt of the drug supply, the study staff members will confirm in writing by signing and dating standard drug accountability forms. An IMP log will be kept current by each site, detailing the date and quantity of investigational product received from and returned to the sponsor. Moreover, detailing the dates and quantities of IMP administered to each subject. This documentation only will be available to an unblinded monitor to verify drug accountability during the study. Any unused IMP and empty vials will be accounted for and returned to the sponsor or destroyed at site in a confidential manner.
At the end of the study, all used and unused supplies (see paragraph above) will be returned to the sponsor or destroyed by the investigator in a confidential manner, after assessment of drug accountability.

6 INVESTIGATIONAL PLAN

6.1 Overall trial design

This is a double blinded, block-randomized, active- and placebo controlled, dose escalation phase 2 trial, assessing immunogenicity, safety and tolerability of MV-CHIK. 320 healthy male and female subjects aged 18-55 years will be randomized to one of six treatment groups (A, B, C. D, M1 or M2) differing in dosage and scheduling of vaccinations.

Treatment plan:
Group A + B: two doses of 0.3 mL MV-CHIK low dose $5x10^4$ (± 0.5 log) $TCID_{50}$/ dose vs. two doses of 0.5 mL control vaccine (e.g Priorix®)
Group C + D: two doses of 0.3 mL MV-CHIK high dose $5x10^5$ (± 0.5 log) $TCID_{50}$/ dose vs. two doses of 0.5 mL control vaccine
Group M1 + M2: after one dose of 0.5 mL control vaccine, two doses of 0.3 mL MV-CHIK high dose $5x10^5$ (± 0.5 log) $TCID_{50}$/ dose at different time points

Table 1 Treatment groups and vaccination schedule

Group	Vaccine	Vaccine on day	Placebo on day
A	MV-CHIK low dose or control vaccine	0 + 28	196
B	MV-CHIK low dose or control vaccine	28 + 196	0
C	MV-CHIK high dose or control vaccine	0 + 28	196
D	MV-CHIK high dose or control vaccine	28 + 196	0
M1	control vaccine and	-28	168 + 196
	MV-CHIK high dose	0 + 28	
M2	control vaccine and	-28	0 + 28
	MV-CHIK high dose	168 + 196	
	Total Number of Subjects		

All subjects of group A, B, C and D will receive three i.m. injections on study day 0, 28 and 196. Subjects of group A and B will receive MV-CHIK low dose or control-vaccine Priorix® (or equivalent measles vaccine) and subjects of group C and D will be treated with MV-CHIK high dose or control-vaccine (Priorix® or equivalent measles vaccine).

All subjects of group A, B, C and D additionally will be randomized to one of two treatment sequences; group A and C will receive MV-CHIK or control-vaccine Priorix® on study day 0 and 28 followed by placebo on day 196, and group B and D receive placebo on day 0 and MV-CHIK or Priorix® on day 28 followed by an additional vaccination of the same product on day196 (boosting vaccination).

All subjects of the measles booster group M1 and M2 will receive five i.m. injections on study day -28, 0, 28, 168 and 196. The first vaccination will be Priorix® (or equivalent measles vaccine) on study day -28. Group M1 will receive MV-CHIK high dose vaccinations on day 0 and day 28 and placebo on day 168 and 196. Group M2 will receive placebo on day 0 and 28 and MV-CHIK high dose on day 168 and on day 196.
All subjects will be followed for safety and immunogenicity evaluation until day 224.

The estimated study duration per subject will be 33-37 weeks (~8 months), respectively. Overall study duration estimates to be 3 years from study initiation until reporting.

6.2 Discussion of design

The double-blind design will allow the most objective scoring of any adverse events. Block-randomization is considered to be necessary because of the limited group sizes.
Priorix® will be used as a control-vaccine because MV is the backbone of MV-CHIK. In case of unavailability or shortness of supplies also MMR-Vax-Pro or an equal measles vaccine may be used as control-vaccine.
Saline, serving as placebo control will be used to allow the assessment of absolute differences in signs and symptoms induced by MV-CHIK on an individual level and in order to maintain blinding of the sequence.
The sequence of vaccine injection on days 0 and 28 will allow assessment of the immunogenicity of two shots of the MV-CHIK vaccine 4 weeks apart. This sequence would be more realistic, if MV-CHIK will be used as a traveler vaccine.
The sequence of vaccine injection on days 28 and 196 will provide information on the immunogenicity of MV-CHIK after a single shot up to 196 days, which is also relevant for travelers. It will also allow testing of the immunogenicity of two shots of MV-CHIK (boosting vaccination after 24 weeks) in case the interval of 28 days between vaccinations is suboptimal for immunogenicity.

The shedding of infectious measles virus particles from a subset of immunized study subjects will be determined. Samples for shedding will only be collected from a subset of study subjects (of treatment groups A-D) that are enrolled at the study site in AKH Vienna. Body fluids including saliva, urine and whole blood will be collected at Visits 1, 2, and 5. In addition, the subjects will be asked to return to the study site on days 7, 10 and 14 after the first immunization for collection of saliva, urine and whole blood.

The induction of Chikungunya virus specific T cells is a critical step in the generation of a functional immune response. Only subjects (of all treatment groups) that are enrolled at study sites in Vienna will be included in a T cell investigation. For this purpose, peripheral blood monocyte cells (PBMC) from whole blood will be collected from the subjects. The frequency of Chikungunya virus specific T cells will be analyzed in vitro.

6.3 Randomization

At Visit 0, eligible subjects will be assigned to one of six treatment groups in timely order and receive a three-digit randomization number using randomization envelopes provided by data management. The site will also be provided with emergency envelopes for unblinding in case of emergency and if knowledge of the treatment assignment is mandatory for emergency treatment.

Treatment groups A, B, C and D:

All subjects will receive three i.m. injections on study day 0, 28 and 196. Subjects randomly allocated to group A or B will receive either MV-CHIK low dose or a control-vaccine (Priorix® or MMR-Vax-Pro® or equal measles vaccine). Subjects randomly allocated to group C or D will receive MV-CHIK high dose or a control-vaccine. Block randomization will be used with a randomization ratio of 1:6 (control-vaccine : MV-CHIK).

All subjects of treatment groups A, B, C and D additionally will be randomized to one of two treatment sequences. Group A + C will receive vaccinations (MV-CHIK or control vaccine) on day 0 and 28 and placebo injection on day 196 whereas groups B + D receive placebo injection on day 0 and vaccinations (MV-CHIK or control vaccine) on day 28 and 196.

Measles booster group M1 and M2:

All subjects will receive five i.m. injections and the first injection on day -28 will be the control vaccine (Priorix® or MMR-Vax-Pro® or an equal measles vaccine). Subjects will be randomly allocated to receive MV-CHIK on day 0 and on 28 (and placebo on day 168 and 196), or to receive MV-CHIK on day 168 and on 196 (and placebo on day 0 and 28). Randomization ratio between the two booster groups will be M1 : M2 = 1 : 1

Randomization of 320 subjects to one of the six treatment groups:

Group A: MV-CHIK low : control-vaccine = 60 : 10

Group B: MV-CHIK low : control-vaccine = 60 : 10

Group C: MV-CHIK high : control-vaccine = 60 : 10

Group D: MV-CHIK high : control-vaccine = 60 : 10

Group M1:M2 MV-CHIK high day 0+28 : MV-CHIK high day 168+196 = 20 : 20

6.4 Blinding

This study will be conducted in a double-blind manner in regard to assignment to treatment groups A, B, C or D. An assignment to the measles booster groups M1 and M2 will be apparent to both subject and study personnel, but the vaccination sequence will also be kept double-blind (the allocation to M1 or to M2 is unknown). The vaccine will be prepared by authorized personnel otherwise not involved in the conduct of the study or in the assessment of safety or efficacy after randomization.

7 STUDY POPULATION

7.1 Number of Centers and Subjects (planned and analyzed)

- Multi-center study:
  - Department of Clinical Pharmacology, Medical University of Vienna (Austria)
  - Hansa Sanatorium GmbH of Graz (Austria)
  - Institute for specific Prophylaxis and Tropical Medicine, Vienna (Austria)
  - Berlin Centre for Travel & Tropical Medicine, Berlin (Germany)
  - Department of Tropical Medicine and Infectious Diseases, Rostock University Medical Center, Rostock (Germany)
  - University Medical Centre Hamburg-Eppendorf, Bernhard Nocht Centre for Clinical Trials (BNCCT), Hamburg (Germany)
- 320 healthy volunteers, aged between 18 and 55 years, randomized in 6 treatment groups; four groups (A-D) will be vaccinated on day 0, 28 and 196 and two groups (M1+M2) will be vaccinated on day -28, 0, 28, 168 and 196.

