ModernaTX, Inc.

Protocol mRNA-1893-P201

A Phase 2, Randomized, Observer-Blind, Placebo-Controlled, Dose Confirmation Study to Evaluate the Safety, Tolerability, and Immunogenicity of Zika Vaccine mRNA-1893 in Adults Aged 18 Through 65 Years and Living in Endemic and Non-Endemic Flavivirus Areas

Statistical Analysis Plan

SAP Version 2.0
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DOCUMENT HISTORY

Version	Date	Description of main modifications
1.0	19 January 2022	Original Version (Version 1.0)
2.0	19 April 2023	Version 2.0 This version inlcudes clarification to multiple sections. • Removed analyses using RVP assay across the document. • Updated analysis for NS1-binding antibody at Section 3.3. • Added actual treatment determination rule to Section 5.3. • Clarified immunogenicity assay window for the definition of PP set. • Updated analyses for safety analysis stages, study disposition categories, analysis subgroups, and study duration definition to Section 6.2. • Added analyses for SMQ to Section 6.3.1 • Clarified the grading of vital sign measurements in Section 6.3.3 • Updated analysis window for vital signs in Table 3.

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List of Abbreviations

Abbreviation	Definition
AE	Adverse event
AESI	Adverse event of special interest
ANCOVA	Analysis of Covariance
AR	Adverse reaction
bAb	Binding antibody
BMI	Body mass index
CFR	Code of Federal Regulations
CI	Confidence interval
CMI	Cell-mediated immunity
CMV	Cytomegalovirus
CRA	Clinical research associate
CRO	Contract research organization
CSP	Clinical study protocol
CSR	Clinical study report
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
eCRF	Electronic case report form
EDC	Electronic Data Capture
eDiary	Electronic diary
ELISA	Enzyme-linked immunosorbent assay
ELISpot	Enzyme-linked immunosorbent spot
EOS	End of Study
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMC	Geometric mean concentration
GMFR	Geometric mean fold-rise
GMT	Geometric mean titer
GMR	Geometric mean ratio
IA	Interim analysis
ICF	Informed consent form
ICH	International Council for Harmonisation
Ig	Immunoglobulin
IM	Intramuscular(ly)
IP	Investigational product
IRT	Interactive Response Technology
LLOQ	Lower limit of quantification
LTFU	Lost to follow-up
MAAE	Medically attended adverse event
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	Messenger RNA

Abbreviation	Definition
nAb	Neutralizing antibody
NS1	Nonstructural protein 1
PP	Per-protocol
PRNT	Plaque reduction neutralization test
PT	Preferred term
RT-PCR	Reverse transcription polymerase chain reaction
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Statistical Analysis System
SCR	Seroconversion rate
SD	Standard deviation
SOA	Schedule of Assessments
SOC	System organ class
TEAE	Treatment-emergent adverse event
ULOQ	Upper limit of quantification
WHO	World Health Organization
WHODD	World Health Organization drug dictionary
ZIKV	Zika virus

1. Introduction

This statistical analysis plan (SAP), which describes the planned analyses for Study mRNA-1893-P201, is based on the most recent approved clinical study protocol (CSP), Version Amendment 3, dated 09-Dec-2021. The most recent approved electronic case report form (eCRF) Version 5, dated 07-Oct-2021.

In addition to the information presented in the statistical analysis plan section of the protocol (Section 9) which provides the principal features of analyses for this study, this SAP provides statistical analysis details/data derivations. It also documents modifications or additions to the analysis plan that are not "principal" in nature and result from information that was not available at the time of protocol finalization.

Study mRNA-1893-P201 is a Phase 2, randomized, observer-blind, placebo-controlled, dose confirmation study to evaluate the safety, tolerability, and immunogenicity of Zika vaccine mRNA-1893 in adults aged 18 through 65 years and living in endemic and non-endemic flavivirus areas.

PPD Biostatistics and programming team, designee of Moderna Biostatistics and Programming department, will perform the statistical analysis of the safety, reactogenicity, and immunogenicity data; Statistical Analysis System (SAS) Version 9.4 or higher will be used to generate all statistical outputs (tables, figures, listings, and datasets). The SAP will be finalized and approved prior to the interim analysis database lock when all participants completed their Day 57 visit. If the methods in this SAP differ from the methods described in the protocol, the SAP will prevail.

