CLINICAL STUDY PROTOCOL

A PHASE 1, RANDOMIZED, PLACEBO-CONTROLLED, DOSE-RANGING STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF VAL-181388 IN HEALTHY ADULTS IN A NON-ENDEMIC CHIKUNGUNYA REGION

PROTOCOL NO. VAL-181388-P101

Sponsor:	Moderna Therapeutics, Inc. 200 Technology Square Cambridge, MA 02139
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IND No.	17166
Version of Protocol:	4.0
Date of Protocol:	30 August 2018

CONFIDENTIAL

The study will be conducted according to the International Council for Harmonisation harmonised tripartite guideline E6(R1): Good Clinical Practice.

Signature Page

PROTOCOL TITLE: A Phase 1, Randomized, Placebo-Controlled, Dose-Ranging Study to Evaluate the Safety and Immunogenicity of VAL-181388 in Healthy Adults in a Non-endemic Chikungunya Region

PROTOCOL NUMBER: VAL-181388-P101

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Moderna Therapeutics, Inc.

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Moderna Therapeutics, Inc.

Investigator Protocol Agreement Page

I agree to conduct the study as outlined in the protocol entitled "A Phase 1, Randomized, Placebo-Controlled, Dose-Ranging Study to Evaluate the Safety and Immunogenicity of VAL-181388 in Healthy Adults in a Non-endemic Chikungunya Region" in accordance with the guidelines and all applicable government regulations including US Title 21 of the Code of Federal Regulations Part 54. I have read and understand all sections of the protocol.

Signature of Investigator	Date

Printed Name of Investigator

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Protocol Synopsis

Protocol Number:	**VAL-181388-P101**
Title:	A Phase 1, Randomized, Placebo-Controlled, Dose-Ranging Study to Evaluate the Safety and Immunogenicity of VAL-181388 in Healthy Adults in a Non-endemic Chikungunya Region
Study Phase:	1
Study Site:	One site in the United States
Objectives:	Primary: • To assess the safety of VAL-181388 relative to placebo. Secondary: • To determine the immunogenicity of 3 dose levels of VAL-181388 to inform the choice of dose for further development of this vaccine. Immunogenicity assessment will be based on changes from baseline in the following: o Serum neutralizing antibody titers to chikungunya virus (CHIKV) o Serum binding antibody titers to CHIKV-specific proteins Exploratory: • To assess the cross-reactivity of serum antibodies to related viruses, including alphaviruses and flaviviruses • To explore other assays to characterize the immune response to CHIKV
Study Design and Methodology:	This is a Phase 1, first-in-human, randomized, observer-blinded, placebo-controlled, dose escalation study to evaluate the safety and immunogenicity of 3 dose levels of VAL-181388 in healthy adult subjects (18 to 49 years of age, inclusive). VAL-181388 is a messenger RNA (mRNA)-based vaccine being developed for prevention of disease associated with CHIKV infection. Chikungunya virus is a positive-sense, single-stranded RNA virus, of the alphavirus family, in the genus of the togavirus family. It can cause disease in humans and other mammals. It is an arbovirus that is transmitted by a mosquito vector (*Aedes spp*). Currently there is no approved vaccine to protect against this disease, which has recently spread through tropical regions. This is a 2-part study, with Part A including dose escalation and safety and immune testing through 28 days following the final vaccination. Once subjects complete Part A they will transition to Part B. Part B is a continued safety follow-up through 12 months and an assessment of

immunogenicity and immune persistence at approximately 6 and 12 months after final vaccination.

Part A

In Part A, sequential dose escalation of VAL-181388 is planned in 3 dose level cohorts (25, 50, and 100 µg) with each subject receiving 2 vaccinations separated by 28 days. Subjects will be randomly assigned to receive either VAL-181388 or placebo.

For each cohort, a sentinel safety group of 4 subjects will be enrolled who will be randomly assigned to VAL-181388 or placebo (3:1) and followed for 7 days after the first vaccination with review of reactogenicity and safety laboratory results prior to randomizing the remainder of the cohort. An internal safety team (IST) will oversee the safety of the trial and will review blinded safety data during Part A of the study to ensure adherence to the protocol, monitor safety laboratory test results and reactogenicity, and raise concerns to the Safety Monitoring Committee (SMC) should there be safety issues or operational challenges that could affect subject safety. Only after review by the IST of the blinded safety data (reactogenicity, safety laboratory results, and adverse events [AEs]) through 7 days after the first vaccination of the sentinel safety lead-in for each cohort planned, will approval be given to allow randomization of the remainder of that cohort. The SMC will approve escalation to the next higher dosing cohort after review of blinded safety data of the currently dosed cohort through 7 days following the second vaccination and any cumulative safety data of all cohorts as the trial advances. The SMC will also be convened (ad hoc meeting) if a pause rule is triggered.

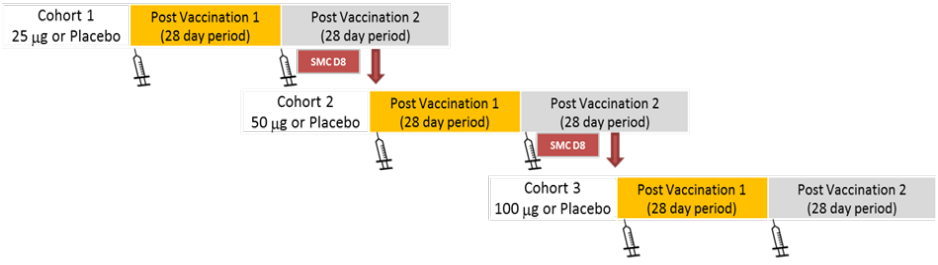
Dose Level Cohort Assignments: VAL-181388 (or placebo) administered IM at Visit 1 and a second dose administered 28 days later (Visit 4).

Cohort 1 (N = 20): 25 µg VAL-181388 or placebo (ratio = 3:1)

Cohort 2 (N = 20): 50 µg VAL-181388 or placebo (ratio = 3:1)

Cohort 3 (N = 20): 100 µg VAL-181388 or placebo (ratio = 3:1)

VAL-181388 Administration and Dose Escalation Schema



VAL-181388 (or placebo) will be administered as an intramuscular (IM) injection (0.5 mL) into the deltoid muscle, preferably in the non-dominant arm, as a 2-dose schedule; the first dose will be given at Visit 1 followed by a second dose 28 days later. VAL-181388 accountability, dose preparation, and administration will be performed by unblinded pharmacy personnel or unblinded designees who will not participate in any other aspect of the study. The remainder of the site staff and all subjects will remain blinded to treatment assignment.

Consent and Screening will occur over a 28-day period prior to randomization. A total of 20 subjects will be randomly assigned to each dose level cohort, 4 subjects (3 VAL-181388 and 1 placebo) to the sentinel safety group and 16 subjects (12 VAL-181388 and 4 placebo) to the expansion group, resulting in 15 subjects being randomly assigned to VAL-181388 and 5 subjects being randomly assigned to placebo (3:1 ratio overall). Subjects meeting all eligibility criteria will receive the first vaccination at Visit 1. Each subject will be monitored in the clinic for at least 1 hour following each vaccination for safety. Assessments will include vital sign measurements and any immediate reactogenicity. Of note, on the day of scheduled vaccination, if a subject is noted to have a systolic or diastolic blood pressure, heart rate, or respiratory rate measurement that shows Grade 2 or higher toxicity after 2 measurements (even after relaxing or resting); has an acute illness; or has an oral temperature >38°C, the subject should not receive a vaccination on that day and will need to return on subsequent days for their vaccination.

All subjects will return to the clinic at 7 (+3), 17 (±3), and 28 (+7) days following each vaccination. In addition, the sentinel safety group will return to the clinic at 1, 2, and 21 (+3) days following the first vaccination. A second vaccination will occur 28 days after the first vaccination (+7 days).

Subjects will be instructed on recording solicited (local and systemic reactogenicity events) and unsolicited AEs, temperature, and medications (prescription or over-the-counter) on their memory aid (ie, diary card). Subjects will also be asked to call/return to the clinic within 24 hours if reactogenicity reaches Grade 3 or higher during the first 7 days following vaccination. As standard practice, a reminder call will be made to the subject by the site at least once during the first 7 days following each vaccination to answer any questions and ensure that the memory aid is being completed correctly and consistently.

Safety assessments will include toxicity grading of solicited (local and systemic reactogenicity events) and unsolicited AEs, vital sign measurements, physical examination findings, and clinical laboratory test results for hematology, serum chemistry, coagulation, and urinalysis.

All unsolicited AEs will be collected and additional specific categories will include serious adverse events (SAEs); AEs of special interest (AESIs); and medically attended AEs. Subjects will self-report (using a memory aid) solicited (local and systemic reactogenicity events) and unsolicited AEs and medication usage (over-the-counter and prescription) through 28 days after each vaccination. In addition, subjects will be instructed to measure their oral temperature daily for the first 7 days post vaccination and record that measure on the memory aid. At applicable clinic visits, the investigator will review the entries on the memory aid with the subject to ensure consistency.

Safety laboratory tests will be performed at Screening, on vaccination days (before dosing), and at 7 (+3) days after each vaccination. At 17 (±3) days following each vaccination and at 28 (+7) days after the second vaccination, safety laboratory tests will be performed for coagulation and liver function tests only. The sentinel safety group will also have coagulation and liver function tests collected at 21 (+3) days after the first vaccination. Additional safety laboratory tests may be conducted at any time by the investigator if deemed necessary.

Blood samples for immunogenicity will be collected on dosing days (pre-dose) and at 28 (+7) days after the final vaccination.

Subjects unable to complete their second vaccination due to noncompliance with the visit schedule and not due to a cohort pause will still be required to follow the original visit and testing schedule. Specifically, these subjects will be asked to undergo blood collection to comply with a second immune test at 56 (+10) days (Visit 7) following the first vaccination.

To allow cohort advancement, the following data from the highest dosed cohort are expected to be reviewed by the SMC: 1) toxicity scoring of safety laboratory test results through 7 days following the second vaccination (through Visit 5); 2) toxicity scoring of vital signs and reactogenicity through 7 days following the first and second vaccination (through Visit 5); 3) all unsolicited AEs (with grading and attribution). Data will be prepared by dose cohort and timing relative to each vaccination. As safety data on all cohorts accumulate, the SMC also review ongoing AEs as well as cumulative safety laboratory test results, reactogenicity, and AEs across vaccine doses for subjects remaining in Part A of the study, thereby providing a cumulative and comprehensive review of safety. Should a cohort pause occur due to pre-specified criteria, the SMC will be convened for an unscheduled (ad hoc) meeting and be provided with specific safety data related to the trigger of the pause.

Part A is concluded for each subject when they return to the clinic for Visit 7 (28 days after final vaccination). At that time, subjects will transition to Part B of the study.

Part A: Overview of Visits

Study visit	0	1	1a[a]	1b[a]	2	3	3a[a]	4	5	6	7
Vaccination day		X						X			
Days relative to most recent vaccination	NA	0	1	2	7	17	21	28	7	17	28
Window allowance	+28	0	0	0	+3	±3	+3	+7	+3	±3	+7
Screening	X										
Physical examination[b]	X	X[c]			X		X	X[c]	X		X
Safety laboratory tests	X	X[c]			X	X	X	X[c]	X	X	X
Vital sign measurements	X	X[d]	X	X	X		X	X[d]	X		X
Immune laboratory tests		X[c]						X[c]			X
Study drug administration[e]		X						X			
Review of concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Review of safety (solicited and unsolicited)		X[d]	X	X	X	X	X	X[d]	X	X	X

a. Applies to sentinel safety group only.
b. Symptom-directed after Screening.
c. Obtained before study drug administration.
d. Obtained before and after study drug administration.
e. Vaccination cannot occur if systolic or diastolic blood pressure, heart rate, or respiratory rate measurements show Grade 2 or higher toxicity after 2 measurements; if a subject has an acute illness; or if a subject has an oral temperature >38°C.

Part B

To monitor for longer-term safety and immune persistence, each subject will be entered into a continued blinded follow-up period (Part B). This period will be conducted such that subjects and safety monitors will remain blinded to treatment assignment. Part B of the study is initiated for a subject once they have returned for Visit 7 (28 days following the second vaccination).

Once entered into Part B, each safety contact will occur by telemedicine (eg, telephone, text message, internet) every 28 (±7) days, and blood samples for immune persistence will be collected from each subject on Visit 12 (±15 days) and Visit 19 (+15 days). Each safety contact will

capture outcomes of any AESI or SAE that remains unresolved since the last visit or is newly identified through scripted query.

The telemedicine visits may require additional data through medically attended visits, in addition to medications and vaccinations taken by the subject during this time. Subjects will have consented during study enrollment to allow access to additional medical records needed to complete Part B, thereby allowing the blinding of the treatment assignment to be maintained.

Part B: Overview of Visits

Study visit	8	9	10	11	12	13	14	15	16	17	18	19
Study day from 1st dose if 2nd dose not completed	84	112	140	168	196	224	252	280	308	336	364	392
Study day from 2nd dose, if completed	56	84	112	140	168	196	224	252	280	308	336	364
Window allowance	±7	±7	±7	±7	±15	±7	±7	±7	±7	±7	±7	+15
Safety contact	X	X	X	X		X	X	X	X	X	X	
Immune samples					X							X
Serious adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events of special interest	X	X	X	X	X	X	X	X	X	X	X	X
End of Study												X

When all subjects have completed their final contact (approximately 12 months after their last vaccination), immune testing is completed, and all queries resolved, the database will be locked and analyzed and a final clinical study report will be provided to regulators.

Inclusion criteria:

Each subject must meet all of the following criteria during the screening period to be enrolled in this study:

1. The subject is male or female between 18 and 49 years of age, inclusive.
2. The subject has a body mass index between 18 and 35 kg/m^2, inclusive.
3. The subject is considered by the investigator to be in good general health as determined by medical history, clinical laboratory assessments, vital sign measurements, and physical examination findings at Screening.
4. Female subjects must be non-pregnant and non-lactating and meet one of the following criteria: A) post-menopausal (defined as amenorrhea for 12 consecutive months without an alternative medical cause or documented serum follicle-stimulating hormone

level in the post-menopausal range); B) surgically sterile (ie, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy). NOTE: procedures and laboratory results must be confirmed in the medical record, by physical examination, or by official written confirmation of a procedure; or C) if of childbearing potential, agrees to be heterosexually inactive from at least 21 days prior to enrollment and through 3 months after the final vaccination or agrees to consistently use any of the following methods of contraception from at least 21 days prior to enrollment and through 3 months after the final vaccination: condoms (male or female) with spermicide, diaphragm with spermicide, cervical cap with spermicide, intrauterine device, oral or patch contraceptives, Norplant®, Depo-Provera®, or other Food and Drug Administration-approved contraceptive method which is designed to protect against pregnancy. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

5. Male subjects must agree to consistently use appropriate contraception and to refrain from sperm donation through 3 months after the final vaccination.
6. The subject understands and agrees to comply with the study procedures and provides written informed consent before any study procedures are performed.
7. The subject has access to a consistent and reliable means of telephone contact, which may be in the home, workplace, or by personal mobile electronic device.
8. The subject agrees to stay in contact with the study site for the duration of the study, to provide updated contact information as necessary, and has no current plans to move from the study area for the duration of the study.

Exclusion criteria:

Subjects meeting any of the following criteria will be excluded from the study:

1. The subject has any ongoing significant chronic illness requiring medical or surgical care. Asymptomatic conditions or findings (eg, mild hypertension, dyslipidemia) that are not associated with evidence of end-organ damage are not exclusionary provided that they are being appropriately managed and are clinically stable in the

opinion of the investigator (ie, unlikely to result in symptomatic illness within the time-course of this study).

2. The subject is a female of childbearing potential and has a positive pregnancy test at Screening or on the day of vaccination.
3. The subject has any abnormal screening laboratory result meeting the criteria listed below.
    a. Elevated liver function tests (aspartate aminotransferase, alanine aminotransferase, total or direct bilirubin, or alkaline phosphatase), elevated creatinine, or reduced platelets with a toxicity score ≥ Grade 1 at Screening. Re-testing of these parameters is not allowed.
    b. Other than a) above, any safety laboratory test result (urine or serum) with:
        i. a toxicity score ≥ Grade 2
        ii. a toxicity score of ≥ Grade 1 deemed clinically significant
4. The subject has participated in another investigational study involving any investigational product (ie, study drug, biologic, device) within 60 days, or 5 half-lives, whichever is longer, before planned date of first vaccination.
5. The subject has received any live attenuated or inactive vaccines within 4 weeks prior to enrollment, or plans to receive any vaccine during the active vaccination period (through 4 weeks after their last planned vaccination).
6. The subject has received (at any time) a vaccine for CHIKV, dengue, Yellow Fever, tick-borne encephalitis, or Japanese encephalitis.
7. The subject has a history of confirmed or suspected CHIKV infection, has lived in a CHIKV-endemic area for >1 year, or has cumulatively spent more than 30 days in a CHIKV-endemic area within the last 5 years.
8. The subject has reported previously participating in an investigational study involving lipid nanoparticles.
9. The subject has a history of hypersensitivity or serious reactions (eg, anaphylaxis, urticaria, other significant reaction) to previous vaccinations.
10. The subject has any known or suspected autoimmune disease or immunosuppressive condition, acquired or congenital, as determined by medical history and/or physical examination.
11. The subject has a history of arthritis (including inflammatory arthritis) or arthralgia.
12. The subject has a neurologic disorder (eg, history of seizures, Guillain-Barre syndrome, dementia, vasculitis, or any known congenital or acquired disorder).

13. The subject received immunoglobulins and/or any blood products within the 3 months preceding the administration of the study drug or plans to receive such products at any time during the study.
14. The subject has had chronic administration (defined as more than 14 continuous days) of an immunosuppressant or other immune-modifying drug within 6 months prior to vaccine administration or plans to receive any products during the active vaccination period (through 4 weeks after their last planned vaccination). An immunosuppressant dose of a glucocorticoid will be defined as a systemic dose of ≥10 mg of prednisone per day or equivalent. The use of topical, inhaled, and nasal glucocorticoids will be permitted.
15. The subject is currently receiving antipyretic or analgesic medication on a daily or every other day basis (a daily dose of ≤100 mg of aspirin given under the guidance of a physician is not a contraindication to enrollment).
16. The subject has any acute illness at the time of enrollment (defined as the presence of a moderate or severe illness with or without fever, or an oral temperature >38.0°C on the planned day of vaccination). In such cases, subjects may be re-evaluated during the screening period for resolution of the illness to allow at least 3 days of wellness prior to the planned vaccination.
17. The subject has any significant disorder of coagulation (acquired or hereditary) requiring ongoing or intermittent treatment. Subjects receiving prophylactic antiplatelet medications, eg, low-dose acetylsalicylic acid (≤100 mg/day or equivalent), and without clinically apparent bleeding tendency, are eligible.
18. The subject has a history of idiopathic urticaria.
19. The subject has a history of alcohol abuse or drug addiction within 1 year before the planned day of dose administration (self-reported).
20. The subject has a positive test result for drugs of abuse at Screening.
21. The subject has any abnormality or permanent body art (eg, tattoo) that would obstruct the ability to observe local reactions at the injection site (deltoid region).
22. The subject has any condition that, in the opinion of the investigator, would pose a health risk to the subject if enrolled or could interfere with evaluation of the study drug or interpretation of study results (including neurologic or psychiatric conditions deemed likely to impair the quality of safety reporting).
23. The subject has a positive test result for hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus types 1 or 2 antibodies.