7.2 Inclusion criteria

1. Signed informed consent obtained before any trial-related activities. (Trial activities are any procedures that would not have been performed during normal management of the subject).
2. 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
3. Available for the duration of the trial
4. Healthy men or women aged ≥18 and ≤55 years
5. In female subjects either childbearing potential terminated by surgery or one year post-menopausal, or a negative urine pregnancy test during screening and the willingness not to become pregnant during the entire study and up to 6 months after the last vaccination by practicing reliable methods of contraception as specified in protocol section 8.3.6.
6. Normal findings in medical history and physical examination **or** the investigator considers **all** abnormalities to be clinically irrelevant
7. Normal laboratory values or the investigator considers all abnormalities to be clinically irrelevant (unless otherwise specified in exclusion criteria)

7.3 Exclusion criteria

Any of the following will exclude a subject from the study:

1. Participation in another clinical study within the past month in which the subject has been exposed to an investigational product (pharmaceutical product or placebo or device) or planned concurrent participation in another clinical study during the study period
2. History of immunodeficiency, known human immunodeficiency virus (HIV) infection, current hepatitis B/C infection
3. Drug addiction including alcohol dependence
4. Inability or unwillingness to avoid more than the usual intake of alcohol during the 48 hours after vaccination (not more than 20g alcohol per day, which equals ½ L beer or ¼ L of wine)
5. Persons who are accommodated in an institution on court or official order.
6. Persons in direct relationship with the sponsor, an Investigator or other study site staff. Direct relationship includes relatives or close dependents (children, spouse/partner, siblings or parents), as well as employees (site or sponsor)

7. Non-study licensed vaccines: vaccination within 4 weeks prior to first vaccination or planning to receive any non-study vaccine during the study period
8. Measles vaccination or booster within the last 5 years or during the clinical study
9. Prior receipt of any Chikungunya vaccine
10. Blood donations during 1 month prior to Screening Visit and throughout the study
11. Recent infection (within 1 week prior to Screening Visit) (If non-serious, can be basis for temporary deferral)
12. Relevant history of renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, hematological, endocrine, inflammatory or neurological diseases, that in the opinion of the investigator may interfere with the aim of the study
13. History of neoplastic disease (excluding non-melanoma skin cancer that was successfully treated) within the past 5 years or a history of any hematological malignancy
14. History of autoimmune disease (e.g. rheumatoid arthritis, systemic lupus erythematosus (SLE), autoimmune thyroid disease)
15. History of moderate or severe arthritis or arthralgia within the past 3 months prior to Screening Visit
16. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the participant to understand and cooperate with the study protocol
17. 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 or exacerbated by ingredients of the control vaccine (i.e. neomycin (sulfate), gelatin, sorbitol, mannitol, and sucrose)
18. History of anaphylaxis to drugs or major allergic reactions in general, which the investigator considers may compromise the safety of the volunteers
19. Clinically relevant abnormal laboratory values indicative of physical illness:
    - Hematology: hemoglobin, hematocrit, erythrocyte count, differential white blood count, platelets
    - Chemistry: creatinine (>1.7 mg/dL), potassium, sodium, calcium, AST/ALT >= 2.6 ULN, alkaline phosphatase, bilirubin
    - Coagulation parameter: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen according to the evaluation of the principle investigator
    - Urinalysis according to the evaluation of the principle investigator

20. Use of medication during 2 weeks before the first vaccination and throughout the study, which the investigator considers may affect the validity of the study except hormonal

contraception in female subjects; prior to taking any medication during 72 h prior to the first vaccination, the study center should be consulted

21. Immunosuppressive drugs: use of corticosteroids (excluding topical preparations) or immunosuppressive drugs within 30 days prior to vaccination, or anticipated use during the trial
22. Receipt of blood products or immunoglobulins within 120 days prior to Screening Visit or anticipated receipt of any blood products or immunoglobulin during the trial
23. Pregnancy (positive pregnancy test at screening or during study phase), lactation or unreliable contraception in female subjects with child-bearing potential (for details please refer to section 8.3.6)
24. Subjects with any condition which in the opinion of the investigator makes the subject unsuitable for inclusion
25. Individuals who are living and/or working with severely immunocompromised people, children under 15 months old or pregnant women.
26. Inability or unwillingness to provide informed consent and to abide by the requirements of the study
27. Refusal to allow storage of specimens for future research.
28. Regular blood plasma donations, (in-)frequent blood product donations, tissue or organ donation, as well as sperm donation
29. double contraception without barrier method (condom) up to 6 months after the last vaccination

7.4 Premature discontinuation

Subjects have the right to withdraw consent to participation in the clinical study at any time for any reason without naming it.

It will be documented whether or not each subject completed the clinical trial. If, for a subject, trial treatment or observations have been discontinued, the reason will be recorded. Volunteers have to be withdrawn for the following reasons:

- voluntary subject withdrawal for any reason
- at the discretion of the investigator
- if an adverse event (including a concomitant illness) develops, which is considered by the investigator incompatible with the continuation of the study
- if the administration of a drug, which is not permitted by the exclusion criteria, is necessary
- subject failure to comply with the requirements of the protocol
- violation of exclusion criteria 1, 7, 8, 10. 19, 20, 21, 22 and 23 during the study

For any subject discontinuing the study before schedule, the investigator will:

- ask the subject to take part, as far as possible, in the early termination visit in order to examine the subject's health conditions and perform the required blood sampling for the clinical blood tests.
- complete the eCRF, indicating in the final visit, the date of termination, the date and time of the last dose administration and the reason for discontinuing the study
- arrange for alternative medical care for the withdrawn subject, if necessary.
- record in the eCRF any follow-up if the subject was withdrawn due to an AE.

All subjects who are discontinued prior to Visit 0 will be replaced.

8 TRIAL SCHEDULE

The estimated study duration will be 33-37 weeks (~8 months) for each subject. The overall study duration estimates to be 3 years from study initiation until reporting.

8.1 Screening Procedures

8.1.1 Screening Visit VS (1 to 7 days prior to Visit 0)

Subjects will be informed about the aims, procedures and possible risks of the study and will be asked to sign the informed consent form for inclusion in the trial.
The screening visit will be performed for each volunteer within one week before Visit 0. For pre-study assessment, all volunteers will be checked for inclusion/exclusion criteria, undergo a physical examination and evaluation of vital signs (systolic and diastolic blood pressure, pulse and body temperature). The subjects will be asked if they experience any kind of arthralgia and blood and urine will be collected for clinical laboratory assays (HIV, hepatitis B/C, hematology, coagulation parameters, clinical chemistry and urinalysis). HIV tests that were performed up to 30 days before screening and where results are available are acceptable. Medical history, vaccination history (covering the last three years prior to screening), prior medication within 30 days prior to screening and demographic data will be recorded. A urine pregnancy test will be performed in women of childbearing potential.
If the subjects are eligible for the clinical study, volunteers will be asked to return to the study site within 7 days after the screening visit.

8.2 Treatment Procedures

8.2.1 Study day -28 (±5 days) /Visit 0 (or day 0 /Visit 1 for group A-D)

Subjects, who do not have to be in a fasted state, are requested to return within 7 days after screening visit, at the study site where inclusion and exclusion criteria will be checked once again and their medical history and concomitant medication will be updated, if necessary. A urine pregnancy test will be performed in women of childbearing potential. Symptom-directed physical exam, system-based assessment (only if necessary according to 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 if they experience any kind of arthralgia. Blood samples for baseline immunogenicity 1 and measles antibody titer will be drawn from an appropriate forearm or cubital vein. All study sites except the two sites in Vienna will collect serum samples for cross neutralization and passive transfer analyses (immunogenicity 2).

Then subjects will be randomized and will receive their 1st vaccination according to the assigned treatment group.

- Group A, B, C and D will perform Visit 0 = Visit 1 procedures on one day (study day 0, just one visit).
- Group M1 and M2 will perform Visit 0 and Visit 1 separated on two days, 28 days apart (study day -28 and study day 0, two visits)

All subjects will be observed for 1 hour after each vaccination before discharge from study site. One hour after vaccination local tolerability and adverse events, if applicable, will be recorded.

