# CLINICAL STUDY PROTOCOL

# A PHASE 1, RANDOMIZED, OBSERVER-BLIND, PLACEBO-CONTROLLED, DOSE-RANGING STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF ZIKA VACCINE mRNA-1893 IN HEALTHY FLAVIVIRUS SEROPOSITIVE AND SERONEGATIVE ADULTS

## PROTOCOL NO. mRNA-1893-P101

**Sponsor:** ModernaTX, Inc.
200 Technology Square
Cambridge, MA 02139

**Sponsor Contact:** PPD
ModernaTX, Inc.
500 Technology Square
Cambridge, MA 02139
Telephone: PPD

**Version of Protocol:** Final 3.0

**Date of Protocol:** 17 March 2020

## CONFIDENTIAL

The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without the expressed, written consent of ModernaTX, Inc.

The study will be conducted according to the International Council for Harmonisation harmonized tripartite guideline E6(R2): Good Clinical Practice.

# Signature Page

**PROTOCOL TITLE:** A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Dose-Ranging Study to Evaluate the Safety, Tolerability, and Immunogenicity of Zika Vaccine mRNA-1893 in Healthy Flavivirus Seropositive and Seronegative Adults

**PROTOCOL NUMBER:** mRNA-1893-P101

**See eSignature and date signed on last page of document.**

PPD

Date

ModernaTX, Inc.

**See eSignature and date signed on last page of document.**

PPD

Date

ModernaTX, Inc.

# Investigator Protocol Agreement Page

I agree to conduct the study as outlined in the protocol entitled "A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Dose-Ranging Study to Evaluate the Safety, Tolerability, and Immunogenicity of Zika Vaccine mRNA-1893 in Healthy Flavivirus Seropositive and Seronegative Adults" 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

## Table of Contents

## List of Tables

**List of Figures**

# Summary of Changes

| New Version Number / Date | Section(s) in Which Change(s) Occurred | Brief Description of Change(s) | Justification |
|---|---|---|---|
| V2.0/17JUL2019 | 3.2.3.2: Contraception and Pregnancy Avoidance Procedures | Clarification of the requirements for male contraception timing to match eligibility requirements. | Administrative Update |
| V2.0/17JUL2019 | 3.4.2.2 Table 3 – Pause Rules | Updated pause rule #6 to remove the timeframe around collection of any ≥ Grade 3 unsolicited adverse events and abnormal laboratory results | Per FDA request |
| V2.0/17JUL2019 | 3.5.6: Solicited Safety Measurements | Clarification of the number of days diary data will be collected post vaccination | Administrative Update |
| V2.0/17JUL2019 | Table 5 Footnote i | Clarification of collection of Diary Data | Administrative Update |
| V3.0/17MAR2020 | 3.4.5: Blinding and 3.6.8: Interim Analysis | Update Blinding section and Interim Analysis to clarify for whom and when unblinded data will be accessible | Statistical Analysis Update |
| V3.0/17MAR2020 | Synopsis; 3.6.2 Secondary Endpoints; 3.6.3 Exploratory Endpoints; 3.6.6.1 Immunogenicity Analyses. | Wording changes to take into consideration new level of Lower Limit of Quantification (LLOQ) during assay performance | Change impacting data analysis |
| V3.0/17MAR2020 | Table 5: Schedule of Events and throughout the document | Day 29 and Day 57 Visit Windows | Updated visit windows due to potential impact of COVID-19 |
| V3.0/17MAR2020 | 3.5.8 Clinical Laboratory Testing | Clarification of the requirements of when to repeat out of range lab testing | Administrative Update |

# Protocol Synopsis

**Protocol Number:** **mRNA-1893-P101**

**Title:** A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Dose-Ranging Study to Evaluate the Safety, Tolerability, and Immunogenicity of Zika Vaccine mRNA-1893 in Healthy Flavivirus Seropositive and Seronegative Adults

**Study Phase:** 1

**Study Sites:** Clinical sites in both endemic and nonendemic Zika regions of the United States or its territories.

**Objectives:**

Primary:

- To evaluate the safety, tolerability, and reactogenicity of a 2-dose vaccination schedule of mRNA-1893 Zika vaccine, given 28 days apart, across a range of dose levels in flavivirus-seronegative and flavivirus-seropositive participants compared with placebo.

Secondary:

- To evaluate the immunogenicity of a 2-dose vaccination schedule of mRNA-1893 Zika vaccine, given 28 days apart as measured by Zika virus (ZIKV)-specific neutralization assay (Plaque Reduction Neutralization Test [PRNT]).

Exploratory:

- To evaluate the immunogenicity of a 2-dose vaccination schedule of mRNA-1893 Zika vaccine, given 28 days apart, as measured by enzyme-linked immunosorbent assay (ELISA) and additional neutralization assays.
- To assess the occurrence of flavivirus infection throughout the course of participation in the trial.

**Study Design and Methodology:**

This is a Phase 1, randomized, observer-blind, placebo-controlled, dose-ranging study to evaluate the safety, tolerability, and immunogenicity of Zika vaccine mRNA-1893 administered to healthy flavivirus-seropositive and seronegative adult participants (18 to 49 years of age, inclusive). The study will comprise a Screening Phase (up to 28 days), a Vaccination Phase (up to 57 days), and a Long-Term Follow-up Phase (up to 12 months after last vaccination). Participants will have approximately 8 clinic visits with an additional 13 safety telephone calls. Study duration will be approximately 13 months for each participant. Participants will provide written informed consent before any study-specific procedures are performed.

A total of 120 participants (30 participant per cohort) will be enrolled into 1 of 4 mRNA-1893 dose cohorts (10, 30, 100, or 250 µg). Within each cohort, eligible participants will be randomly assigned to mRNA-1893 or placebo (4:1 mRNA-1893 to placebo) and administered the study vaccine as a 0.5-mL intramuscular (IM) injection on a 2-dose vaccination schedule, 28 days apart (Day 1 and Day 29). Participants will be stratified by baseline flavivirus serostatus (seropositive and seronegative).

For each dose cohort (Cohorts 1 through 4), Safety Oversight will be performed 7 days after the first 5 participants (all seronegative) are randomly assigned and receive their first study vaccination (Day 1). Once safety is confirmed, the remaining 25 participants in the dose level cohort will be randomly assigned to dosing. Seven days after all 30 participants in the cohort have received the first study vaccination, a blinded IST that is not directly involved in the day-to-day activities of the study, will review all available safety data for the currently dosed cohort and any cumulative safety data of all cohorts as the trial advances to determine the acceptability to escalate to the next

mRNA-1893 dose level. The 30 participants in the first cohort will continue to receive their second study vaccine dose on Day 29 (+14 days).

The blinded IST will oversee the safety of the trial and will review safety data to ensure adherence to the protocol, will monitor safety laboratory test results and reactogenicity, and may request input from the SMC should the study meet pause rules or for any other study events that could potentially affect participant safety. The IST will approve escalation to the next higher dosing cohort after review of blinded safety data of the currently dosed cohort through 7 days after the first vaccination and any cumulative safety data of all cohorts as the trial advances.

At each vaccination visit (Day 1 and Day 29), a diary card will be provided to the participant and study staff will provide training on its proper use. Participants will record daily body temperature, any solicited local (injection site) and systemic AEs (solicited AEs), any unsolicited AEs, and any concomitant medications and vaccinations on the day of each vaccination and on 7 subsequent days. Participants will be instructed to return the completed diary card to the Investigator at the subsequent planned study visit (Day 8 and Day 36).

Participants will record on the same diary card daily any unsolicited AEs experienced, daily body temperature, and concomitant medications and vaccinations (excluding vitamins and minerals) received from 7 through 28 days after each vaccination. Participants will be instructed to return the completed diary card to the Investigator at Day 29 and Day 57.

All concomitant medications and vaccinations (excluding vitamins and minerals) received and SAEs, MAAEs, AESIs, and

AEs leading to withdrawal from vaccine dosing or from the study will be collected from Day 1 until Month 13 (EOS Visit). Other safety assessments will include clinical laboratory test results (hematology, serum chemistry, and coagulation); vital sign measurements; and physical examination findings. Blood samples for immunogenicity assessments will be collected the day of each vaccination (Days 1 and 29 before vaccination), 28 (+14) days after each vaccination, and during the Long-Term Follow-up Phase at Month 7 (± 14 days) and Month 13 (± 14 days).

Once all subjects from a cohort have completed the Vaccination Phase through Day 57 (-7/+14 days), the database will be locked for that cohort and safety and immunogenicity data will be analyzed through 28 days following the second vaccination by an unblinded statistician. As dose escalation occurs, cumulative analyses will be included for each subsequent data lock to allow for all prior dosing cohorts to be analyzed by dose assignment, and in aggregate for mRNA-1893 exposure. Immunogenicity and safety data, including mean group analyses of change from baseline, where applicable, will be summarized for each dose group. These data are required to inform decisions on dose selection for this and other development programs using the same messenger RNA (mRNA) platform.

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

| | |
|---|---|
| **Study Population:** | Participants (males and females 18 to 49 years of age, inclusive), will be included in the study if they are in good health as determined by medical history, clinical laboratory assessments, vital sign measurements, a physical examination at screening and per investigator judgement. Negative pregnancy tests will be required at screening and before vaccine administration for female participants of childbearing potential. Flavivirus serostatus (positive or negative) will be determined by ELISA or other commercially available serological assay. The full lists of inclusion and exclusion criteria are provided in Section 3.2.1 and Section 3.2.2, respectively. |
| **Safety Assessments:** | Safety assessments will include monitoring and recording of solicited AEs (local and systemic reactogenicity events) and unsolicited AEs, serious AEs (SAEs), AEs of special interest (AESIs), AEs leading to study withdrawal, medically attended AEs (MAAEs); clinical laboratory test results (hematology, serum chemistry, and coagulation); vital sign measurements; and physical examination findings. |
| **Immunogenicity Assessments:** | Immunogenicity assessments will include the following:<br>• Serum neutralizing antibodies (nAb) against ZIKV<br>• Serum binding antibodies (bAb) against ZIKV |
| **Vaccine, Dosage, and Route of Administration:** | mRNA-1893 is a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine encoding the full pre-membrane and envelope (prME) structural polyproteins of ZIKV. mRNA-1893 consists of an mRNA Drug Substance formulated with LNPs. mRNA-1893 is provided as a sterile liquid for injection at a concentration of 0.5 mg/mL in 100 mM Tris buffer, 7% propylene glycol, and 1 mM diethylenetriamine-pentaacetic acid (DTPA). |

mRNA-1893 (10, 30, 100, or 250 µg) and placebo 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. The second dose of vaccine or placebo will be administered preferably in the same arm used for the first dose.

The placebo is 0.9% sodium chloride injection, United States Pharmacopeia (USP) or British Pharmacopeia (BP).

**Sample Size:**

A total of 120 participants (30 participant per cohort) are planned for enrollment in the study and random assignment to study dosing. The sample size is considered sufficient to meet the study objective of identifying a dose and establishing initial safety results in a population of healthy adults in both endemic and nonendemic Zika regions. Formal sample size calculations were not performed.

**Statistical Methods:**

**Safety:** Reactogenicity will be summarized by dosing group (10, 30, 100, or 250 µg mRNA-1893 or placebo), vaccination (first or second dose), duration, and severity. Adverse events will be coded by preferred term and system organ class using MedDRA and summarized by dose group, vaccination (first or second dose), and overall. Adverse events will also be summarized by severity and relationship to the study vaccine. Descriptive statistics will be presented, and the difference in the proportion of participants with AEs will be provided, comparing each dose level with placebo pooled across all cohorts. Individual participant listings will be provided for all AEs, AEs leading to study withdrawal, AESIs, MAAEs, and SAEs.

Safety data from clinical laboratory test results and vital sign measurements will be graded by severity scoring and analyzed by dose 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 serology, urine drug screening, physical examinations, and pregnancy tests will be listed for all participants randomly assigned to receive study vaccine.

Medical history data for all participants randomly assigned to receive study vaccine will be presented by participant in a listing. Baseline demographic and background variables will be summarized by dosing group for all randomly assigned participants. The number of participants who enroll in the study and the number and percentage of participants who complete the study will be presented. Frequency and percentage of participants who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will also be summarized.

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

**Immunogenicity:** The following secondary immunogenicity outcome measures and the 95% confidence intervals, where appropriate, will be summarized by dose group and by visit:

- Serum neutralizing antibodies (nAb titers) against ZIKV by PRNT:
- Geometric mean titer (GMT) of nAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
- GMT of nAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.

- GMT of nAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
- Percentage of participants who seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as a change of PRNT from below the lower limit of quantification (LLOQ) to a PRNT equal to or above LLOQ, or a multiplication by at least 4 in subjects with pre-existing PRNT titers.
- Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
- Proportion of initially seropositive participants with a 2-fold or 4-fold increase in nAb titers as compared with baseline as measured by PRNT.

The following exploratory immunogenicity outcome measures and the 95% confidence intervals, where appropriate, will be summarized by dosing group and by visit:

- GMT of nAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- GMT of nAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- GMT of nAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7,

and Month 13 as measured by additional neutralization assay.

- Percentage of participants who seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as change of nAb titer (by additional neutralization assay) from below the LLOQ to a titer equal to or above LLOQ, or a multiplication by at least 4 in pre-existing neutralizing titer.
- Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- Proportion of initially seropositive participants with a 2-fold or 4-fold increase in nAb as compared with baseline as measured by additional neutralization assay.
- GMT of bAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- GMT of bAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- GMT of bAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- Percentage of participants that seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57,

from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as a change of binding antibody titer from below the LLOQ to a binding antibody titer equal or above the LLOQ, or a multiplication by at least 4 in pre-existing bAb titers.

- Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- Proportion of initially seropositive participants with a 2-fold or 4-fold increase in bAb as compared with baseline as measured by ELISA binding assay.
- IgG and IgM antibodies against envelope- and NS1-based antigens present in serum collected at baseline and at end of study measured as antibody binding by ELISA or equivalent methodology to flaviviruses.