24. The subject has a history of active cancer (malignancy) in the last 10 years. An exception is a subject with adequately treated nonmelanomatous skin carcinoma, who may participate in the study.
25. The subject has donated blood or blood products >450 mL within 30 days of dosing.
26. The subject has any screening vital sign measurement (systolic or diastolic blood pressure, heart rate, or respiratory rate) that shows greater than or equal to Grade 2 toxicity after 2 measurements.
27. The subject is an employee or first-degree relative of the Sponsor, PPD, or study site personnel.

Part A Safety Assessments:

- Solicited AEs (local and systemic reactogenicity events) collected for 7 days following each vaccination with toxicity grading.
- Unsolicited AEs collected for 28 days following each vaccination. Additional classification if serious, medically attended, or an AESI.
- Safety laboratory test results (serum chemistry, hematology, coagulation, and urinalysis) with toxicity grading
- Vital sign measurements with toxicity grading and physical examination findings

Part B Safety Assessments

- Serious AEs and AESIs through 1 year (or until resolved, whichever comes first) following the last vaccination

Part A Immunogenicity Testing:

- Neutralizing serum antibody titers to CHIKV (baseline and 28 days after each vaccination)
- Serum binding antibody titers (immunoglobulin G [IgG]) to CHIKV-specific proteins (baseline and 28 days after each vaccination)
- Exploratory antibody assays may be performed with excess serum to assess for cross-reactivity to related viruses at the discretion of the Sponsor
- Additional exploratory assays based upon current research may be performed with excess serum to better characterize the immune response to the CHIKV proteins at the discretion of the Sponsor

Part B Immunogenicity Testing:	• Neutralizing serum antibody titers to CHIKV (at 6 and 12 months after the last vaccination) • Serum binding antibody titers (IgG) to CHIKV-specific proteins (at 6 and 12 months after the last vaccination) • Exploratory antibody assays may be performed with excess serum to assess for cross-reactivity to related viruses at the discretion of the Sponsor • Additional exploratory assays based upon current research may be performed with excess serum to better characterize the immune response to the CHIKV proteins at the discretion of the Sponsor
Study Drug, Dosage, and Route of Administration	25, 50, or 100 µg VAL-181388 or placebo (0.9% Sodium Chloride Injection, USP) will be prepared as outlined in the pharmacy manual and administered via IM injection (0.5 mL) into the deltoid muscle on designated vaccination days, preferably in the non-dominant arm.
Sample Size:	Approximately 60 subjects are planned to be randomized, 20 per dose level. Formal sample size calculations were not performed as this is an observational safety and immunogenicity study with no formal null hypotheses being tested.
Statistical Methods:	**Safety:** Data for subjects receiving placebo will be pooled across all dose levels for all presentations. Reactogenicity will be summarized by dose levels (25, 50, or 100 µg or placebo), vaccination (first or second), duration, relationship to study vaccine, and severity. Adverse events will be coded by preferred term and system organ class using the Medical Dictionary of Regulatory Activities and summarized by part, treatment, vaccination (first or second), and overall. Adverse events will also be summarized by severity and relationship to study vaccine. Descriptive statistics will be presented and the difference in the proportion of AEs will be provided comparing each dose level with placebo recipients pooled across all dose levels. Adverse events for subjects in the safety cohort for each dose level will be provided in a separate subject listing. Adverse events leading to withdrawal, AESIs, medically attended AEs, and SAEs will be provided by subject in a listing. Safety data from clinical laboratory test results and vital sign measurements will be graded by severity and analyzed by treatment group and vaccination (first or second). Absolute and change from baseline values will be provided according to the toxicity table, along with mean, median, and standard deviation. Results of hematology, serum chemistry, coagulation, and urinalysis assessments; urine drug screen; and pregnancy testing will be listed for all subjects randomly assigned to receive study treatment.

Medical history data for all subjects randomly assigned to receive study treatment will be presented by subject in a listing.

Baseline demographic and background variables will be summarized by treatment group and cohort for all subjects. The number of subjects who enroll in the study and the number and percentage of subjects who complete the study will be presented. Frequency and percentage of subjects who withdraw or discontinue from the study, and the reason for withdrawal or discontinuation, will also be summarized.

Prior and concomitant medication will be listed (with start and stop dates) for each subject and summarized by common medical dictionary coding. Any vaccinations that occur during the trial conduct will also be captured and summarized.

Immunogenicity: The following immunogenicity outcome measures (for serum neutralizing antibody titers and serum binding antibody titers to CHIKV-specific proteins) and their 95% confidence intervals, where appropriate, will be summarized by dose level cohort and combined placebo, and by days post-vaccination:

- Geometric mean titer (GMT) at baseline (pre-vaccination at Visit 1) and at post-dose time points
- Geometric mean ratio$_{Post/Pre}$: the ratio of post-vaccination GMT to pre-vaccination (Visit 1) GMT of subjects who have a baseline sample and post-vaccination results at post-dose time points
- Seroresponse: The proportion of subjects in each treatment group who either had an undetectable titer at baseline and detectable titer after vaccination, or detectable titer at baseline and at least a 4-fold increase (of baseline titer) after vaccination will be calculated.

Following completion of each dose level cohort in Part A, the database will be locked for that cohort and safety and/or immune testing results through 28 days following the final vaccination will be analyzed. As dose escalation occurs, cumulative analyses will be included for each subsequent data lock to allow for all prior dose level cohorts to be analyzed by dose level and in aggregate for VAL-181388 exposure. Immunogenicity and safety data, including mean group analyses of change from baseline, where applicable, will be summarized for each dose level cohort and combined placebo group. These analyses will be performed by an unblinded team, independent of the study team. All study personnel and participants other than the third-party statistician will remain blinded to treatment allocation. These data will be provided to designated Sponsor representatives only. They will inform decisions on this and other development programs using the same mRNA platform.

Data may be unblinded to the Sponsor and dosing adjustments may be made during the conduct of the study should the data reveal the need to adjust dosing due to either safety or immune response.

An interim analysis of safety, reactogenicity, and immunogenicity data collected from Day 1 to Month 7 will be conducted and will be reported on a treatment assignment level. Access to individual listings will be restricted to identified Sponsor members and the clinical research organization unblinded statistician. Study sites will remain blinded. This analysis will provide information regarding short-term antibody persistence.

The final analysis of safety and immunogenicity data collected from Visit Day 1 through the end of the study will be performed as soon as data are cleaned and locked. The results of this analysis will be presented in a clinical study report including individual data listings.

Additional information can be found in the statistical analysis plan.

Date of Protocol: 30 August 2018

List of Abbreviations

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
CARPA	complement activation-related pseudoallergy
CBER	Center for Biologics Evaluation and Research
CFR	Code of Federal Regulations
CHIKV	chikungunya virus
DAIDS	Division of AIDS
eCRF	electronic case report form
ECSA	East Central South African
EOS	end-of-study
FDA	Food and Drug Administration
GMT	geometric mean titer
HA	hemagglutinin
IB	investigator's brochure
ICF	informed consent form
ICH	International Council for Harmonisation
ID	intradermal
IFN	interferon
IgG	immunoglobulin G
IM	intramuscular
IRB	institutional review board
IST	internal safety team
IV	intravenous
LLOQ	lower limit of quantification
LNP	lipid nanoparticle
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	messenger RNA
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level

Abbreviation	Definition
ORF	open reading frames
SAE	serious adverse event
SMC	Safety Monitoring Committee
ULOQ	upper limit of quantification

1 Introduction

1.1 Background

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus posing a significant public health problem in tropical and subtropical regions. While chikungunya has been present in Africa for centuries, it has recently caused outbreaks and epidemics in new regions reflecting the increasing distribution of the *Aedes* mosquito. A chikungunya epidemic beginning in 2004 in Kenya, which spread to the Indian Ocean islands and to India, and was exported to nearly all regions of the world via infected travelers, resulted in millions of infected individuals and brought chikungunya to the attention of the western world. As of April 2016, chikungunya cases had been reported in 103 countries and territories around the world, including 46 countries and territories throughout the Americas (Centers for Disease Control and Prevention 2016). There are an estimated 3 million cases of chikungunya globally.

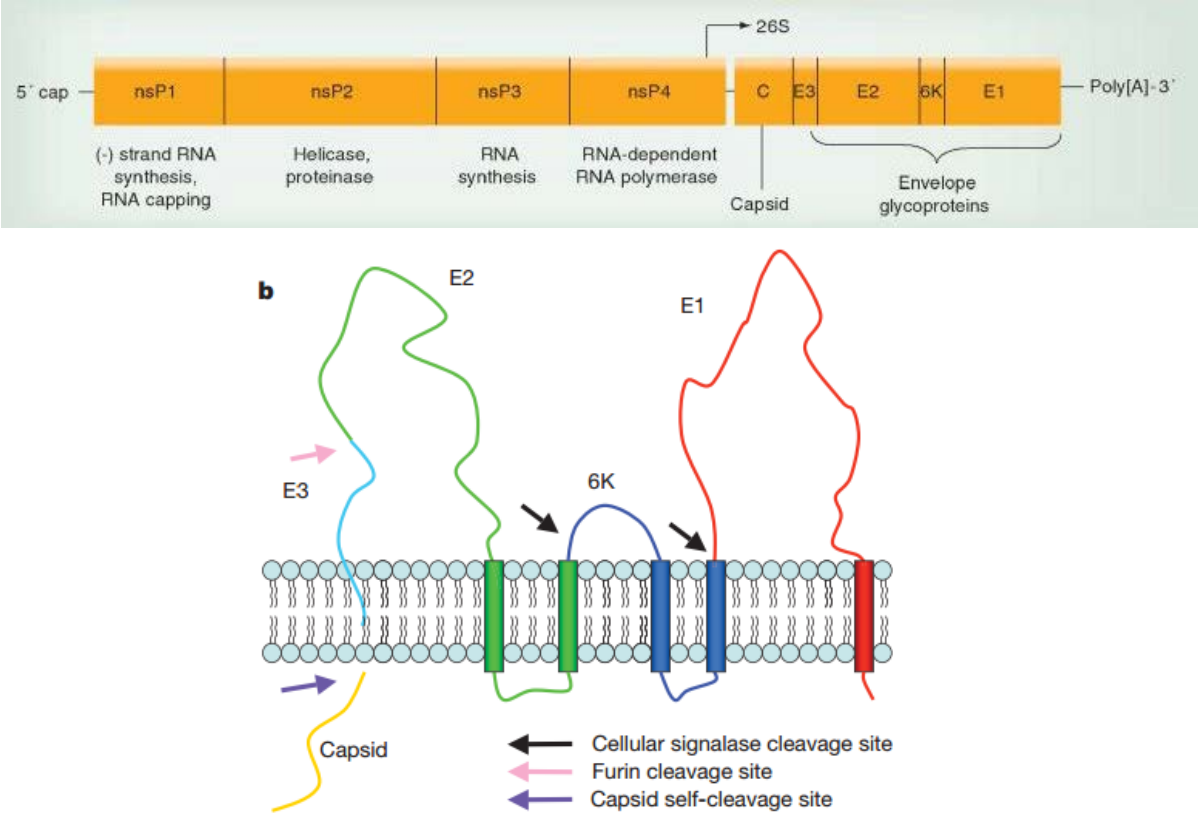
Chikungunya virus infection causes chikungunya, characterized by an acute onset of fever, rash, myalgia, and debilitating polyarthralgia (Schwartz and Albert 2010; Weaver et al 2012), from which it derives its name which means “that which bends up” when translated from Makonde (language spoken by an ethnic group in southeast Tanzania and northern Mozambique) (Halstead 2015). It is rarely fatal, but neurological sequelae such as Guillain-Barre syndrome and chronic arthritides have been increasingly recognized.

Since its identification in 1952 (Robinson 1955), CHIKV isolates from around the world have been grouped into three broad genotypes which correspond to geographic origin: (1) Asian, (2) West African, and (3) East Central South African (ECSA) (Weaver et al 2012). Isolates from the Indian Ocean region since 2000 are closely related to the ECSA lineage, and many harbor mutations that augment replication and transmission in *Aedes albopictus* mosquitos (Tsetsarkin et al 2011; Weaver et al 2012). The *Aedes aegypti* mosquito is the classical vector for CHIKV and is widely distributed across equatorial countries, and the jump to *Aedes albopictus* puts additional regions such as the United States and Europe at risk, as this species is found in these more temperate climates and is considered a strong viral vector for spread of disease (Weaver and Lecuit 2015).

Chikungunya virus is an alphavirus of the *Togaviridae* family with a positive-strand RNA genome of 11.8 kilobases. Like other alphaviruses, the genome is capped and polyadenylated, and encodes 2 open reading frames (ORFs). The 5′ ORF (2/3 of the genome) encodes 4

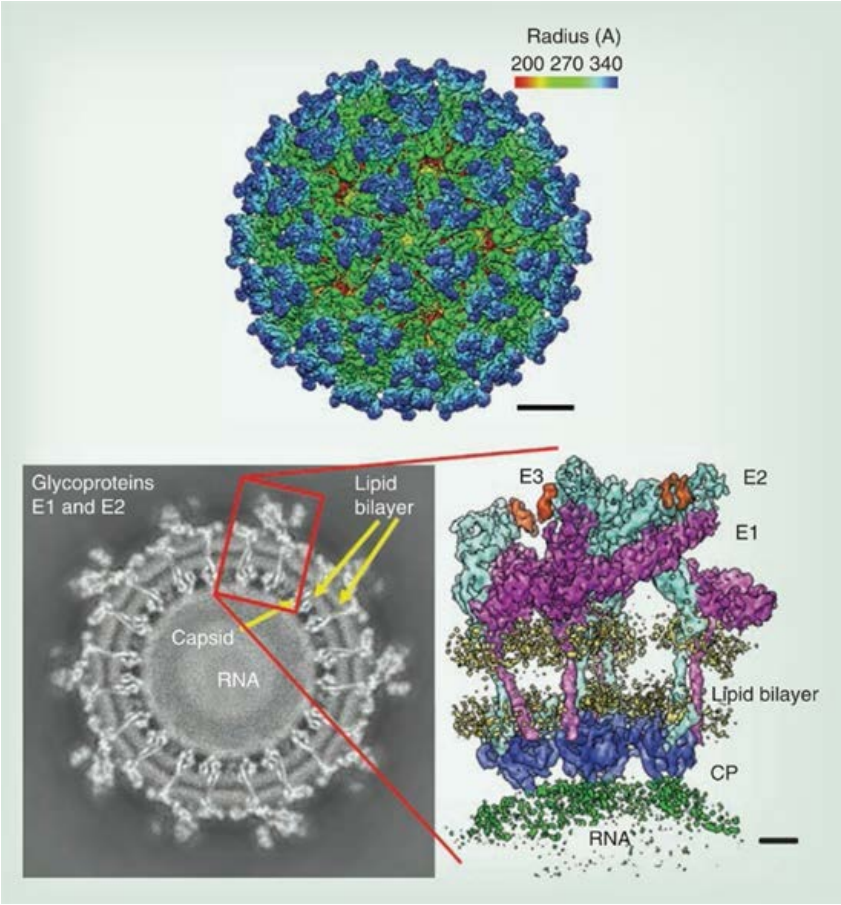
nonstructural proteins required for viral replication and the 3′ ORF encodes the structural proteins. The structural proteins are expressed as a single polyprotein from a subgenomic promoter and are cleaved by viral and cellular proteases into capsid (C) and envelope glycoproteins E3, E2, 6K, and E1 (Figure 1-1) (Kuhn 2007). The mature chikungunya virion is 70 nm in diameter and contains 240 heterodimers of E1/E2 arranged as 80 trimeric spikes on its surface (Figure 1-2) (Strauss JH and Strauss EG 1994; Zhang et al 2011). The E1 protein serves as the fusion protein and the E2 as the attachment protein.

Figure 1-1 Chikungunya Virus Genome and Post-Translational Processing of Structural Proteins



Top panel: Organization of the CHIKV genome, including structural and nonstructural polyproteins and 26S subgenomic promoter (Weaver et al 2012). Bottom panel: Threading of the chikungunya virus structural polyprotein through the endoplasmic reticulum and processing by proteases (Li et al 2010).

Figure 1-2 Structure of Alphavirus Virion



Abbreviation: CP, capsid protein.
Cryo-electron microscopic reconstruction of the alphavirus virion (Zhang et al 2011, Weaver et al 2012).

Although the detailed mechanism of protection against CHIKV infection in humans is not completely understood, it is clear that neutralizing antibodies play an important role (Couderc et al 2009). Natural CHIKV infection of humans induces high neutralizing antibody titers (Lanciotti et al 2007) and immunoglobulin G (IgG) levels correlate with viral clearance and protection (Kam et al 2012a, Kam et al 2012b). Human CHIKV-specific neutralizing monoclonal antibodies have also been isolated and shown to protect animals from experimental CHIKV infection (Smith et al 2015). The majority of these monoclonals recognize the E2 protein and are able to cross-neutralize CHIKV from all genotypes, including currently circulating ECSA strains (Smith et al 2015). Passive immunotherapy of convalescent sera has also been shown to protect animals from CHIKV infection (Partidos et al 2011).

Further details on messenger RNA (mRNA)-based therapeutics, as well as the physical, chemical, and pharmaceutical properties of VAL-181388 can be found in the investigator's brochure (IB).

1.2 Nonclinical Studies in Development of VAL-181388

1.2.1 Nonclinical Efficacy

The species selected for nonclinical studies to demonstrate efficacy and safety of VAL-181388 are mice and rats. Mice are susceptible to CHIKV disease in the first few weeks of life, but adult wild-type mice are resistant (Couderc et al 2008), which makes testing of prophylactic vaccines challenging. Adult inbred mice with a partial or complete defect in the interferon (IFN) pathway are widely used as animal models for CHIKV research. Inoculation of these strains with CHIKV results in high levels of viremia and nearly systemic virus dissemination to other tissues, with high viral loads (Couderc et al 2008; Partidos et al 2011; Gardner et al 2012; Plante et al 2015). After initial replication in the liver of sensitive mice, the virus primarily targets muscle, joint, and skin fibroblasts, and viral RNA has been shown to persist in joint-associated tissue for months (Couderc et al 2008; Hawman et al 2013). These findings are relevant to human disease, as similar tissue and cell tropisms have been observed in CHIKV-infected patients (Couderc et al 2008), although detailed, comprehensive human biopsy evaluations have not been performed. Mice with severe disease also develop a central nervous system infection (Couderc et al 2008), as do some humans. One particularly sensitive animal model is the AG129 mouse, which has null mutations in the IFN-α/-β and -γ receptors (van der Broek et al 1995). Inoculation of AG129 mice with 1×10^4 plaque-forming units of attenuated CHIKV vaccine strain 181/25 via the intradermal (ID) route results in rapid onset of clinical signs of disease, including ruffled fur and hunched posture, followed by death within 3 to 4 days (Partidos et al 2011). Although these mice are deficient in IFN signaling, they are able to mount B- and T-cell responses. Neutralizing antibodies are believed to be the primary effectors of protection against CHIKV, and indeed immune serum can afford protection in this model (Partidos et al 2011). A number of CHIKV candidate vaccines have been evaluated in AG129 and related mouse models carrying a deficiency in only IFN-α/-β receptor signaling (A129) and have been shown to elicit neutralizing antibodies that protect from a lethal CHIKV challenge (Partidos et al 2011; Plante et al 2011; Brandler et al 2013; Metz et al 2013). Some of these candidate vaccines have also demonstrated efficacy in nonhuman primate challenge models (Akahata et al 2010; Roy et al 2014) and have induced neutralizing antibody responses in humans (Edelman et al 2000; Chang et al 2014; Ramsauer et al 2015). Based on these

findings, the AG129 mouse is considered an appropriate model for efficacy evaluation of CHIKV vaccines.