Subjects will be discharged after the dispense and the instructions for the use of the subject diaries took place (subjects are asked to report any adverse events in the diary for 7 days after vaccination). Only if the investigating physician considers the situation to be safe for the subjects, they will be allowed to leave the department and will be asked to return to study site after 28 days (± 5 days).

AKH Vienna only:
Virus shedding samples (urine, saliva and whole blood) will be collected from a subset of subjects randomized to treatment group A-D. Subjects participating in these shedding analyses additionally will be asked to return to the study site 7, 10 and 14 days (±1 day each) after their 1st vaccination for collection of saliva, urine and whole blood samples.

Vienna sites only:
For T cell assays a total of 24-30 mL blood (3x 10 mL citrate tubes) will be collected from all subjects enrolled at these sites.

8.2.2 Study day 0 / Visit 1

Subjects of the treatment groups A-D have performed these Visit 1 procedures together with Visit 0 on one day.
Subjects of group M1 and M2 will return to the site for this outpatient visit 28 (±5 days) days after Visit 0. The inclusion/exclusion criteria and concomitant medication will be checked again. A symptom-directed physical exam, system-based assessment (only if necessary according to symptom-directed physical exam), assessment of arthralgia and evaluation of vital signs as well as body temperature will be recorded. Blood samples for immunogenicity 1 and measles antibody titer serum sample will be obtained and all study sites except the two sites in Vienna will collect serum samples for cross neutralization and passive transfer analyses (immunogenicity 2). From all subjects enrolled in Vienna blood samples will be

collected for T cell assays. A urine pregnancy test will be performed in women of childbearing potential. Subject diaries of group M1 and M2 will be collected and checked for completeness. Subjects of treatment group M1 and M2 will be assessed for local tolerability of the first vaccination and will then receive their 2nd injection. They will be observed for 1 hour after vaccination before discharge from study site. One hour after vaccination local tolerability and adverse events will be recorded, if applicable.
Subjects will be discharged after dispensing and explanation of the subject diaries, only if the investigating physician considers it to be safe for the subjects to leave the department and are asked to return to study site after 28 days (± 5 days).

8.2.3 Study day 28 (±5 days) / Visit 2

All subjects will return to the site for an outpatient visit 28 days (±5 days) after Visit 1. The inclusion/exclusion criteria will be checked again. Blood samples for immunogenicity 1 and measles antibody titer serum sample will be obtained and all study sites except the two sites in Vienna will collect serum samples for cross neutralization and passive transfer analyses (immunogenicity 2). Symptom-directed physical exam, system-based assessment (only if necessary according to symptom-directed physical exam) and evaluation of vital signs as well as body temperature will be recorded. A urine pregnancy test will be performed in women of childbearing potential. Concomitant medication will be assessed. Subject diaries will be collected and checked for completeness.

Assessment of local tolerability of the previous injection site will take place. In addition, the subjects will be asked if they experience any kind of arthralgia.

All subjects will be vaccinated (groups A, B, C, D receive 2nd injection and group M1, M2 receive 3rd injection) and observed for 1 hour after vaccination before discharge from study site. One hour after vaccination local tolerability and adverse events, if applicable, will be recorded.

Subject diaries will be dispensed. The subjects are asked to report any adverse events in the diary for 7 days after vaccination. Adverse events and concomitant medication will be recorded, if applicable.

The subjects are asked to return to study site after 28 days for their next visit.

<u>AKH Vienna only:</u>
Virus shedding samples (urine, saliva and whole blood) will be collected from a subset of subjects randomized to treatment group A-D.
Vienna sites only:
For T cell assays a total of 24-30 mL blood (3x 10 mL citrate tubes) will be collected from all subjects enrolled at these sites.

8.2.4 Study day 56 (±5 days) / Visit 3

At Visit 3 all subjects will return to the study site for an outpatient visit. During this visit inclusion/exclusion criteria will be checked again, a symptom-directed physical exam and system-based assessment (only if necessary according to symptom-directed physical exam) will be performed, vital signs as well as body temperature will be recorded. Blood samples will be collected for clinical chemistry, hematology and coagulation parameters. In addition, a urine sample will be collected for urinalysis. The subjects will be asked if they experience any kind of arthralgia. The investigator will document local tolerability (of previous vaccination site), AEs and concomitant medication. Subject diaries will be collected and checked for completeness.

Blood samples for immunogenicity 1 and for measles antibody titer will be obtained and a urine pregnancy test will be performed in women of childbearing potential. All study sites except the two sites in Vienna will collect serum samples for cross neutralization and passive transfer analyses (immunogenicity 2).

<u>Vienna sites only:</u>

For T cell assays a total of 48-60 mL blood (6x 10 mL citrate tubes) will be collected from all subjects enrolled at these sites.

Subjects of group M1 and M2 will be asked to return to the study site for Visit 4 on study day 168 (± 10 days).

Subjects of treatment groups A, B, C and D do not have to come for Visit 4 and will be asked to return to the study site for Visit 5 on study day 196 (± 10 days).

8.2.5 Study day 168 (±10 days) / Visit 4, M1 + M2 only

Only subjects of treatment groups M1 and M2 will return to the study sites for this outpatient Visit 4. Inclusion/exclusion criteria will be checked again, symptom-directed physical exam, system-based assessment (only if necessary according to symptom-directed physical exam) and evaluation of vital signs as well as body temperature will be recorded. Assessment of arthralgia and local tolerability (of previous vaccination site) will take place. Blood samples for immunogenicity 1 analyses will be obtained, a urine pregnancy test will be performed in women of childbearing potential and all study sites except the two sites in Vienna will collect serum samples for cross neutralization and passive transfer analyses (immunogenicity 2). Adverse events and concomitant medication will be recorded, if applicable. Subjects of treatment group M1 and M2 will receive the 4th vaccination and will be observed for 1 hour after vaccination before discharge from study site. One hour after vaccination local tolerability and adverse

events, if applicable, will be recorded. Subject diaries will be dispensed and the subjects will be asked to report any adverse events in their diary for 7 days after vaccination.

Subjects will be asked to return to the study site on day 196 (± 10 days) for Visit 5.

Vienna sites only:

For T cell assays a total of 24-30 mL blood (3x 10 mL citrate tubes) will be collected from all subjects enrolled at these sites.

8.2.6 Study day 196 (± 10 days) / Visit 5

All subjects will return to the study sites for an outpatient visit. The inclusion/exclusion criteria will be checked again. Symptom-directed physical exam, system-based assessment (only if necessary according to symptom-directed physical exam) and evaluation of vital signs as well as body temperature will be recorded. For subjects of group M1 and M2 an assessment of local tolerability (of previous vaccination site) will be done and subject diaries will be collected and checked for completeness. Blood samples for immunogenicity 1 will be obtained, a urine pregnancy test will be performed in women of childbearing potential and all study sites except the two sites in Vienna will collect serum samples for cross neutralization and passive transfer analyses (immunogenicity 2). In addition, the subjects will be asked if they experience any kind of arthralgia. Adverse events and concomitant medication will be recorded, if applicable.
Subjects of all groups will receive their last vaccination (group A-D the 3rd and group M1 + M2 the 5th vaccination) and will be observed for 1 hour after each vaccination before discharge from study site. One hour after vaccination local tolerability and adverse events, if applicable, will be recorded.
The last subject diary will be dispensed and the subjects will be asked to report any adverse events in the diary for 7 days after vaccination. Adverse events and concomitant medication will be recorded, if applicable.

Subjects will be asked to return to the study site after 28 days on study day 224 (± 10 days) for the last Visit 6.

Vienna sites only:

For T cell assays a total of 24-30 mL blood (3x 10 mL citrate tubes) will be collected from all subjects enrolled at these sites.

AKH Vienna only:

Virus shedding samples (urine, saliva and whole blood) will be collected from a subset of subjects randomized to treatment group A-D.

8.2.7 Study day 224 (±10 days) / Visit 6

At Visit 6 all subjects will return to the study site for an outpatient study completion visit. Symptom-directed physical exam, system-based assessment (only if necessary according to symptom-directed physical exam) and evaluation of vital signs as well as body temperature will be recorded and an assessment of local tolerability (of previous vaccination site) will be performed. Blood samples will be collected for analysis on clinical chemistry, hematology and coagulation parameters. In addition, a urine sample will be collected for urinalysis. Blood samples for immunogenicity 1 will be obtained at all study sites. Sites outside of Vienna additionally will collect serum samples for cross neutralization and passive transfer analyses (immunogenicity 2). A urine pregnancy test will be performed and a pregnancy test kit will be provided to all women of childbearing potential. In addition, the subjects will be asked if they experience any kind of arthralgia. Adverse events and concomitant medication will be recorded, if applicable. Subject diaries will be collected and checked for completeness.