**Date of Protocol:** 17 March 2020

# List of Abbreviations

| Abbreviation | Definition |
|---|---|
| AE | adverse event |
| AESI | adverse event of special interest |
| bAb | serum binding antibodies |
| BP | British Pharmacopoeia |
| CFR | Code of Federal Regulations |
| CRO | contract research organization |
| CS | clinically significant |
| DSPC | 1,2-distearoyl-sn-glycero-3-phosphocholine |
| DTPA | diethylenetriamine-pentaacetic acid |
| eCRF | electronic case report form |
| ELISA | enzyme-linked immunoabsorbent assay |
| EOS | end of study |
| FDA | Food and Drug Administration |
| GLP | Good Laboratory Practice |
| GMT | geometric mean titer |
| IB | investigator's brochure |
| ICF | informed consent form |
| ICH | International Council for Harmonisation |
| Ig | immunoglobulin |
| IM | intramuscular |
| IRB | institutional review board |
| IRT | interactive response technology |
| IST | internal safety team |
| IV | intravenous |
| LLOQ | lower limit of quantification |
| LNP | lipid nanoparticle |
| LOQ | limit of quantification |
| MAAE | medically attended adverse event |
| MedDRA | Medical Dictionary for Regulatory Activities |

| Abbreviation | Definition |
|---|---|
| mRNA | messenger RNA |
| nAb | serum neutralizing antibodies |
| NCS | not clinically significant |
| NHP | nonhuman primate |
| PEG2000-DMG | 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 |
| prME | pre-membrane and envelope structural protein of ZIKV |
| PRNT | Plaque Reduction Neutralization Test |
| SAE | serious adverse event |
| SM-102 | heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy) hexyl) amino)octanoate |
| SMC | Safety Monitoring Committee |
| TEAE | treatment-emergent adverse event |
| ULOQ | upper limit of quantification |
| USP | United States Pharmacopoeia |
| WHO | World Health Organization |
| WNL | within normal limits |
| ZIKV | Zika virus |
| ZPIV | Zika purified inactivated vaccine |

# Glossary of Terms

| Term | Definition |
|---|---|
| Adequate contraception | Adequate contraception is defined as consistent and correct use of a Food and Drug Administration-approved contraceptive method in accordance with the product label. For example:<br>• Barrier method (such as condoms, diaphragm, or cervical cap) used in conjunction with spermicide<br>• Intrauterine device<br>• Hormonal contraceptive in the form of a pill or patch<br>• Medroxyprogesterone injection (Depo-Provera®)<br>• Etonogestrel implant (Nexplanon®)<br>• Sterilization of a female participant's monogamous male partner prior to entry into the study<br>• Note: periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. |
| Grade 1 laboratory abnormality with potential clinical significance | A Grade 1 laboratory parameter that cannot be explained or that is judged by the investigator to be potentially clinically significant. A list of laboratory-specific normal ranges and associated toxicity grades is provided in Appendix 3. |
| Grade 1 laboratory abnormality without potential clinical significance | A Grade 1 laboratory parameter that can be explained by a condition that is not related to vaccination and does not increase the risk for an adverse outcome of vaccination. A list of laboratory-specific normal ranges and associated toxicity grades is provided in Appendix 3. |
| Menopause | Natural menopause is defined as the permanent cessation of menstrual periods, determined retrospectively after a woman has experienced 12 months of amenorrhea without any other obvious pathological or physiological cause. Menopause occurs at a median age of 51.4 years. |
| Protocol amendment | The International Council for Harmonisation defines a protocol amendment as "A written description of a change(s) to or formal clarification of a protocol." It may include a change that affects the safety of participants, scope of the investigation, study design, or scientific integrity of the study. |
| Solicited adverse event | Local and systemic reactogenicity events. |

| Term | Definition |
|---|---|
| Unsolicited adverse event | Any adverse event (AE) reported by a participant that is either (1) not specified as a solicited AE in the protocol or (2) specified as a solicited AE in the protocol but its onset occurs outside of the protocol-defined post-vaccination period for reporting solicited AEs (i.e., for the 7 days after each vaccination). |

# 1 INTRODUCTION

## 1.1 Background

Zika virus (ZIKV), first discovered in 1947, is a single-stranded RNA flavivirus, which is transmitted to humans by a mosquito vector (mainly *Aedes aegypti* but other *Aedes* mosquitoes are believed to be competent vectors) or by person-to-person spread, mainly through sexual transmission. In the 60 years after its discovery, ZIKV remained a relatively obscure pathogen, associated with only sporadic cases of human infection that were largely asymptomatic or resulted in a mild febrile illness (Weaver et al 2016). In the last decade, however, there has been rapid geographic spread into the Pacific Islands and South America, where ZIKV outbreaks have been larger, more frequent, and more severe (Lazear and Diamond 2016). Most concerning are infections in pregnant women, particularly during the first and second trimesters, which have resulted in a wide range of birth defects, including microcephaly, intrauterine growth restriction, and spontaneous abortion (Brasil et al 2016).

The devastating consequences of ZIKV infection, including congenital Zika syndrome, pregnancy loss and complicated neurological sequelae such as Guillain-Barre syndrome, led the World Health Organization (WHO) to declare a Public Health Emergency of International Concern on 01 February 2016 (Heymann et al 2016) and to call on the global research and development community to prioritize the development of preventive and therapeutic solutions (WHO 2016a). Although the WHO declared an end to its global health emergency regarding the spread of ZIKV in November 2016, the long-term need for a ZIKV vaccine remains (WHO 2019) a priority need under the Blueprint Plan of Action (WHO 2016b). Currently, there is no approved vaccine to protect against this disease.

ModernaTX, Inc. has developed a proprietary messenger RNA (mRNA)-based vaccine platform. This is based on the principle and observations that antigens can be produced in vivo by delivery and uptake of the corresponding mRNA by cells. The mRNA then undergoes intracellular ribosomal translation to endogenously express the protein antigen(s) encoded by the vaccine mRNA. This mRNA-based vaccine does not enter the cellular nucleus or interact with the genome and is nonreplicating, and expression is transient. mRNA vaccines thereby offer a mechanism to stimulate endogenous production of structurally intact

protein antigens in a way that mimics wild type viral infection and are able to induce highly targeted immune responses against infectious pathogens such as ZIKV.

mRNA-1893 is a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine directed against the pre-membrane and envelope (prME) structural protein of ZIKV. mRNA-1893 consists of an mRNA Drug Substance that is formulated with LNPs composed of 4 lipids: heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino) octanoate (SM-102); cholesterol; 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); and 1-monomethoxypolyethyleneglycol-2,3-dimyristylglycerol with polyethylene glycol of average molecular weight 2000 (PEG2000-DMG). mRNA-1893 is provided as a sterile liquid for injection at a concentration of 0.5 mg/mL in 100 mM Tris buffer, 7% propylene glycol, and 1 mM diethylenetriamine-pentaacetic acid (DTPA).

## 1.2 Nonclinical Studies in Development of mRNA-1893

In support of development of mRNA-1893 for prophylaxis against ZIKV infection, nonclinical immunogenicity, biodistribution, and safety studies have been completed with mRNA-1893 or similar mRNA-based vaccines formulated in SM-102-containing LNPs.

In a non-Good Laboratory Practice (GLP) in vivo dose-range finding study, immunogenicity (binding and neutralizing antibody titers) to the mRNA-1893 encoded protein was evaluated in female C57BL/6 mice at 4 doses (15, 10, 2, and 0.4 µg) following intramuscular (IM) injection on Day 1 and Day 29. mRNA-1893 generated strong neutralizing and binding antibody titers at multiple doses. Mice dosed with 15 and 10 µg mRNA-1893 generated higher neutralizing and binding antibody titers than mice dosed with 0.4 µg mRNA-1893. Mice dosed with 15, 10, and 2 µg mRNA-1893 generated higher binding antibody titers compared with mice dosed with 0.4 µg mRNA-1893.

The immunogenicity and efficacy of mRNA-1893 were evaluated in a non-GLP study in the rhesus macaque ZIKV infection model. A total of 40 monkeys were randomly assigned to 1 of 7 groups to receive mRNA-1893 (200, 100, 50, 10, or 2 µg), Zika purified inactivated vaccine (ZPIV [400 antigenic units]), a formalin-inactivated ZIKV strain PRVABC59 shown to be immunogenic in human, or buffer control. Dose administration occurred via IM injection on Day 0 and Day 28. Following blood collections on Day 56, animals were challenged by subcutaneous injection with a target dose of $1 \times 10^5$ plaque-forming units of

ZIKV strain PRVABC59. Overall, administration of mRNA-1893 by IM injection (2 doses) was clinically well tolerated, with no mRNA-1893-related mortality, clinical observations, or changes in body weight. Animals in the buffer control group developed viremia that persisted for 3 to 7 days, whereas mRNA-1893– and ZPIV-treated animals were either negative for ZIKV RNA at all time points evaluated or had sporadic instances of low levels of ZIKV RNA at a single time point after ZIKV challenge. Detection of ZIKV RNA was inversely related to detection of ZIKV-neutralizing antibodies prior to ZIKV challenge. mRNA-1893 and/or ZPIV-treated animals had detectable neutralizing antibody titers in the ZIKV microneutralization assay at Days 28 and 56 as well as at the end of study (EOS; Day 77, 21 days after ZIKV challenge), whereas animals in the buffer control group did not have ZIKV-neutralizing titers until after ZIKV challenge on Day 77.

Overall, these results demonstrate that mRNA-1893 generates strong immunogenic response in mice and nonhuman primates (NHPs) and prevents viremia, with either no or low levels of ZIKV RNA upon challenge in the rhesus macaque ZIKV infection model.

To evaluate the generalized tissue distribution and tissue half-life of mRNA-1893, the biodistribution of mRNA-1647, a similar mRNA-based vaccine formulated in SM-102–containing LNPs, was evaluated in male rats. mRNA-1647 is a novel mRNA-based cytomegalovirus vaccine formulated in a mixture of the same 4 lipids as mRNA-1893. The biodistribution of mRNA-based vaccines formulated in LNPs is predicted to be driven by the LNP characteristics. Therefore, mRNAs that are within an LNP of the same composition (e.g., mRNA-1893 and mRNA-1647) are expected to distribute similarly. Overall, only a relatively small fraction of the administered mRNA-1647 dose distributed to distant tissues, and the mRNA constructs did not persist past 1 to 3 days in tissues other than the vaccination site, lymph nodes, and spleen.

The safety and tolerability of 3 dose levels of mRNA-1893 administered by IM injection were evaluated in a GLP-compliant, repeat-dose (1 month; 3 doses) toxicity study in Sprague Dawley rats followed by a 2-week recovery period. Animals were administered mRNA-1893 at doses of 10, 30, or 96 µg/dose or phosphate-buffered saline via IM injection on Days 1, 15, and 29. The results of this study indicated that administration of mRNA-1893 was clinically tolerated in rats up to 96 µg/dose with no mortality or changes in body weight or food consumption. Starting at 10 µg/dose, dose-dependent clinical signs

(swelling/firmness/redness/scabs) at the injection site, changes in clinical pathology parameters and cytokines concentrations, and an increase in body temperature were observed and were consistent with an inflammatory reaction. Dose-dependent effects were observed at the vaccination site, spleen, liver, and seminal vesicle of animals given mRNA-1893. Additional microscopic findings in the iliac, inguinal, and popliteal lymph nodes or their perinodal tissue; perineural tissue of the sciatic nerve; spleen (extramedullary hematopoiesis); and bone marrow of animals given mRNA-1893 were considered to be an extension, a secondary response, or a reactive response to the vaccination site inflammation. At the end of the 2-week recovery period, all changes were partially or fully recovered.

In GLP-compliant studies, SM-102, the novel lipid component of the LNP formulation, was not genotoxic when tested in a bacterial reverse mutation (Ames) test or an in vitro micronucleus test. An in vivo micronucleus study in Sprague Dawley rats showed that a similar mRNA-based vaccine formulated in SM-102-containing LNPs (mRNA-1706, which encodes the ZIKV pre-membrane and envelope polypeptide [different from the sequence encoded in mRNA-1893]), induced statistically significant increases in micronucleated immature erythrocytes in male rats at both 24 and 48 hours and in female rats at 48 hours only; however, there was no clear dose response, and the increases were generally weak and associated with minimal bone marrow toxicity. These observations indicate that the risk to humans after IM administration is low due to minimal systemic exposure.

A detailed review of non-clinical experience with mRNA-1893 Zika vaccine is provided in the investigator's brochure (IB).

## 1.3 Clinical Studies With mRNA-1893

No clinical studies with mRNA-1893 have been conducted to date.

## 1.4 Rationale for Study

No vaccines have yet been licensed for prophylaxis of ZIKV infection, although several vaccines have advanced to early clinical development with promising safety and immunogenicity data (Richner and Diamond 2018).

The Sponsor previously advanced the ZIKV-vaccine mRNA-1325 to clinical testing. The mRNA-1325 trial enrolled 90 participants across 3 dose levels (74 participants received

mRNA-1325, 16 participants received placebo). While the vaccine was well tolerated, the immunogenicity objective at doses up to 100 µg was not met. Despite a safety profile that permitted additional dose escalation, current development efforts are focused on the next generation vaccine, mRNA-1893, that contains an engineered sequence shown to be 20 times more potent in NHP Zika challenge studies (data on file).

The Sponsor believes that on the basis of preclinical data, mRNA-1893 has the potential to achieve safety and efficacy levels comparable to virus-like particle or live-attenuated vaccine approaches, with the additional benefit of a rapid and scalable manufacturing platform.

The purpose of this Phase 1, first-in-human, randomized, placebo-controlled, dose-ranging study is to evaluate the safety, tolerability, and immunogenicity of mRNA-1893 Zika vaccine in healthy adult participants in endemic and nonendemic Zika regions. The primary objective of this study is to assess the safety, tolerability, and reactogenicity profile of a range of doses of mRNA-1893 Zika vaccine compared with placebo. The study will also evaluate immunogenicity by assessing changes from baseline in ZIKV-specific neutralizing antibody titers. The safety profile and immune response(s) will be compared between flavivirus-seropositive and flavivirus-seronegative participants to indicate the ability of the selected dose to safely protect populations in both endemic and nonendemic Zika regions.

## 1.5 Rationale for Dose Selection

Doses of mRNA-1893 ranging from 10 to 250 µg will be tested in a dose-escalating fashion starting with 10 µg (Cohort 1), then moving to 30 µg (Cohort 2), then 100 µg (Cohort 3), and eventually 250 µg (Cohort 4). This dose selection is based on preclinical data and clinical experience with other infectious disease programs. A dose as low as 2 µg has been shown to be protective in a NHP challenge model.

## 1.6 Rationale for Study Design

The study is designed to generate the safety and immunogenicity data required to select the optimal dose of mRNA-1893 for further development in both endemic and nonendemic populations. Preclinical and clinical data support the expectation that a 2-dose vaccination schedule, 1 month apart, will induce a protective immune response. The 2-dose schedule will be explored at a range of doses in 2 subpopulations: endemic and nonendemic. The

nonendemic population will be represented by participants recruited from the general population who are flavivirus seronegative. The endemic population will be represented by participants recruited from endemic Zika regions who are seropositive for flavivirus (including dengue, West Nile, and Zika).