The efficacy of the CHIKV C-E3-E2-6k-E1 mRNA vaccine and E2 and E1 mRNA vaccines (mRNA encodes envelope glycoproteins E2 or E1, respectively) given in various combinations of route of administration (ID or intramuscular [IM]), number of vaccinations (1 or 2), and dose (2 µg or 10 µg) was determined in female AG129 mice (lacking the IFN-α/β and -γ receptors). The C-E3-E2-6k-E1 mRNA vaccine provided full protection against the attenuated CHIKV strain 181/clone 25 (181/25) infection regardless of the route of administration, number of vaccinations, or dose. Two vaccinations of the E2 mRNA vaccine were also efficacious with either route of administration or dose, but a single vaccination was not effective with any combination of route of administration and dose. The E1 mRNA vaccine was only efficacious after 2 vaccinations of 10 µg mRNA.

Neutralizing antibody titers were measured in female 129S6 mice that received either 2 µg or 10 µg of the CHIKV C-E3-E2-6k-E1 mRNA vaccine administered via the IM route either once or twice. Higher CHIKV neutralizing antibody titers were observed with higher vaccine dose levels and with greater number of vaccinations. The resulting CHIKV-specific antibodies neutralized all 3 CHIKV strains tested (37997, LaReunion, and Centers for Disease Control Caribbean), representing all major CHIKV lineages.

The efficacy of lower doses of VAL-181388 against a lethal challenge of CHIKV 181/25 was studied in female AG129 mice that were vaccinated via the IM route in various combinations of number of vaccinations (1 or 2) and dose (0.4 µg, 2 µg, or 10 µg). Following a lethal challenge with CHIKV 181/25 at either Day 56 or Day 112, IM vaccination with VAL-181388 provided full protection for mice that received 2 µg or 10 µg VAL-181388, while mice that received 0.4 µg VAL-181388 had survival rates similar to those in mice that received the negative control phosphate-buffered saline (ie, all mice were deceased by Day 5 after CHIKV challenge). Serum samples were tested for reactivity in an enzyme-linked immunosorbent assay for mouse IgG against CHIKV structural glycoproteins E1 and E2 and CHIKV lysate. For the Day 56 serum samples, antibody titers were both dose- and schedule-dependent, with higher titers in mice vaccinated twice compared to those vaccinated once. For Day 112 serum samples, antibody titers were increased in mice that received either 2 µg or 10 µg VAL-181388. Chikungunya virus neutralizing antibody titers were also measured in Day 56 and Day 112 serum samples. VAL-181388 induced high titers of CHIKV-specific neutralizing antibodies for

all 3 CHIKV strains tested in vitro (37997, LaReunion, and Centers for Disease Control Caribbean) at Day 56 and for CHIKV strain 37997 at Day 112 (the only strain tested at Day 112), and the neutralizing antibody response was both dose- and schedule-dependent, with the highest titer observed in mice vaccinated with two 10 µg doses.

The efficacy of VAL-181388 vaccine against a lethal challenge of CHIKV 181/25 was demonstrated in female AG129 mice. Mice were vaccinated via the IM route twice (prime and boost) with either 2 µg or 10 µg of VAL-181388 or a phosphate-buffered saline control. Mice that received VAL-181388 were fully protected. Results of enzyme-linked immunosorbent assay analysis demonstrated that 2 vaccinations with either 2 µg or 10 µg VAL-181388-induced high antibody titers against CHIKV E1 and E2 glycoproteins and CHIKV lysate, with the highest titers against CHIKV lysate.

Together, these studies have shown that the VAL-181388 vaccine is effective in producing an anti-CHIKV immune response in mice that is robust, both dose- and schedule-dependent, and protective against CHIKV challenge.

In support of VAL-181388, the immunogenicity of similar mRNA-based influenza vaccines VAL-339851 (encoding hemagglutinin [HA] from the H7N9 influenza strain) and VAL-506440 (encoding HA from the H10N8 influenza strain) was assessed in cynomolgus monkeys to provide immunogenicity data in a larger animal species. Both vaccines utilize the same lipid nanoparticle (LNP) composition as VAL-181388 but contain different mRNA sequences. Vaccinations were given in various combinations of route of administration (ID or IM), number of vaccinations (1 or 2), and dose (0.2 mg or 0.4 mg). Anti-HA antibodies for VAL-339851 and VAL-506440 were detected starting on Day 15 for both routes of administration. The peak level for anti-HA antibodies against VAL-339851 was reached by Days 29 to 36. The peak level for anti-HA antibodies against VAL-506440 was reached by Days 29 to 36 for animals dosed via the ID route and by Days 36 to 43 for animals dosed via the IM route. Both the 0.2 mg and 0.4 mg doses achieved high levels of hemagglutination inhibition titers against H7 and H10 in cynomolgus monkeys, and ID administration was as effective as IM administration.

1.2.2 Distribution

Pharmacokinetic and drug metabolism studies are not normally performed for vaccines. However, because VAL-181388 is composed of an mRNA complexed with an LNP matrix, the

Sponsor has completed a distribution study with the similar mRNA-based influenza vaccine, VAL-506440 (encoding HA antigen from the H10N8 influenza strain), to assess the tissue distribution and persistence of the mRNA component.

VAL-181388 consists of an mRNA Drug Substance (CX-000314) coding for the structural polyprotein (capsid and envelope glycoproteins E3, E2, 6k, and E1) of CHIKV combined with LNP components. Ultimately, the distribution of the mRNA is driven by the LNP components encapsulating the mRNA. Thus, using the same matrix, with the same route of administration, distribution is anticipated to be identical for any comparably sized mRNAs. Unlike DNA, mRNA has a finite lifetime within cells (measured in hours), does not replicate or integrate, and is not anticipated to persist long-term following IM delivery. Hence, a single dose pharmacokinetic biodistribution study was performed in male mice with the similar mRNA-based influenza vaccine, VAL-506440, which uses the same LNP composition as VAL-181388.

Following a single IM dose in male mice, the peak plasma concentration of VAL-506440 was achieved at 2 hours after dosing and was followed by an apparent bi-exponential decline. The half-life of the mRNA was estimated at just under 10 hours, and the maximum concentration and area under the concentration versus time curve from the start of dose administration to the time after dosing at which the last quantifiable concentration was observed for VAL-506440 were 5470 pg/mL and 35,500 pg•h/mL, respectively.

VAL-506440 was broadly distributed into all analyzed tissues at 2 hours after dosing (bone marrow, brain, heart, kidney, liver, lung, lymph nodes [proximal and distal], gastrointestinal tract, stomach, spleen, testes, and the injection site), but with varying concentrations. When estimable, the half-life was variable among the tissues and ranged from 3.5 to 28.0 hours.

With the exception of the muscle injection site, VAL-506440 mRNA levels were the highest in the proximal lymph nodes, followed by the distal lymph nodes, the spleen, and the liver, whereas the lowest exposures were observed in the jejunum, the brain, the stomach, and the heart. The relatively low level of exposure in the brain suggests limited penetration of VAL-506440 across the blood-brain barrier.

Overall, only a relatively small fraction of the administered VAL-506440 mRNA distributed to distant tissues, and it did not persist notably past 1 to 3 days in tissues other than the injection

site, the spleen, the liver, and the lymph nodes, tissues noted for their potential to take up and retain exogenous DNA.

No absorption, metabolism, excretion, or drug-drug interaction studies have been performed.

1.2.3 Toxicology

1.2.3.1 Single-Dose Toxicity

No single-dose toxicity studies were performed with VAL-181388.

1.2.3.2 Repeat-Dose Toxicity

The Sponsor has completed a repeat-dose IM toxicology study with VAL-181388 in rats, which is composed of mRNA complexed with an LNP matrix. Additional genetic toxicology studies have examined the mutagenic potential of the novel lipid excipients PEG2000-DMG and MC3, and an in vivo genetic bone marrow mutagenicity study has been performed with the similar mRNA-based influenza vaccine, VAL-506440, in support of VAL-181388. Results of these supportive studies are contained in the IB.

The rat was chosen as the nonclinical species for the toxicity studies as it is an accepted species by the Organisation for Economic Co-operation and Development, the European Medicines Agency, and the US Food and Drug Administration (FDA) for the development of vaccines, and is a species with which the testing laboratories have extensive experience. The Sprague Dawley rat is considered a relevant species for safety evaluation with respect to desired and potential exaggerated immunological responses induced by vaccination with mRNA-based vaccines.

Toxicological evaluation of the novel excipients in the LNP matrix (namely MC3 and PEG2000-DMG) requires the selection of species that reliably predict the occurrence of complement activation. Complement activation is observed after intravenous (IV) administration of a variety of compounds including liposomal drugs, contrast agents, pegylated proteins, and antibodies (Szebeni 2014). The complement cascade is relatively conserved across species, and therefore the rat is likely to be sensitive to potential complement activation following administration of the LNP matrix. However, systemic complement activation is unlikely to be relevant for a product that is administered via the IM route where minimal systemic exposure is expected.

In a repeat-dose study in rats, VAL-181388 was administered via IM injection to groups of male and female Sprague Dawley rats at doses of 10, 40, or 160 µg/rat on Days 1, 22, and 43. Overall, VAL-181388 was clinically well tolerated up to 40 µg/dose, with more pronounced injection site reactions and systemic inflammatory responses observed at the 160 µg/dose. No mortality occurred during the course of the study.

There were no VAL-181388-related changes in food consumption or ophthalmology. Lower body weight gain was observed during dosing weeks while the gain was higher during the off-dose weeks resulting in no effect on the overall body weight. VAL-181388-related clinical signs were observed at the injection site/area and were noted at all dose levels. Hematologic and clinical chemistry changes were consistent with administration of an immunostimulatory vaccine.

Intramuscular dosing with VAL-181388 produced transient increases in inflammatory cytokines IP-10, IL-1β, and MCP-1α, which were consistent with localized administration of an immunostimulatory vaccine. With regard to the specific immune response to the vaccine, significant dose-dependent anti-CHIKV antibody responses were noted at all dose levels. Responses were higher on Day 71 when compared with Day 44 results for animals dosed at greater than or equal to the 40 µg/dose.

The majority of macroscopic and microscopic findings observed were at or adjacent to the primary injection sites (subcutaneous inflammation, muscle fiber degeneration/necrosis) with other changes attributed (spleen, thymus, bone marrow, adrenal glands) to test item-induced stress or compensatory changes in both microscopic and clinical pathology.

Most of the changes were either totally or partially resolved following a 4-week recovery period. However, increases in prothrombin time for females at the 160 µg/dose correlating with partial recovery of microscopic findings seen at the injection sites, inguinal and popliteal lymph nodes, and/or sciatic nerve were observed.

Based on these findings, the Sponsor has determined the no observed adverse effect level (NOAEL) to be 40 µg/dose for the IM route of administration in male and female rats.

1.2.4 Genotoxicity

The Sponsor has also evaluated the safety of the LNP composition of VAL-181388 in a series of genetic toxicology studies with the individual lipid components and in a study with the

similar mRNA-based influenza vaccine, VAL-506440, composed of an identical LNP composition as was used for VAL-181388. Neither the PEG2000-DMG nor the MC3 lipids produced any signs of genotoxicity or increased mutation frequency in either the Ames bacterial reverse mutation test or in a mammalian cell mutation study with human peripheral blood lymphocytes.

In the mouse micronucleus in vivo mutation assay, VAL-506440 produced some toxicity and mortality in mice following IV dosing at doses ranging from 1.5 to 10 mg/kg. It must be noted that this test is designed to use a maximum tolerated dose (MTD) level via the systemic IV route, and these higher doses were also associated with significant bone marrow toxicity. Based on the known properties of the lipids, these findings are likely attributable to a complement activation-related pseudoallergy (CARPA) response (Szebeni 2014) specific to IV systemic administration of LNPs such as those in both VAL-181388 and VAL-506440. The doses of VAL-506440 used in this in vivo genetic toxicology study represented the MTD levels, were several fold greater than the 10-µg doses typically administered IM or ID to mice, and are therefore anticipated to represent significantly greater systemic/plasma exposure than is expected via IM administration at the doses used in the Phase 1 clinical study with VAL-181388.

At these doses, increased incidence of micronucleated immature erythrocytes indicative of chromosome damage was observed. However, since these doses were also associated with mortalities and severe clinical signs, they may have far exceeded the limit of tolerance in this assay. No substantial increases in the incidence of micronucleated immature erythrocytes, or substantial decreases in the proportion of immature erythrocytes, were observed at any of the other VAL-506440 doses in males, nor in the females tested at the MTD of 1 mg/kg. Hence, despite notable toxicity at these extremely high doses evaluated, VAL-506440 was considered equivocal for the induction of chromosome damage in mouse immature erythrocytes. Given that the Sponsor's vaccines consist of mRNA complexed with LNPs, and LNPs have been shown to be associated with complement-mediated toxicity specifically following IV administration, these findings would appear to represent an artefactual response to high IV doses of LNPs.

Further details on all nonclinical research on VAL-181388 can be found in the IB.

1.3 Clinical Studies

No clinical studies have been conducted with VAL-181388. No reproductive or developmental toxicity studies have been completed to date with VAL-181388. Women of childbearing potential are included in this Phase 1 clinical study. They must agree to be heterosexually inactive from at least 21 days prior to enrollment and through 3 months after the final vaccination or agree to consistently use protocol-specified approved methods of contraception from at least 21 days prior to enrollment and through 3 months after the final vaccination.

VAL-181388 has not yet been administered to humans. Thus, information on possible risks and adverse reactions associated with IM administration of VAL-181388 is derived from:

- Animal studies with VAL-181388, LNP components, or the similar mRNA-based influenza vaccine, VAL-506440
- Published clinical data on other mRNA-based investigational medicinal products and products encapsulated in LNPs of similar composition as the LNP formulation of VAL-181388
- The Sponsor's recent experience with mRNA-based influenza vaccines, VAL-506440 and VAL-339851, using the same LNP matrix as VAL-181388

Subjects receiving VAL-181388 are likely to experience mild-to-moderate injection site reactions as observed in studies in animals and generally observed and expected for other IM-administered vaccines. These local reactions may consist of pain, swelling, and erythema, which are transient and can be dose dependent. Possible mild to moderate systemic reactions, which are also transient, include fever, fatigue, chills, headache, myalgias, and arthralgias. In addition, other adverse events (AEs) that have been generally associated with approved IM-administered vaccines have included mild hematological and clinical chemistry abnormalities, which are usually reversible.

Subjects receiving VAL-181388 could experience signs and symptoms compatible with a CARPA response, which has been observed in the administration of approved liposomal products, contrast agents, pegylated proteins, and antibodies (Szebeni 2014), as well as for small interfering RNA products formulated in LNPs (Coelho et al 2013; Fitzgerald et al 2014). The signs and symptoms of CARPA resemble those of an acute hypersensitivity reaction. These signs can also be seen preclinically in toxicology species. However, complement activation is

far less likely to be associated with clinical signs for LNP products such as VAL-181388 that are administered IM at a much lower dose on a mg/kg basis than other clinical entities that cause CARPA in humans and nonclinical species.

Moderna Therapeutics, Inc. currently has mRNA-based vaccines in 2 ongoing Phase 1 studies using different mRNAs but the same lipid composition as VAL-181388. The first is Study VAL-506440-P101 with VAL-506440 (encoding the HA antigen from the H10N8 influenza strain) taking place in Germany; the second is VAL-339851-P101 with VAL-339851 (encoding the HA antigen from the H7N9 influenza strain), taking place in the United States. As of August 2016, over 200 subjects have received at least 1 dose of 25, 50, 75, 100, or 400 µg, and over 150 subjects have received 2 doses of 25, 50, or 100 µg. Vaccination has been given by both IM and ID routes. Both studies remain blinded for safety and are ongoing. However, grouped observational review of the data is possible, and summaries of safety have been reviewed by study-specific independent safety review committees prior to dose expansion and dose escalation. The reports of these committees have been submitted to the respective regulatory authorities, the Paul Ehrlich Institute in Germany, and the FDA. No vaccine-related serious adverse events (SAEs) have been observed and all AEs have resolved without intervention. The most common AEs have been local reactions of pain, redness, and occasional induration, and mild to moderate non-specific systemic AEs such as headache and malaise. The majority of these events are mild or moderate in grade and the frequency and severity of these expected AEs appears to be dose related. In study VAL-339851-P101 (H7N9), one subject had asymptomatic severe (Grade 3) elevation of ALT and AST which resolved. Minimal to mild elevations in LFTs have been observed in additional subjects without a consistent trend. Based on preliminary data, the safety experience to date can be summarized as follows:

- There have been no unexpected clinically significant AEs or persistent AEs observed, with the exception of a single case of reversible and asymptomatic Grade 3 elevation of transaminases.

- Local and systemic events are dose related and comparable in frequency and nature to adjuvanted non-live vaccines.

- While these studies are still enrolling subjects as of November 2016, the safety profile of IM doses of VAL-506440 from 25 to 100 µg (prime and boost) and for VAL-339851 from 25 to 50 µg appears tolerable.

1.4 Rationale for Study

There are currently no effective therapies or approved vaccines to treat or prevent chikungunya, and effective mosquito control has proven challenging, even in higher income countries. Mosquito nets have limited effectiveness against the daytime-biting *Aedes* mosquito. Therefore, there is a need for a safe and effective prophylactic vaccine.

Of the new approaches currently in development, virus-like particle vaccines and live-attenuated vaccines show the greatest potential from preclinical data (Wang et al 2008; Akahata et al 2010; Darwin et al 2011; Brandler et al 2013). However, there are significant potential drawbacks with these 2 vaccine approaches. The virus-like particle-based vaccines are typically challenging to manufacture, particularly within the context of a disease of greatest relevance to developing countries where it is desirable to keep the technology simple and cost of goods low. The live-attenuated vaccines, on the other hand, carry the well-known safety concerns normally associated with this type of technology (potential of break-through infections and possible central nervous system sequelae, depending on the chosen virus backbone). Based on encouraging preclinical data with VAL-181388 and other similar mRNA-based vaccine candidates in the development pipeline, the Sponsor believes that VAL-181388 has the potential to be a tolerable and effective prophylactic vaccine against CHIKV.

The purpose of the VAL-181388-P101 study is to evaluate the safety and immunogenicity of VAL-181388 in healthy adult subjects in a non-endemic chikungunya region. The primary objective is to characterize the safety profile of VAL-181388 versus placebo. The study will also evaluate immunogenicity by assessing serum neutralizing antibody titers and serum binding antibody titers to CHIKV proteins.