Vienna sites only:

For T cell assays a total of 24-30 mL blood (3x 10 mL citrate tubes) will be collected from all subjects enrolled at these sites.

8.2.8 Study day 286 (±10 days) / Visit 7

All women of childbearing potential will be followed up 3 months after the last vaccination to ensure that no pregnancy occurred within this period. This will be done via a phone call on study day 286 (or in case of prematurely discontinuation of the study for any reason, 3 months after the subject received the last vaccination). The subject will be asked about the result of the provided pregnancy urine test.

Additionally, subjects will be advised to immediately inform the investigator of pregnancies up to 6 months after the last vaccination also if this occurs after this follow up call.

If a subject has become pregnant since the last visit this has to be reported as defined under 14.7 and will be followed until pregnancy outcome.

8.2.9 Early Termination Visit

Subjects, who are withdrawn or terminated the study prematurely for any reason, will undergo the following investigations if possible: blood sampling for immunogenicity 1, measles antibody titer, clinical chemistry, hematology, blood coagulation and all study sites except the two sites in Vienna serum sampling for cross neutralization and passive transfer analyses (immunogenicity 2). A urine sample will be obtained for urinalysis and pregnancy test in women of childbearing potential, symptom-directed physical exam, system-based assessment (only if

necessary according to symptom-directed physical exam) and evaluation of vital signs, body temperature and local tolerability (of previous vaccination site) will take place. The subjects will be asked if they experience arthralgia. Adverse events and concomitant medication will be recorded, if applicable. Subject diaries will be collected and checked for completeness. A urine pregnancy test kit will be provided to all women of childbearing potential, to be used 3 months after the last vaccination, and a follow up call will be performed by the site to enquire the test result.

Vienna sites only:

For T cell assays a total of 24-30 mL blood (3x 10 mL citrate tubes) except on day 56, 48-60 mL blood (6x 10 ml citrate tubes), will be collected from all subjects enrolled at these sites.

8.3 Collection of Samples

8.3.1 Immunogenicity 1 and measles antibody titer samples (all sites)

From all subjects 2x 8 - 10 mL blood will be collected on days 0, 28, 56, 196 and 224 (from treatment group A, B, C and D) and additionally on day -28 and 168 for treatment group M1 and M2, to isolate serum for determination of immune response against Chikungunya by Plaque reduction neutralization assay ($PRNT_{50}$) and Enzyme linked immunosorbent assay (ELISA). Measles antibody titer and CHIK antibody titer will be determined from the same blood collection for day -28, 0, 28 and 56 by ELISA.

Table 3 Immunogenicity 1 samples (16-20 mL per visit) - schedule for collection

Group	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	early termination
A, B, C, D		X, Y	X, Y	X, Y		X	X	X
M1 + M2	X, Y	X, Y	X, Y	X, Y	X	X	X	X

Y = measles antibody ELISA, will be tested in the immunogenicity 1 samples

8.3.2 Measles virus shedding (AKH Vienna only)

Measles virus shedding will be analyzed in a subset of subjects randomized to treatment group A, B, C or D in the study center of AKH Vienna. Subjects will be asked if they are willing to participate in the virus shedding observation, which implies three additional site visits for sample collection. The participating subjects will sign a separate informed consent that describes the additional visits and procedures of sample collection. A minimum of 30 and a maximum of 50 subjects will be included in the shedding subset.

Besides the regular visits, these subjects additionally will return to the study site on day 7 (±1 day), 10 (±1 day), and 14 (±1 day) after the first injection. In addition, samples of saliva, urine and whole blood (2-3 mL) will be collected at Visit 1, 2, and 5.

The samples will be analyzed by quantitative PCR (by Envigo, Huntingdon, UK) to detect measles virus RNA. Any PCR positive sample will be further tested in an in vitro infectivity assay for the presence of infectious virus.

Table 4 Shedding samples (2-3 mL) - schedule for collection

sample	**Visit 1** day 0	day 7 (±1 day)	day 10 (±1 day)	day 14 (±1 day)	**Visit 2** day 28	**Visit 5** day 196
urine	X	X	X	X	X	X
saliva	X	X	X	X	X	X
whole blood	X	X	X	X	X	X

8.3.3 T cell analysis (Vienna sites only)

At the study sites in Vienna all subjects will participate in the T cell analysis. For this purpose, a total of 24-30mL blood will be collected in 3x 10 mL citrate tubes for all time points except on day 56, 48-60 mL blood will be collected in 6x 10 ml citrate tubes. The time points for blood draw are depicted in Table 5 and in section 1.3. PBMCs will be isolated from fresh blood (within 8 hours of blood draw) and frozen 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 immunizations. The functional T cell assays will be performed by the Department of Virology, Medical University of Vienna.

Table 5 T cell analysis (24-30 mL and once on day 56, 48-60 mL) - schedule for collection

Group	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	early termination
A, B, C, D		X	X	X		X	X	X
M1 + M2		X	X	X	X	X	X	X

8.3.4 Immunogenicity 2 (all sites except sites in Vienna)

From all subjects enrolled at study sites outside of Vienna 16-20 mL blood will be collected per visit to isolate serum for immunogenicity 2 analyses. These analyses will include testing for cross neutralization immunogenicity and passive transfer. The time points for blood draw are depicted in Table 6 and in section 1.3.

Table 6 Immunogenicity 2 samples (16-20 mL per visit) - schedule for collection

Group	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	early termination
A, B, C, D		X	X	X		X	X	X
M1, M2	X	X	X	X	X	X	X	X

8.3.5 Safety parameters – urine and blood sample collection

Blood samples will be collected from all subjects during the screening visit for determination of hepatitis B surface Ag, hepatitis B core Ab, hepatitis C Ab and HIV Ab performed by the local labs.

For hematology analyses, blood chemistry, coagulation parameters and urinalysis (specified in section 11.2.6) samples will be taken during the screening visit, on study day 56 and during study completion visit on day 224.

8.3.6 Pregnancy Test – Contraception

Females of childbearing potential must have a negative urine pregnancy test at the screening visit and within 24 hours prior to each vaccination, with a negative result documented. Urine pregnancy tests in female subjects of childbearing potential will be performed throughout the study conduct up to Visit 6 or early termination. All subjects and their respective partners must practice at least two acceptable methods of birth control (whereby one has to be the use of condoms) up to 6 months after the last vaccination. Acceptable methods of birth control include hormonal contraceptives (oral, injected, transdermal, started 3 months before study participation) or intra-uterine devices (hormone loop, loop) in combination with condoms. Also acceptable are sterilization of the partner or abstinence. Non-childbearing potential includes being surgically sterilized or postmenopausal with no menstrual bleeding for at least one year prior to the study. Even in case of non-childbearing potential the use of condoms represents the only way of protection against potential transmission of MV-CHIK during all kind of sexual intercourses.

8.4 Treatment duration

Subjects allocated to treatment groups A-D will have a total study duration (including screening) of approximately 231 days including vaccinations on study days 0, 28 and 196. Subjects allocated to treatment group M1 and M2 will have a total study duration (including screening) of approximately 259 days including vaccinations on study day -28, 0, 28, 168 and 196.

9 TREATMENT OF SUBJECTS

9.1 Investigational Treatment

9.1.1 Dose and dosing schedule

All subjects will be randomized and receive i.m. vaccinations according to the assigned treatment group A, B, C, D, M1 or M2.

Vaccination concentration

Group A + B: MV-CHIK low dose $5x10^4$ (± 0.5 log) $TCID_{50}$ or control-vaccine and placebo

Group C + D: MV-CHIK high dose $5x10^5$ (± 0.5 log) $TCID_{50}$ or control-vaccine and placebo

Group M1 + M2: MV-CHIK high dose $5x10^5$ (± 0.5 log) $TCID_{50}$ and control-vaccine and placebo

Table 7 Vaccination time points

Group	Visit 0 day -28	Visit 1 day 0	Visit 2 day 28	Visit 3 day 56	Visit 4 day 168	Visit 5 day 196	Visit 6 day 224	Visit 7 day 286
A, C	V/C		V/C			P		
B, D	P		V/C			V/C		
M1	C	V	V		P	P		
M2	C	P	P		V	V		

V = MV-CHIK vaccine (high or low dose), C = control-vaccine, P = placebo

The investigational medicinal products (IMPs) are described in detail in section 5.1, dosage and sequence ad 1.2, 1.3, 6.1 and Table 1, route of administration in section 5.3 and treatment period in section 8.4

9.2 Prior and concomitant therapy

Permitted prior and concomitant therapy

Any prior vaccination within the last three years prior screening and any medication within 30 days prior screening have to be documented. Any medication taken during the study up to Visit 6 (study day 224) has to be reported to the investigator and has to be documented.