In order to explore vaccine safety, dosing will begin with 10 µg (Cohort 1) and escalate to 30, 100, and 250 µg. For each dose cohort, 5 flavivirus seronegative participants will be randomly assigned to dosing (4:1 mRNA-1893 to placebo) and followed for 7 days after the first vaccination, with safety oversight to confirm no pause rules were met (Section 3.4.2.2; Section 3.5.6) before randomly assigning the remaining 25 participants in the cohort. In addition, starting with the 10 µg-dose cohort, 7 days after all participants have received the first study vaccination, a blinded internal safety team (IST) will review all safety data for the cohort and determine the acceptability of escalating to the next mRNA-1893 dose level. The IST will approve escalation to the next higher dosing cohort after review of blinded safety data of the currently dosed cohort participants through 7 days after the first vaccination and any cumulative safety data of all cohorts as the trial advances.

Pause rules have been pre-established to trigger ad hoc unblinded Safety Monitoring Committee (SMC) reviews. The SMC is composed of independent experts with relevant expertise that will meet upon request of the IST, if required.

Immunogenicity against ZIKV will be assessed before vaccination (baseline; Day 1) and 28 (+14) days after each vaccination and assessed for persistence at Month 7 and at Month 13 (12 months after the last vaccination) with assays that are relevant for the field, in particular, neutralization assays that may correlate with protection. A 12-month Long-Term Follow-up Phase after the last vaccine administration will be performed to assess if any serious AEs (SAEs), medically attended AEs (MAAEs), and AEs of special interest (AESIs) have occurred during the study. This is comparable to the assessments performed by the Sponsor across its mRNA vaccine candidates in early development.

# 2 STUDY OBJECTIVES

## 2.1 Primary Objective

The primary objective of the study is to evaluate the safety, tolerability, and reactogenicity of a 2-dose vaccination schedule of mRNA-1893 Zika vaccine, given 28 days apart, across a range of dose levels in flavivirus-seronegative and flavivirus-seropositive participants compared with placebo.

## 2.2 Secondary Objectives

The following is the secondary objective of the study:

- To evaluate the immunogenicity of a 2-dose vaccination schedule of mRNA-1893 Zika vaccine, given 28 days apart as measured by ZIKV-specific neutralization assay (Plaque Reduction Neutralization Test [PRNT]).

## 2.3 Exploratory Objectives

The following are the exploratory objectives of the study:

- To evaluate the immunogenicity of a 2-dose vaccination schedule of mRNA-1893 Zika vaccine, given 28 days apart, as measured by enzyme-linked immunoabsorbent assay (ELISA) and additional neutralization assays.
- To assess the occurrence of flavivirus infection throughout the course of participation in the trial.

# 3 INVESTIGATIONAL PLAN

## 3.1 Study Design

This is a Phase 1, randomized, observer-blind, placebo-controlled, dose-ranging study to evaluate the safety, tolerability, and immunogenicity of Zika vaccine mRNA-1893 administered to healthy flavivirus-seropositive and seronegative adult participants (18 to 49 years of age, inclusive). A total of 120 participants (30 participants per cohort) will be enrolled into 1 of 4 mRNA-1893 dose cohorts (10, 30, 100, or 250 µg). Within each cohort, eligible participants will be randomly assigned to mRNA-1893 or placebo (4:1 mRNA-1893 to placebo) and administered the study vaccine as an IM injection on a 2-dose vaccination schedule, 28 days apart (Day 1 and Day 29). Participants will be stratified by baseline flavivirus serostatus (seropositive and seronegative).

The study will comprise a Screening Phase (up to 28 days), a Vaccination Phase (up to 57 days), and a Long-Term Follow-up Phase (up to 12 months after last vaccination) (Table 5). Participants will have approximately 8 clinic visits with an additional 13 safety telephone calls. Study duration will be approximately 13 months for each participant. Participants will provide written informed consent before any study-specific procedures are performed.

Study vaccine dosing will begin with Cohort 1 (10 µg mRNA-1893 or placebo), followed sequentially by Cohort 2 (30 µg mRNA-1893 or placebo), Cohort 3 (100 µg mRNA-1893 or placebo), and Cohort 4 (250 µg mRNA-1893 or placebo). Vaccine accountability, dose preparation, and vaccine administration will be performed by unblinded pharmacy personnel, who will not participate in any other aspects of the study. The remainder of the study staff and all participants will remain blinded to dosing assignment.

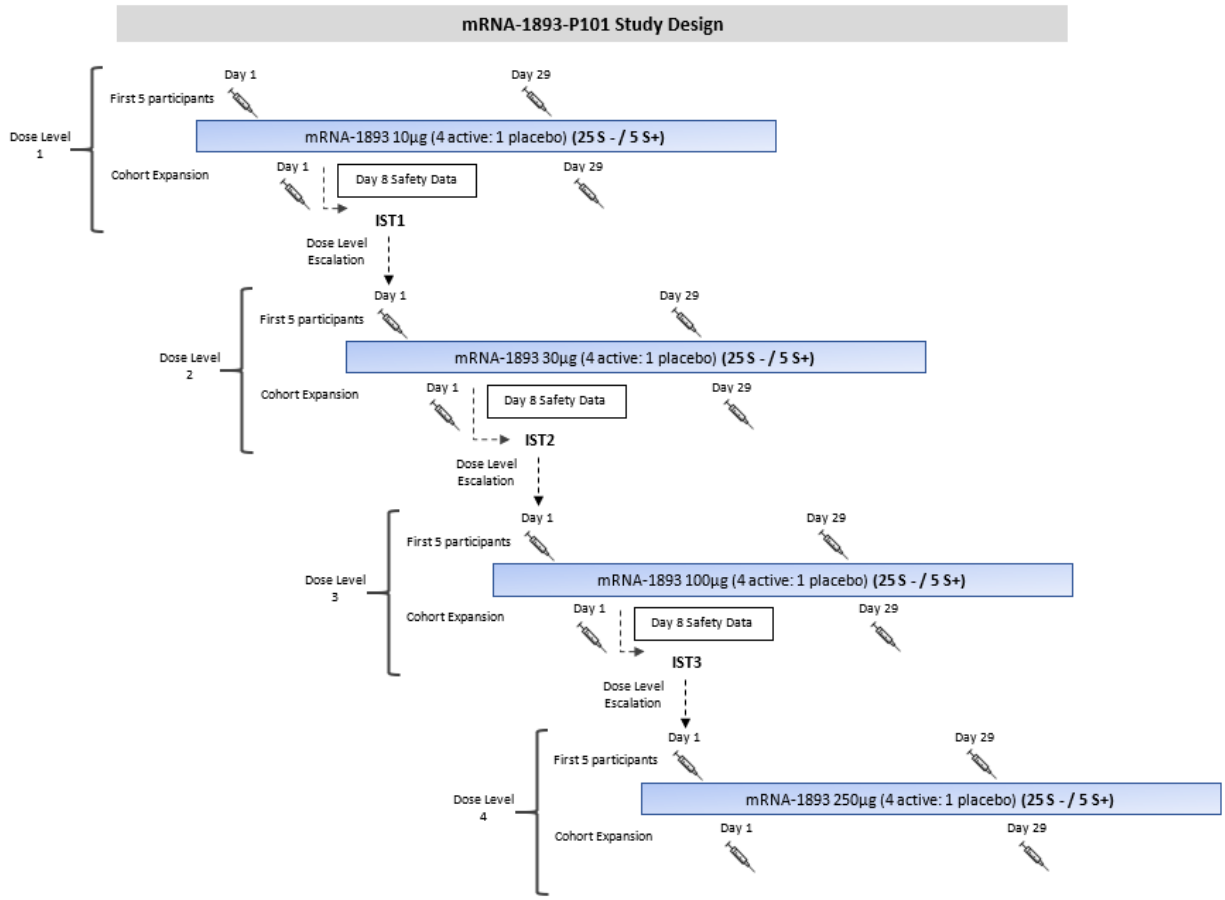
For each dose cohort (Cohort 1 through 4), Safety Oversight will be performed 7 days after the first 5 participants (all seronegative) are randomly assigned and receive the first study vaccination (Day 1). Once safety is confirmed, the remaining 25 participants in the dose level cohort will be randomly assigned to dosing. Seven days after all 30 participants in the cohort have received the first study vaccination, a blinded IST that is not directly involved in the day-to-day activities of the study, will review all available safety data for the currently dosed

cohort and any cumulative safety data of all cohorts as the trial advances to determine the acceptability to escalate to the next mRNA-1893 dose level. The 30 participants in the first cohort will continue to receive their second study vaccine dose on Day 29 (+14 days).

The blinded IST will oversee the safety of the trial and will review safety data to ensure adherence to the protocol, will monitor safety laboratory test results and reactogenicity, and may request input from the SMC should the study meet pause rules or for any other study events that could potentially affect participant safety. The IST will approve escalation to the next higher dosing cohort after review of blinded safety data of the currently dosed cohort through 7 days after the first vaccination and any cumulative safety data of all cohorts as the trial advances.

A diagram of the dosing schema by cohort is shown in Figure 1.

**Figure 1: Dosing Schema by Cohort**




Abbreviations: IST = internal safety team; S- = flavivirus seronegative; S+ = flavivirus seropositive.

Note: For each dose cohort (Cohorts 1 through 4), Safety Oversight will be performed 7 days after the first 5 participants (all flavivirus-seronegative) are randomly assigned and receive their first study vaccination (Day 1) before randomly assigning the remaining 25 participants in the cohort. Seven days after all 30 participants in the cohort have received the first study vaccination and review of all available safety data has been performed (ie, no safety concerns and pause rules have not been met), the IST will determine the acceptability to escalate to the next mRNA-1893 dose level.

During the Vaccination Phase, participants will be instructed to contact the Investigator immediately should they develop any untoward signs or symptoms or any medical occurrence that leads to an unplanned visit to a health care practitioner, hospitalization, or an emergency room visit. In case of MAAEs, if the participant presents with clinical symptoms

that may suggest a dengue infection, a blood sample should be collected for testing of dengue infection. In addition, participants will be instructed to contact the site within 24 hours, in the event of any severe Grade 3 local and systemic reactogenicity AE (solicited AEs; Section 3.5.6) or any episode of rash within 6 days after study vaccination.

At each vaccination visit (Day 1 and Day 29), a diary card will be provided to the participant and study staff will provide training on its proper use. Participants will record daily body temperature, any solicited local (injection site) and systemic AEs (solicited AEs), any unsolicited AEs, and any concomitant medications and vaccinations on the day of each vaccination and on 6 subsequent days. Participants will be instructed to return the completed diary card to the Investigator at the subsequent planned study visit (Day 8 and Day 36).

Participants will record on the same diary card daily any unsolicited AEs experienced and concomitant medications and vaccinations (excluding vitamins and minerals) received from 7 through 28 days after each vaccination. Participants will be instructed to return the completed diary card to the Investigator at Day 29 and Day 57.

All concomitant medications and vaccinations (excluding vitamins and minerals) received, and SAEs, MAAEs, AESIs, and AEs leading to withdrawal from vaccine dosing or from the study will be collected from Day 1 until Month 13 (EOS Visit). Other safety assessments will include clinical laboratory test results (hematology, serum chemistry, and coagulation); vital sign measurements; and physical examination findings.

Blood samples for immunogenicity assessments will be collected the day of each vaccination (Days 1 and 29 before vaccination), 28 (+14) days after each vaccination, and during the Long-Term Follow-up Phase (Month 7 and Month 13).

Detailed information on all statistical analysis of data is presented in Section 3.6.6.

The complete Schedule of Events is shown in Table 5.

## 3.2 Selection of Study Population

Healthy male or female participants will be enrolled at clinical sites in both endemic and nonendemic Zika regions of the United States or its territories. A total of 120 participants (30 participants per cohort) are planned to be randomly assigned to receive study dosing.

### 3.2.1 Inclusion Criteria

Each participant must meet all of the following criteria during the Screening Phase and at Day 1, unless noted otherwise, to be enrolled in this study:

1. Male or female 18 to 49 years of age, inclusive.

2. Understands and agrees to comply with the study procedures and provides written informed consent.

3. In the opinion of the investigator, can and will comply with the requirements of the protocol (e.g., complete diary cards, return for follow-up visits, be available for safety telephone calls).

4. Is in good general health, as determined by medical history, clinical laboratory assessments, vital sign measurements, and physical examination at screening.

5. For flavivirus-seropositive group, has positive flavivirus test results (including dengue, West Nile, and Zika) as determined by ELISA or other commercially available serological assay.

6. For flavivirus-seronegative group, has negative flavivirus test results (including dengue, West Nile, and Zika) as determined by ELISA or other commercially available serological assay.

7. Female participants of childbearing potential may be enrolled in the study, if the participant: (1) has a negative urine pregnancy test at Screening, Day 1, and before second vaccine administration, (2) has practiced adequate contraception (Glossary of Terms) or is abstaining from all activities that could lead to pregnancy for 30 days before the first vaccination, and (3) has agreed to continue adequate contraception through 3 months after the last vaccination.

8. Female participants of nonchildbearing potential may be enrolled in the study. Nonchildbearing potential is defined as bilateral tubal ligation > 1 year before screening, bilateral oophorectomy, hysterectomy, or menopause (Glossary of Terms). The

follicle-stimulating hormone level may be measured at the discretion of the investigator to confirm menopausal status.

9. Male participants must agree to practice adequate contraception (Glossary of Terms) from the time of the first vaccination and through 3 months after the last vaccination. Males must also agree to refrain from donation of sperm from the time of first vaccination until 3 months following the last vaccination.

### 3.2.2 Exclusion Criteria

Participants meeting any of the following criteria during the Screening Phase and at Day 1, unless noted otherwise, will be excluded from the study:

1. Has any acute or chronic, CS disease, as determined by medical history, physical examination, clinical laboratory assessments, and vital sign measurements. Asymptomatic conditions or findings (e.g., mild hypertension or dyslipidemia) are not exclusionary if they are being appropriately managed, are clinically stable, and are unlikely to progress within the study period, in the opinion of the investigator.

2. Has received (at any time) a vaccine for dengue, Japanese encephalitis, tick-borne encephalitis, West Nile, Yellow Fever, or Zika.

3. Has a neurologic disorder (e.g., history of seizures, Guillain-Barré syndrome, dementia, vasculitis, or any known congenital or acquired disorder).

4. Has a body mass index that is $\leq 18$ or $\geq 35$ kg/m$^2$.

5. If female of childbearing potential, is pregnant or lactating or has not adhered to an adequate contraception method from at least 30 days before the first dose of study vaccine through 3 months after the last vaccination.