In this document, subject and participant are used interchangeably; injection of IP, injection, and dose are used interchangeably; vaccination group, treatment group, and treatment arm are used interchangeably.

2. Study Objectives

2.1. Primary Objective

The primary objectives are the following:

- To evaluate the safety, tolerability, and reactogenicity of 2 dose levels of mRNA-1893 Zika vaccine, administered in a 1-dose (CCI µg) or 2-dose schedule (CCI µg or CCI µg dose level) given 28 days apart, in comparison to a placebo control.

- To evaluate the immunogenicity of 2 dose levels of mRNA-1893 Zika vaccine, administered in a 1-dose (CCI µg) or 2-dose schedule (CCI µg or CCI µg dose level) given 28 days apart, as measured by Zika virus (ZIKV)-specific neutralization assay (plaque reduction neutralization [PRNT]), in comparison to a placebo control at Day 57.

2.2. Secondary Objectives

The secondary objectives are the following:

- To evaluate the immunogenicity of 2 dose levels of mRNA-1893, as measured by ZIKV-specific neutralization assay (PRNT and microneutralization [MN]), in comparison to a placebo control, in flavivirus-seronegative participants at baseline and in flavivirus-seropositive participants at baseline.

2.3. Exploratory Objectives

The exploratory objectives are the following:

- To evaluate the duration of the humoral immune response of 2 dose levels of mRNA-1893 at Days 85, 196, 364, 532, and 700, as measured by ZIKV-specific neutralization assay (PRNT and MN), in comparison to a placebo control, in flavivirus-seronegative participants at baseline and in flavivirus-seropositive participants at baseline.
- To evaluate the humoral immunogenicity of 2 dose levels of mRNA-1893, as measured by ligand binding assay, in comparison to a placebo control, in flavivirus-seronegative participants at baseline and in flavivirus-seropositive participants at baseline.
- To evaluate the ZIKV-specific cellular immune response of 2 dose levels of mRNA-1893 in comparison to a placebo control in flavivirus-seronegative participants at baseline and flavivirus-seropositive participants at baseline (cell-mediated immunity [CMI] subset of participants).
- To assess the occurrence of flavivirus infections throughout the entire course of participation in the study in comparison to a placebo control.

3. Study Endpoints

3.1. Primary Endpoints

The primary safety objective will be evaluated by the following safety endpoints:

- Solicited local and systemic adverse reactions (ARs) through 7 days after each investigational product (IP) injection.
- Unsolicited adverse events (AEs) through 28 days after each IP injection.
- Medically attended AEs (MAAEs) throughout the entire study period.
- Serious AEs (SAEs) and adverse events of special interest (AESIs) throughout the entire study period.

The primary immunogenicity objective will be evaluated by either:

- Geometric mean titer (GMT) of ZIKV-specific neutralizing antibodies (nAbs) at Day 57, as measured by PRNT, in all participants, in initially flavivirus-seronegative, and in initially flavivirus-seropositive participants.
- Seroconversion from Day 1 to Day 57.

Seroconversion is defined as either an increase in ZIKV-specific nAb titer from below the lower limit of quantification [LLOQ] to a titer equal to or above LLOQ, or an increase of at least 4-fold in ZIKV-specific nAb titer in participants with pre-existing nAb titers [as measured by PRNT].

3.2. Secondary Endpoints

The secondary objective will be evaluated by the following endpoints:

- GMT of ZIKV-specific nAbs at Days 1, 8, 29, 36, and 57 (Day 57 only for MN), as measured by PRNT and MN.
- GMT of ZIKV-specific nAbs in initially flavivirus-seronegative participants at Days 1, 8, 29, 36, and 57 (Day 57 only for MN), as measured by PRNT and MN.
- GMT of ZIKV-specific nAbs in initially flavivirus-seropositive participants at Days 1, 8, 29, 36, and 57 (Day 57 only for MN), as measured by PRNT and MN.