Forbidden prior and concomitant therapy

Non-study licensed vaccines: vaccinations within 4 weeks prior to first vaccination and within 4 weeks after each study dose are not allowed.

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

9.3 Treatment Compliance

The study medication will be prepared at site and administered during the study visits. Each treatment will be documented in the subject's charts, the eCRF and an IMP Inventory Log (section 5.8)

10 ASSESSMENT OF IMMUNOGENICITY

10.1 Methods of Immunogenicity Measurements

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

Humoral systemic immune response will be determined by anti-chikungunya neutralization assay. The 50% plaque reduction neutralization titer ($PRNT_{50}$) will be measured to identify immunogenicity in sera of all subjects on days 0, 28, 56, 196 and 224 (treatment group A, B, C and D) and additionally on day -28 and 168 for treatment group M1 and M2. The assay will be validated and performed by SRI International (Harrisonburg, VA/USA).

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. CHIK-Ab will be determined on days 0, 28, 56, 196 and 224 (treatment group A, B, C and D) and additionally on day -28 and 168 for treatment group M1 and M2. Measles-Ab will be measured on days 0, 28 and 56 (treatment group A, B, C and D) and additionally on day -28 for treatment group M1 and M2. The assays will be validated and performed by the Department of Virology at the Medical University of Vienna, Austria.

T cell assay

Samples of a subset of subjects (all subjects enrolled in Vienna) will be analyzed for T cell immune response. PBMC from whole blood will be isolated to determine the number of functional, Chikungunya virus specific T cells on days 0, 28, 56, 196 and 224 (treatment group A, B, C and D) and additionally on day 168 for treatment group M1 and M2 (section 8.3.2). PBMC isolation and in vitro T cell assays will be performed by the Department of Virology, at the Medical University of Vienna. The functional T cell assays will be at research grade and will not be validated.

11 ASSESSMENT OF SAFETY

11.1 Data Safety Monitoring Board (DSMB) review

As the safety and tolerability profile of the vaccine was acceptable in a Phase 1 study in 42 healthy subjects, no major reactogenicity or safety findings are expected. Nevertheless, an independent external data safety monitoring board (DSMB) will be established by the study sponsor. The committee members will be experts in the field and they will not be involved in

the study in any other way. The purpose of the DSMB is to determine whether study or individual subject stopping rules have been met during ad-hoc meetings (teleconferences). The DSMB will periodically review listings and summary tabulations of SAEs, deaths, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination in an unblinded manner. After about 50% of subjects completed Visit 3 (day 56) in groups A, B, C and D, the DSMB will review safety and immunogenicity in order to evaluate the risk-benefit of the MV-CHIK administration and make recommendations to the sponsor regarding the further study conduct and/or protocol modifications if needed.
In addition, the board will also review any SAE and "discontinuations" within a week after the event has been reported. In the case of an unexpected concern about potentially emerging safety problems, when a safety stopping rule is triggered, or when important new information external to the trial arises, a DSMB meeting will be scheduled. The DSMB will review unblinded data.
Detailed objectives, roles and responsibilities of the DSMB will be specified in a separate DSMB charter.

11.2 Post vaccination reactogenicity assessments

Please also refer to Table 2 and section 8.2 for exact time points.

All subjects enrolled in the study and who receive at least one immunization (Priorix® or MV-CHIK) will be followed up for at least 28 days after the last immunization.

Adverse event rates will be used to evaluate the secondary study objective.

Serious adverse events: see below for detailed clarification

Medically attended adverse events: All adverse events, for which the subjects were seeking medical care (i.e. doctor’s office, emergency service, hospital, but not use of self-medication).

11.2.1 Physical examination

At the Screening visit, all subjects will undergo a physical examination, including but not limited to assessment of general appearance and skin, head/ eyes/ ears/ nose/ throat, respiratory system, spleen, liver and lymph nodes.

11.2.2 Symptom-directed physical examination

A symptom-directed physical exam will be performed at all visits, except screening. If a symptom is reported, a system-based assessment will be performed, if needed, for a detailed

check of the affected body system. Any symptom reported, including worsening of existing conditions, will also be recorded as an adverse event.

11.2.3 Vital signs and body temperature

Systolic and diastolic blood pressure and pulse as well as body temperature will be recorded at all visits with the subject at rest in a sitting position.

11.2.4 Local and systemic tolerability

The local tolerability (i.e., injection site reaction) must be inspected and evaluated by the investigator one hour after each vaccination (for treatment group M1 and M2 starting after the first vaccination on day -28 and for groups A-D starting on day 0 after their first injection). Additionally, the local tolerability (i.e., inspection of the previous injection site) will be performed prior to application of the subsequent study treatment, if applicable (for treatment group M1 and M2 this examination starts before the second vaccination on day 0 and for groups A-D on day 28). Furthermore, local tolerability will be evaluated by the subjects after the vaccination in the subject diaries over a period of 7 days after each treatment (see section 0). Grading will be performed according to the respective FDA Guidance for Industry (3), modified, reflecting the guidance of the Brighton Collaboration:

Table 8 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 hospitalization
Tenderness	Mild pain to touch	Pain with movement	Significant pain at rest	ER visit or hospitalization
Erythema/ Redness*	≤ 5 cm	5.1–10 cm	> 10 cm	Necrosis or exfoliate dermatitis
Swelling**:	≤ 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 hospitalization
Induration*	≤ 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

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

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

Diary solicited systemic (i.e., systemic tolerability) AEs include: Nausea/vomiting, headache, fatigue, and myalgia, also to be documented in the eCRF. In addition, body temperature will be assessed daily for 7 days and fever will be graded. Grading will be performed according to the FDA Guidance for Industry (3).

Table 9 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 hospitalization for hypotensive shock
Diarrhea	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 hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
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 hospitalization
Fever (°C) (°F)	38.0-38.4 100.4-101.1	38.5-38.9 101.2-102.0	39.0-40.0 102.1-104.0	>40.0 >104.0

In addition, any signs and symptoms of arthralgia will be carefully assessed. Importantly, subjects will be asked if they experience any signs of limb pain or joint pain, to distinguish symptoms of arthralgia from flu like symptoms including limb pain.

11.2.5 Subject diary

The subjects should complete the diary (local, systemic tolerability and adverse events as well as body temperature) after vaccination for 7 days; solicited local and solicited systemic AEs will be assessed by the subjects themselves by checking the presence of the symptoms listed in the subject diary and appropriate measuring of the size of the affected area (a template will be provided for local reaction grading, as shown in the Appendix). The diary will also have space for recording unsolicited AEs and concomitant medication. The assessments will be recorded once daily into a subject diary. Assessments should occur at the same time each day, starting with the day of vaccination. The first entry should be made 6 hours (+/- 1h) after the vaccination. The subject diary will be verified for completeness by the investigator at the

subject's next visit to the study site. The diaries will be collected at the following visit and new ones will be dispensed at each treatment visit. Any AE recorded in the subject diaries will be entered in the eCRF by the investigator or authorized delegates and will only be analyzed when entered in the eCRF. The investigator will assess the severity of the reported local reaction according to the above table and the severity.

11.2.6 Laboratory parameters

The following laboratory parameters will be assessed at time points specified in Table 2 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).

The following parameters will be analyzed by local laboratories according to the applicable laboratory SOP:

- Hematology: hemoglobin, hematocrit, erythrocyte count, differential white blood count, platelets.
- Chemistry: creatinine (>1.7 mg/dL), potassium, sodium, calcium, AST, ALT (≥2.6 ULN), alkaline phosphatase, bilirubin
- Coagulation parameter: prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen
- Virology: HIV 1/2 Ab if not done within 30 days before screening and HBs-Ag, anti-HBc-Ab and anti-HCV-Ab
- Urinalysis: a standard urine test stick for determining pH-value, glucose, protein, bilirubin, urobilinogen, red blood cells, white blood cells, nitrite, ketone and specific gravity will be used and clinical relevant findings will be noted in the source data and in the eCRF.