6. Has elevated liver function tests, defined as aspartate aminotransferase, alanine aminotransferase, or alkaline phosphatase with a toxicity score of Grade $\geq 1$ at Screening (Appendix 3). Retesting of these parameters is not allowed.

7. Has clinical laboratory test results (hematology, serum chemistry, or coagulation) with a toxicity score of Grade ≥ 1 at Screening (Appendix 3). Retesting of these parameters is allowable once, but the results must be within normal limits (WNL). If results are not WNL, the participant will be excluded from the study.

8. Has a bleeding disorder that would contraindicate IM injections or phlebotomy.

9. Reports a diagnosis of congenital or acquired immunodeficiency (including human immunodeficiency virus [HIV] infection), or autoimmune disease.

10. Has a history of hypersensitivity or severe reactions (e.g., anaphylaxis, urticaria, other significant event requiring medical intervention) to previous vaccinations or any component of the study vaccine.

11. Has a history of idiopathic urticaria.

12. Reports a previous diagnosis of hematologic malignancy or pre-malignancy (leukemia, lymphoma, or lymphoproliferative disorders) or a diagnosis of any other malignancy within the previous 10 years (excluding nonmelanoma skin cancer).

13. Has a medical, psychiatric, or occupational condition that, in the opinion of the investigator, might pose an additional risk to the participant due to participation in the study or would interfere with the evaluation of the study vaccines or the interpretation of study results.

14. Is acutely ill or febrile on the day of screening (Day 0) or randomization (Day 1). Fever is defined as a temperature ≥ 38.0°C/100.4°F by the oral, axillary, or tympanic route. Participants meeting this criterion may be rescheduled for screening at a later date. Afebrile participants with minor illnesses can be enrolled at the discretion of the investigator.

15. Has a history of inflammatory arthritis.

16. Has a history of febrile disease with arthritis or arthralgia within 2 weeks of administration of any study vaccine.

17. Has received an investigational or nonregistered product (drug or vaccine) within 30 days before the first dose of study vaccine or has plans for administration during the study period.

18. Has received or is scheduled to receive an inactivated vaccine within the period from 14 days before, or through 14 days after, administration of any study vaccination.

19. Has received or is scheduled to receive a live virus vaccine administered within the period from 28 days before, or through 28 days after, any dose of study vaccine.

20. Has received chronic administration (defined as > 14 total days) of immunosuppressants or other immune-modifying drugs within 6 months before the first study vaccine dose (for corticosteroids: prednisone ≥ 20 mg/day or equivalent is not permitted). Inhaled, nasal, and topical steroids are allowed.

21. Has received immunoglobulins and/or blood products within the 3 months before the first study vaccine dose or has plans for administration during the study period.

22. Has a positive test result at the Screening Visit for hepatitis B surface antigen, hepatitis C virus antibody, or HIV type 1 or 2 antibodies.

23. Has donated > 450 mL of whole blood or blood products within 30 days of enrollment or plans to do so during the study period.

24. Is an immediate family member or household member of study personnel.

25. Previously participated in an investigational study involving LNPs.

26. Has a positive urine drug screen result at Screening for any of the following nonprescription drugs of abuse: amphetamines, benzodiazepines, cocaine, methadone, opiates, and phencyclidine. A positive test result for any other drug will require investigator approval prior to inclusion of the participant. Positive urine drug screens for amphetamines, benzodiazepines, or opiates will not be exclusionary if the positive result is due to a prescribed concomitant medication, in the opinion of the investigator.

### 3.2.3 Participant Restrictions During the Study

#### 3.2.3.1 General and Dietary

To avoid false positive results for drugs of abuse tested at the Screening Visit, participants should refrain from food or drink containing poppy seeds (e.g., specialty breads and muffins) for 72 hours before the Screening Visit.

#### 3.2.3.2 Contraception and Pregnancy Avoidance Procedures

Female participants who are not of childbearing potential (defined as any female who has experienced menarche and who is NOT permanently sterile or postmenopausal) must be postmenopausal, as defined in Glossary of Terms, or surgically sterile (e.g., hysterectomy, bilateral tubal ligation, or bilateral oophorectomy). NOTE: These procedures and/or laboratory test results must be confirmed by physical examination or be provided through medical documentation. If of childbearing potential, female participants must be practicing a medically approved and highly effective method of contraception (Glossary of Terms).

All female participants of childbearing potential must have a negative urine pregnancy test result at the Screening Visit and before vaccine administration. Women of childbearing potential must agree to be heterosexually inactive or to consistently use acceptable methods of contraception, as defined in Glossary of Terms, from at least 30 days before the first vaccination through 3 months after the last vaccination.

Male participants must agree to practice adequate contraception (Glossary of Terms) from the time of first vaccination through 3 months after the last vaccination. Males must also agree to refrain from donation of sperm from the time of first vaccination through 3 months after the last vaccination.

Periodic abstinence, declaration of abstinence for the duration of the study, and withdrawal are not acceptable methods of contraception for male or female participants.

Female participants of childbearing potential and male participants will be provided with information on acceptable methods of contraception as part of the participant 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 Participants From the Study or Study Dosing

### 3.3.1 Participant Withdrawal From the Study

Participants can withdraw consent and discontinue from the study at any time, for any reason, without prejudice to further treatment the participant may need to receive.

If participant desires to withdraw from the study because of an AE, the investigator will try to obtain agreement to follow up with the participant until the event is considered resolved or stable and will then complete the EOS electronic case report form (eCRF).

Potential reasons for withdrawing a participant from the study include the following:

- SAE
- AE (non-SAE)
- Protocol violation (specify)
- Consent withdrawal (document the reason)
- Lost to follow-up
- Other (specify)

Participants who are withdrawn from the study will be requested to complete the EOS (Visit 20 [Month 13]) assessments. The reason for participant withdrawal or lost to follow-up will be documented, as detailed in Section 3.3.3.

### 3.3.2 Participant Withdrawal From Study Vaccine

Every reasonable attempt will be made to follow up with participants for safety throughout the entire study period, even if further vaccination is withheld or the participant misses one or more visits.

Unless consent is withdrawn, a participant who discontinues from receiving the second dose of study vaccine will remain in the study and complete all safety and immunogenicity procedures required through 1 month after the last vaccination received per the Schedule of Events (i.e., up to Visit 5/Day 29 for subjects receiving only the first dose of vaccine).

Subjects will then enter the 12-month Long-Term Follow-up Phase per the Schedule of Events (Table 5).

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

- Becomes pregnant
- Develops, during the course of the study, symptoms or conditions listed in the exclusion criteria
- Experiences an AE (other than reactogenicity) after vaccination that is considered by the investigator to be possibly or probably related to vaccine and is of Grade 3 (severe) or greater severity (Appendix 3)
- Experiences an AE or SAE that, in the judgment of the investigator, requires study vaccine withdrawal due to its nature, severity, or required treatment, regardless of the causal relationship to vaccine
- Experiences a CS change in clinical laboratory test results, vital sign measurements, or general condition that, in the judgment of the investigator, requires vaccine withdrawal
- Experiences anaphylaxis clearly attributed to study vaccine
- Experiences generalized urticaria related to the study vaccine

The reason(s) for withdrawing further vaccination will be recorded.

### 3.3.3 Handling of Withdrawals

When a participant withdraws from the study, the reason(s) for withdrawal will be recorded by the investigator on the relevant page of the eCRF. These participants will also be requested to complete the EOS (Visit 20 [Month 13]) assessments.

Any participant who fails to return for final assessments will be contacted by the site with a minimum of 3 documented telephone calls, faxes, text messages, or emails and 1 registered mail letter. If participant is not reached, the participant will be defined as lost to follow-up. All attempts to contact the participant must be documented in the participant's source

documents. After the final attempt, the participant will be declared "lost to follow-up" at the EOS Visit.

### 3.3.4 Replacements and Rescreening

Any participant who is withdrawn, who is significantly outside the allowed vaccination window, or who is lost to follow-up from the study may be replaced at the Sponsor's discretion. Rescreening of an eligible subject is allowed if their originally intended dose level closes and their 28-day screening window is surpassed before another dose level opens. The participant will be assigned a new screening number and all screening procedures will be repeated.

## 3.4 Study Dosing Groups

### 3.4.1 Method of Assigning Participants to Dosing Groups

Participants will be randomly assigned (Day 1) in a blinded manner using the centralized Interactive Response Technology (IRT), with approximately 30 participants assigned to each cohort. Study vaccine dosing will begin with Cohort 1 (10 µg mRNA-1893 or placebo), followed sequentially by Cohort 2 (30 µg mRNA-1893 or placebo), Cohort 3 (100 µg mRNA-1893 or placebo), and Cohort 4 (250 µg mRNA-1893 or placebo). Within each cohort, participants will be randomly assigned in a blinded manner to receive either mRNA-1893 vaccine or placebo (4:1 mRNA-1893 vaccine to placebo) using the centralized IRT. Participants will be stratified according to flavivirus baseline serostatus.

In each cohort, the initial 5 participants (all seronegative) will be randomly assigned to vaccine or placebo (4:1 mRNA-1893 vaccine to placebo) before the remaining participants (n=25, 20 seronegative and 5 seropositive) are enrolled in the cohort.

Randomization by the centralized IRT will be in accordance with pre-generated randomization schedules, with only the unblinded pharmacy personnel having controlled access.

Dose group assignment in each cohort and stratification within each cohort is summarized in Table 1.

**Table 1: Dose Group Assignment**

| Cohort | Dose Group Assignment | n | Flavivirus Status Stratification[a] | | Total (Ratio) |
|---|---|---|---|---|---|
| | | | Status | n | |
| 1 | mRNA-1893 10 µg | 24 | + | 4 | 30 (4:1) |
| | | | - | 20 | |
| | Placebo | 6 | + | 1 | |
| | | | - | 5 | |
| 2 | mRNA-1893 30 µg | 24 | + | 4 | 30 (4:1) |
| | | | - | 20 | |
| | Placebo | 6 | + | 1 | |
| | | | - | 5 | |
| 3 | mRNA-1893 100 µg | 24 | + | 4 | 30 (4:1) |
| | | | - | 20 | |
| | Placebo | 6 | + | 1 | |
| | | | - | 5 | |
| 4 | mRNA-1893 250 µg | 24 | + | 4 | 30 (4:1) |
| | | | - | 20 | |
| | Placebo | 6 | + | 1 | |
| | | | - | 5 | |
| | | | | **Total** | **120** |

Abbreviations: - = flavivirus seronegative; + = flavivirus seropositive.

[a] In each cohort, the initial 5 participants enrolled will be flavivirus seronegative and randomly assigned to vaccine or placebo (4:1 mRNA-1893 to placebo) before the remaining participants (n=25, 20 seronegative and 5 seropositive) are enrolled in the cohort.

### 3.4.2 Vaccination Administration

Vaccine dosing will begin with Cohort 1 (10 µg mRNA-1893 or placebo), followed sequentially by Cohort 2 (30 µg mRNA-1893 or placebo), Cohort 3 (100 µg mRNA-1893 or placebo), and Cohort 4 (250 µg mRNA-1893 or placebo).

The vaccine will be administered as an IM injection into the deltoid muscle on a 2-dose vaccination schedule at Day 1 and Day 29 (e.g., at least a 28-day interval between dosing).

Each vaccination will contain 0.5 mL of 10, 30, 100, or 250 µg mRNA-1893 or 0.5 mL of placebo. Preferably, the second dose of vaccine should be administered in the same arm used for the first dose.

The vaccine will be prepared as detailed in the Pharmacy Manual. Unblinded pharmacy personnel, who will not participate in any other aspect of the study, will perform vaccine accountability, dose preparation, and vaccine administration. The investigator or designee will be responsible for oversight of the administration of vaccine according to the procedures stipulated in this study protocol and the Pharmacy Manual.

At each visit when vaccines are administered, participants will be monitored for 60 minutes after vaccine administration; assessments will include vital sign measurements and monitoring for local or systemic reactions (Schedule of Events, Table 5).

Eligibility for subsequent study vaccination is determined by following the criteria outlined in Section 3.4.2.3.

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

Details regarding safety oversight of within-cohort dosing and dose escalation are provided in Section 3.4.2.1. The rules for pausing dosing are provided in Section 3.4.2.2.

#### 3.4.2.1 Dose Escalation

For each dose cohort (Cohorts 1 through 4), Safety Oversight will be performed 7 days after the first 5 participants (all seronegative) are randomly assigned and receive their first study vaccination (Day 1) before randomly assigning the remaining participants in the cohort. Once safety is confirmed (i.e., no safety concerns and pause rules as defined in Section 3.4.2.2 have not been met), the study medical monitor will authorize the random assignment of the remaining 25 participants in the cohort. Seven days after all 30 participants in the cohort have received the first study vaccination, a blinded IST that is not directly involved in the day-to-day activities of the study, will review all available safety data for the currently dosed cohort and any cumulative safety data of all cohorts as the trial advances to determine the

acceptability to escalate to the next mRNA-1893 dose level. The 30 participants in the first cohort will continue to receive their second study vaccine dose on Day 29 (+14 days).

Oversight of safety will be performed in a blinded manner by the contract research organization's (CRO's) medical monitor, the Sponsor's medical monitor, and the individual site principal investigators throughout the study for all participants.

The blinded IST will oversee the safety of the trial and will review safety data to ensure adherence to the protocol, monitor safety laboratory test results and reactogenicity, and they may request input from the SMC should the study meet pause rules (Section 3.4.2.2) or for any other study events that could potentially affect participant safety. The IST 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 first vaccination and any cumulative safety data of all cohorts as the trial advances.

The IST is composed of 3 Sponsor clinicians who are not directly involved in the day-to-day conduct of the study and who are the voting members for decision for dose escalation. Additionally, the investigator, the CRO medical monitor, and the Sponsor medical lead will be nonvoting extended members. A Biomedical Advanced Research and Development Authority representative may also join these meetings as an observer. Details regarding composition, responsibilities, and procedures of the IST are presented in the IST charter.

An independent, unblinded SMC will be consulted at the discretion of the IST or if a pause rule is met (Section 3.4.2.2). The SMC will review all available safety data to adjudicate any potential study pauses and make recommendations on further study conduct, including requesting additional information, recommending stopping the study, recommending changes to study conduct and/or the protocol, or recommending additional operational considerations due to safety issues that arise during the study.

The SMC will be comprised of external independent consultants with relevant expertise. Details regarding composition, responsibilities, and procedures of the SMC are presented in the SMC charter.