1.5 Rationale for Dose Selection

The clinical safety and efficacy of VAL-181388 have yet to be determined. The proposed dose levels of VAL-181388 to be evaluated in this study (25, 50, and 100 µg) are based on several data sources, including nonclinical studies (Section 1.2) using VAL-181388 and the similar mRNA-based influenza vaccine VAL-506440, and ongoing Phase 1 clinical studies with the

Sponsor's other mRNA-based vaccines. The proposed clinical starting dose of 25 μg provides a very large safety margin (285-fold for a human subject weighing 70 kg) over the NOAEL in rats (102.6 μg/kg, based upon a mean body weight of 390 g) and yet still has the potential to elicit a meaningful immune response as demonstrated by nonclinical studies completed in mice and rats. The highest dose proposed for administration, 100 μg, is expected to provide a safety margin of approximately 72-fold over the NOAEL in rats.

1.6 Rationale for Study Design

This study is designed to evaluate the initial safety and immunological outcomes of VAL-181388 for future clinical studies. Since the study will be conducted in non-endemic chikungunya region(s), where subjects are expected to be immunologically naive to the CHIKV components of VAL-181388, it is anticipated that a 2-dose schedule will be required to demonstrate an adequate immune response. To ensure subject safety, dose level cohorts will be sequential in nature, starting with the lowest dose and requiring review by the SMC to advance to the next dose level cohort. For each cohort, a sentinel safety group of 4 subjects will be enrolled who will be randomly assigned to VAL-181388 or placebo (3:1) and followed for 7 days after the first vaccination, with review of reactogenicity and safety laboratory results, prior to randomizing the remainder of the cohort. In addition, pause rules have been pre-established to trigger ad hoc SMC reviews, if required, and an internal safety team (IST) will review (blinded review) clinically significant safety laboratory test results, vital sign measurements, reactogenicity, and any AEs (all with toxicity scoring/grading) during the active vaccination phase of the trial. Immunogenicity will be assessed prior to each vaccination, and at 28 days and 6 and 12 months after the final vaccination. This allows assessment after the first and second vaccination, determination of best dose response, and duration of response through 1 year.

2 Study Objectives

2.1 Primary Objective

The primary objective of this study is to assess the safety of VAL-181388 relative to placebo.

2.2 Secondary Objectives

The secondary objective of the study is to determine the immunogenicity of 3 dose levels of VAL-181388 to inform the choice of dose for further development of this vaccine. Immunogenicity assessment will be based on changes from baseline in the following:

- Serum neutralizing antibody titers to CHIKV
- Serum binding antibody titers to CHIKV-specific proteins

2.3 Exploratory Objectives

The exploratory objectives of the study are to:

- Assess the cross-reactivity of serum antibodies to related viruses, including alphaviruses and flaviviruses
- Explore other assays to characterize the immune response to CHIKV

3 Investigational Plan

3.1 Study Design

This is a Phase 1, first-in-human, randomized, observer-blinded, placebo-controlled, dose escalation study to evaluate the safety and immunogenicity of 3 dose levels of VAL-181388 in healthy adult subjects (18 to 49 years of age, inclusive). VAL-181388 is an mRNA-based vaccine being developed for prevention of disease associated with CHIKV infection.

Chikungunya virus is a positive-sense, single-stranded RNA virus, of the alphavirus family, in the genus of the togavirus family. It can cause disease in humans and other mammals. It is an arbovirus that is transmitted by a mosquito vector (*Aedes spp*). Currently there is no approved vaccine to protect against this disease, which has recently spread through tropical regions.

This is a 2-part study, with Part A including dose escalation and safety and immune testing through 28 days following the final vaccination. Once subjects complete Part A they will transition to Part B. Part B is a continued safety follow-up through 12 months and an assessment of immunogenicity and immune persistence at approximately 6 and 12 months after final vaccination.

Part A

In Part A, sequential dose escalation of VAL-181388 is planned in 3 dose level cohorts (25, 50, and 100 μg) with each subject receiving 2 vaccinations separated by 28 days. Subjects will be randomly assigned to receive either VAL-181388 or placebo.

For each cohort, a sentinel safety group of 4 subjects will be enrolled who will be randomly assigned to VAL-181388 or placebo (3:1) and followed for 7 days after the first vaccination with review of reactogenicity and safety laboratory results prior to randomizing the remainder of the cohort. An IST will oversee the safety of the trial and will review blinded safety data during Part A of the study to ensure adherence to the protocol, monitor safety laboratory test results and reactogenicity, and raise concerns to the SMC should there be safety issues or operational challenges that could affect subject safety. Only after review by the IST of the blinded safety data (reactogenicity, safety laboratory results, and AEs) through 7 days following the first vaccination of the sentinel safety lead-in for each cohort planned, will approval be given to allow randomization of the remainder of that cohort. The SMC will approve escalation to the next higher dosing cohort after review of blinded safety data of the

currently dosed cohort through 7 days following the second vaccination and any cumulative safety data of all cohorts as the trial advances. The SMC will also be convened (ad hoc meeting) if a pause rule is triggered.

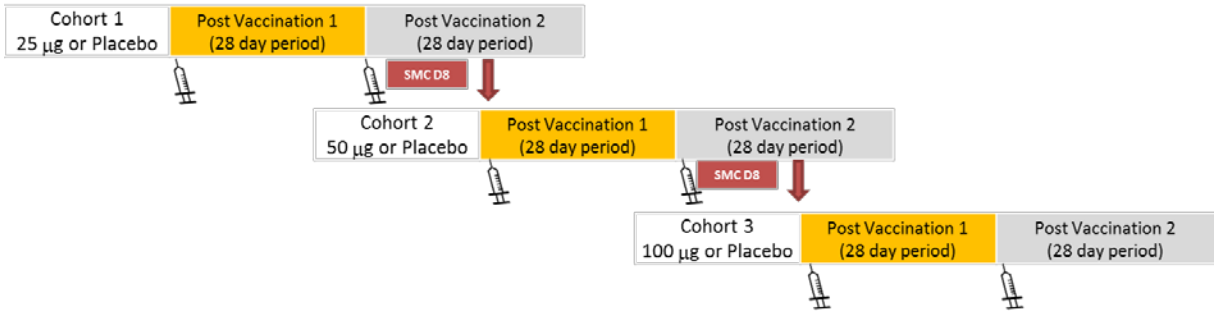
Dose Level Cohort Assignments: VAL-181388 (or placebo) administered IM at Visit 1 and a second dose administered 28 days later (Visit 4).

Cohort 1 (N = 20): 25 µg VAL-181388 or placebo (ratio = 3:1)

Cohort 2 (N = 20): 50 µg VAL-181388 or placebo (ratio = 3:1)

Cohort 3 (N = 20): 100 µg VAL-181388 or placebo (ratio = 3:1)

Vaccine Administration and Dose Escalation Schema



VAL-181388 (or placebo) will be administered as an IM injection (0.5 mL) into the deltoid muscle, preferably in the non-dominant arm, as a 2-dose schedule; the first dose will be given at Visit 1 followed by a second dose 28 days later. VAL-181388 accountability, dose preparation, and administration will be performed by unblinded pharmacy personnel or unblinded designees who will not participate in any other aspect of the study. The remainder of the site staff and all subjects will remain blinded to treatment assignment.

Consent and Screening will occur over a 28-day period prior to randomization. A total of 20 subjects will be randomly assigned to each dose level cohort, 4 subjects (3 VAL-181388 and 1 placebo) to the sentinel safety group and 16 subjects (12 VAL-181388 and 4 placebo) to the expansion group, resulting in 15 subjects being randomly assigned to VAL-181388 and 5 subjects being randomly assigned to placebo (3:1 ratio overall). Subjects meeting all eligibility criteria will receive the first vaccination at Visit 1. Each subject will be monitored in the clinic for at least 1 hour following each vaccination for safety. Assessments will include

vital sign measurements and any immediate reactogenicity. Of note, on the day of vaccination, if a subject is noted to have a systolic or diastolic blood pressure, heart rate, or respiratory rate measurement that shows Grade 2 or higher toxicity after 2 measurements (even after relaxing or resting); has an acute illness; or has an oral temperature >38°C, the subject should not receive a vaccination on that day and will need to return on subsequent days for their vaccination.

All subjects will return to the clinic at 7 (+3), 17 (±3), and 28 (+7) days following each vaccination. In addition, the sentinel safety group will return to the clinic at 1, 2, and 21 (+3) days following the first vaccination. A second vaccination will occur 28 days after the first vaccination (+7 days).

Subjects will be instructed on recording solicited (local and systemic reactogenicity events) and unsolicited AEs, temperature, and medications (prescription or over-the-counter) on their memory aid (ie, diary card). Subjects will also be asked to call/return to the clinic within 24 hours if reactogenicity reaches Grade 3 or higher during the first 7 days following vaccination. As standard practice, a reminder call will be made to the subject by the site at least once during the first 7 days following each vaccination to answer any questions and ensure that the memory aid is being completed correctly and consistently.

Safety assessments will include toxicity grading of solicited (local and systemic reactogenicity events) and unsolicited AEs, vital sign measurements, physical examination findings, and clinical laboratory test results for hematology, serum chemistry, coagulation, and urinalysis. All unsolicited AEs will be collected and additional specific categories will include SAEs; AEs of special interest (AESIs); and medically attended AEs. Subjects will self-report (using a memory aid) solicited (local and systemic reactogenicity events) and unsolicited AEs and medication usage (over-the-counter and prescription) through 28 days after each vaccination. In addition, subjects will be instructed to measure their oral temperature daily for the first 7 days post vaccination and record that measure on the memory aid. At applicable clinic visits, the investigator will review the entries on the memory aid with the subject to ensure consistency.

Safety laboratory tests will be performed at Screening, on vaccination days (before dosing), and at 7 (+3) days after each vaccination. At 17 (±3) days following each vaccination and at 28 (+7) days after the second vaccination, safety laboratory tests will be performed for coagulation and liver function tests only. The sentinel safety group will also have coagulation

and liver function tests collected at 21 (+3) days after the first vaccination. Additional safety laboratory tests may be conducted at any time by the investigator if deemed necessary.

Blood samples for immunogenicity will be collected on dosing days (pre-dose) and at 28 (+7) days after the final vaccination.

Subjects unable to complete their second vaccination due to noncompliance with the visit schedule and not due to a cohort pause will still be required to follow the original visit and testing schedule. Specifically, these subjects will be asked to undergo blood collection to comply with a second immune test at 56 (+10) days (Visit 7) following the first vaccination.

To allow cohort advancement, the following data from the highest dosed cohort are expected to be reviewed by the SMC: 1) toxicity scoring of safety laboratory test results through 7 days following the second vaccination (through Visit 5); 2) toxicity scoring of vital signs and reactogenicity through 7 days following the first and second vaccination (through Visit 5); 3) all unsolicited AEs (with grading and attribution). Data will be prepared by dose cohort and timing relative to each vaccination. As safety data on all cohorts accumulate, the SMC also review ongoing AEs as well as cumulative safety laboratory test results, reactogenicity, and AEs across vaccine doses for subjects remaining in Part A of the study, thereby providing a cumulative and comprehensive review of safety. Should a cohort pause occur due to pre-specified criteria, the SMC will be convened for an unscheduled (ad hoc) meeting and be provided with specific safety data related to the trigger of the pause.

Part A is concluded for each subject when they return to the clinic for Visit 7 (28 days after final vaccination). At that time, subjects will transition to Part B of the study.

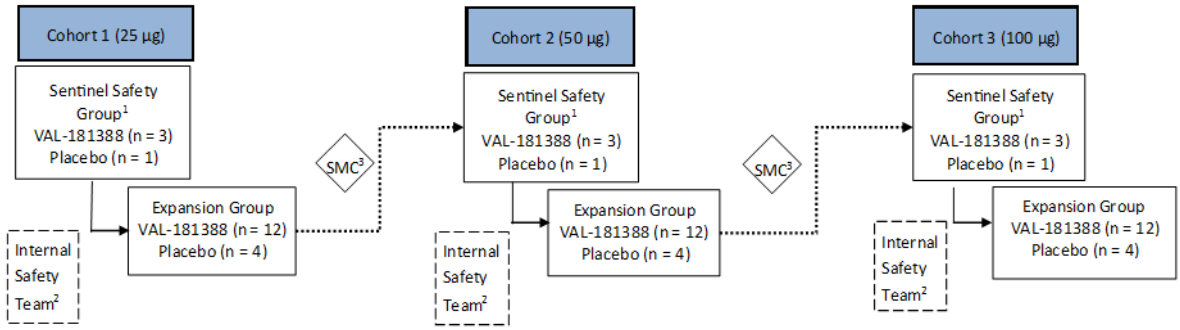
The overview of study visit, safety, and immune assessments is presented in Table 3-1 and the dosing schematic is presented in Figure 3-1.

Table 3-1 Part A: Overview of Visits

Study visit	0	1	1a[a]	1b[a]	2	3	3a[a]	4	5	6	7
Vaccination day		X						X			
Days relative to most recent vaccination	NA	0	1	2	7	17	21	28	7	17	28
Window allowance	+28	0	0	0	+3	±3	+3	+7	+3	±3	+7
Screening	X										
Physical examination[b]	X	X[c]			X		X	X[c]	X		X
Safety laboratory tests	X	X[c]			X	X	X	X[c]	X	X	X
Vital sign measurements	X	X[d]	X	X	X		X	X[d]	X		X
Immune laboratory tests		X[c]						X[c]			X
Study drug administration[e]		X						X			
Review of concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Review of safety (solicited and unsolicited)		X[d]	X	X	X	X	X	X[d]	X	X	X

a. Applies to sentinel safety group only.
b. Symptom-directed after Screening.
c. Obtained before study drug administration.
d. Obtained before and after study drug administration.
e. Vaccination cannot occur if systolic or diastolic blood pressure, heart rate, or respiratory rate measurements show Grade 2 or higher toxicity after 2 measurements; if a subject has an acute illness; or if a subject has an oral temperature >38°C.

Figure 3-1 Dose Level Schematic



Abbreviation: SMC, Safety Monitoring Committee.

1. A sentinel safety group of 4 subjects in each cohort will receive VAL-181388 or placebo (3:1) on Day 1.
2. Review of safety subject data by the internal safety team (IST) through at least 7 days after first vaccination (inclusive of reactogenicity, laboratory testing results, and adverse events) is required before the full cohort can be randomized.
3. Safety assessment for dose escalation by the Safety Monitoring Committee (SMC) will occur when all subjects in a dosing cohort have completed their 7-day post second vaccination visit (inclusive of reactogenicity, adverse events, and laboratory results) and will include safety data accumulation through the entire period for that cohort and all previous dosing cohorts.
4. The IST may perform additional reviews of blinded safety data during the active vaccination phase of the study, and will raise concerns to the SMC if there are findings that could affect subject safety.

Part B

To monitor for longer-term safety and immune persistence, each subject will be entered into a continued blinded follow-up period (Part B). This period will be conducted such that subjects and safety monitors will remain blinded to treatment assignment. Part B of the study is initiated for a subject once they have returned for Visit 7 (28 days after the second vaccination).

Once entered into Part B, each safety contact will occur by telemedicine (eg, telephone, text message, internet) every 28 (±7) days, and blood samples for immune persistence will be collected from each subject on Visit 12 (±15) and Visit 19 (+15) of the study. Each safety contact will capture outcomes of any AESI or SAE that remains unresolved since the last visit or is newly identified through scripted query.

The telemedicine visits may require additional data through medically attended visits, in addition to medications and vaccinations taken by the subject during this time. Subjects will have consented during study enrollment to allow access to additional medical records needed to complete Part B, thereby allowing the blinding of the treatment assignment to be maintained.

The overview of study visit, safety, and immune assessments is presented in Table 3-2.

Table 3-2 Part B: Overview of Visits

Study visit	8	9	10	11	12	13	14	15	16	17	18	19
Study day from 1st dose, if 2nd dose not completed	84	112	140	168	196	224	252	280	308	336	364	392
Study day from 2nd dose, if completed	56	84	112	140	168	196	224	252	280	308	336	364
Window allowance	±7	±7	±7	±7	±15	±7	±7	±7	±7	±7	±7	+15
Safety contact	X	X	X	X		X	X	X	X	X	X	
Immune samples					X							X
Serious adverse events	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events of special interest	X	X	X	X	X	X	X	X	X	X	X	X
End of Study												X

When all subjects have completed their final contact (approximately 12 months after their last vaccination), immune testing is completed, and all queries resolved, the database will be locked and analyzed and a final clinical study report will be provided to regulators.

3.2 Selection of Study Population

Healthy male or female subjects will be enrolled in the US (1 center). Approximately 60 subjects are planned to be randomized.

3.2.1 Inclusion Criteria

Each subject must meet all of the following criteria during the screening period to be enrolled in this study:

1. The subject is male or female between 18 and 49 years of age, inclusive.

2. The subject has a body mass index between 18 and 35 kg/m^2, inclusive.

3. The subject is considered by the investigator to be in good general health as determined by medical history, clinical laboratory assessments, vital sign measurements, and physical examination findings at Screening.

4. Female subjects must be non-pregnant and non-lactating and meet one of the following criteria: A) post-menopausal (defined as amenorrhea for 12 consecutive months without an alternative medical cause or documented serum follicle-stimulating hormone level in the post-menopausal range); B) surgically sterile (ie, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy). NOTE: procedures and laboratory test results must be confirmed in the medical record, by physical examination, or by official written confirmation of a procedure; or C) if of childbearing potential, agrees to be heterosexually inactive from at least 21 days prior to enrollment and through 3 months after the final vaccination or agrees to consistently use any of the following methods of contraception from at least 21 days prior to enrollment and through 3 months after the final vaccination: condoms (male or female) with spermicide, diaphragm with spermicide, cervical cap with spermicide, intrauterine device, oral or patch contraceptives, Norplant®, Depo-Provera®, or other FDA-approved contraceptive method which is designed to protect against pregnancy. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

5. Male subjects must agree to consistently use appropriate contraception and to refrain from sperm donation through 3 months after the final vaccination.

6. The subject understands and agrees to comply with the study procedures and provides written informed consent before any study procedures are performed.

7. The subject has access to a consistent and reliable means of telephone contact, which may be in the home, workplace, or by personal mobile electronic device.

8. The subject agrees to stay in contact with the study site for the duration of the study, to provide updated contact information as necessary, and has no current plans to move from the study area for the duration of the study.

3.2.2 Exclusion Criteria

Subjects meeting any of the following criteria will be excluded from the study:

1. The subject has any ongoing significant chronic illness requiring medical or surgical care. Asymptomatic conditions or findings (eg, mild hypertension, dyslipidemia) that are not associated with evidence of end-organ damage are not exclusionary provided that they are being appropriately managed and are clinically stable in the opinion of the investigator (ie, unlikely to result in symptomatic illness within the time-course of this study).
2. The subject is a female of childbearing potential and has a positive pregnancy test at Screening or on the day of vaccination.
3. The subject has any abnormal screening laboratory result meeting the criteria listed below.
    a. Elevated liver function tests (aspartate aminotransferase, alanine aminotransferase, total or direct bilirubin, or alkaline phosphatase), elevated creatinine, or reduced platelets with a toxicity score ≥ Grade 1 at Screening. Re-testing of these parameters is not allowed.
    b. Other than a) above, any safety laboratory test result (urine or serum) with:
        i. a toxicity score ≥ Grade 2
        ii. a toxicity score of ≥ Grade 1 deemed clinically significant
4. The subject has participated in another investigational study involving any investigational product (ie, study drug, biologic, device) within 60 days, or 5 half-lives, whichever is longer, before planned date of first vaccination.