Table 10 Blood sample collection:

Test	Type of Vials	Volume	Collections
Virology (HIV, HBV, HCV) (all sites)	serum	8 – 10 mL	1x
Immunogenicity 1 and Measles Serology (all sites)	serum	2x 8 - 10 mL	5x (A-D), 7x (M1, M2)
Immunogenicity 2 (all sites except sites in Vienna)	serum	2x 8 -10 mL	5x (A-D), 7x (M1, M2)
T cell Analysis (all sites in Vienna)	(citrate tubes) PBMCs	3x 8-10 mL once 6x 8-10 mL	5x (A-D), 6x (M1, M2)
Shedding whole blood (only AKH Vienna)	EDTA	2 - 3 mL	6x (shedding analysis, A-D only)
Hematology (all sites)	EDTA	3 - 5 mL	3x
Coagulation (all sites)	citrate tubes	3 - 5 mL	3x
Clinical chemistry (all sites)	serum	8 - 10 mL	3x

The total blood volume that will be collected during the entire study:

Sites outside Vienna: treatment group A, B, C, D: 210 – 270 mL
treatment group M1 and M2: 274 – 350 mL

Site AKH in Vienna: treatment group A, B, C, D: 286 – 368 mL
treatment group M1 and M2: 330 – 420 mL

Site Tropical Medicine Vienna: treatment group A, B, C, D: 274 – 350 mL
treatment group M1 and M2: 330 – 420 mL

Laboratory values deviating from the normal ranges will be evaluated by the investigator according to the FDA Guidance for Industry. The values will be graded (mild, moderate, severe) according to the values provided. Clinically relevant deviations have to be reported as adverse events.

NCR = not clinically relevant deviation
CR = clinically relevant deviation
NA = not assessable - laboratory error.

11.3 Safety Stopping Rule

An independent DSMB will be installed to periodically review accruing safety information, and if necessary, to determine whether study or individual subject stopping rules have been met.

The DSMB will periodically review the listing and summary tabulations of SAEs, deaths, solicited AEs, unsolicited AEs and AEs leading to withdrawal from further vaccination. After about 50% of subjects of treatment groups A, B, C and D completed Visit 3 (day 56), the DSMB will review safety and immunogenicity in order to evaluate the risk-benefit of the MV-CHIK administration and make recommendations to the sponsor regarding the further study conduct and/or protocol modifications, if needed. A written DSMB charter will be developed.

Individual subjects

The following subjects will be discontinued from further vaccination:

a. Subjects who develop a severe or life-threatening (grade 3 or 4) suspected adverse reaction (SAR) or laboratory abnormality.
b. Subjects who develop a grade 3 solicited systemic adverse event that occurs without alternative etiology in the 7 days following the study vaccination.
c. Subjects who develop a medical condition for which continued participation, in the opinion of the site principal investigator or appropriate sub-investigator, would pose a risk to the subject or would be likely to confound interpretation of the results.
d. Pregnant subjects.

In case a subject has to discontinue the clinical study prematurely for any reason, he or she should be asked to perform an early termination visit. Additionally, women of childbearing potential will be followed up by a phone call, 3 months after the last vaccination to ensure no pregnancy occurred within this period as confirmed by a urine pregnancy test provided by the investigator.

Treatment group stopping rules

If four or more subjects in a treatment group experience a treatment related grade 3 suspected adverse reaction (SAR) or laboratory abnormality, or one or more subjects in a treatment group experience a treatment related grade 4 SAR or laboratory abnormality, the study will be suspended pending an unblinded safety review of those subjects by the DSMB. This safety review might lead to the recommendation to discontinue a treatment group.

In case a treatment group has to discontinue the clinical study prematurely for any reason, the concerned subjects should be asked to perform an early termination visit. Additionally, women of childbearing potential will be followed up by a phone call, 3 months after the last vaccination to ensure no pregnancy occurred within this period as confirmed by a urine pregnancy test provided by the investigator.

Entire study stopping rules

The study will be stopped (no new enrollments and no additional vaccine administered) pending a full DSMB safety review if any of the following occurs:

a. One or more subjects experience a suspected adverse reaction (SAR) that is both serious and unexpected, based on the reference safety information (Investigator's Brochure).
b. One or more subject's death assessed by the investigator, medical monitor or sponsor as related to investigational product.
c. One or more subjects develop laryngospasm, bronchospasm or anaphylaxis within 24 hours of vaccine administration.
d. One or more subjects develop injection-site ulceration, abscess or necrosis.
e. Two or more subjects experience generalized urticaria within 72 hours after administration of study product.

Upon completion of this review and receipt of the advice of the DSMB, the sponsor will determine if study entry or study dosing should be discontinued (or if a dose level should be dropped) or if study entry and study dosing may continue according to the protocol. The competent authorities and ethical committees will be informed whenever the study is stopped for DSMB review.

In case the entire clinical study has to discontinue prematurely or a dose level has to be dropped for any reason, the concerned subjects should be asked to perform an early termination visit. Additionally, women of childbearing potential will be followed up by a phone call, 3 months after the last vaccination to ensure no pregnancy occurred within this period as confirmed by a urine pregnancy test provided by the investigator.

12 LABELING, STORAGE AND TRANSPORT OF SAMPLES

A detailed Lab Manual will be available with information about labeling and transport of the vials.

12.1 Labeling of samples

Each sample tube will be clearly and unequivocally identifiable by a label resistant to the storage temperature, which contains the following information:
Study code, site number, subject number, visit code and collection date.

12.2 Samples storage

Aliquots of samples will be stored at -95°C to -65°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.

12.3 Transport of samples

Study samples packed in sufficient solid CO_2 will be shipped by an authorized courier from the study site to the sponsor or to an analytical laboratory outside the hospital, if necessary.

13 STATISTICAL METHODS AND SAMPLE SIZE

The data will be analyzed by Assign Data Management and Biostatistics GmbH. A Statistical Analysis Plan (SAP) will be provided before database snapshot/closure describing in more detail how the study results will be evaluated.

A preliminary analysis including safety and immunogenicity data will be performed after all subjects completed Visit 3 (day 56). The goal of this analysis is to provide highly valuable data of the novel Chikungunya vaccine for further development. Results from this analysis will have no impact on the study design and treatment of the subjects in this study. A final analysis will be conducted once the last subject has completed the study.

13.1 Immunogenicity Analysis

The primary immunogenicity analysis will compare the anti-chikungunya $PRNT_{50}$ antibody geometric mean titer (GMT) 28 days after one or two immunizations 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 vaccination 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 for the MV-CHIK groups (pairwise comparisons adjusted for multiple comparisons according to Tukey-Kramer; groups A vs. B, C vs. D, M1 vs. M2), e.g. 28 days after the 2nd MV-CHIK dose, etc. Likewise, SCRs will also be compared between groups. Seroconversion will be defined as anti-Chikungunya $PRNT_{50}$ titers ≥ 10.

Measles ELISA titers will be analyzed as described above. In addition, the effect of pre-existing anti-measles immunity will be assessed: The CHIKV $PRNT_{50}$ titers 28 days after first vaccination, between the four baseline measles titer groups, defined by baseline percentiles

(25%, 25-50%, 50-75%, 75-100%), will be compared. No stratification by dose groups is intended.

13.2 Safety Analysis

Solicited AEs (reactogenicity): Solicited local and solicited systemic AEs will be summarized for the 7 day period, by AE grade (mild, moderate, severe), by group and by dose. Solicited AEs will not be coded into MedDRA or assessed for relatedness. The number and percentage of subjects with AEs will be presented. Subjects who reported data for a solicited event at any time point during the time period summarized would be included in the denominator for that event. Thus, denominators for each solicited event will not include subjects who were missing all data for that event, during the time period summarized. P-values for AE rates for MV-CHIK vs. Priorix® (or equal control-vaccine), for the same schedule, will be provided in summary tables (pairwise comparisons).

Unsolicited AEs: Separately, the number and percentage of subjects with unsolicited treatment emergent adverse events (TEAEs), medically-attended AEs (MAEs) and serious adverse events (SAEs) will be presented for each treatment group overall and by system organ class/preferred term, and by AE grade, and relatedness. All SAEs and MAEs are also included in TEAE tables. Arthritis AEs, AEs of special interest (AESI), will be included in the TEAE tables. For unsolicited AEs, all subjects entered into the study who receive at least one vaccination will be included in the safety analysis based on the product received (“as treated”), for the time period summarized. P-values for AE rates for MV-CHIK vs. Priorix®, for the same schedule, will be provided in summary tables (pairwise comparisons).