#### 3.4.2.2 Pause Rules

Study pause rules will be continuously monitored during all phases of the study by the investigators, study medical monitor, Sponsor medical lead, IST, and SMC (as warranted). If the investigator, study medical monitor, Sponsor medical lead, IST, or SMC request that the study be paused due to a safety concern, further study vaccine administration in the affected cohort and at higher dose levels will be suspended, but all other planned procedures relating to safety, reactogenicity, and immunogenicity assessments will continue as described in the protocol.

The occurrence of any of the events listed in Table 2, regardless of dosing group, will result in immediate suspension of dosing and enrollment in the cohort. An unscheduled meeting of the SMC will be convened to assess specific data concerns and to make recommendations.

The Sponsor will notify the Center for Biologics Evaluation and Research within 48 hours in the event of a study pause.

**Table 2: Pause Rules Based on the Occurrence of a Single Adverse Event and Adjudicated by the SMC**

| Pause Rule | Adverse Event | Number of Participants per Dose Cohort |
|---|---|---|
| 1 | Any serious adverse event that cannot be reasonably attributed to a cause other than vaccination | ≥ 1 |
| 2 | Any Grade 4 adverse event that cannot be reasonably attributed to a cause other than vaccination | ≥ 1 |
| 3 | Any systemic immediate hypersensitivity reaction within 60 minutes after the study vaccination | ≥ 1 |

Abbreviation: SMC = Safety Monitoring Committee.

Table 3 summarizes safety events that will pause study dosing based on defined threshold levels, which are aggregate incidences relative to the number of exposed participants within a dosing group. An unblinded statistician may be consulted to determine if a pause rule has been met.

**Table 3: Pause Rules Based on the Occurrence of Adverse Events in a Proportion of Participants**

| Pause Rule | Adverse Event | Number of Participants per Dose Cohort[a] |
|---|---|---|
| 4 | Any Grade 3 solicited local adverse event lasting more than 24 hours in an mRNA-1893 cohort[b] within the 7-day (Days 1-8) post-vaccination period | ≥ 20% and ≥ 2 participants/group |
| 5 | Any Grade 3 solicited general adverse event lasting more than 24 hours in an mRNA-1893 cohort[b] that cannot be reasonably attributed to a cause other than vaccination, within the 7-day (Days 1-8) post-vaccination period | ≥ 20% and ≥ 2 participants/group |
| 6 | Any ≥ Grade 3 unsolicited adverse event in an mRNA-1893 cohort[b] that cannot be reasonably attributed to a cause other than vaccination<br>OR<br>Any ≥ Grade 3 laboratory abnormality[c] in an mRNA-1893 cohort[b] that cannot reasonably be attributed to a cause other than vaccination | ≥ 20% and ≥ 2 participants/group |

[a] For the first 5 participants in each dose level cohort, the pause rule will be considered met if 2 of the first 5 participants experience the same event regardless of dosing group.

[b] The rate of adverse events and laboratory abnormalities will be computed based on the number of exposed participants who have provided safety data (i.e., have completed a post-dosing visit for assessment of safety).

[c] Grading of laboratory parameters will be based on the US Food and Drug Administration Guidance for Industry "Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials" (DHHS 2007).

If a pause is triggered in the study, each participant's clinic visits will continue until the next scheduled vaccination visit. If a pause affects a participant's vaccination visit, the window for that participant's vaccination visit will be suspended until the pause is lifted and vaccination can resume. Once the pause is lifted at a site, vaccination should be reinstated as soon as possible. Clinic visits should thereafter (Day 36 [+3 days], Day 57 [-7/+14 days], Month 7 [± 14 days], and Month 13 [± 14 days]) be scheduled as if participants had received their second vaccination within the 28 (+14) day window. The Dose 2 window may be extended to 28 (+14) days at the discretion of the principal investigator and with approval of the study medical monitor on a case by case basis for participants whose window is affected by a study pause.

If a pause is prolonged such that the second vaccination takes place outside the 28 (+14) day window, the Sponsor has the option to add additional participants in a dosing cohort to achieve the original number of participants planned for dosing within the 28 (+14) day window. If a participant is in the Screening Phase for more than 28 days as the result of a pause, the participant may be rescreened for study eligibility (and will receive a new screening number) as long as the participant continues to provide consent to participate in the study.

#### 3.4.2.3 Contraindications to Subsequent Vaccination

Prior to receiving a second vaccination, participants will be reassessed to ensure that they continue to meet eligibility requirements as outlined below.

The following events constitute absolute contraindications to any further administration of study vaccines. If any of these events occur during the study, the participant must not receive additional doses of vaccine but will be encouraged to continue study participation for safety through 12 months following last vaccination (Section 3.3.2).

- Anaphylaxis or systemic hypersensitivity reaction following the administration of vaccine
- Any SAE judged to be related to study vaccine
- Any AESI related to study vaccine (Appendix 2)
- Pregnancy
- Any CS medical condition that, in the opinion of the Investigator, poses an additional risk to the participant if he/she continues to participate in the study

The following events constitute contraindications to administration of study vaccine at certain points in time, and if any of these events occur at the time scheduled for vaccination, the participant may be vaccinated at a later date, within the time window specified in the Schedule of Events (Table 5), or the participant may be withdrawn from dosing at the discretion of the Investigator (Section 3.3):

- Acute moderate or severe infection with or without fever at the time of vaccination
- Fever, defined as body temperature ≥ 38.0°C (100.4°F) at the time of vaccination

Participants with a minor illness without fever, as assessed by the investigator, can be administered study vaccines. Participants with a fever of 38.0°C (100.4°F) or higher will be recontacted within the time window acceptable for participation and reevaluated for eligibility.

### 3.4.3 Identity of Investigational Product

mRNA-1893 is a novel LNP-encapsulated mRNA-based vaccine directed against the prME structural protein of ZIKV. mRNA-1893 consists of CX-005809, an mRNA Drug Substance that is formulated into LNPs composed of 4 lipids: SM-102; cholesterol; DSPC; and PEG2000-DMG. mRNA-1893 is provided as a sterile liquid for injection at a concentration of 0.5 mg/mL in 100 mM Tris buffer, 7% propylene glycol, and 1 mM DTPA.

The placebo is 0.9% sodium chloride injection, United States Pharmacopeia (USP) or British Pharmacopeia (BP).

### 3.4.4 Management of Clinical Supplies

#### 3.4.4.1 Study Vaccine Packaging and Storage

The Sponsor will provide the investigator and study site with adequate quantities of mRNA-1893. The placebo (0.9% sodium chloride injection, USP or BP) is commercially available and will be supplied by the Sponsor or CRO. mRNA-1893 will have all required labeling per regulations. mRNA-1893 will be supplied to the pharmacy in an unblinded manner. Each vial will be individually labeled for future participant identification purposes.

mRNA-1893 will be supplied as a sterile liquid for injection at a concentration of 0.5 mg/mL. The sterile product will be provided in 2-mL glass vials with a 0.85-mL fill volume. The unblinded study site pharmacy personnel will prepare a single dose (0.5 mL) for each participant based on the cohort and randomization assignment. A pharmacy manual will be available, and training provided.

mRNA-1893 vaccine 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, vaccine accountability study staff (e.g., the unblinded pharmacy personnel) 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 vaccine accountability study staff (e.g., unblinded pharmacy personnel) should have access to the products used in this study.

The site is responsible for reporting any mRNA-1893 vaccine that was not temperature controlled during shipment or during storage to the unblinded site (pharmacy) monitor. Such mRNA-1893 will be retained for inspection by the unblinded monitor and disposed of according to approved methods.

#### 3.4.4.2 Study Vaccine Accountability

It is the investigator's responsibility that the unblinded pharmacy personnel maintain accurate records of receipt of all mRNA-1893 vaccine, including dates of receipt. In addition, accurate records will be kept regarding when and how much mRNA-1893 is dispensed and used by each participant in the study. Reasons for departure from the expected dispensing regimen must also be recorded. To satisfy regulatory requirements regarding vaccine accountability, all mRNA-1893 vaccine will be reconciled and retained until study conclusion. At that time, mRNA-1893 vaccine will be destroyed or returned to the Sponsor according to applicable regulations.

### 3.4.5 Blinding

This is an observer-blind study. The Sponsor, investigator, study participants, site monitors, and study staff will be blinded to the study vaccine administered until trial end, with the following exceptions:

- Unblinded pharmacy personnel (of limited number) will be assigned to vaccine accountability procedures and will prepare and administer mRNA-1893 (or placebo) to all participants. The designee(s) will have no study functions other than study vaccine management, documentation, accountability, preparation, and administration. They will not be involved in participant evaluations and will not reveal the study

vaccine identity to either the participant or the study site personnel involved in the conduct of the study unless this information is necessary in the case of an emergency.

- An unblinded study monitor, not involved in other aspects of monitoring, will be assigned as the vaccine accountability monitor. They will have responsibilities to ensure that the site is following all proper vaccine 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, including evaluation of the pause rules criteria summarized in Table 3 (Section 3.4.2.2). The interim analyses of immunogenicity data will be performed as outlined in Section 3.6.8.
- The Sponsor will remain blinded to individual treatment assignment throughout the Vaccination Phase through Visit 9 (28 days post last vaccination) for each vaccine cohort/dose level of the study. Once the Vaccination Phase for each vaccine cohort/dose level is completed, at the time of the Day 57 Interim Analysis pre-identified Sponsor team members will be unblinded to review treatment level results and individual listings and will remain unblinded. Study sites will remain blinded to individual treatment assignments until trial end.

The dosing assignment will be concealed by having the unblinded designee(s) (e.g., unblinded pharmacy personnel) prepare the study vaccine in a secure location that is not accessible to other study staff. The syringe used for administration will maintain the blind at the time of vaccination (i.e., a sleeve will be used, as the vaccine substance will be distinguishable in appearance between the mRNA-1893 and placebo). Only pharmacy staff will conduct the vaccination procedure. Once the vaccination is completed, the blinded study staff will perform further assessments and interact with the participants. Access to the randomization code will be strictly controlled at the pharmacy.

### 3.4.6 Breaking the Blind

A participant or participants may be unblinded in the event of an SAE or other severe event, or if there is a medical emergency requiring the identity of the product to be known to

properly treat a participant. If a participant becomes seriously ill or pregnant during the study, the blind will be broken if knowledge of the administered vaccine will affect that participant's dosing options. In the event of a medical emergency requiring identification of the vaccine administered to an individual participant, the investigator will make every attempt to contact the Sponsor medical lead 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 interim analyses as outlined in Section 3.6.8.

### 3.4.7 Dosing Compliance

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

Participants who 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 as described in the protocol. Unless consent is withdrawn, a participant who discontinues from receiving the second dose of study vaccine will remain in the study and complete all safety and immunogenicity procedures required through 1 month after the last vaccination received per the Schedule of Events (i.e., up to Day 29 for subjects receiving only the first dose of vaccine). Subjects will then enter the 12-month Long-Term Follow-up Phase per the Schedule of Events (Table 5).

Subjects will enter the Long-Term Follow-up Phase starting at Month 3 and will complete protocol-specified procedures, including further blood sampling for immunogenicity at Month 7 and Month 13 (Table 5).

The study site is responsible for ensuring participants comply with the study windows allowed. If a participant misses a visit, every effort should be made to contact the participant and complete a visit within the defined visit window. If a participant exceeds their

post-vaccination visit in excess of 28 days from the scheduled visit or misses a vaccination schedule by more than 28 days (i.e., 28 days beyond the scheduled time of vaccination), then that visit will be classified as a missed visit and the participant will continue with subsequent study visits. All safety requirements of the missed visit will be captured and included in the subsequent visit (e.g., clinical laboratory testing, diary card review for reactogenicity, immune testing, as applicable).

### 3.4.8 Prior and Concomitant Medications

#### 3.4.8.1 Prior Medications and Therapies

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

#### 3.4.8.2 Concomitant Medications and Therapies

Concomitant medications include all medications (including vaccinations received outside of the study) that the participant takes from the time of signing the informed consent form (ICF) through the follow-up visit at Month 13. At each study visit, the site personnel should question the participant about concomitant medications use, including any vaccinations received, and record use in the eCRF.

The use of the following concomitant medications and/or vaccines will not require withdrawal of the participant from the study but may determine a participant's evaluability in the per-protocol analysis (analysis sets are described in Section 3.6.5):

- Any investigational or nonregistered product (drug or vaccine) other than the study vaccine used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e., more than 14 days in total) during the study period. For corticosteroids, this will mean that prednisone ≥ 20 mg/day or the equivalent is not permitted. Inhaled, nasal, and topical steroids are allowed.

- Long-acting immune-modifying drugs administered at any time during the study period (e.g., infliximab).
- A vaccine not foreseen by the study protocol administered during the period from 30 days before through 30 days after each study vaccination, except for any licensed influenza vaccine that was administered ≥ 15 days before or after any study vaccination.
- Immunoglobulins and/or any blood products administered during the study period.
- Any hepatotoxic drugs administered chronically (i.e., more than 14 days in total) (Appendix 4).
- Analgesics/antipyretics may be used post-vaccination for symptomatic relief of reactogenicity symptoms.

Concomitant medications (including vaccinations) will be coded using the WHO Drug Dictionary. If a participant takes a prohibited drug therapy, the investigator and the CRO medical monitor will make a joint decision about continuing or withholding further vaccination of the participant based on the time the medication was administered, the drug's pharmacology and pharmacokinetics, and whether use of the medication will compromise the participant's safety or interpretation of the data. It is the investigator's responsibility to ensure that details regarding the concomitant medications are adequately recorded in the eCRF.

## 3.5 Study Procedures

Before performing any study procedures, all potential participants will sign an ICF (as detailed in Section 4.3). Participants will undergo study procedures at the time points specified in the Schedule of Events (Table 5).

A participant also can be seen for an unscheduled visit at any time during the study. An unscheduled visit may be prompted by reactogenicity issues, new or ongoing AEs, or abnormal clinical laboratory test results. The site also has the discretion to make reminder telephone calls or send text messages to inform the participant about visits, request further laboratory assessments, review diary card content requirements, or follow up on ongoing or outstanding issues.

### 3.5.1 Safety Telephone Calls

A safety telephone call is a telephone call made to the participant by trained site personnel. This call will follow a script, which will facilitate the collection of relevant safety information. The participant will be interviewed according to the script, about occurrence of AEs, MAAEs, SAEs, AESI, or AEs leading to study withdrawal and concomitant medications associated with those events will be collected.

Additionally, in the Vaccination Phase of the study, the site will telephone the participant for safety monitoring on each of the 2 days after study vaccination (i.e., Day 2 and Day 3 after the first vaccination and Day 30 and Day 31 after the second vaccination). The purpose of this safety telephone call is to identify any rash or Grade 3 local and systemic reactogenicity AEs (solicited AEs) that may occur within 48 hours after study vaccination and to ensure that they are recorded in the eCRF and reported to the medical monitor in a timely manner.