5. The subject has received any live attenuated or inactive vaccines within 4 weeks prior to enrollment, or plans to receive any vaccine during the active vaccination period (through 4 weeks after their last planned vaccination).
6. The subject has received (at any time) a vaccine for CHIKV, dengue, Yellow Fever, tick-borne encephalitis, or Japanese encephalitis.
7. The subject has a history of confirmed or suspected CHIKV infection, has lived in a CHIKV-endemic area for >1 year, or has cumulatively spent more than 30 days in a CHIKV-endemic area within the last 5 years.
8. The subject has reported previously participating in an investigational study involving LNPs.
9. The subject has a history of hypersensitivity or serious reactions (eg, anaphylaxis, urticaria, other significant reaction) to previous vaccinations.
10. The subject has any known or suspected autoimmune disease or immunosuppressive condition, acquired or congenital, as determined by medical history and/or physical examination.
11. The subject has a history of arthritis (including inflammatory arthritis) or arthralgia.
12. The subject has a neurologic disorder (eg, history of seizures, Guillain-Barre syndrome, dementia, vasculitis, or any known congenital or acquired disorder).
13. The subject received immunoglobulins and/or any blood products within the 3 months preceding the administration of the study drug or plans to receive such products at any time during the study.
14. The subject has had chronic administration (defined as more than 14 continuous days) of an immunosuppressant or other immune-modifying drug within 6 months prior to vaccine administration or plans to receive any products during the active vaccination period (through 4 weeks after their last planned vaccination). An immunosuppressant dose of a glucocorticoid will be defined as a systemic dose of ≥10 mg of prednisone per day or equivalent. The use of topical, inhaled, and nasal glucocorticoids will be permitted.
15. The subject is currently receiving antipyretic or analgesic medication on a daily or every other day basis (a daily dose of ≤100 mg of aspirin given under the guidance of a physician is not a contraindication to enrollment).
16. The subject has any acute illness at the time of enrollment (defined as the presence of a moderate or severe illness with or without fever, or an oral temperature >38.0°C on the planned day of vaccination). In such cases, subjects may be re-evaluated during the

screening period for resolution of the illness to allow at least 3 days of wellness prior to the planned vaccination.

17. The subject has any significant disorder of coagulation (acquired or hereditary) requiring ongoing or intermittent treatment. Subjects receiving prophylactic antiplatelet medications, eg, low-dose acetylsalicylic acid (≤100 mg/day or equivalent), and without clinically apparent bleeding tendency, are eligible.
18. The subject has a history of idiopathic urticaria.
19. The subject has a history of alcohol abuse or drug addiction within 1 year before the planned day of dose administration (self-reported).
20. The subject has a positive test result for drugs of abuse at Screening.
21. The subject has any abnormality or permanent body art (eg, tattoo) that would obstruct the ability to observe local reactions at the injection site (deltoid region).
22. The subject has any condition that, in the opinion of the investigator, would pose a health risk to the subject if enrolled or could interfere with evaluation of the study drug or interpretation of study results (including neurologic or psychiatric conditions deemed likely to impair the quality of safety reporting).
23. The subject has a positive test result for hepatitis B surface antigen, hepatitis C virus antibody, or human immunodeficiency virus types 1 or 2 antibodies.
24. The subject has a history of active cancer (malignancy) in the last 10 years. An exception is a subject with adequately treated nonmelanomatous skin carcinoma, who may participate in the study.
25. The subject has donated blood or blood products >450 mL within 30 days of dosing.
26. The subject has any screening vital sign measurement (systolic or diastolic blood pressure, heart rate, or respiratory rate) that shows greater than or equal to Grade 2 toxicity after 2 measurements.
27. The subject is an employee or first-degree relative of the Sponsor, PPD, or study site personnel.

3.2.3 Subject Restrictions During the Study

3.2.3.1 General and Dietary

To avoid false positive drugs of abuse screening results, no food or drink containing poppy seeds (eg, specialty breads and muffins) will be allowed for 72 hours before the screening visit.

3.2.3.2 Contraception and Pregnancy Avoidance Procedures

Female subjects must be post-menopausal (defined as amenorrhea for 12 consecutive months without an alternative medical cause or documented serum follicle-stimulating hormone level in the post-menopausal range) or surgically sterile (ie, hysterectomy, bilateral tubal ligation, or bilateral oophorectomy). NOTE: procedures and laboratory test results must be confirmed in the medical record, by physical examination, or by official written confirmation of a procedure; or, if of childbearing potential, practicing a medically approved and highly effective method of contraception (defined as those which result in a low failure rate [ie, less than 1% per year] when used consistently and correctly).

All female subjects of childbearing potential must have a negative pregnancy test at Screening and before dosing with study drug. Women of childbearing potential must agree to be heterosexually inactive from at least 21 days prior to enrollment and through 3 months after the final vaccination or agree to consistently use any of the following methods of contraception from at least 21 days prior to enrollment and through 3 months after the final vaccination: condoms (male or female) with spermicide, diaphragm with spermicide, cervical cap with spermicide, intrauterine device, oral or patch contraceptives, Norplant®, Depo-Provera®, or other FDA-approved contraceptive method which is designed to protect against pregnancy. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Male subjects must agree to consistently use appropriate contraception and to refrain from sperm donation through 3 months after the final vaccination.

Subjects will be provided with information on acceptable methods of contraception as part of the subject informed consent process and will be asked to sign a consent form stating that they understand the requirements for avoidance of pregnancy.

3.3 Withdrawal of Subjects From the Study

3.3.1 Reasons for Withdrawal

Subjects can withdraw consent and discontinue from the study at any time, for any reason, without prejudice to further treatment.

The investigator can also withdraw a subject upon the request of Moderna Therapeutics, Inc. or if Moderna Therapeutics, Inc. terminates the study. Upon occurrence of a serious or intolerable AE, the investigator will confer with the Sponsor or designee. If a subject is discontinued because of an AE, the event will be followed until it is resolved or until stable. In the event of a safety concern that results in withdrawal from further vaccination, the subject will be continued in the trial for safety evaluations.

Every reasonable attempt will be made to follow withdrawn subjects for safety. The reason for subject withdrawal will be documented.

The investigator, in consultation with the Sponsor's medical monitor, **may** withdraw a subject from further vaccination if the subject experiences any of the following:

1. Becomes pregnant;

2. Develops, during the course of the study, symptoms or conditions listed in the exclusion criteria;

3. Experiences an AE (other than reactogenicity) after vaccination that is considered by the investigator to be related to treatment and is of Grade 3 (severe) or higher severity;

4. Experiences an AE or SAE that, in the judgment of the investigator, requires study drug withdrawal due to its nature, severity, or required treatment, regardless of the causal relationship to treatment;

5. Experiences a clinically significant change in clinical laboratory test results, vital signs, or general condition that, in the judgment of the investigator, requires treatment withdrawal;

6. Experiences anaphylaxis clearly attributed to study vaccine; or

7. Experiences generalized urticaria related to the study vaccine.

The reason for withdrawing from further vaccination will be recorded. In addition, if a subject refused further vaccination they will be continued in the trial with all planned visits and assessments. The recording of the reason for vaccination refusal will be documented.

3.3.2 Handling of Withdrawals

When a subject withdraws from the study, the reason(s) for withdrawal will be recorded by the investigator on the relevant page of the electronic case report form (eCRF). These subjects will also be requested to complete all Visit 7 assessments. Any subject who fails to return for final assessments will be contacted by the site with a minimum of 3 telephone call attempts, followed by a certified letter.

Lost to follow-up is defined by the inability to reach the subject after a minimum of 3 documented telephone calls, faxes, text messages, or emails as well as lack of response by subject to 1 registered mail letter. All attempts should be documented in the subject's medical records.

3.3.3 Replacements

If a subject is withdrawn, who is significantly outside the allowed vaccination window, or is lost to follow-up from the study, the decision on whether or not to replace the subject will be made by the Sponsor in conjunction with the principal investigator.

3.4 Study Treatments

3.4.1 Method of Assigning Subjects to Treatment Groups

Subjects will be randomly assigned in a sequential manner to a dose level cohort after all entry criteria have been satisfied on Visit 1. A randomization table will be pre-generated, and only the unblinded pharmacy personnel will have controlled access. Within each dose level cohort, 20 subjects will be randomly assigned to receive either VAL-181388 (25, 50, or 100 µg) or placebo in an overall ratio of 3:1. The first 4 subjects within each dose level cohort will be randomly assigned to receive VAL-181388 (25, 50, or 100 µg) or placebo in a 3:1 ratio. Only after review by the IST of the blinded safety data (reactogenicity, safety laboratory results, and AEs) through 7 days following the first vaccination of the sentinel safety lead-in for each cohort planned, will approval be given to allow randomization of the remainder of that cohort. The remaining 16 subjects within each dose level cohort will be randomly assigned to receive either VAL-181388 (25, 50, or 100 µg) or placebo in a 3:1 ratio.

3.4.2 Treatments Administered

Up to 60 subjects will receive 1 of the following treatments based on the treatment to which they are randomly assigned. Dose level cohorts will advance in a sequential manner after review of all safety data through 7 days after the final vaccination of the previous dose level cohort by the SMC (inclusive of reactogenicity, safety laboratory test results, and AEs). All vaccines will be administered as a 2-injection schedule (28 days apart), into the deltoid muscle (IM), preferably in the non-dominant arm:

- Cohort 1 (N = 20): 25 µg VAL-181388 or placebo (ratio = 3:1)
- Cohort 2 (N = 20): 50 µg VAL-181388 or placebo (ratio = 3:1)
- Cohort 3 (N = 20): 100 µg VAL-181388 or placebo (ratio = 3:1)

The clinic will be appropriately staffed, trained on emergency resuscitation, and have stocked available rescue medications (such as epinephrine, steroids, antihistamines, and IV fluids) should any severe reaction (eg, anaphylactoid or profound urticaria) occur which requires immediate intervention.

3.4.2.1 Dose Escalation

Internal Safety Team

An IST will oversee the safety of the trial and will review blinded safety data during Part A of the study to ensure adherence to the protocol, monitor safety laboratory test results and reactogenicity, and raise concerns to the SMC should there be safety issues or operational challenges that could affect subject safety. Pause rules have been pre-established to trigger ad-hoc SMC reviews, if required (Section 3.4.2.2), and the IST will review (blinded review) safety laboratory test results, vital sign measurements, reactogenicity, and any AEs (all with toxicity grading) during the active vaccination phase of the trial. In addition, recruitment rates, timing of vaccination sequence, and window adherence will be reported to the IST. The chairperson of the IST may request that additional data be provided to the SMC that relates to the safety of subjects (eg, study conduct, quality issues, or safety concerns).

The 4 subjects in the sentinel safety group will receive VAL-181388 or placebo at Visit 1. The IST will review blinded safety data through at least 7 days after vaccination, inclusive of

reactogenicity, laboratory results, and any AEs from all subjects in the sentinel safety group. The IST is responsible for opening randomization to the remainder of the dose level cohort for that dose level after review of the safety data.

At minimum, the IST will be composed of the Sponsor's study medical monitor lead, the contract research organization's medical monitor, and the principal investigator. The pharmacovigilance officer will oversee safety aspects of the study and will participate as needed. Additional ad hoc members may be included as needed.

Safety Monitoring Committee

Dose escalation will proceed following review of blinded safety data by the SMC of the entire dose level cohort through 7 days following the second vaccination. In addition, the SMC will review prior dose level cohort reactogenicity and accumulated AEs at the time of the scheduled safety review to allow the SMC an aggregate view of overall safety. The SMC safety data assessment will include the following blinded summary data: 1) toxicity scoring of safety laboratory test results through 7 days following the second vaccination (through Visit 5); 2) toxicity scoring of vital signs and reactogenicity through 7 days following the first and second vaccination (through Visit 5); 3) all unsolicited AEs (with grading and attribution). Data will be prepared by dose cohort and timing relative to each vaccination. As safety data on all cohorts accumulate, the SMC also review ongoing AEs as well as cumulative safety laboratory test results, reactogenicity, and AEs across vaccine doses for subjects remaining in Part A of the study, thereby providing a cumulative and comprehensive review of safety. Should a cohort pause occur due to pre-specified criteria, the SMC will be convened for an unscheduled (ad hoc) meeting and be provided with specific safety data related to the trigger of the pause. If no pause rules (Section 3.4.2.2) are triggered or any other safety concerns are identified, the SMC may recommend escalation to the next dose level.

In addition, the Sponsor will submit available safety data for the 25 and 50 µg dose cohorts and recommendations of the SMC to the Center for Biologics Evaluation and Research (CBER) prior to initiation of the 100 µg cohort.

The SMC may recommend advancement to the next dose level, request additional information prior to providing a recommendation, recommend stopping the study, recommend changes to study conduct and/or the protocol, or recommend additional operational considerations due to safety issues that arise during study conduct. The Sponsor has the authority to accept (or

amend) the SMC recommendations, to stop the study at any time, to request a cohort pause for safety concerns (to be addressed by the SMC), and to request additional consultation during study conduct for either safety concerns or operational issues. The SMC will also be convened should a pause rule (Section 3.4.2.2) be triggered or at the request of the IST, the investigator, or the Sponsor.

The SMC will be comprised of 3 voting and independent members who are medical doctors familiar with Phase 1 clinical trial and safety review and independent from the trial. In addition, a biostatistician not associated with the trial will prepare the safety data (non-voting member), and a coordinator not associated with the trial will organize and record decisions of the SMC (non-voting member). The SMC may decide to include a non-medical person to weigh in on ethical issues if the need arises.

3.4.2.2 Pause Rules

During the active vaccination period, if any of the following events occur and are assessed to be potentially VAL-181388 related, the study will be paused for further enrollment and vaccination and an unscheduled SMC will be convened to assess specific data concerns and make recommendations:

- Two or more severe reactogenicity events of the same nature (whether systemic or local) within a dose cohort following any single (eg, the first, the second) vaccination
- Two or more Grade 3 (or greater) laboratory toxicities within a cohort and of the same nature
- One or more SAEs assessed as vaccine related, including any systemic hypersensitivity reaction

An unscheduled SMC may also be convened (with vaccination pausing if deemed necessary), by the investigator, IST, or Sponsor, if any other significant safety or tolerability issues are identified in the comprehensive review of available data that warrant further evaluation before additional subjects are dosed. This may include emerging nonclinical data, clinically relevant AEs, or relevant data from other sources indicating safety concerns even if the event(s) per se does not meet the criteria specified in this section.

Should a pause be triggered in the study, then each subject's visits will continue until the next scheduled vaccination visit. Should a pause affect a subject's vaccination visit, then the window for that subject's vaccination visit will be suspended until the pause is lifted and vaccination can resume. Once the pause is lifted at the site, vaccination should be reinstated as soon as possible. Visits should thereafter be scheduled as if they had received their second vaccination within the 28 (+7) day time window.

Should a pause be prolonged such that the vaccination sequence cannot establish a 28 (+ 7) day time window, the Sponsor has the option to add additional subjects in a dosing cohort to achieve the original number of subjects planned to be dosed within the 28 (+7) day time window. If a subject is in the screening period during this pause and more than 28 days has transpired, they may be rescreened for study eligibility as long as they continue to provide consent to participate in the study.

The objectives of the study are to characterize safety, tolerability, and immunogenicity of multiple ascending doses of VAL-181388. Thus, this protocol is written with some flexibility to reflect the dose-finding nature of this Phase 1 clinical study. Modifications to the dose level may be required to achieve the scientific goals of the study and/or to ensure appropriate safety monitoring of the study subjects. Identity of Investigational Product

VAL-181388 is composed of 1.6 mg/mL of CX-000314, the VAL-181388 mRNA drug substance, formulated in a mixture of 4 lipids, including MC3 (DLin-MC3-DMA, a novel ionizable lipid), cholesterol, DSPC (1,2-Distearoyl-sn-glycero-3-phosphocholine), and PEG2000-DMG (1,2-Dimyristoyl-sn-glycerol, methoxypolyethyleneglycol), to form an mRNA-lipid complex (LNP) in 7.0% propylene glycol, 1 mM DTPA (diethylenetriaminepentaacetic acid), 100 mM Tris buffer.

The placebo is 0.9% Sodium Chloride Injection, USP or BP.

3.4.3 Management of Clinical Supplies

3.4.3.1 Study Drug Packaging and Storage

Moderna Therapeutics, Inc. will provide the investigator and study site with adequate quantities of VAL-181388. The placebo (0.9% Sodium Chloride Injection, USP or BP) is commercially available and will be supplied by the study site. VAL-181388 will be labeled "for clinical trial use" and have all required labeling per regulations. VAL-181388 will be

supplied to the pharmacy in an unblinded manner. Each vial will be individually labeled for future subject identification purposes.

VAL-181388 Injection will be supplied in 2-mL glass vials with a 0.5-mL fill volume at a concentration of 1.6 mg/mL. The unblinded study site pharmacy personnel will prepare a single dose for each subject based on the dose level cohort and randomization assignment. A single vial may be used for multiple subjects in a given day as long as the storage conditions are met and the vaccine is not refrozen. A pharmacy manual will be available and training provided to ensure pharmacy staff can comply with all vaccine storage, preparation, administration, and drug accountability procedures.

VAL-181388 must be stored in a secure area with limited access (unblinded pharmacy staff only), protected from moisture and light, and be stored at −20°C ± 5°C. The freezer should have an automated temperature recording and alert system. There must be an available back-up freezer. The freezers must be connected to a back-up generator. The pharmacy must have in place a 24-hour alert system that allows for rapid response in case of freezer malfunctioning. In addition, drug accountability study personnel (eg, the unblinded pharmacy staff) are required to keep a temperature log to establish a record of compliance with these storage conditions. The placebo will be stored according to the instructions on the product label and must also comply with storage in a restricted access area. Only drug accountability personnel should have access to the products used in this study.

The site is responsible for reporting any VAL-181388 that was not temperature controlled during shipment or during storage to the unblinded site (pharmacy) monitor and to the Moderna Therapeutics, Inc. Clinical Supply Manager. Such VAL-181388 will be retained for inspection by the unblinded monitor and next steps discussed with the Moderna Clinical Supply Manager. VAL-181388 determined to be questionable or unsuitable may be quarantined or disposed of upon approval by Moderna according to approved methods.

3.4.3.2 Study Drug Accountability

It is the investigator's responsibility to ensure that that the unblinded pharmacy personnel maintain accurate records of receipt of all VAL-181388, including dates of receipt. In addition, accurate records will be kept regarding when and how much VAL-181388 is dispensed and used by each subject in the study (including documentation of each dilutional record used to prepare the vaccine for injection). Reasons for departure from the expected dispensing regimen

must also be recorded. To satisfy regulatory requirements regarding drug accountability, all VAL-181388 will be reconciled and retained until study conclusion. At that time, VAL-181388 will be destroyed or returned to the Sponsor according to applicable regulations.