Unsolicited AEs and concomitant diseases will be coded using the MedDRA coding dictionary; concomitant medications will be coded using the WHO Drug Dictionary.

Solicited and unsolicited AEs (local and systemic), MAE, SAE and AESI (arthralgia) will be recorded up to Visit 6 (day 224).

Safety analyses will be performed by means of descriptive measures. Laboratory values will be assigned a grade. Changes in laboratory values and grades as well as vital signs from study entry to end of treatment/follow up will be analyzed descriptively.

13.3 Determination of Sample Size

Sample size for this study is determined on grounds of feasibility and common practice in similar trials.

13.4 Sample Size Justification

No formal sample size calculation has been performed for this phase 2 trial, but the sample size is typical for phase 2 trials, and in our experience, has been useful to assess the immunogenicity of vaccines.

Results of the predecessor study MV-CHIK-101 allow to assess the usefulness of the chosen sample size:

In phase I study MV-CHIK-101, a GMT of 7.3 (LOG_{10} SD 0.254) at day 28 in the placebo group and a GMT 13.7 (LOG_{10} SD 0.515) in the lowest dose group (MV-CHIK 0.05 mL = 1.5 $x10^4$ $TCID_{50}$) was observed. Both higher dose groups (MV-CHIK 0.25 mL = 7.5 $x10^4$ $TCID_{50}$; 1 mL = 3 $x10^5$ $TCID_{50}$) demonstrated higher GMTs of 48 (LOG_{10} SD 0.466) and 45.7 (LOG_{10} SD 0.579).

In the present phase II study, the sample size of 60 subjects in the MV-CHIK dose group vs. 10 subjects in the corresponding control group within each dose group A-D will allow for a detection of a GMT of 15.4 (MV-CHIK) vs. 7.3 (placebo) with a LOG_{10} SD of 0.6 vs. 0.26 and statistical power of 80% (two-sided alpha=5%, unequal-variance t-test). Between dose groups, the sample size of 60 subjects per group will allow for a comparison of a GMT of 45.7 vs. 22.4 (LOG_{10} SD 0.6 for both groups) with a statistical power of 80% (two-sided alpha=5%, equal-variance t-test).

Since the magnitude of GMTs and LG10 DS in this assessment is comparable to the observed results in study MV-CHIK-101, and the statistical methodology applied in the current study has a higher statistical power then pairwise t-tests, the chosen sample size seems adequate.

13.5 Analysis Populations

13.5.1 Safety Population

All safety analyses for TEAEs will be based on the safety population, defined as subjects who entered into the study and received at least one vaccination. All analysis based on the Safety Population will be carried out using the actual treatment received. The denominators for reactogenicity rates are determined separately as discussed in section 0 (Safety Analysis).

13.5.2 Modified Intent-to-Treat (mITT) Population

The exploratory immunogenicity analyses will be based on the modified ITT population. The modified intent-to-treat (mITT) analysis population is defined to include all subjects randomized who receive at least one vaccination.

13.5.3 Per-Protocol (PP) Population

As a primary analysis, immunogenicity will be assessed on the per-protocol (PP) population. The PP population includes subjects who have received at least one vaccination. Subjects with protocol deviations that could have an impact on immune response will be excluded from the PP Population. 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 vaccination, 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 study medication

These criteria for potential protocol violation have been 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.

14 ADVERSE EVENTS (AEs)

14.1 Definition

14.1.1 Adverse Event (AE)

An AE is any undesirable medical event occurring to a subject in a clinical trial, whether or not considered related to the trial product(s). This includes events not seen at baseline or worsened if present at baseline. The following should not be recorded as AEs, if recorded at screening (on Screening Form or eCRF):

- Pre-planned procedure, unless the condition for which the procedure was planned, has worsened since baseline.
- Pre-existing conditions found as a result of screening procedures.

14.1.2 Clinical Laboratory Adverse Event

A clinical laboratory AE is any clinical laboratory abnormality that suggests a disease and/or organ toxicity and is of a severity, which requires active management (i.e. changes of dose, discontinuation of drug, more frequent follow-up or diagnostic investigation).

14.1.3 Serious Adverse Event (SAE)

An SAE is any adverse experience that results in any of the following outcomes:

- death
- a life-threatening experience
- in-patient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect.

Important medical events that may not result in death, are life-threatening*, or require hospitalization will be considered a serious adverse event when they may jeopardize the health of the subject or patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

* The term life threatening in the definition of serious adverse event refers to an event in which the subject 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 was more severe.

14.1.4 Non-Serious Adverse Event

A non-serious adverse event is any AE, which does not fulfill the definition of an SAE.

14.1.5 Medically-attended events (MAEs)

Medically-attended events (MAEs) refer to non-serious and serious events leading to an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits.

14.2 Severity Assessment Definitions

Mild: Transient symptoms, no interference with the subject’s daily activities

Moderate: Marked symptoms, moderate interference with the subject's daily activities
Severe: Considerable interference with the subject's daily activities, unacceptable.

14.3 Relationship to Trial Drug Assessment 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;

For reporting purposes, the categories "Definitely", "Probably" and "Possibly" will be summarized as "Suspected" Adverse Reactions. AEs with missing causality assessment will be regarded as possibly related unless further specified.

The sponsor will classify all related SAEs as either expected or unexpected:
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 it differs because of greater severity or greater specificity

All SAEs that are considered unexpected and suspected to be related to the IMP qualify for a SUSAR (Suspected Unexpected Serious Adverse Reaction) and require expedited reporting.

14.4 Outcome Categories and Definitions

Recovered
Stabilized: An AE is stabilized 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 protocol.
Recovered with sequelae: As a result of the SAE, the subject is suffering from persistent or significant disability/incapacity (e.g. became blind, deaf, paralyzed). Any AE recovered with sequelae should be rated as an SAE since an SAE criterion is fulfilled.
Not Recovered
Ongoing at final examination
Died

14.5 Collection, Recording and Reporting of Adverse Events

All events meeting the definition of an Adverse Event must be collected and reported starting with randomization (Visit 0/ day -28) until the end of the protocol required post-treatment follow-up period (Visit 6 28 days after the last vaccination).
At each contact to the site (visit or telephone, excluding safety visits) where the subject is not seeing the investigator or his staff (e.g. visits to the laboratory), the subject must be asked about adverse events. All adverse events either observed by the investigator or reported by the subject must be recorded by the investigator and evaluated.
The subjects will be asked in general: “How are you?” as well as specifically: “Have you experienced any problems since the last contact?”.
The investigator should record the diagnosis, if available. If no diagnosis is available, the investigator should record each sign and symptom as individual adverse events.
The investigator must record all adverse events in the eCRF. One single adverse event line must be used per adverse event, from start to resolution. For serious adverse events, the Serious Adverse Event Report Form must also be completed.

The investigator must report all SAEs with the completed SAE Report Form within 24 hours after learning of its occurrence to:

Assign Safety Desk: Fax: 0043 512 281514 77
Email: SafetyDesk@assigndmb.com

For urgent questions please call the 24-hours Safety Hotline: **0043 676 844033 835**

Under certain circumstances the first notification can be done by phone; nevertheless, a written Serious Adverse Event Report Form has to be submitted for confirmation to Assign Safety Desk. Reporting timelines are applicable also if the SAE is not felt to be treatment-related. The monitor must be informed accordingly.
The original copy of the SAE Report Form and the fax confirmation sheet must be kept with the documentation at the study site.
Please note: Each SAE has also to be documented in the eCRF as a serious adverse event in the corresponding AE section.

The sponsor is responsible to fulfill the requirements of expedited reporting of all SUSARs (Suspected Unexpected Serious Adverse Reaction) to the Competent Authorities, Ethics Committees and principal investigators concerned according to national and European law and regulations.

14.6 Follow-up of Adverse Events

During and following a subject's participation in a clinical trial, the investigator/institution will ensure that adequate medical care is provided to the subject for any adverse events, including clinically significant laboratory values related to the trial. The investigator/institution will inform the subject when medical care is needed for adverse event(s) of which the investigator becomes aware. All non-serious adverse events classified as severe or definitely/possibly/probably related to the trial product must be followed until 28 days after the administration of MV-CHIK or control (vaccine or placebo). All serious adverse events must be followed until the subject has recovered, stabilized, recovered with sequelae or died.
All other adverse events must be followed until the subject has recovered or stabilized or until the end of the protocol required post-treatment follow-up whichever comes first, and until all adverse event related queries for the subject have been resolved.
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 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.