The timing of the safety telephone calls is provided in Table 5.

All safety information described by the participant must be documented in source documents and not documented on the script used for the safety telephone contact.

### 3.5.2 Completion of Diary Card

At each vaccination visit (Day 1 and Day 29), study staff will provide a diary card to participants and training on its proper use. The participant will use the diary card for recording AE information; this information will be subject to potential change based on further questioning and/or follow-up by study staff.

Each participant will be instructed to complete the diary card to including the following:

- Solicited local and systemic reactogenicity AEs, as defined in Section 3.5.6, that occur on the day of each vaccine administration and during the 7 days after vaccine administration, with appropriate documentation to allow severity scoring by the investigator.
- Daily oral body temperature measurement should 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 should be recorded.

- Measurement, as applicable, for solicited local AEs; the size measurements will be performed using the ruler provided by the study site.
- All medications (excluding vitamins and minerals) taken on the day of each vaccine administration and during the 28 days after vaccine administration.
- Any unsolicited AE (Section 3.5.7) during the 28 days after each vaccine administration

During clinic visits, study staff will review the diary card information regarding solicited and unsolicited AEs, body temperature, and concomitant medications. This information will be recorded in the participant's source documents and the eCRF.

Diary cards will be the only source documents allowed for solicited systemic and local AEs (including body temperature measurements). The following additional rules apply to documentation of safety information collected using Diary Cards:

- No corrections or additions to the Diary Card will be allowed after it is delivered to the site.
- Any blank or illegible fields on the Diary Card must be described as missing data.
- The site staff must enter all readable entries in the Diary Cards into the eCRF.
- Any new safety information reported during the site visit (including a solicited reaction) must not be written into the Diary Card and must be described in the source documents as verbally reported event. Any adverse reaction reported in this manner must be described as an unsolicited reaction and therefore entered on the adverse event eCRF.

Any unreturned Diary Cards will be requested from the subject through telephone call(s) or other suitable method.

### 3.5.3 Immunogenicity Assessments

Blood samples for the following immunogenicity assessments will be collected at the time points indicated in the Schedule of Events (Table 5):

- Serum neutralizing antibodies (nAb) against ZIKV
- Serum binding antibodies (bAb) against ZIKV

Sample aliquots will be designed to ensure that backup samples are available and that adequate vial volumes may allow further 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.

### 3.5.4 Total Blood Volume

The approximate blood volumes to be collected from each participant during the study are provided in Table 4.

**Table 4: Total Blood Volume**

| Assessment | Blood Volume per Sample | Scheduled Number of Collections[a] | Total Amount of Scheduled Blood |
|---|---|---|---|
| **Clinical Laboratory Assessments** | | | |
| Hematology | 2 mL | 6 | 12 mL |
| Serum chemistry | 6.5 mL | 6 | 39 mL |
| Coagulation | 3 mL | 6 | 18 mL |
| Flavivirus screening by ELISA[b] | 12 - 16 mL | 1 | 12 - 16 mL |
| Serology[c] | 6 mL | 1 | 6 mL |
| **Immunogenicity Assessments** | | | |
| Serum neutralizing antibodies against Zika by neutralization assays | 8 mL | 5 | 40 mL |
| Serum binding antibodies against Zika by ELISA | 4 mL | 5 | 20 mL |
| IgG and IgM antibodies against envelope- and NS1-based antigens by ELISA | 4 mL | 5 | 20 mL |
| **Participant Total** | | | **167 - 171 mL** |

Abbreviations: IgG = immunoglobulin G; IgM = immunoglobulin M; ELISA = enzyme-linked immunosorbent assay; HIV = human immunodeficiency virus; NSI = nonstructural protein 1.

[a] Additional blood collections may be required at the discretion of the investigator to follow up on abnormal results.

[b] Flavivirus testing by ELISA will include testing for dengue, West Nile, and Zika.

[c] Serology testing will include hepatitis B surface antigen, hepatitis C virus antibody, HIV virus type 1 and 2, Separate counseling and consent will be provided for HIV testing.

### 3.5.5 Safety Assessments

Safety assessments will include monitoring and recording of solicited AEs (local and systemic reactogenicity events) and unsolicited AEs, SAEs, AESI, AEs leading to study withdrawal, and MAAEs; clinical laboratory test results (hematology, serum chemistry, and coagulation); vital sign measurements; and physical examination findings. If an MAAE occurs that evokes clinical symptomatology consistent with a flavivirus infection, serology and polymerase chain reaction testing against dengue will be uniformly collected.

### 3.5.6 Solicited Safety Measurements

The term "reactogenicity" refers to selected signs and symptoms (AEs) occurring after vaccine administration that the participants are systemically asked to record. Participants will record such occurrences in a diary card (Section 3.5.2) on the day of each vaccine administration and for the 6 days after vaccine administration. Participants will be instructed to call or return to the clinic within 24 hours if a reactogenicity score reaches Grade 3 or greater during the first 6 days after vaccination.

The following AEs are included on the diary card:

- Solicited local AEs:
  - Injection site pain
  - Injection site erythema
  - Injection site induration/swelling
- Solicited systemic AEs:
  - Body temperature (oral)
  - Generalized myalgia (muscle ache or pain)
  - Generalized arthralgia (joint ache or pain)
  - Headache
  - Fatigue/malaise (unusual tiredness)
  - Nausea/vomiting
  - Chills
  - Rash

Appropriate reactogenicity measurements will be taken using the tools provided by Sponsor (oral thermometer and measuring device; Section 3.5.2). The investigator will later review, confirm, and grade reactogenicity according to the Guidance for Industry: Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventative vaccine clinical trials (DHHS 2007; Table 6).

In case of any rash episode observed within 6 days after study vaccination, the participants will be instructed to contact the study site within 24 hours. During subject evaluation, the investigator should categorize the rash as one of the following:

- Rash no longer present and history not consistent with urticaria.
- Rash no longer present but history is consistent with urticaria.
- Rash present but clinical findings are not consistent with urticaria. Alternative diagnosis should be specified as an AE.
- Rash present and clinical findings consistent with urticaria.

If a solicited local or systemic reactogenicity AE continues beyond 7 days after vaccine administration, it will also be recorded as an AE in the eCRF from 8 days after vaccination until the AE has resolved or stabilized (Section 3.5.7.1.1).

Other solicited reactions will include the following:

- Use of analgesics and/or antipyretics will be recorded as absent or present, and participant will indicate if use was for treatment or prophylaxis.
- To improve recall before the next visit, participants will record the incidence of unsolicited AEs and any medication (prescription and over the counter [excluding vitamins and minerals]) using the diary card during the 28 days after each vaccine administration.

All AEs necessitating an unscheduled physician visit or medical attention or leading to withdrawal from the study will also be collected throughout the study, as detailed in Section 3.5.7.

### 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 vaccine 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. Participants will be instructed to record any AEs (solicited or unsolicited) in the diary card.

A treatment-emergent AE (TEAE) is defined as any event not present before exposure to vaccine 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 vaccine 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 vaccine 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 vaccine. Adverse reactions are a subset of all suspected adverse reactions for which there are reasons to conclude that the drug caused the event.

An unsolicited AE is any AE reported by the participant that is either (1) not specified as a solicited AE in the protocol or (2) specified as a solicited AE in the protocol, but its onset occurs outside of the protocol-defined post-vaccination period for reporting solicited AEs (i.e., for the 7 days after each vaccination; Section 3.5.6 for additional information on solicited safety measures).

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the IB or is not listed at the specificity or severity that has been observed with the vaccine being tested or, if an IB is not required or available, it 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.

Unsolicited AEs will also be evaluated by the investigator for the coexistence of any of the other following conditions:

- Medically attended AE, defined as an AE that leads to an unscheduled visit to a healthcare practitioner

Any solicited AE (Section 3.5.6) that meets any of the following criteria must be entered as an AE in the eCRF:

- Solicited local or systemic AE leading to the participant withdrawing from the study or the participant being withdrawn from the study by the investigator
- Solicited local or systemic AE lasting beyond 7 days post-vaccination
- Solicited local or systemic AE that leads to participant withdrawal from vaccine
- Solicited local or systemic AE that otherwise meets the definition of an SAE
- Laboratory test result or vital sign measurements with a toxicity score of Grade 3 or greater

#### 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
- Is life-threatening
- 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 participant 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 participant 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. The reporting requirements for SAEs are discussed in Section 3.5.7.3.

#### 3.5.7.1.3 Adverse Events of Special Interest

Adverse events of special interest are a subset of AEs that include potentially immune-mediated medical conditions (autoimmune or auto-inflammatory diseases) that may have the theoretical potential for association with novel vaccines. All participants enrolled in the study will be monitored for new onset of AESI from Day 1 through Month 13. The occurrence of any of these AEs will be treated as an SAE, meeting the criterion of a "medically important event."

The list of AESI is presented in Appendix 2.

A diagnosis of an AESI will be reported to the Sponsor in an expedited manner, similar to an SAE (Section 3.5.7.3). The AESI diagnosis, as well as any medications taken to treat the condition, will be recorded in the participant's eCRF.

#### 3.5.7.1.4 Pregnancy

To ensure participant safety, each pregnancy in a participant after study vaccination must be reported to the Sponsor or designee within 72 hours of the site learning of its occurrence. If the participant agrees to submit this information, the pregnancy must be followed to determine the outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. This follow-up should occur even if intended duration of the Long-Term Follow-up Phase for the study has ended.

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, SAEs and AESI are recorded in the eCRF and reported to the Sponsor. Unsolicited AEs will be assessed during the Vaccination Phase from the time of the first dose administration at Day 1 through Day 57 (±7 days). Serious AEs, MAAEs, and AESI will be assessed from Day 1 through Month 13 (EOS Visit), as specified in the Schedule of Events (Table 5). Any AEs occurring before receipt of the vaccine will be analyzed separately from TEAEs.

At every clinic visit or telephone contact, participants will be asked a standard question to elicit any medically-related changes in their well-being according to the scripts provided. Participants 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 participant observations, data from clinical laboratory test results, physical examination findings, or other documents relevant to participant safety classified as an AE will be documented on the AE page of the eCRF.

#### 3.5.7.3 Reporting Adverse Events

All unsolicited AEs reported or observed during the study will be recorded on the AE page of the eCRF. Information to be collected includes dose group assignment and dose, type of event, time of onset, investigator-specified assessment of severity and relationship to vaccine, time of resolution of the event, seriousness, as well as any required treatment or evaluations, and outcome. The unsolicited 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 (NCS). The Medical Dictionary for Regulatory Activities (MedDRA) will be used to code all unsolicited AEs.

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

Any AE or AESI considered serious by the investigator or that 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 or AESI). The investigator will assess whether there is a reasonable possibility that the vaccine caused the SAE or AESI. The Sponsor will be responsible for notifying the relevant regulatory authorities of any SAE or AESI 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.

If the eCRF is unavailable at the time of the SAE or AESI, the following contact information is to be used for SAE reporting:

SAE Mailbox: PPD

SAE Hotline (USA and Canada): PPD

SAE Fax line (USA and Canada): PPD

#### 3.5.7.4 Assessment of Severity

The severity (or intensity) of an AE refers to the extent to which it affects the participant's daily activities. When applicable, the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (DHHS 2007) will be used to categorize local and systemic reactogenicity events (solicited AEs), clinical laboratory test results, and vital sign measurements observed during this study. Specific criteria for clinical and laboratory abnormalities are presented in Appendix 3 (Table 6 and Table 7, respectively) and will be graded if outside of the reference range for the laboratory utilized.

The determination of severity for all unsolicited AEs not listed in Toxicity Grading Scale should be made by the investigator based upon medical judgment and the definitions of severity as follows:

- Mild: These events do not interfere with the participant's daily activities.

- Moderate: These events cause some interference with the participant's daily activities but do not require or require limited medical intervention.
- Severe: These events prevent the participant's daily activity and require intensive therapeutic intervention.

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.

In the case where an AE meets the definition of a serious AE (SAE), refer to Section 3.5.7.1.2.

#### 3.5.7.5 Assessment of Causality

The investigator's assessment of an AE's relationship to vaccine 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 (i.e., whether there is a reasonable possibility that the vaccine caused the event) for all AEs, AESIs, and SAEs. The relationship will be characterized using the following classification:

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

#### 3.5.7.6 Follow-up of Adverse Events

All AEs, SAEs, and AESI must be reported in detail on the appropriate page of the eCRF and followed until the event is resolved or stable or judged by the investigator to be NCS.

### 3.5.8 Clinical Laboratory Testing

Clinical laboratory tests will be performed by the central laboratory, unless otherwise specified. Blood and urine will be collected at the time points indicated in the Schedule of Events (Table 5). Fasting is not required before collection of laboratory samples.

Clinical laboratory results will be scored for toxicity using the grading scale provided in Appendix 3 (Table 7). Of note, the laboratory test value itself may not be the AE classification. Each AE will be classified by its most specific term (e.g., renal insufficiency, bronchitis) and supporting evidence (e.g., laboratory test value) will not be classified as an AE. The following hematology, serum chemistry, and coagulation assessments will be performed:

| | |
|---|---|
| Hematology: | Hemoglobin, platelet count, red blood cell count, and total and differential white blood cell count |
| Serum chemistry: | Alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin (total and direct), blood urea nitrogen, creatinine, random glucose, and total protein |
| Coagulation: | Prothrombin time and partial thromboplastin time |

Additional tests include the following:

- Urine pregnancy testing will be performed on site by the local laboratory for all female participants of childbearing potential. Testing will be performed at the Screening Visit, before each vaccine administration, and as needed at unscheduled visits.
- If not documented in a female participant's medical records, a follicle-stimulating hormone test may be performed at the Screening Visit, as necessary and at the discretion of the investigator, to confirm postmenopausal status.
- Hepatitis B surface antigen, Hepatitis C virus antibody, and HIV virus (types 1 and 2) antibody at screening using commercially available kits

- Flaviviruses as determined by ELISA (including dengue, West Nile, and Zika) at screening
- A urine screen for drugs of abuse performed by the local laboratory for amphetamines, benzodiazepines, cocaine, methadone, opiates, and phencyclidine (Exclusion Criterion 26; Section 3.2.2).

Should clinical laboratory testing at 7 days post-vaccination result in a Grade 2 or greater toxicity score, then repeat testing must be performed within the next 7 days and may include an unscheduled visit. Should the participant's laboratory test value not return to baseline (i.e., toxicity grade at Visit Day 1), then periodic testing may be needed until the abnormality is deemed to be associated with a new stable AE or determined to be NCS by the investigator.