3.4.4 Blinding

This is an observer-blinded study. The investigator, study subjects, site monitors, and study site personnel will be blinded to the study drug administered, with the following exceptions:

- Unblinded pharmacy personnel (of limited number) will be assigned to perform drug accountability procedures and to prepare and administer VAL-181388 (or placebo) to all subjects. The unblinded pharmacy personnel will have no other study functions other than study drug management, documentation, accountability, preparation, and administration. They will not be involved in subject evaluations and will not reveal the study drug identity to either the subject or study site personnel involved in the conduct of the study, except in the case of an emergency.

- An unblinded study monitor, not involved in other aspects of monitoring, will be assigned as the drug accountability monitor. They will have responsibilities to ensure the site is following all proper drug accountability, preparation, and administration procedures.

- An unblinded statistician will provide a descriptive analysis of safety and immunological endpoints after the completion of each dosing cohort. The interim analyses of immunogenicity data will be performed as outlined in Section 3.6.7.

The treatment assignment will be concealed by having the unblinded pharmacy personnel prepare the study drug in a secure location that is not accessible to other study personnel. The syringe used will maintain the blind at the time of vaccination (eg, a sleeve will be used should the vaccine substance be distinguishable in appearance between the VAL-181388 and placebo). The unblinded pharmacy personnel will conduct the vaccination procedure. Once the vaccination is completed, the blinded study staff will take over further assessments and interactions with the subjects. Access to the randomization code will be strictly controlled at the pharmacy.

3.4.5 Breaking the Blind

A subject or subjects may be unblinded in the event of an SAE or other event, or if there is a medical emergency requiring the identity of the drug to be known to properly treat a subject. If a subject becomes seriously ill or pregnant during the study, the blind will be broken only if knowledge of the administered study drug will affect that subject's treatment options. In the event of a medical emergency requiring identification of the study drug administered to an individual subject, the investigator will make every attempt to contact the medical monitor to explain the need for opening the code within 24 hours of opening the code. The investigator will be responsible for documenting the time, date, reason for the code break, and the names of the personnel involved.

In addition to the aforementioned situations where the blind may be broken, the data will also be unblinded to a statistical team at specified time points for each data lock as outlined in Section 3.6.7. At the interim analysis of safety, reactogenicity, and immunogenicity data collected from Day 1 to Month 7, access to individual listings (unblinded) will be available to a restricted number of identified Sponsor team members and the clinical research organization unblinded statistician. Study sites will remain blinded.

3.4.6 Treatment Compliance

All doses of study drug will be administered at the study site under direct observation of study site personnel and appropriately recorded (date and time) in the eCRF. Site personnel will confirm that the subject has received the entire dose of study drug. If a subject does not receive study drug or does not receive all of the planned doses, the reason for the missed dose will be recorded in the source documents.

Subjects that miss the second vaccination due to noncompliance with the visit schedule and not due to a cohort pause will still be required to follow the original visit and testing schedule. Specifically, these subjects will be asked to undergo blood collection to comply with a second immune test at 56 (+10) days (Visit 7) following the first vaccination.

The study site is responsible for ensuring subjects comply with the study windows allowed. Should a subject miss a visit, every effort will be made to contact the subject and achieve a visit within the defined visit window. If a subject exceeds their post-vaccination visit in excess of 28 days from the scheduled visit or misses a vaccination schedule by more than 28 days (eg, 28 days beyond the scheduled time of vaccination) then that visit will be classified as a missed

visit and the subject will continue with subsequent visits for Parts A and B of the study. All safety requirements of the missed visit will be captured and included in the follow-up visit (eg, safety laboratory testing, memory aid review for reactogenicity, as applicable).

3.4.7 Prior and Concomitant Medications

3.4.7.1 Prior Medications and Therapies

Information about prior medications (including any prescription or over-the-counter medications, vaccines, or blood products) taken by the subject within the 30 days before providing informed consent (or as designated in the inclusion/exclusion requirements) will be recorded in the subject's eCRF.

3.4.7.2 Concomitant Medications and Therapies

In Part A of the study, concomitant medications include all medications (including vaccination outside of trial) taken by the subject from the time of signing the informed consent form (ICF) through 28 days after the second vaccination (Visit 7) and will be recorded in the eCRF. In Part B, receipt of immunomodulators (including vaccines), immunosuppressants, or other concomitant medications that could potentially impact immune response will be collected through the end-of-study (EOS) visit.

Subjects are prohibited from receiving immunoglobulins and/or any blood products within the 3 months preceding the administration of the study drug or at any time during the study. Acetaminophen may be allowed at the discretion of the investigator. A daily dose of ≤100 mg of aspirin given under the guidance of a physician is not a contraindication to enrollment.

To allow accurate assessment of analgesic/antipyretic use during the 7 days after each vaccination, subjects will be inquired directly as to use (absent or present) and if used will be inquired for treatment or prophylaxis.

Chronic administration (defined as more than 14 continuous days) of an immunosuppressant or other immune modifying drug within 6 months prior to vaccine administration or any such products during the active vaccination period (through 4 weeks after their last planned vaccination) is prohibited. An immunosuppressant dose of a glucocorticoid will be defined as a systemic dose of ≥10 mg of prednisone per day or equivalent. The use of topical, inhaled, and nasal glucocorticoids will be permitted.

Receipt of any other licensed vaccines (inactivated or live vaccines) within 4 weeks prior to dose administration or planned within 4 weeks from any dose administration is an exclusion criteria.

Concomitant medications (including vaccinations) will be coded using the WHO Drug Dictionary. If prohibited drug therapy is taken, a joint decision will be made by the investigator and the Sponsor to continue or withhold further vaccination of the subject based on the time the medication was administered and its pharmacology and pharmacokinetics, and whether the use of the medication will compromise the subject safety or interpretation of the data. It is the investigator's responsibility to ensure that details regarding the concomitant medication are adequately recorded in the eCRF.

3.5 Study Procedures

Before performing any study procedures, all potential subjects will sign an ICF. The investigator must address all questions raised by the subject before the subject signs the consent form. The investigator will also sign the ICF. Subjects will undergo study procedures at the time points specified in the schedule of events (Table 6-1 and Table 6-2).

At any time during the study a subject can be seen for an unscheduled visit. This may be prompted by abnormal laboratory test results, reactogenicity issues, or new or ongoing AEs. The site also has the discretion to make reminder telephone calls or text messages to inform the subject on visits, request further laboratory assessments, review memory aid content requirements, and follow-up on ongoing or outstanding issues. As standard practice, a reminder call should be made to the subject by the site at least once during the first 7 days following each vaccination to ensure that the memory aid is being completed correctly and consistently.

Specific activities at each visit are outlined in Table 6-1 and Table 6-2 and may include the following:

- Confirm subject identification and contact information
- Record vital signs (toxicity graded) and perform physical examination
- Review memory aid
- Grade reactogenicity

- Assess for new AEs and follow up on any outstanding AEs (including grading and attribution)
- Review any new laboratory test results (toxicity graded) and identify any laboratory tests that should be repeated
- Record any concomitant medications and vaccinations since last visit
- Collect blood samples as indicated by visit
- Vaccinate (Visits 1 and 4 only)
- Schedule follow up appointments
- Complete all eCRFs
- Enroll subject in the continued blinded follow up period (Part B) (after subject has returned for Visit 7 [28 days following the second vaccination] in Part A)

3.5.1 Safety Contact

Once entered into Part B, each safety contact will occur by telemedicine (eg, telephone, text message, internet), and blood samples for immune persistence will be collected from each subject at the time points indicated in Table 6-2.

Safety contacts must be performed by appropriately trained study site staff. If the initial contact is unsuccessful, the study site staff should make a total of 3 attempts for each scheduled safety contact. All attempts to contact the subjects will be recorded in the source documents. The safety contact is considered missed if there is a failure to contact the subject by the time of the succeeding visit (every 28 days plus window allowance).

These contacts will follow a script, which will facilitate the collection of relevant safety information. Each safety contact will capture outcomes of any AESI or SAE that remains unresolved since the last visit or is newly identified through scripted query. Additional data may be requested through medically attended visits, and medications and vaccination will be recorded as part of the medical intake for each telemedicine visit. All safety information described by the subject must be documented in the source documents and not documented on the script used for the safety contact.

3.5.2 Completion of Memory Aid

The memory aid utilized will be a diary card (paper or electronic device) and is a method to record systematic collection of AE information from the subject; this information is subject to potential change based on further questioning and/or follow-up by study staff. Each subject will be instructed to complete a memory aid to describe:

- Solicited local and systemic AEs (Section 3.5.6) occurring (with appropriate documentation to allow severity grading by the investigator) during the day of each dose administration and for the following 7 days
    - Daily oral body temperature measurement is to be performed at approximately the same time each day using the thermometer provided by the study site. If body temperature is taken more than once in a given day, only the highest temperature reading will be recorded.
    - For solicited local AEs that require measurement, the measurement of size will be performed using the ruler provided by the study site.
- All medications (excluding vitamins and minerals) taken during the day of each vaccine administration and for the following 28 days
- Any unsolicited AE during the first 28 days following each vaccination

Study staff will review the information regarding solicited and unsolicited AEs, temperatures, and concomitant medications during the clinic visit. This information will be recorded in the subject's source documents and the eCRF.

3.5.3 Immunogenicity Assessments

Blood samples for immunogenicity assessment will be collected at the time points indicated (Table 6-1 and Table 6-2). Sample aliquots will be designed so as to ensure that back-up samples are available and adequate vial volumes will allow for testing needs. The actual time and date of each sample collected will be recorded in the eCRF and unique sample identification will be utilized to maintain the blind at the laboratory at all times and to allow for automated sample tracking and housing. Handling and preparation of the samples for

analysis, as well as shipping and storage requirements will be provided in a separate laboratory manual.

Immunogenicity endpoints are provided in Section 3.6.2.

3.5.4 Total Blood Volume

The approximate blood volumes to be collected from each subject during the study are provided in Table 3-3.

Table 3-3 Total Blood Volume

Assessment	Blood Volume per Sample	Scheduled Number of Collections[a]	Total Amount of Scheduled Blood
Clinical Laboratory Assessments			
Hematology	2 mL	5	10 mL
Serum chemistry	6 mL	8 (9 if sentinel)	48 mL (54 mL if sentinel)
Coagulation	4.5 mL	8 (9 if sentinel)	36 mL (40.5 mL if sentinel)
Serology[b]	6 mL	1	6 mL
Immunogenicity Assessments			
Serum neutralizing assay	7 mL	5	35 mL
Serum binding assay	4 mL	5	20 mL
Exploratory antibody assays	10 mL	5	50 mL
Subject Total			**205 mL (215.5 mL if sentinel)**

[a] Additional blood collections may be required at the discretion of the investigator to follow up on abnormal results. Subjects in the sentinel safety group will have 1 additional collection for serum chemistry and coagulation samples; thus, the total volume for these subjects will be 215.5 mL.

[b] Serology testing will include hepatitis B surface antigen, hepatitis C virus antibody, and HIV virus type 1 and 2 antibodies. A separate counseling and consenting for HIV testing will occur.

3.5.5 Safety Assessments

Safety assessments will include monitoring and recording of the following:

- Solicited (local and systemic reactogenicity events) and unsolicited AEs
- SAEs
- AESIs

- Clinical laboratory test results including hematology, serum chemistry, coagulation, and urinalysis
- Vital sign measurements
- Physical examination findings

3.5.6 Solicited Safety Measurements

The term "reactogenicity" refers to selected signs and symptoms (AEs) occurring after dose administration, to be collected by the subject during the day of each dose administration and for the following 7 days using self-reporting and the memory aid. Subjects will be instructed to call or return to the clinic within 24 hours if reactogenicity reaches Grade 3 or higher during the first 7 days following vaccination.

The following AEs are included in the memory aid.

Solicited local AEs include:

- injection site induration/swelling
- injection site tenderness
- injection site erythema/redness
- injection site pain

Solicited systemic AEs include:

- body temperature (oral)
- generalized myalgia (muscle ache or pain)
- generalized arthralgia (joint ache or pain)
- headache
- fatigue/malaise (unusual tiredness)

- nausea/vomiting
- diarrhea

The AEs will be recorded by subjects daily using the memory aid; where appropriate reactogenicity measurements will be taken using the tools provided (oral thermometer and measuring device). The investigator will later review, confirm, grade, and attribute AEs including reactogenicity, as absent (Grade 0), mild (Grade 1), moderate (Grade 2), severe (Grade 3), or potentially life threatening (Grade 4). The Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (CBER 2007; Table 6-3) will be used to grade reactogenicity reactions. If a solicited local or systemic AE continues beyond 7 days after dose administration, it will also be recorded as an AE in the eCRF from 8 days after vaccination until resolved or stable.

Other solicited reactions:

Use of analgesics and/or antipyretics will be recorded as absent or present, and it will be indicated if they were used for treatment or for prophylaxis.

Solicited local and systemic AEs (including body temperature) will be collected using the memory aid during the day of each dose administration (at approximately 6 hours) and for the following 7 days. To improve recall for the next visit, subjects will record incidence of unsolicited AEs and medication using the memory aid until approximately 28 days after each vaccine administration.

All AEs necessitating an unscheduled physician visit, medical attention, or leading to withdrawal from the study will also be collected throughout Part A of the study, and all AEs will be monitored until resolution or, if the AE becomes chronic, a cause is identified. If an AE is unresolved at the conclusion of Part A of the study, a clinical assessment will be made by the investigator and the medical monitor to determine whether or not continued follow-up of the AE is warranted. The relationship of the study drug to any AE and any SAE will be determined by the investigator as related or not related. All AEs resulting in withdrawal of the subjects from the study will be documented in the source documents and in the eCRF.

For Part B of the study, a script will be utilized to query for any new SAEs or AESIs and proper documentation will be obtained to allow an assessment of relatedness to vaccination or study

participation. All SAEs and AESIs will be followed to resolution, to chronic stable state, or until the last subject visit for Part B occurs. At that time, any ongoing AEs will receive their final classification so that the safety database can be locked.

A tabulation of all SAEs and AESIs, categorized by the Medical Dictionary for Regulatory Activities (MedDRA) preferred terms and assessed relationship to study drug, will be performed.

3.5.7 Unsolicited Safety Measurements

3.5.7.1 Adverse Events

The investigator is responsible for reporting all AEs that are observed or reported during the study, regardless of their relationship to study drug or their clinical significance. If there is any doubt as to whether a clinical observation is an AE, the event should be reported.

3.5.7.1.1 Definitions

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Subjects will be instructed to record any AEs (solicited or unsolicited) in the memory aid.

A treatment-emergent AE is defined as any event not present before exposure to study drug or any event already present that worsens in intensity or frequency after exposure.

A suspected adverse reaction is any AE for which there is a reasonable possibility that the study drug caused the AE. For the purposes of investigational new drug safety reporting, “reasonable possibility” means that there is evidence to suggest a causal relationship between the study drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a study drug. An adverse reaction is any AE caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there are reasons to conclude that the drug caused the event.

An AE or suspected adverse reaction is considered “unexpected” if it is not listed in the IB or at the specificity or severity that has been observed with the study drug being tested; or, if an IB is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the IB

referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the IB listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse events will also be evaluated by the investigator for the coexistence of any of the other following conditions:

- Medically attended AE: an AE that leads to an unscheduled visit to a healthcare practitioner

Any solicited AE that meets any of the following criteria will be entered onto the AE page of the eCRF:

- Solicited local or systemic AE leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator
- Solicited local or systemic AE lasting beyond 7 days duration
- Solicited local or systemic AE that lead to subject withdrawal from study drug
- Solicited local or systemic AE that otherwise meets the definition of an SAE

3.5.7.1.2 Serious Adverse Events

An AE or suspected adverse reaction is considered "serious" (SAE) if, in the view of either the investigator or Sponsor, it results in any of the following outcomes:

- Death
- Life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- Congenital anomaly or birth defect

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An AE or suspected adverse reaction is considered "life threatening" if, in the view of either the investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

3.5.7.1.3 Adverse Events of Special Interest

Certain AESIs are evaluated after the administration of immunostimulatory agents. All subjects enrolled in the study will be monitored for AESIs from enrollment through the EOS visit. The occurrence of any of these AEs will be treated as an SAE, meeting the criterion of a "medically important event."

The list of AESIs is presented in Appendix 2.

A diagnosis of an AESI will be reported to the Sponsor in an expedited manner similar to an SAE. The AESI diagnosis, as well as any medications taken to treat the condition, will be recorded in the subject's eCRF.

3.5.7.1.4 Pregnancy

The investigator is required to inform the Sponsor about any unexpected case of pregnancy occurring during the study (for female subjects as well as for partners of male subjects), monitor the pregnancy until delivery and report whatever outcome to the Sponsor (no later than 1 week after becoming aware of the pregnancy).

Pregnancy report forms will be distributed to the study site to be used for this purpose.

The investigator must immediately (within 24 hours of awareness) report to the Sponsor any case of pregnancy resulting in an abnormal outcome (miscarriage or newborn with congenital abnormality and/or stillbirth) according to the procedures described for SAEs.

3.5.7.2 Eliciting and Documenting Adverse Events

The investigator is responsible for ensuring that all AEs and SAEs are recorded in the eCRF and reported to Moderna Therapeutics, Inc. In Part A of the study, AEs will be assessed from the time of the first dose administration at Visit 1 through Visit 7. However, for the time period after the ICF is signed until before receiving the study drug, AEs will only be recorded when they are defined as one or more of the following: SAEs, AESIs, or AEs leading to study withdrawal. In Part B of the study, AESIs and SAEs will be assessed from Visit 8 through Visit 19. Any AEs occurring before receipt of the study drug will be analyzed separately from TEAEs.

At every clinic visit or telephone contact, subjects will be asked a standard question to elicit any medically related changes in their well-being according to the scripts provided. Subjects will also be asked if they have been hospitalized, had any accidents, used any new medications, or changed concomitant medication regimens (both prescription and over-the-counter medications).

In addition to subject observations, data from clinical laboratory test results, physical examination findings, or other documents relevant to subject safety classified as an AE will be documented on the AE page of the eCRF.

3.5.7.3 Reporting Adverse Events

All AEs reported or observed during the study will be recorded on the AE page of the eCRF. Information to be collected includes drug treatment and dose, type of event, time of onset, dosage, investigator-specified assessment of severity and relationship to study drug, time of resolution of the event, seriousness, as well as any required treatment or evaluations, and outcome. The AEs resulting from concurrent illnesses, reactions to concurrent illnesses, reactions to concurrent medications, or progression of disease states must also be reported. All AEs will be followed until they are resolved or stable or judged by the investigator to be not clinically significant. The MedDRA will be used to code all AEs.

Any medical condition that is present at the time that the subject is screened but does not deteriorate should not be reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

Any AE considered serious by the investigator or which meets SAE criteria (Section 3.5.7.1.2) must be reported to the Sponsor immediately (within 24 hours of becoming aware of the SAE). The investigator will assess whether there is a reasonable possibility that the study drug caused the SAE. The Sponsor will be responsible for notifying the relevant regulatory authorities of any SAE as outlined in the 21 US Code of Federal Regulations (CFR) Parts 312 and 320. The investigator is responsible for notifying the institutional review board (IRB) directly.