The original copy of the SAE form including any follow-up information and the fax confirmation sheet must be kept with the documentation at the study site.

14.7 Reporting of Pregnancies

Women must not become pregnant during the entire clinical study period and 6 months after the last vaccination. If a subject becomes pregnant within this period, she must immediately inform the investigator of 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. The first notification can be done via phone (24-hours safety hotline: 0043 676 844033 835), nevertheless a written Pregnancy Report Form must be submitted for confirmation. If the pregnancy occurs during the study the subject should attend follow-up visits as planned. In addition, subjects who become pregnant during the study (and who received at least one dose) or subjects who become pregnant within 6 months after the last vaccination

(even in case of withdrawal or early termination for any reason) will be followed until pregnancy outcome, even if this occurs after the study is completed.

15 QUALITY CONTROL AND QUALITY ASSURANCE

15.1 Source Data and Records

Source data are defined as all information related to clinical findings, observations, or other activities in the study, written down in original records or certified copies of original records. The investigator will permit study-related monitoring, audits, IRB/IEC review and regulatory inspections, by providing direct access to source data/records. Source records should be preserved for the maximum period of time required by local regulations.

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

- Date of subject's study entry and termination, study identification
- Documentation of informed consent procedure
- Date of each study visit and study related correspondence
- 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
- Dosing dates
- Subject number
- Completed subject diaries

Source data entries must be made in accordance with local requirements. Signed and dated copies of the laboratory result reports have to be kept within the subject's files.

15.2 Periodic Monitoring

A designated monitor will inspect the eCRFs at regular intervals throughout the study to verify completeness, accuracy and consistency of the data, protocol adherence and adherence to Good Clinical Practice guidelines. The monitor should 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 Assign Clinical Research GmbH (acquired by Celerion), Hainburger Straße 33, 1030 Wien, telephone number +43 1 403 38 05

15.3 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 main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected and that all data relevant for the assessment of safety and efficiency of the investigational product have appropriately been reported to the sponsor.

15.4 Confidentiality of Subject's Data

The investigator will exercise all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of subjects' identities. On eCRFs or any other documents submitted to the sponsor, subjects will only be identified by subject number. Documents not for submission to the sponsor, e.g. subject identification log and original informed consent forms, will be maintained by the investigator in strict confidence.

16 DATA HANDLING AND RECORD KEEPING

16.1 Information of Investigators

An Investigational Drug Brochure (IB) containing all important data relating to the safe use of the investigational product will be supplied to the investigator prior to study start.

The investigator will be kept informed on new data as the study proceeds.

16.2 Data collection

All visits and assessments are entered into an interactive electronic Case Report Form (eCRF). A subset of the eCRFs will be source document verified following guidelines established before study onset and detailed in the Monitoring Plan. Maintenance of the eCRF and the study database will be performed by the data management center at Assign Data Management and Biostatistics GmbH.

16.3 Electronic Case Report Forms (eCRFs)

16.3.1 eCRF entries

eCRF entries and corrections will only be performed by the investigator or study site staff authorized by the investigator. Each user is informed by data management of the clinical study web-site internet address and is allocated to a user account with a personal password for access. The personal password must be kept confidentially and must only be used by the person to whom it was assigned. For additional authorized users at the site, a new user account needs to be requested 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.
All visit data need to be recorded in the database as soon as possible after each visit.

16.3.2 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 fulfill particular quality criteria, a message will specify the type of problem or syntax error 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).
Computerized 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 authorized staff.
As long as an eCRF page is not locked, required data changes can be conducted by the investigator or authorized site staff at any time.
In case of queries requiring data changes on locked pages, the investigator or authorized site staff will provide an unambiguous query answer and will explicitly authorize data management to perform the changes to be made; if the query answer is ambiguous or the request to data management for performing the data changes is inexplicit, the eCRF data will not be changed by data management.

Corrections of eCRF data may be performed by authorized staff only. The person performing the changes in the eCRF may be required to electronically confirm the changes made.

16.3.3 eCRF entry validation

The principal investigator or the authorized 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.

16.4 Investigator File

16.4.1 Maintenance

The investigator is responsible for maintaining all records to enable the conduct of the study to be fully documented. The records should include a protocol, study approval letters, all original informed consent forms, drug dispensing and accountability logs, and all correspondence pertaining to the study.

16.4.2 Archiving and destruction

All study related documents should be kept by the investigator for the maximum period of time required by local regulations. No study document should be destroyed without prior written agreement between the investigator and the sponsor. Should the investigator elect to assign the study documents to another party, or move them to another location, the sponsor must be notified.

16.5 Provision of Additional Information

On request, the investigator will supply the sponsor with additional data relating to the study, or copies of relevant source records, duly data protected. In case of particular issues or governmental queries, it is also necessary to have access to the complete study records provided, so that the subject's confidentiality is protected in accordance with applicable regulations.

17 CHANGES IN THE CONDUCT OF THE STUDY

17.1 Protocol Amendments

Proposed amendments must be submitted to the appropriate Ethics Committees. Amendments may be implemented only after EC approval has been obtained.

17.2 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 EC and CA in writing in accordance with local requirements. Documentation will be submitted for filing in Central File and investigator File.

18 REPORTING AND PUBLICATION

18.1 Clinical Study Report

After the completion of the study, a clinical study report will be prepared by the sponsor or delegate in accordance with relevant guidelines.

18.2 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.

19 ETHICS

The trial will be conducted in accordance with the Declaration of Helsinki for biomedical research involving human subjects.

19.1 Informed Consent Form of Trial Subjects (AMG § 38)

In obtaining and documenting informed consent, the investigator must comply with the applicable regulatory requirement(s) and adhere to ICH guideline for GCP, the requirements in the Declaration of Helsinki and the EU directive 2005/28/EG (24, 25, 26).

Prior to any trial-related activity, the investigator or an authorized physician must give the subject oral and written information about the trial in a form that the subject can read and understand.

A voluntary, signed and dated Informed Consent Form will be obtained from the subject prior to any trial-related activity. The subject must have consented to participate after the nature, scope and possible consequences of the clinical trial have been explained in a form understandable to him.

The written informed consent must be signed by the person, who conducted the informed consent.

If information becomes available that may be relevant to the subject's willingness to continue participating in the trial, the investigator must inform the subject in a timely manner, and a revised written informed consent must be obtained.

19.2 Independent Ethics Committees (AMG §§ 40, 41)

Prior to commencement of the trial the protocol, any amendments, Subject Information/Informed Consent Form, any other written information to be provided to the subject, subject recruitment procedures (e.g. advertisements), if any, investigator's Brochure (IB), package insert (if marketed product), information about payments and compensation available to subjects if not mentioned in the subject information, the investigator's current CV and/or other documentation evidencing qualifications, and other documents as required by the local Independent Ethics Committee (IEC) should be submitted. The submission letter should clearly identify (by including version no. and/or date of the document) which documents have been submitted to the IRB/IEC. Written approval/favorable opinion must be obtained from the IEC prior to commencement of the trial.

During the trial, the investigator must promptly report the following to the IEC: Updates to IB, unexpected SAEs where a causal relationship cannot be ruled out, amendments to the protocol, 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/favorable opinion, unless necessary to eliminate immediate hazards to the subjects.

19.3 Regulatory Authorities

The Regulatory Authorities will receive the protocol, amendments, reports on SAEs and related relevant safety information, including the Final Report according to EU Directive and national regulations.

19.4 Insurance (AMG § 32(2))

All subjects participating in this clinical trial will be insured through Themis Bioscience GmbH. An insurance will be contracted by the assigned CRO.

20 DEVIATIONS FROM THE PROTOCOL

20.1 Relevant Protocol Deviations

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 IRB/Ethics Committee's established procedures in accordance with applicable regulations.

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

A complete evaluation should be recorded at the time of the subject's withdrawal, including an explanation of why the subject is withdrawing.

20.3 Subsequent Therapy

Not applicable.

21 RETENTION OF CLINICAL TRIAL DOCUMENTS

Subject notes must be kept for the maximum time period as permitted by the hospital, institution or private practice. Other source documents and the investigator's trial file must be retained for at least 15 years or longer in accordance with local regulation. However, the Subject Identification Codes must be kept for at least 15 years. The investigator must agree to archive the documentation pertaining to the trial in an archive after completion or discontinuation of the trial, if not otherwise notified. If any modifications become necessary or desirable, these will be documented in writing; major changes require the approval of all investigators and the ethics committee.

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23 APPENDICES

Appendix 1: Template distributed to subjects to assess local reaction