Values that fall within the normal laboratory range will automatically be classified as normal and receive a toxicity score of zero. All values that have a toxicity score of Grade 1 or greater will also be evaluated by the investigator and classified as CS or NCS (Glossary of Terms for the definition of laboratory abnormalities with and without potential clinical significance). Investigators should use their clinical judgment when considering the clinical significance of any abnormal laboratory findings. All laboratory test values with a toxicity score of Grade 3 or greater will be entered as AEs.

Any additional laboratory test value that is determined to be CS will also be recorded as an AE, should the test result 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 participant with additional assessments until the value has reached the reference range or the value at baseline (i.e., toxicity grade at Visit Day 1) 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 pressure, heart rate, respiratory rate, and body temperature (preferred route is oral). The participant will be seated for at least 5 minutes before all measurements are taken. Vital signs will be measured at the time points indicated in the Schedule of Events (Table 5). On Visits Day 1 and Day 29, vital

sign measurements will be collected once before vaccine administration and at least 1 hour after vaccine administration (before participants are discharged from the clinic).

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.

If any of the vital sign measurements meet the toxicity grading criteria for clinical abnormalities (Table 6) of Grade 3 or greater, the abnormal value and grade will be documented on the AE page of the eCRF (unless there is another known cause of the abnormality that would result in an AE classification). The investigator will continue to monitor the participant with additional assessments until the vital sign value has reached the reference range, the vital sign value at baseline, is considered stable, or until the investigator determines that follow-up is no longer medically necessary.

### 3.5.10 Physical Examinations

A full physical examination will be performed at the Screening Visit and a symptom-directed (targeted) physical examination will be performed at all other scheduled time points as indicated in the Schedule of Events (Table 5). 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 abnormal clinical laboratory test results. Before vaccination and at 7 days post-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 calculated, at screening only.

## 3.6 Statistical Analysis Plans

### 3.6.1 Primary Endpoints

The following are the primary endpoints of the study:

- Frequency and grade of each solicited local and systemic reactogenicity AE during a 7-day follow-up period after each vaccination

- Frequency and grade of any unsolicited AEs during the 28-day follow-up period after each vaccination
- Frequency of any MAAE, SAE, and AESI from Day 1 to the EOS Visit at Month 13

### 3.6.2 Secondary Endpoints

The following are the secondary endpoints of the study:

- Serum nAb titers against ZIKV by PRNT:
  - GMT of nAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
  - GMT of nAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
  - GMT of nAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
  - Percentage of participants who seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as a change of PRNT from below the LLOQ to a PRNT equal to or above LLOQ, or a multiplication by at least 4 in subjects with pre-existing PRNT titers.
  - Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
  - Proportion of initially seropositive participants with a 2-fold or 4-fold increase in nAb titers as compared with baseline as measured by PRNT.

### 3.6.3 Exploratory Endpoints

The following are the exploratory endpoints of the study:

- Geometric mean titer (GMT) of nAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.

- GMT of nAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- GMT of nAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- Percentage of participants who seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as change of nAb titer (by additional neutralization assay) from below the LLOQ to a titer equal to or above LLOQ, or a multiplication by at least 4 in pre-existing neutralizing titer.
- Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- Proportion of initially seronegative participants with a 2-fold or 4-fold increase in nAb as compared with baseline as measured by additional neutralization assay.
- GMT of bAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- GMT of bAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- GMT of bAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- Percentage of participants that seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as a change of binding antibody titer from below LLOQ to a binding antibody titer equal or above the LLOQ, or a multiplication by at least 4 in pre-existing bAb titers.
- Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- Proportion of initially seropositive participants with a 2-fold or 4-fold increase in bAb as compared with baseline as measured by ELISA binding assay.

- IgG and IgM antibodies against envelope- and NS1- based antigens present in serum collected at baseline and at end of study measured as antibody binding by ELISA or equivalent methodology to flaviviruses.

### 3.6.4 Sample Size Calculations

A total of 120 participants (30 participant per cohort) are planned for enrollment in the study and random assignment to study dosing. The sample size is considered sufficient to meet the study objective of identifying a dose and establishing initial safety results in a population of healthy adults in both endemic and nonendemic Zika regions. With 24 subjects receiving mRNA-1893 in each dose level, there is a 92% probability to observe at least 1 subject with an AE if the true incidence of the AE is 10% and a 70.8% probability if the true incidence of the AE is 5%.

### 3.6.5 Analysis Sets

The All Enrolled Participants Set will include all participants who signed the ICF.

The Safety Set will include all participants who received at least 1 dose of vaccine (mRNA-1893 or placebo). Participants will be analyzed according to the vaccine actually received.

The Per-Protocol Set will be determined for each visit and will include all participants who did not have a major protocol violation, received vaccine within the acceptable vaccination window (i.e., received full dose[s] of assigned vaccine), had blood collection within acceptable visit windows, and had a pre-vaccination and at least 1 post-vaccination serum sample available for testing. All participants in the Per-Protocol Set will be analyzed according to the vaccine the participant was randomly assigned to receive and not according to vaccine actually received. Participants who missed the second vaccination may still be included in the Per-Protocol Set for Visit 5 immunogenicity data analysis. If the second vaccination occurred outside of the window, the subsequent data may need to be excluded from the Per-Protocol Set analysis.

The Intent-to-Treat Set will include all participants who were randomized to the study, regardless of protocol violations, unacceptable visit windows, missed vaccination, or missing data. The Intent-to-Treat Set will used for supportive analyses.

### 3.6.6 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 participants excluded from an analysis set will be presented in the data listings but not included in the calculation of summary statistics.

Data from participants who received placebo will be pooled across cohorts for all dosing.

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

#### 3.6.6.1 Immunogenicity Analyses

The following secondary immunogenicity outcome measures and the 95% confidence intervals, where appropriate, will be summarized by dose group and by visit:

- Serum neutralizing antibodies (nAb titers) against ZIKV by PRNT:
  - GMT of nAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
  - GMT of nAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
  - GMT of nAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.
  - Percentage of participants who seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as a change of PRNT from below LLOQ to a PRNT equal to or above LLOQ, or a multiplication by at least 4 in subjects with pre-existing PRNT titers.
  - Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by PRNT.

- Proportion of initially seropositive participants with a 2-fold or 4-fold increase in nAb titers as compared with baseline as measured by PRNT.

The following exploratory immunogenicity outcome measures and the 95% confidence intervals, where appropriate, will be summarized by dosing group and by visit:

- GMT of nAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- GMT of nAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- GMT of nAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- Percentage of participants who seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as change of nAb titer (by additional neutralization assay) from below the LLOQ to a titer equal to or above LLOQ, or a multiplication by at least 4 in pre-existing neutralizing titer.
- Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by additional neutralization assay.
- Proportion of initially seropositive participants with a 2-fold or 4-fold increase in nAb as compared with baseline as measured by additional neutralization assay.
- GMT of bAb against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- GMT of bAb in initially seronegative participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- GMT of bAb in initially seropositive participants against ZIKV at Day 1, Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- Percentage of participants that seroconverted from Day 1 (baseline) to Day 29, from Day 1 to Day 57, from Day 1 to Month 7, and from Day 1 to Month 13. A seroconversion is defined as a change of binding antibody titer from below LLOQ to

a binding antibody titer equal or above the LLOQ, or a multiplication by at least 4 in pre-existing bAb titers.

- Proportion of initially seronegative participants with a seroresponse at Day 29, Day 57, Month 7, and Month 13 as measured by ELISA binding assay.
- Proportion of initially seropositive participants with a 2-fold or 4-fold increase in bAb as compared with baseline as measured by ELISA binding assay.
- IgG and IgM antibodies against envelope- and NS1- based antigens present in serum collected at baseline and at end of study measured as antibody binding by ELISA or equivalent methodology to flaviviruses.

#### 3.6.6.2 Safety Analyses

Reactogenicity will be summarized by dosing group (10, 30, 100, or 250 µg mRNA-1893 or placebo), vaccination (first or second dose), duration, and severity. Adverse events will be coded by preferred term and system organ class using MedDRA and summarized by dose group, vaccination (first or second dose), and overall. Adverse events will also be summarized by severity and relationship to the study vaccine. Descriptive statistics will be presented, and the difference in the proportion of participants with AEs will be provided, comparing each dose level with placebo pooled across all cohorts. Individual participant listings will be provided for all AEs, AEs leading to study withdrawal, AESI, MAAEs, and SAEs.

Safety data from clinical laboratory test results and vital sign measurements will be graded by severity scoring and analyzed by dose 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 serology, urine drug screening, physical examinations, and pregnancy tests will be listed for all participants randomly assigned to receive study vaccine.

Medical history data for all participants randomly assigned to receive study vaccine will be presented by participant in a listing.

Baseline demographic and background variables will be summarized by dosing group for all randomly assigned participants. The number of participants who enroll in the study and the

number and percentage of participants who complete the study will be presented. Frequency and percentage of participants who withdraw or discontinue from the study and the reason for withdrawal or discontinuation will also be summarized.

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

### 3.6.7 Handling of Missing Data

For GMT calculation, antibody values reported as below the lower LOQ (LLOQ) will be replaced by 0.5 × LLOQ. Values that are greater than the upper LOQ (ULOQ) will be converted to the ULOQ. Missing data will not be imputed.

### 3.6.8 Interim Analyses

Once all subjects from a cohort have completed the Vaccination Phase through Visit 9, the database will be locked for that cohort and safety and immune test results will be analyzed through 28 days following the second vaccination by an unblinded statistician. As dose escalation occurs, cumulative analyses will be included for each subsequent data lock to allow for all prior dosing cohorts to be analyzed by dose assignment, and in aggregate for mRNA-1893 exposure. Immunogenicity and safety data, including mean group analyses of change from baseline, where applicable, will be summarized for each dose group. These data are required to inform decisions on dose selection for this and other development programs using the same mRNA platform. Participant-level dose assignment will not be released to the participants or to those individuals involved directly in managing or assessing safety until the study is completed.

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 International Council for Harmonisation (ICH) harmonized tripartite guideline E6(R2): 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 participant 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 pursuant 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 participant 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 participant, 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(R2) guidelines require that approval be obtained from an IRB before participation of human participants in research studies. Before study onset, the protocol, informed consent, advertisements to be used for the recruitment of study participants, and any other written information regarding this study to be provided to the participant must be approved by the IRB. Documentation of all IRB approvals and of the IRB compliance with the ICH E6(R2) 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 Participant Consent

Written informed consent in compliance with US Title 21 CFR Part 50 shall be obtained from each participant before he or she enters the study or before any unusual or nonroutine procedure that involves risk to the participant is 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 participants must sign the revised form.

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

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 further characterize the immune response to the ZIKV, additional assay development, and the immune response across flaviviruses.

The investigator or designee will provide a copy of the ICF to the participant. The original form shall be maintained in the participant'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 AESI according to the timeline 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(R2) 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 (Food and Drug Administration) 1572, fully executed, and all updates on a new fully executed Form FDA 1572,
- Curriculum vitae for the principal investigator and each subinvestigator 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 participant, 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(R2). 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 participants 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 participants, 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(R2) 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 or her 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 vaccine. 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 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 dose assignment. A separate unblinded study monitor will be responsible for vaccine 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(R2) 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, or other regulatory agency 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 participant, 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 participants are enrolled into an amended protocol.

### 5.2.2 Protocol Violations and Deviations

The investigator or designee must document and explain in the participant'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 participants 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 participants or a small group of participants and do not involve inclusion/exclusion or primary endpoint criteria. A protocol violation occurs when the participant or investigator does not adhere to the protocol, resulting in a significant additional risk to the participant. Protocol violations can include nonadherence to inclusion or exclusion criteria, enrollment of the participant without prior Sponsor approval, or nonadherence to FDA regulations or ICH E6(R2) 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 EOS is defined as the date on which the last participant 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 harmonized 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 will have the opportunity to review complete study results.

A final clinical study report will contain all data collected through the Long-Term Follow-up Phase.

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

# 6 APPENDICES

## 6.1 Appendix 1: Schedule of Events

The Schedule of Events is presented in Table 5.

## Table 5: Schedule of Events

| Procedure | Screening Phase | Vaccination Phase | | | | | | | | | Long-Term Follow-up Phase | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Visit** | **Screening** | **Day 1** | **Day 2** | **Day 3** | **Day 8** | **Day 29** | **Day 30** | **Day 31** | **Day 36** | **Day 57 (Month 2)** | **Months 3, 4, 5, 6** | **Month 7** | **Months 8, 9, 10, 11, 12** | **Month 13 (EOS)[a]** |
| **Visit Number** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10-** | **14** | **15-19** | **20** |
| **Type of Visit** | **C** | **C** | **SC** | **SC** | **C** | **C** | **SC** | **SC** | **C** | **C** | **SC** | **C** | **SC** | **C** |
| **Window Allowance** | **-28/0** | **0** | **0** | **0** | **+3** | **+14** | **0** | **0** | **+3** | **-7/+14** | **±7** | **±14** | **±7** | **±14** |
| Informed consent | X | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Inclusion/exclusion criteria | X | X | – | – | – | – | – | – | – | – | – | – | – | – |
| Baseline demographics | X | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Medical and prior medication history | X | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Concomitant medications, including vaccinations | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Vital sign measurements[b] | X | X | – | – | – | X | – | – | – | – | – | – | – | – |
| Physical examination[c] | X | X | – | – | X | X | – | – | X | X | – | (X) | – | (X) |
| Serology screening[d] | X | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Serology flavivirus screening by ELISA[e] | X | – | – | – | – | – | – | – | – | – | – | – | – | – |
| Urine drug screen[f] | X | – | – | – | – | – | – | – | – | – | – | – | – | – |