The following contact information is to be used for SAE reporting:

SAE Hotline: PPD
SAE Fax line: PPD

3.5.7.4 Assessment of Severity

The severity (or intensity) of an AE refers to the extent to which it affects the subject's daily activities and will be classified as mild (Grade 1), moderate (Grade 2), severe (Grade 3), or potentially life threatening (Grade 4) using the following criteria:

- Mild (Grade 1): These events do not interfere with the subject's daily activities.
- Moderate (Grade 2): These events cause some interference with the subject's daily activities but do not require medical intervention.
- Severe (Grade 3): These events prevent the subject's daily activity and require medical intervention.
- Life threatening (Grade 4): These events require an emergency room visit or hospitalization.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. An AE characterized as intermittent requires documentation of onset and duration of each episode.

The Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (CBER 2007) will be used to categorize solicited

reactogenicity, safety laboratory test results, and vital sign measurements observed during this study. Specific criteria for clinical and laboratory abnormalities are presented in Table 6-3 and Table 6-4, respectively, and will be graded if outside of the reference range for the laboratory utilized. Of note, the laboratory test value itself may not be the AE classification. Each AE will be classified by its most specific term (eg, renal insufficiency, bronchitis) and supporting evidence (eg, laboratory test value, x-ray) will not be classified as an AE per se.

3.5.7.5 Assessment of Causality

The investigator's assessment of an AE's relationship to study drug is part of the documentation process but is not a factor in determining what is or is not reported in the study.

The investigator will assess causality (ie, whether there is a reasonable possibility that the study drug caused the event) for all AEs and SAEs. The relationship will be characterized using the following classification:

- Not related: There is not a reasonable possibility of Sponsor's product relationship: Subject did not receive the Sponsor's product OR temporal sequence of the AE onset relative to administration of the Sponsor's product is not reasonable OR the AE is more likely explained by another cause than the Sponsor's product.
- Related: There is a reasonable possibility of Sponsor's product relationship: There is evidence of exposure to the Sponsor's product. The temporal sequence of the AE onset relative to the administration of the Sponsor's product is reasonable. The AE is more likely explained by the Sponsor's product than by another cause.

3.5.7.6 Follow-Up of Adverse Events

All AEs must be reported in detail on the appropriate page of the eCRF and followed until the AE is resolved or stable or judged by the investigator to be not clinically significant in Part A of the study. For Part B of the study, only AESIs and SAEs will be recorded. The investigator may request an unscheduled visit at any time, if warranted.

3.5.8 Clinical Laboratory Testing

Clinical laboratory tests will be performed by the central laboratory, unless specified otherwise. Blood and urine will be collected in Part A at the time points indicated in the schedule of events (Table 6-1). Fasting is not required before collection of laboratory samples.

The following hematology, serum chemistry, coagulation, and urinalysis assessments will be performed. The results will be toxicity graded using Table 6-4.

Hematology:	Hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelet count, red blood cell count, and total and differential leukocyte count
Serum Chemistry:	Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, amylase, lipase, bilirubin (total and direct), blood urea nitrogen, creatinine, random glucose, potassium, sodium, total protein, albumin, and calcium Females subjects only: β-human chorionic gonadotropin (childbearing potential) and follicle-stimulating hormone (post-menopausal)
Coagulation:	Prothrombin time, partial thromboplastin time
Urinalysis:	pH, protein, glucose, ketone, bilirubin, urobilinogen, blood, nitrite, leucocytes, and specific gravity

A pregnancy test (β-human chorionic gonadotropin) will be performed on all female subjects who have reproductive potential at Screening and before each dose administration (urine or serum). Only pregnancy tests at Screening and for unscheduled visits will be sent to the central laboratory. Pregnancy testing prior to each study vaccination will be performed by the local laboratory. A follicle-stimulating hormone test will be performed at Screening, as necessary, to confirm post-menopausal status in female subjects, if not documented in the subject's medical records.

Human immunodeficiency virus (types 1 or 2) antibody, hepatitis B surface antigen, and hepatitis C virus antibody will be assessed at Screening.

A urine screen for drugs of abuse will be performed by the local laboratory at Screening for alcohol, opiates, cocaine, phencyclidine, amphetamines, benzodiazepines, and methadone.

Should safety laboratory testing result in a Grade 2 or higher toxicity, repeat testing must occur within the next 10 days; this may include an unscheduled visit. Should the subject's laboratory value not return to baseline then periodic testing may be needed until the abnormality is deemed to be associated with a new stable AE or determined to be not clinically significant by the investigator.

Those values that fall within the normal range of the laboratory will automatically be classified as normal. All values that have a toxicity of Grade 1 or higher will also be evaluated by the investigator and classified as "abnormal clinically significant (CS)", or "abnormal not clinically significant (NCS)". Investigators should use their clinical judgment when considering the clinical significance of any abnormal laboratory findings. All laboratory test values with a toxicity of Grade 3 or higher will be entered as AEs. Any additional laboratory test value that is determined to be clinically significant will also be recorded as an AE, should that be considered the primary diagnosis. In such instances, the abnormal value and grade will be documented on the AE page of the eCRF. The investigator will continue to monitor the subject with additional assessments until the values have reached the reference range or the values at Screening or until the investigator determines that follow-up is no longer medically necessary. The only exception to this rule would be a laboratory test value that is associated with an identified ongoing AE where that event would be the classifying AE.

3.5.9 Vital Sign Measurements

Vital sign measurements will include systolic and diastolic blood pressures, heart rate, respiratory rate, and body temperature (which is classified as a solicited systemic event). The subject will be seated for at least 5 minutes before all measurements are taken. Vital signs will be measured in Part A at the time points indicated in the schedule of events (Table 6-1).

When procedures overlap and are scheduled to occur at the same time point, the order of procedures should be vital sign measurements and then the blood collection.

Of note, on the day of vaccination, if a subject is noted to have a systolic or diastolic blood pressure, heart rate, or respiratory rate measurement that shows Grade 2 or higher toxicity after 2 measurements (even after relaxing or resting); has an acute illness; or has an oral temperature >38°C, the subject should not receive a vaccination on that day and will need to return on subsequent days for their vaccination.

If any of the vital sign measurements meet the toxicity grading criteria for clinical abnormalities (Table 6-3) of Grade 3 or higher, the abnormal value and grade will be documented on the AE page of the eCRF (unless there is another known cause of the abnormality and that would result in an AE classification). The investigator will continue to monitor the subject with additional assessments until the value has reached the reference range,

the value at Screening, is considered stable, or until the investigator determines that follow-up is no longer medically necessary.

3.5.10 Physical Examinations

In Part A, a full physical examination will be performed at Screening and a symptom-directed (targeted) physical examination will be performed at all other scheduled time points indicated in the schedule of events (Table 6-1). The full examination will include assessment of skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities. Interim physical examinations will be performed at the discretion of the investigator, if necessary, to evaluate AEs or clinical laboratory abnormalities. Prior to vaccination and at 7 days following vaccination, a physical evaluation of the arm that was vaccinated and the associated lymph nodes should be evaluated.

Height and weight will be measured and body mass index will be calculated at Screening only.

3.6 Statistical Analysis Plans

3.6.1 Safety Endpoints

The following are the safety (primary) endpoints:

Part A:

- Solicited AEs (local and systemic reactogenicity events) collected for 7 days following each vaccination with toxicity grading
- Unsolicited AEs collected for 28 days following each vaccination; additional classification if serious, medically attended, or an AESI
- Safety laboratory test results (serum chemistry, hematology, coagulation, and urinalysis) with toxicity grading
- Vital sign measurements with toxicity grading and physical examination findings

Part B:

- Serious AEs and AESIs through 1 year (or until resolved, whichever comes first) following the last vaccination

3.6.2 Immunogenicity Endpoints

The immunogenicity assessments are as follows:

Part A:

- Neutralizing serum antibody titers to CHIKV proteins (baseline and 28 days after each vaccination)
- Serum antibody titers (IgG) to CHIKV proteins (baseline and 28 days after each vaccination)
- Exploratory antibody assays may be performed with excess serum to assess for cross-reactivity to related viruses at the discretion of the Sponsor
- Additional exploratory assays based upon current research may be performed with excess serum to better characterize the immune response to the CHIKV proteins at the discretion of the Sponsor

Part B

- Neutralizing serum antibody titers to CHIKV (at 6 and 12 months after the last vaccination)
- Serum binding antibody titers (IgG) to CHIKV-specific proteins (at 6 and 12 months after the last vaccination)
- Exploratory antibody assays may be performed with excess serum to assess for cross-reactivity to related viruses at the discretion of the Sponsor
- Additional exploratory assays based upon current research may be performed with excess serum to better characterize the immune response to the CHIKV proteins at the discretion of the Sponsor

3.6.3 Sample Size Calculations

Approximately 60 subjects are planned to be randomized, 20 per dose level. Formal sample size calculations were not performed as this is an observational safety and immunogenicity study with no formal null hypotheses being tested.

3.6.4 Analysis Sets

The All Enrolled Subjects set will include subjects who signed the ICF. The All Enrolled Subjects set will only be used for descriptive purposes.

The Safety set will include all subjects who receive at least 1 dose of study drug (VAL-181388 or placebo). All subjects in the Safety set will be analyzed according to the study drug actually received and not according to the study drug the subject was randomly assigned to receive, in the event there is a discrepancy.

The Per-Protocol set will include all subjects who did not observe a major protocol violation, received vaccine within the acceptable vaccination window (their full dose(s) of assigned study drug), had blood collection within accepted visit windows, and had a pre-vaccination and at least one serum sample from the post-vaccination testing period available for testing. All subjects in the Per-Protocol set will be analyzed according to the study drug the subject was randomly assigned to receive and not according to what was actually received, in the event there is a discrepancy. In the case where there is not a paired sample for the specific time point that data will not be included in the analysis.

The Intent-to-Treat set will provide supportive analyses. For the Intent-to-Treat set, all subjects who were randomly assigned to the study will be included regardless of protocol violations, exceeded visit windows, missed vaccination, or missing data.

3.6.5 Statistical Analysis

Details of all statistical analyses will be described in a statistical analysis plan. All data collected will be presented in data listings. Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics.

Data from subjects receiving placebo will be pooled across dose level cohorts for all presentations.

For categorical variables, frequencies and percentages will be presented. Continuous variables will be summarized using descriptive statistics (number of subjects, mean, median, SD, minimum, and maximum).

3.6.5.1 Immunogenicity Analyses

The following immunogenicity outcome measures (for serum neutralizing antibody titers and serum binding antibody titers to CHIKV-specific proteins) and their 95% confidence intervals, where appropriate, will be summarized by dose level cohort and combined placebo, and by days post-vaccination:

- Geometric mean titer (GMT) at baseline (pre-vaccination at Visit 1) and at post-dose time points
- Geometric mean ratio$_{Post/Pre}$: the ratio of post-vaccination GMT to pre-vaccination (Visit 1) GMT of subjects who have a baseline sample and post-vaccination results at post-dose time points
- Seroresponse: The proportion of subjects in each treatment group who either had an undetectable titer at baseline and detectable titer after vaccination, or detectable titer at baseline and at least a 4-fold increase (of baseline titer) after vaccination will be calculated

3.6.5.2 Safety Analyses

Data for subjects receiving placebo will be pooled across all dose levels for all presentations. Reactogenicity will be summarized by dose levels (25, 50, or 100 µg or placebo), vaccination (first or second), duration, relationship to study vaccine, and severity. Adverse events will be coded by preferred term and system organ class using MedDRA and summarized by part, treatment, vaccination (first or second), and overall. Adverse events will also be summarized by severity and relationship to study vaccine. Descriptive statistics will be presented and the difference in the proportion of AEs will be provided comparing each dose level with placebo recipients pooled across all dose levels. Adverse events for subjects in the safety cohort for each dose level will be provided in a separate subject listing. Adverse events leading to withdrawal, AESIs, medically attended AEs, and SAEs will be provided by subject in a listing.

Safety data from clinical laboratory test results and vital sign measurements will be graded by severity and analyzed by treatment group and vaccination (first or second). Absolute and change from baseline values will be provided according to the toxicity table, along with mean, median, and standard deviation. Results of hematology, serum chemistry, coagulation, and urinalysis assessments; urine drug screen; and pregnancy testing will be listed for all subjects randomly assigned to receive study treatment.

Medical history data for all subjects randomly assigned to receive study treatment will be presented by subject in a listing.

Baseline demographic and background variables will be summarized by treatment group and cohort for all subjects. The number of subjects who enroll in the study and the number and percentage of subjects who complete the study will be presented. Frequency and percentage of subjects who withdraw or discontinue from the study, and the reason for withdrawal or discontinuation, will also be summarized.

Prior and concomitant medication will be listed (with start and stop dates) for each subject and summarized by common medical dictionary coding. Any vaccinations that occur during the trial conduct will also be captured and summarized.

3.6.6 Handling of Missing Data

For GMT calculation, antibody values reported as below the lower limit of quantification (LLOQ) will be replaced by 0.5 × LLOQ. For fold-rise, values <LLOQ will be replaced by 0.5 × LLOQ. Values that are greater than the upper limit of quantification (ULOQ) will be converted to the ULOQ. Missing results will not be imputed.

3.6.7 Interim Analyses

Following completion of each dose level cohort in Part A, the database will be locked for that cohort and safety and/or immune testing results through 28 days following the final vaccination will be analyzed. As dose escalation occurs, cumulative analyses will be included for each subsequent data lock to allow for all prior dose level cohorts to be analyzed by dose level and in aggregate for VAL-181388 exposure. Immunogenicity and safety data, including mean group analyses of change from baseline, where applicable, will be summarized for each dose level cohort and combined placebo group. These analyses will be performed by an unblinded team, independent of the study team. All study personnel and participants other than the third-party statistician will remain blinded to treatment allocation. These data will be provided to designated Sponsor representatives only. They will inform decisions on this and other development programs using the same mRNA platform.

An interim analysis of safety, reactogenicity, and immunogenicity data collected from Day 1 to Month 7 will be conducted and will be reported on a treatment assignment level. Access to individual listings will be restricted to identified Sponsor members and the contract research

organization unblinded statistician. Study sites will remain blinded. This analysis will provide information regarding short-term antibody persistence. The final analysis of safety and immunogenicity data collected from Visit Day 1 through the end of the study will be performed as soon as data are cleaned and locked. The results of this analysis will be presented in a clinical study report including individual data listings.

Additional information can be found in the statistical analysis plan.

3.7 Data Quality Assurance

All aspects of the study will be monitored for compliance with applicable government regulations with respect to current ICH harmonised tripartite guideline E6(R1): Good Clinical Practice and current standard operating procedures. The eCRFs will be utilized and accessed through iMedidata® via the internet. This electronic data capture system is validated and compliant with US Title 21 of CFR Part 11. Each person involved with the study will have an individual identification code and password that allow for record traceability. There may be an internal quality review audit of the data and additional reviews by the clinical monitor.

Due to safety review requirements, no more than 3 business days should transpire between subject data availability (visits, laboratory test results, etc) and data entry. As a quality measure, timeliness of data entry and data query resolution will be reported to the IST. Other issues of data quality that may hinder safety review or pose a concern with patient safety will also be reported to the IST with appropriate awareness to the SMC if needed.

4 Investigator Obligations

The following administrative items are meant to guide the investigator in the conduct of the study and may be subject to change based on industry and government standard operating procedures, working practice documents, or guidelines. Changes will be reported to the IRB but will not result in protocol amendments.

4.1 Confidentiality

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain subject confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the subject, except as necessary for monitoring and auditing by the Sponsor, its designee, relevant regulatory authority, or the IRB.

The investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

4.2 Institutional Review

Federal regulations and the ICH E6(R1) guidelines require that approval be obtained from an IRB before participation of human subjects in research studies. Before study onset, the protocol, informed consent, advertisements to be used for the recruitment of study subjects, and any other written information regarding this study to be provided to the subject must be approved by the IRB. Documentation of all IRB approvals and of the IRB compliance with the ICH E6(R1) guidelines will be maintained by the site and will be available for review by the Sponsor or its designee.

All IRB approvals should be signed by the IRB chairman or designee and must identify the IRB name and address, the clinical protocol by title or protocol number or both and the date approval or a favorable opinion was granted.

4.3 Subject Consent

Written informed consent in compliance with US Title 21 CFR Part 50 shall be obtained from each subject before he or she enters the study or before any unusual or non-routine procedure that involves risk to the subject are performed. If any institution-specific modifications to study-related procedures are proposed or made by the site, the consent should be reviewed by the Sponsor or its designee or both before IRB submission. Once reviewed, the investigator will submit the ICF to the IRB for review and approval before the start of the study. If the ICF is revised during the course of the study, all active participating subjects must sign the revised form.

Before recruitment and enrollment, each prospective subject will be given a full explanation of the study and be allowed to read the approved ICF. Once the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give his or her consent to participate in the study by signing the ICF. A separate counseling and consent for HIV testing will occur.

The ICF will also explain that excess serum from immunogenicity testing may be used for future research which may be performed at the discretion of the Sponsor to better characterize the immune response to the CHIKV protein (inclusive of additional assay development and the immune response across alphaviruses and flaviviruses).

The investigator or designee will provide a copy of the ICF to the subject. The original form shall be maintained in the subject's medical records at the site.

4.4 Study Reporting Requirements

By participating in this study, the investigator agrees to submit reports of SAEs and AESIs according to the time line and method outlined in this protocol. In addition, the investigator agrees to submit annual reports to his or her IRB as appropriate.

4.5 Financial Disclosure and Obligations

The investigator is required to provide financial disclosure information to allow the Sponsor to submit the complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigator must provide to the Sponsor a commitment to

promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

Neither the Sponsor, PPD, nor the study site is financially responsible for further testing or treatment of any medical condition that may be detected during the screening process. In addition, in the absence of specific arrangements, neither the Sponsor, PPD, nor the study site is financially responsible for further treatment of the disease under study.

4.6 Investigator Documentation

Prior to beginning the study, the investigator will be asked to comply with ICH E6(R1) 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- IRB approval,
- An original investigator-signed investigator agreement page of the protocol,
- Form FDA 1572, fully executed, and all updates on a new fully executed Form FDA 1572,
- Curriculum vitae for the principal investigator and each sub-investigator listed on Form FDA 1572. Current licensure must be noted on the curriculum vitae. The curriculum vitae will be signed and dated by the principal investigators and subinvestigators at study start-up, indicating that they are accurate and current,
- Financial disclosure information to allow the Sponsor to submit complete and accurate certification or disclosure statements required under 21 CFR 54. In addition, the investigators must provide to the Sponsor a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year after the completion of the study,
- An IRB-approved ICF, samples of site advertisements for recruitment for this study, and any other written information about this study that is to be provided to the subject, and
- Laboratory certifications and reference ranges for any local laboratories used by the site, in accordance with 42 CFR 493.

4.7 Study Conduct

The investigator agrees that the study will be conducted according to the principles of ICH E6(R1). The investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations. The study will be conducted in compliance with the protocol, current Good Clinical Practice guidelines – adopting the principles of the Declaration of Helsinki – and all applicable regulatory requirements.

4.8 Data Collection

4.8.1 Case Report Forms and Source Documents

As part of the responsibilities assumed by participating in the study, the investigator agrees to maintain adequate case histories for subjects treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include laboratory reports and similar sources.

Electronic case report forms are accessed through iMedidata® via the internet. This electronic data capture system is validated and compliant with 21 CFR 11. Each person involved with the study will have an individual identification code and password that allows for record traceability. Thus, the system, and subsequently any investigative reviews, can identify coordinators, investigators, and individuals who have entered or modified records, as well as the time and date of any modifications. There may be internal quality review audit of the data and additional reviews by the clinical monitor.

Each eCRF is presented as an electronic copy, allowing data entry by site personnel, who can add and edit data, add new subjects, identify and resolve discrepancies, and view records. This system provides immediate direct data transfer to the database, as well as immediate detection of discrepancies, enabling site coordinators to resolve and manage discrepancies in a timely manner.

4.9 Adherence to Protocol

The investigator agrees to conduct the study as outlined in this protocol in accordance with ICH E6(R1) and all applicable guidelines and regulations.

4.10 Reporting Adverse Events

By participating in this study, the investigator agrees to submit reports of SAEs according to the time line and method outlined in this protocol. In addition, the investigator agrees to submit annual reports to his IRB as appropriate. The investigator also agrees to provide the Sponsor with an adequate report, if applicable, shortly after completion of the investigator's participation in the study.

4.11 Investigator's Final Report

Upon completion of the study, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB with a summary of the study's outcome, and the Sponsor and regulatory authority(ies) with any reports required.

4.12 Records Retention

Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the study drug. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by an agreement with the Sponsor. It is the Sponsor's responsibility to inform the investigator/institution as to when these documents no longer need to be retained.

4.13 Publications

After interim analyses or at completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. In these cases, the Sponsor will be responsible for these activities and will work with the investigators to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues. The Sponsor has final approval authority over all such issues.

Data are the property of the Sponsor and cannot be published without their prior authorization, but data and publication thereof will not be unduly withheld.

5 Study Management

5.1 Monitoring

5.1.1 Monitoring of the Study

The clinical monitor, as a representative of the Sponsor, is obligated to follow the study closely. In doing so, the monitor will visit the investigator and study facility at periodic intervals, in addition to maintaining necessary telephone and letter contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation, and discussion of the conduct of the study with the investigator and staff. The monitor will be blinded to treatment assignment. A separate unblinded study monitor will be responsible for drug accountability.

All aspects of the study will be carefully monitored by the Sponsor or its designee for compliance with applicable government regulation with respect to current ICH E6(R1) guidelines and standard operating procedures.

5.1.2 Inspection of Records

The investigator and institution involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspections by providing direct access to all study records. In the event of an audit, the investigator agrees to allow the Sponsor, their representatives, the FDA, the Department of Defense or federal representatives, or other regulatory agencies access to all study records.

The investigator should promptly notify the Sponsor of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the Sponsor.

5.2 Management of Protocol Amendments and Deviations

5.2.1 Modification of the Protocol

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the subject, must be reviewed and approved by the Sponsor or designee. Amendments to the protocol must be submitted in writing to the investigator's IRB for approval before subjects are enrolled into an amended protocol.

5.2.2 Protocol Violations and Deviations

The investigator or designee must document and explain in the subject's source documentation any deviation from the approved protocol. The investigator may implement a deviation from, or a change to, the protocol to eliminate an immediate hazard to study subjects without prior IRB approval. As soon as possible after such an occurrence, the implemented deviation or change, the reasons for it, and any proposed protocol amendments should be submitted to the IRB for review and approval, to the Sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended or unanticipated departure from the procedures or processes approved by the Sponsor and the IRB and agreed to by the investigator. Deviations usually have an impact on individual subjects or a small group of subjects and do not involve inclusion/exclusion or primary endpoint criteria. A protocol violation occurs when the subject or investigator do not adhere to the protocol, resulting in a significant, additional risk to the subject. Protocol violations can include non-adherence to inclusion or exclusion criteria, enrollment of the subject without prior Sponsor approval, or non-adherence to FDA regulations or ICH E6(R1) guidelines.

Protocol violations and deviations will be documented by the clinical monitor throughout the course of monitoring visits. The investigator will be notified in writing by the monitor of violations and deviations. The IRB should be notified of all protocol violations and deviations, if appropriate, in a timely manner.

5.3 Study Termination

Although the Sponsor has every intention of completing the study, they reserve the right to discontinue it at any time for clinical or administrative reasons.

The end of the study is defined as the date on which the last subject completes the last visit (includes the EOS visit and any additional long-term follow-up). Any additional long-term follow-up that is required to monitor the resolution of a finding or AE may be reported through an amendment to the clinical study report.

5.4 Final Report

Whether the study is completed or prematurely terminated, the Sponsor will ensure that clinical study reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The Sponsor will also ensure that clinical study reports in marketing applications meet the standards of the ICH harmonised tripartite guideline E3: Structure and content of clinical study reports.

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the clinical study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and have the opportunity to review complete study results.

A final clinical study report will contain all data collected through 28 days following the second vaccination of all dosing cohorts (Part A) and all data through the extension safety period (12-month follow-up after the second vaccination) and will include additional information on immune persistence.

Upon completion of the clinical study report(s), the Sponsor will provide the investigator(s) with the final approved clinical study report(s).

6 Appendices

6.1 Appendix 1: Schedule of Events

The schedule of events for Part A is presented in Table 6-1, and the schedule of events for Part B is presented in Table 6-2.

Table 6-1 Part A Schedule of Events

Procedure	**Screening**	**Treatment Period**									
Study visit	**0**	**1**	**1a[a]**	**1b[a]**	**2**	**3**	**3a[a]**	**4**	**5**	**6**	**7**
Vaccination day		**X**						**X**			
Days relative to most recent vaccination	NA	0	1	2	7	17	21	28	7	17	28
Window allowance	+28	0	0	0	+3	±3	+3	+7	+3	±3	+7
Informed consent	X										
Inclusion/Exclusion criteria	X										
Medical history	X										
Physical examination[b]	X	X[c]			X		X	X[c]	X		X
Vital sign measurements[d]	X	X[e]	X	X	X		X	X[e]	X		X
Serology[f]	X										
Clinical laboratory testing	X[g]	X[c,g]			X[g]	X[h]	X[h]	X[c,g]	X[g]	X[h]	X[h]
Urine drug screen[i]	X										
Pregnancy test (all female subjects of childbearing potential)	X	X[c]						X[c]			
Follicle-stimulating hormone (female subjects only)[j]	X										
Randomization		X									
Blood sample for serum neutralizing antibody titers[k]		X[c]						X[c]			X
Blood sample for serum antibody titers to CHIKV protein[k]		X[c]						X[c]			X
Blood sample for exploratory antibody assay[k]		X[c]						X[c]			X
Study drug administration		X						X			
Reactogenicity		X[e]	X	X	X		X	X[e]	X		X
Subject memory aid completion: solicited local and systemic AEs, oral body temperature, and medications taken[l]		X Visit 1 through Visit 2						X Visit 4 through Visit 5			

Procedure	**Screening**	**Treatment Period**									
Study visit	**0**	**1**	**1a[a]**	**1b[a]**	**2**	**3**	**3a[a]**	**4**	**5**	**6**	**7**
Vaccination day		**X**						**X**			
Days relative to most recent vaccination	NA	0	1	2	7	17	21	28	7	17	28
Window allowance	+28	0	0	0	+3	±3	+3	+7	+3	±3	+7
Subject memory aid completion: any unsolicited AEs and related medications[l]		X (Visit 1 through Visit 7)									
Collection of memory aids								X			X
Adverse events assessment[m]	X	X	X	X	X	X	X	X	X	X	X
Prior and concomitant medications[n]	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: AE, adverse event; CHIKV, chikungunya virus; NA, not applicable.

a. This visit is required for sentinel safety group subjects only.
b. Full physical examination at Screening; symptom-directed (targeted) physical examination at all other scheduled time points. Interim physical examinations will be performed at the discretion of the investigator, if necessary. Height and weight will be measured and body mass index calculated at Screening only.
c. Assessment to be performed before study drug administration.
d. Vital sign measurements (systolic and diastolic blood pressures, heart rate, respiratory rate, and body temperature) on Visits 1 and 4 will be collected once before study drug administration and at least 1 hour after study drug administration (before subjects are discharged). Vaccination cannot occur if systolic or diastolic blood pressure, heart rate, or respiratory rate measurements show Grade 2 or higher toxicity after 2 measurements; if a subject has an acute illness; or if a subject has an oral temperature >38°C.
e. Assessment to be performed before and after study drug administration.
f. Serology testing will include hepatitis B surface antigen, hepatitis C virus antibody, and human immunodeficiency virus type 1 and 2 antibodies.
g. Hematology, serum chemistry, coagulation, and urinalysis assessments.
h. Safety laboratory tests for albumin, bilirubin (total and direct), prothrombin time, partial thromboplastin time, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase.
i. The drug screen will include alcohol, opiates, cocaine, phencyclidine, amphetamines, benzodiazepines, and methadone.
j. To confirm post-menopausal status, as needed.
k. Excess serum from immunogenicity may be used for future research at the discretion of the Sponsor to better characterize the immune response to the CHIKV protein.
l. Subjects will be instructed on how to complete the memory aid prior to discharge from the clinic at Visit 1. Subjects will be instructed to call or return to the clinic within 24 hours if reactogenicity reaches Grade 3 or higher during the first 7 days following vaccination.

[m.] Adverse events will be assessed from the time of study drug administration on Visit 1 through Visit 7. However, for the time period after the informed consent form is signed until before receiving the study drug, AEs will only be recorded when they are defined as one or more of the following: serious AEs, AEs of special interest, AEs leading to study withdrawal.

[n.] Prior medications taken by the subject within the 30 days before providing informed consent will be collected. Concomitant medications include all medications (including vaccinations outside of the trial) taken by the subject from the time of signing the informed consent and through Visit 7.

Table 6-2 Part B Schedule of Events

Procedure												
Study visit	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19[a]**
Study day from 1st dose, if 2nd dose not completed	84	112	140	168	196	224	252	280	308	336	364	392
Study day from 2nd dose, if completed	56	84	112	140	168	196	224	252	280	308	336	364
Window allowance	±7	±7	±7	±7	±15	±7	±7	±7	±7	±7	±7	+15
Type of visit	SC	SC	SC	SC	LV[b]	SC	SC	SC	SC	SC	SC	LV[b]
Safety contact[c]	X	X	X	X		X	X	X	X	X	X	
Blood sample for serum neutralizing antibody titers[d]					X							X
Blood sample for serum antibody titers to CHIKV proteins[d]					X							X
Blood sample for exploratory antibody assays[d]					X							X
Adverse events assessment[e]	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications[f]	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: CHIKV, chikungunya virus; LV, laboratory visit; SC; safety contact.

[a] Visit 19 will be considered the end of study visit.

[b] Blood samples for immune persistence will be collected from each subject using a method that maintains the blind (eg, home visits, locally contracted laboratories).

[c] Each safety contact will occur by telemedicine (eg, telephone, text message, internet).

[d] Excess serum from immunogenicity may be used for future research at the discretion of the Sponsor to better characterize the immune response to the CHIKV protein and/or to assess for cross-reactive antibody responses to other related viruses.

[e] Each safety contact will capture outcomes of any adverse event of special interest or serious adverse event that remains unresolved since the last visit or is newly identified through scripted query. Additional data may be requested through medically attended visits, and medications and vaccination will be recorded as part of the medical intake for each telemedicine visit.

[f] Receipt of immunomodulators (including vaccines), immunosuppressants, other concomitant medications that could potentially impact immune response will be collected through Visit 19.

6.2 Appendix 2: Adverse Events of Special Interest

The following is a list of Adverse Events of Special Interest:

Gastrointestinal disorders:

- Celiac disease
- Crohn's disease
- Ulcerative colitis
- Ulcerative proctitis

Liver disorders:

- Autoimmune cholangitis
- Autoimmune hepatitis
- Primary biliary cirrhosis
- Primary sclerosing cholangitis

Metabolic diseases:

- Addison's disease
- Autoimmune thyroiditis (including Hashimoto thyroiditis)
- Diabetes mellitus type I
- Grave's or Basedow's disease

Musculoskeletal disorders:

- Antisynthetase syndrome
- Dermatomyositis

- Juvenile chronic arthritis (including Still's disease)
- Mixed connective tissue disorder
- Polymyalgia rheumatic
- Polymyositis
- Psoriatic arthropathy
- Relapsing polychondritis
- Rheumatoid arthritis
- Scleroderma, including diffuse systemic form and CREST syndrome
- Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis
- Systemic lupus erythematosus
- Systemic sclerosis

Neuro-inflammatory disorders:

- Acute disseminated encephalomyelitis, including site specific variants (eg, non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis)
- Cranial nerve disorders, including paralyses/paresis (eg, Bell's palsy)
- Guillain-Barré syndrome, including Miller Fisher syndrome and other variants
- Immune-mediated peripheral neuropathies and plexopathies, including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, and polyneuropathies associated with monoclonal gammopathy
- Multiple sclerosis
- Narcolepsy

- Optic neuritis
- Transverse myelitis

Skin disorders:

- Alopecia areata
- Autoimmune bullous skin diseases, including pemphigus, pemphigoid and dermatitis herpetiformis
- Cutaneous lupus erythematosus
- Erythema nodosum
- Morphoea
- Lichen planus
- Psoriasis
- Sweet’s syndrome
- Vitiligo

Vasculitides:

- Large vessels vasculitis including: including Takayasu's arteritis and giant cell arteritis/temporal arteritis
- Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg−Strauss syndrome (allergic granulomatous angiitis), Buerger’s disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch−Schönlein purpura, Behcet's syndrome, leukocytoclastic vasculitis

Others:

- Antiphospholipid syndrome
- Autoimmune hemolytic anemia
- Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis)
- Autoimmune myocarditis/cardiomyopathy
- Autoimmune thrombocytopenia
- Goodpasture syndrome
- Idiopathic pulmonary fibrosis
- Pernicious anemia
- Raynaud's phenomenon
- Sarcoidosis
- Sjögren's syndrome
- Stevens-Johnson syndrome
- Uveitis

6.3 Appendix 3: Toxicity Grading Scale Tables

The toxicity grading scales for clinical and laboratory abnormalities are presented in Table 6-3 and Table 6-4, respectively. Note that for laboratory abnormalities, grading only occurs if the values reside outside of the normal values established by the clinical laboratory. For study-specific laboratory normal ranges and associated toxicity grades, refer to the laboratory manual.

Table 6-3 Tables for Clinical Abnormalities

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	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness*	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling**	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

Abbreviation: ER, emergency room.

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

** Induration/swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Source: Guidance for industry – Toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for clinical abnormalities (CBER 2007).

Vital Signs *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Tachycardia (beats per minute)	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia (beats per minute)**	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) (mm Hg)	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) (mm Hg)	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) (mm Hg)	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory rate (breaths per minute)	17 – 20	21 – 25	> 25	Intubation

Abbreviation: ER, emergency room.

Note that fever is classified under systemic reactions for grading purposes.

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

** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Source: Guidance for industry – Toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for clinical abnormalities (CBER 2007).

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) * (°F) *	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Nausea/vomiting	No interference with activity or 1 to 2 episodes/ 24 hours	Some interference with activity or > 2 episodes/ 24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 g/ 24 hours	4 – 5 stools or 400 – 800 g/ 24 hours	6 or more watery stools or > 800 g/ 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/Malaise (unusual tiredness)	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Generalized myalgia (muscle ache or pain)	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Generalized arthralgia (joint ache or pain)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Abbreviations: ER, emergency room; IV, intravenous.

* Oral temperature; no recent hot or cold beverages or smoking.

Sources: Guidance for industry – Toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for clinical abnormalities (CBER 2007), Division of AIDS (DAIDS) Grading the Severity of Adult and Pediatric Adverse Events (DAIDS 2014).

Table 6-4 Tables for Laboratory Abnormalities

Serum Chemistry*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – hyponatremia (mEq/L)	132 – 134	130 – 131	125 – 129	< 125
Sodium – hypernatremia (mEq/L)	144 – 145	146 – 147	148 – 150	> 150
Potassium – hyperkalemia (mEq/L)	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – hypokalemia (mEq/L)	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – hypoglycemia (mg/dL)	65 – 69	55 – 64	45 – 54	< 45
Glucose – hyperglycemia Random (mg/dL)	110 – 125	126 – 200	> 200	Insulin requirements or hyperosmolar coma
Blood urea nitrogen (mg/dL)	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine (mg/dL)	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia (mg/dL)	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia (mg/dL)	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Albumin – hypoalbuminemia (g/dL)	2.8 – 3.1	2.5 – 2.7	< 2.5	–
Total Protein – hypoproteinemia (g/dL)	5.5 – 6.0	5.0 – 5.4	< 5.0	–
Alkaline phosphate; increase by factor	1.1 – 2.0 × ULN	2.1 – 3.0 × ULN	3.1 – 10 × ULN	> 10 × ULN
Liver function tests –ALT and AST; increase by factor	1.1 – 2.5 × ULN	2.6 – 5.0 × ULN	5.1 – 10 × ULN	> 10 × ULN
Bilirubin – when accompanied by any increase in liver function test; increase by factor	1.1 – 1.25 × ULN	1.26 – 1.5 × ULN	1.51 – 1.75 × ULN	> 1.75 × ULN
Bilirubin – when liver function test is normal; increase by factor	1.1 – 1.5 × ULN	1.6 – 2.0 × ULN	2.0 – 3.0 × ULN	> 3.0 × ULN
Pancreatic enzymes – amylase and lipase	1.1 – 1.5 × ULN	1.6 – 2.0 × ULN	2.1 – 5.0 × ULN	> 5.0 × ULN

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ULN, upper limit of the normal range.

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

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

Source: Guidance for industry – Toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for laboratory abnormalities (CBER 2007).

Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (female) (g/dL)	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (female) change from Baseline value (g/dL)	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (male) (g/dL)	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (male) change from Baseline value (g/dL)	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC increase (cell/mm^3)	10 800 – 15 000	15 001 – 20 000	20 001 – 25 000	> 25 000
WBC decrease (cell/mm^3)	2500 – 3500	1500 – 2499	1000 – 1499	< 1000
Lymphocytes decrease (cell/mm^3)	750 – 1000	500 – 749	250 – 499	< 250
Neutrophils decrease (cell/mm^3)	1500 – 2000	1000 – 1499	500 – 999	< 500
Eosinophils (cell/mm^3)	650 – 1500	1501 – 5000	> 5000	Hypereosinophilic
Platelets decreased (cell/mm^3)	125 000 – 140 000	100 000 – 124 000	25 000 – 99 000	< 25 000
PT; increase by factor	1.0 – 1.10 × ULN	1.11 – 1.20 × ULN	1.21 – 1.25 × ULN	> 1.25 × ULN
PTT; increase by factor	1.0 – 1.2 × ULN	1.21 – 1.4 × ULN	1.41 – 1.5 × ULN	> 1.5 × ULN

Abbreviations: PT, prothrombin time; PTT, partial thromboplastin time; ULN, upper limit of normal; WBC, white blood cell.

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Source: Guidance for industry – Toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for laboratory abnormalities (CBER 2007).

Urine *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 – 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Source: Guidance for industry – Toxicity grading scale for heathy adult and adolescent volunteers enrolled in preventative vaccine clinical trials; Tables for laboratory abnormalities (CBER 2007).

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