| Procedure | Screening Phase | Vaccination Phase | | | | | | | | | Long-Term Follow-up Phase | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Visit** | **Screening** | **Day 1** | **Day 2** | **Day 3** | **Day 8** | **Day 29** | **Day 30** | **Day 31** | **Day 36** | **Day 57 (Month 2)** | **Months 3, 4, 5, 6** | **Month 7** | **Months 8, 9, 10, 11, 12** | **Month 13 (EOS)[a]** |
| **Visit Number** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10-** | **14** | **15-19** | **20** |
| **Type of Visit** | **C** | **C** | **SC** | **SC** | **C** | **C** | **SC** | **SC** | **C** | **C** | **SC** | **C** | **SC** | **C** |
| **Window Allowance** | **-28/0** | **0** | **0** | **0** | **+3** | **+14** | **0** | **0** | **+3** | **-7/+14** | **±7** | **±14** | **±7** | **±14** |
| Urine pregnancy test[g] | X | X | – | – | – | X | – | – | – | – | – | – | – | – |
| Assessment of eligibility/contraindications for subsequent vaccination[h] | – | X | – | – | – | X | – | – | – | – | – | – | – | – |
| Randomization | – | X | – | – | – | – | – | – | – | – | – | – | – | – |
| Blood sample for clinical laboratory tests (hematology, serum chemistry, and coagulation) (Section 3.5.8) | X | X | – | – | X | X | – | – | X | X | – | – | – | – |
| Diary card training | – | X | – | – | – | X | – | – | – | – | – | – | – | – |
| Distribute diary card for collection of solicited and unsolicited AEs[i] | – | X | – | – | – | X | – | – | – | – | – | – | – | – |
| Vaccination administration | – | X | – | – | – | X | – | – | – | – | – | – | – | – |
| 60-minute post-vaccination assessment[j] | – | X | – | – | – | X | – | – | – | – | – | – | – | – |

| Procedure | Screening Phase | Vaccination Phase | | | | | | | | | Long-Term Follow-up Phase | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study Visit** | **Screening** | **Day 1** | **Day 2** | **Day 3** | **Day 8** | **Day 29** | **Day 30** | **Day 31** | **Day 36** | **Day 57 (Month 2)** | **Months 3, 4, 5, 6** | **Month 7** | **Months 8, 9, 10, 11, 12** | **Month 13 (EOS)[a]** |
| **Visit Number** | **0** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10-** | **14** | **15-19** | **20** |
| **Type of Visit** | **C** | **C** | **SC** | **SC** | **C** | **C** | **SC** | **SC** | **C** | **C** | **SC** | **C** | **SC** | **C** |
| **Window Allowance** | **-28/0** | **0** | **0** | **0** | **+3** | **+14** | **0** | **0** | **+3** | **-7/+14** | **±7** | **±14** | **±7** | **±14** |
| Blood sample collection for vaccine-induced immunogenicity assay[k] | – | X[l] | – | – | – | X[l] | – | – | – | X | – | X | – | X |
| Post-vaccination SC[m] | – | – | X | X | – | – | X | X | – | – | X | – | X | – |
| Return and review diary card | – | – | – | – | X | X | – | – | X | X | – | – | – | – |
| Record unsolicited AEs | – | X | X | X | X | X | X | X | X | X | – | – | – | – |
| Record SAEs, MAAEs, and AEs of special interest[n] | – | X | X | X | X | X | X | X | X | X | X | X | X | X |

Abbreviations: –, not collected; AE, adverse event; C, clinic visit; AE, adverse event; AESI, adverse event of special interest; ELISA, enzyme-linked immunosorbent assay; EOS, end of study; IgG, immunoglobulin G; IgM, immunoglobulin M; MAAE, medically attended adverse event; NA, not applicable; SAE, serious adverse event; SC, safety telephone call; SOE, schedule of events.

[a] Unless consent is withdrawn, a participant who discontinues from receiving the second dose of study vaccine will remain in the study and complete all safety and immunogenicity procedures required through 1 month after the last vaccination received per the SOE (i.e., up to Day 29 for subjects receiving only the first dose of vaccine). Subjects will then enter the 12-month Long-Term Follow-up Phase per the SOE.

[b] Vital sign measurements will include systolic and diastolic blood pressure, heart rate, respiratory rate, and body temperature. Oral temperature is preferred route. The participant will be seated for at least 5 minutes before all measurements are taken. Vital sign measurements will be collected once before dose administration and at least 1 hour after dose administration (before participants are discharged from the clinic) on Visits Day 1 and Day 29. 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.

[c] A full physical examination, including height and weight (and body mass index calculation), will be performed at screening (Section 3.5.10); symptom-directed (targeted) physical examinations will be performed at all other scheduled time points. Interim physical examinations will be performed at the discretion of the investigator. Investigator may perform targeted physical examinations at Month 7 and Month 13 at their discretion. A physical examination of the arm that was vaccinated and the associated lymph nodes should be evaluated before each vaccination and at 7 days post-vaccination.

[d] Serology testing will include hepatitis B virus surface antigen, hepatitis C virus antibody, human immunodeficiency virus type 1 and 2 antibodies.

[e] Flavivirus screening by ELISA will include screening for dengue, West Nile, and Zika.

[f] Urine drug screen will include amphetamines, benzodiazepines, cocaine, methadone, opiates, and phencyclidine (Exclusion Criterion 26, Section 3.2.2).

[g] Urine pregnancy testing only for women of childbearing potential (performed at the local laboratory) at the Screening Visit, prior to vaccination administration and as needed at unscheduled visits.

[h] Participants are assessed for eligibility/contraindications for second vaccination (Section 3.4.2.3).

[i] At each vaccination visit (Day 1 and Day 29), study staff will provide a diary card to participants and training on its proper use. The participant will use the diary card (Section 3.5.2) for recording information on solicited AEs (Section 3.5.6) during the 6 days after vaccine administration, unsolicited AEs (Section 3.5.7) during the 28 days after each vaccination.

[j] At each visit when vaccine injections are administered, participants will be monitored for 60 minutes after vaccine administration; assessments will include vital sign measurements and monitoring for local or systemic reactions.

[k] For all participants, blood samples for immunogenicity testing will be collected for the analysis of vaccine-induced immunogenicity (Section 3.5.3). At EOS, blood samples will also be used to test for flavivirus seroconversion.

[l] Sample collection before study vaccination.

[m] During the Vaccination Phase, all participants will receive a post-vaccination safety telephone call at 24 hours and 48 hours after vaccinations (Day 2 and Day 3 after first vaccination and Day 30 and Day 31 after second vaccination). Trained site personnel will inquire about the presence of solicited and unsolicited AEs and concomitant medication use and will remind participants to keep their diary cards up to date (Section 3.5.2).

[n] The investigator must report all SAEs, MAAEs, and AESI within 24 hours of awareness (Section 3.5.7.3).

## 6.2 Appendix 2: Adverse Events of Special Interest

The following is a list of AESI:

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
- Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome), and undifferentiated spondyloarthritis
- Systemic lupus erythematosus
- Systemic sclerosis (with limited or diffuse cutaneous involvement)

Neuroinflammatory disorders:

- Acute disseminated encephalomyelitis, including site-specific variants (e.g., encephalitis, encephalomyelitis, myelitis, myeloradiculoneuritis, cerebellitis)
- Cranial nerve disorders, including paralyses/paresis (e.g., Bell's palsy)
- Immune-mediated peripheral neuropathies and plexopathies, including Guillain-Barré syndrome, Miller Fisher syndrome and other variants, chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy, and polyneuropathies associated with monoclonal gammopathy
- Multiple sclerosis
- Myasthenia gravis (including Lambert-Eaton myasthenic syndrome)
- Narcolepsy
- Optic neuritis
- Transverse myelitis

Skin disorders:

- Alopecia areata
- Autoimmune bullous skin diseases, including pemphigus, pemphigoid, and dermatitis herpetiformis

- Cutaneous lupus erythematosus
- Erythema nodosum
- Morphea
- Lichen planus
- Psoriasis
- Sweet’s syndrome
- Vitiligo

Vasculitides:

- Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis
- Medium-sized and/or small vessels vasculitis including the following: 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 positive vasculitis (type unspecified), Henoch-Schönlein purpura, Behcet’s syndrome, and leukocytoclastic vasculitis

Other:

- 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

Abbreviations: AESI, adverse event of special interest; IgA, immunoglobulin A.
Source: Tavares Da Silva et al 2013

## 6.3 Appendix 3: Toxicity Grading Scale Tables

The toxicity grading scales for clinical and laboratory abnormalities are presented in Table 6 and Table 7, respectively. Note that for laboratory abnormalities, grading only occurs if the values are 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: 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 (DHHS 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.

* Participant 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 participant 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 (DHHS 2007).

| **Systemic (General)** | **Mild (Grade 1)** | **Moderate (Grade 2)** | **Severe (Grade 3)** | **Potentially Life-threatening (Grade 4)** |
|---|---|---|---|---|
| Fever (°C) *<br>(°F) * | 38.0 – 38.4<br>100.4 – 101.1 | 38.5 – 38.9<br>101.2 – 102.0 | 39.0 – 40<br>102.1 – 104 | > 40<br>> 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 (DHHS 2007). Division of AIDS Grading the Severity of Adult and Pediatric Adverse Events (DHHS 2014).

## Table 7: 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<br>Fasting (mg/dL)<br>Random (mg/dL) | <br>100 - 110<br>110 – 125 | <br>111 - 125<br>126 – 200 | <br>>125<br>> 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 |
| Magnesium – hypomagnesemia (mg/dL) | 1.3 – 1.5 | 1.1 – 1.2 | 0.9 – 1.0 | < 0.9 |
| Phosphorous – hypophosphatemia (mg/dL) | 2.3 – 2.5 | 2.0 – 2.2 | 1.6 – 1.9 | < 1.6 |
| CPK (mg/dL) | 1.25 – 1.5X ULN | 1.6 – 3.0X ULN | 3.1 – 10X ULN | > 10X ULN |
| 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 x 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 |
| Cholesterol | 201 – 210 | 211 – 225 | > 226 | - |
| 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 mEq/L) should be recorded as a Grade 4 hyponatremia event if the participant 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 (DHHS 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 | 1,5001 – 20,000 | 20,001 – 25,000 | > 25,000 |
| WBC decrease (cell/mm$^3$) | 2,500 – 3,500 | 1,500 – 2,499 | 1,000 – 1,499 | < 1,000 |
| Lymphocytes decrease (cell/mm$^3$) | 750 – 1,000 | 500 – 749 | 250 – 499 | < 250 |
| Neutrophils decrease (cell/mm$^3$) | 1,500 – 2,000 | 1,000 – 1,499 | 500 – 999 | < 500 |
| Eosinophils (cell/mm$^3$) | 650 – 1,500 | 1,501 – 5,000 | > 5,000 | 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 |
| Fibrinogen increase (mg/dL) | 400 – 500 | 501 – 600 | > 600 | - |
| Fibrinogen decrease (mg/dL) | 150 – 200 | 125 – 149 | 100 – 124 | < 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC) |

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 (DHHS 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 | 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 (DHHS 2007).

## 6.4 Appendix 4: List of Hepatotoxic Drugs

Cardiovascular

- Amiodarone
- Atorvastatin
- Hydralazine
- Methyldopa
- Quinidine
- Simvastatin
- Ticlopidine

Antimicrobial

- Amoxicillin-clavulanate
- Didanosine
- Efavirenz
- Erythromycin
- Flucloxacillin
- Interferon alpha/Peginterferon
- Isoniazid
- Ketoconazole
- Minocycline
- Nevirapine
- Nitrofurantoin
- Pyrazinamide
- Rifampin
- Sulfamethoxazole/Trimethoprim
- Sulfasalazine
- Sulfonamides
- Telithromycin

Antineoplastic/Immunosuppressives

- Azathioprine/6-Mercaptopurine
- Busulfan
- Floxuridine
- Flutamide
- Gold salts
- Infliximab
- Interferon beta
- Methotrexate
- Propylthiouracil
- Thioguanine

Neurologic

- Carbamazepine
- Chlorpromazine
- Dantrolene
- Disulfiram
- Phenytoin
- Valproate

Nonsteroidal antiinflammatory

- Diclofenac
- Ibuprofen
- Nimesulide
- Sulindac

Miscellaneous

- Allopurinol
- Anabolic steroids
- Contraceptives
- Halothane

Source: Björnsson 2016

# 7 REFERENCE LIST

Björnsson ES. Hepatotoxicity by drugs: the most common implicated agents. Int J Mol Sci. 2016;17(2):224.

Brasil P, Pereira JP Jr, Moreira ME, Ribeiro Nogueira RM, Damasceno L, Wakimoto M, et al. Zika virus infection in pregnant women in Rio de Janeiro. N Engl J Med. 2016;375(24):2321-34.

Department of Health and Human Services (DHHS), Food and Drug Administration, Center for Biologics Evaluation and Research (US). Guidance for industry: Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventative vaccine clinical trials. September 2007 [cited 2019 Apr 10] [10 screens]. Available from: https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf.

Department of Health and Human Services (DHHS), National Institutes of Health, National Institute of Allergy and Infectious Diseases, Division of AIDS. Division of AIDS (DAIDS) table for grading the severity of adult and pediatric adverse events. Version 2.0. November 2014. [cited 2019 April 13]. Available from: https://rsc.niaid.nih.gov/sites/default/files/daids-ae-grading-table-v2-nov2014-highlighted-changes.pdf.

Heymann DL, Hodgson A, Sall AA, Freedman DO, Staples JE, Althabe F, et al. Zika virus and microcephaly: why is this situation a PHEIC? Lancet. 2016;387(10020):719-21.

Lazear HM, Diamond MS. Zika virus: new clinical syndromes and its emergence in the Western Hemisphere. J Virol. 2016;90(10):4864-75.

Richner JM, Diamond MS. Zika virus vaccines: immune response, current status, and future challenges. Curr Opin Immunol. 2018;53:130-6.

Tavares Da Silva F, De Keyser F, Lambert PH, Robinson WH, Westhovens R, Sindic C. Optimal approaches to data collection and analysis of potential immune mediated disorders in clinical trials of new vaccines. Vaccine. 2013;31(14):1870-6.

Weaver SC, Costa F, Garcia-Blanco MA, Ko AI, Ribeiro GS, Saade G, et al. Zika virus: history, emergence, biology, and prospects for control. Antiviral Res. 2016;130:69-80.

World Health Organization [Internet]. WHO and experts prioritize vaccines, diagnostics and innovative vector control tools for Zika R&D. 09 March 2016a [cited 2019 April 12]; [1 screen]. Available from: http://www.who.int/mediacentre/news/notes/2016/research-development-zika/en.

World Health Organization [Internet]. An R&D blueprint for action to prevent epidemics: plan of action. May 2016b [cited 2019 April 12]; [48 screens]. Available from: https://www.who.int/blueprint/about/r_d_blueprint_plan_of_action.pdf?ua=1.

World Health Organization [homepage on the Internet]. Zika virus disease; c2019 [cited 2019 April 12]. Available from: https://www.who.int/emergencies/diseases/zika/en/.

Signature Page for VV-CLIN-000302 v4.0

| | |
|---|---|
| Approval | PPD<br>31-Mar-2020 19:27:59 GMT+0000 |

| | |
|---|---|
| Approval | PPD<br>01-Apr-2020 17:51:05 GMT+0000 |

Signature Page for VV-CLIN-000302 v4.0