- Geometric mean fold-rise (GMFR) of ZIKV-specific nAbs in all participants, in initially flavivirus-seronegative and in initially flavivirus-seropositive participants at Days 8, 29, 36, and 57, as measured by PRNT and MN.
- Seroconversion from Day 1 (baseline) to Days 8, 29, 36, and 57 (only for MN), as measured by PRNT and MN.
- Seroresponse at Days 8, 29, 36, and 57 (Day 57 only for MN) in flavivirus-seronegative participants at baseline.
- Increase from baseline of 2-fold or 4-fold in ZIKV-specific nAb titers at Days 8, 29, 36, and 57, as measured by PRNT and MN, in flavivirus-seropositive participants at baseline.

Seroresponse is defined as an increase in ZIKV-specific nAb titer [as measured by PRNT or MN] from below the LLOQ to greater than or equal to the LLOQ.

3.3. Exploratory Endpoints

The exploratory endpoints are the following:

- GMT of ZIKV-specific nAbs at Days 85, 196, 364, 532, and 700, as measured by PRNT and MN.

  GMT of ZIKV-specific nAbs at Days 85, 196, 364, 532, and 700, as measured by PRNT and MN, in flavivirus-seronegative participants at baseline.
- GMT of ZIKV-specific nAbs at Days 85, 196, 364, 532, and 700, as measured by PRNT and MN, in flavivirus-seropositive participants at baseline.
- GMFR of ZIKV-specific nAbs in all participants, in initially flavivirus-seronegative, and in initially flavivirus-seropositive participants at Days 85, 196, 364, 532, and 700, as measured by PRNT and MN.
- Seroconversion from Day 1 (baseline) to Days 85, 196, 364, 532, and 700, as measured by PRNT and MN.
- Seroresponse at Days 85, 196, 364, 532, and 700, as measured by PRNT and MN, in flavivirus-seronegative participants at baseline.
- Increase from baseline of 2-fold or 4-fold in ZIKV-specific nAb titers at Days 85, 196, 364, 532, and 700, as measured by PRNT and MN, in flavivirus-seropositive participants at baseline.

- Proportion of all participants, of baseline flavivirus-seronegative participants, and of baseline flavivirus-seropositive participants with a ZIKV-specific nAb titer equal or greater than a nAb titer defined as a protective level in an animal model, at baseline, and Days 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by PRNT.
- Proportion of all participants, of baseline flavivirus-seronegative participants, and of baseline flavivirus-seropositive participants with a ZIKV-specific nAb titer equal or greater than a nAb titer defined as a protective level in an animal model, at baseline, and Days 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by MN.
- Geometric mean concentration (GMC) of ZIKV-specific serum ligand binding antibodies (bAbs) at Days 1, 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by ligand binding assay.
- GMC of ZIKV-specific bAbs at Days 1, 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by ligand binding assay, in flavivirus-seronegative participants at baseline.
- GMC of ZIKV-specific bAbs at Days 1, 8, 29, 36, 57, 85, 196, 364, 532 and 700, as measured by ligand binding assay, in flavivirus-seropositive participants at baseline.
- Seroconversion from Day 1 (baseline) to Days 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by ligand binding assay.
- Seroresponse at Days 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by ligand binding assay, in flavivirus-seronegative participants at baseline.
- Increase from baseline 2-fold or 4-fold in bAb concentrations at Days 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by ligand binding assay, in flavivirus-seropositive participants at baseline.
- Frequency of ZIKV-specific T cells at Days 1 (baseline), 29, 36, 57, 196, 364, 532, and 700, as measured by intracellular cytokine staining assay and sequencing (CMI subset of baseline flavivirus-seronegative and -seropositive participants).
- Frequency of ZIKV-specific B cells at Days 1 (baseline), 29, 36, 57, 196, 364, 532, and 700, as measured by ELISpot (CMI subset of baseline flavivirus-seronegative and -seropositive participants).

- Additional assays to profile the T- and B-cell responses may also be performed (CMI subset of baseline flavivirus-seronegative and -seropositive participants).
- Seroconversion (ZIKV NS1-specific bAbs) from Day 1 (baseline) to Days 29, 57, 85, 196, 364, 532, and 700

Seroconversion for ZIKV NS1-specific bAbs is defined as the change to a positive ZIKV NS1-specific bAbs status from a baseline negative ZIKV NS1-specific bAbs status.

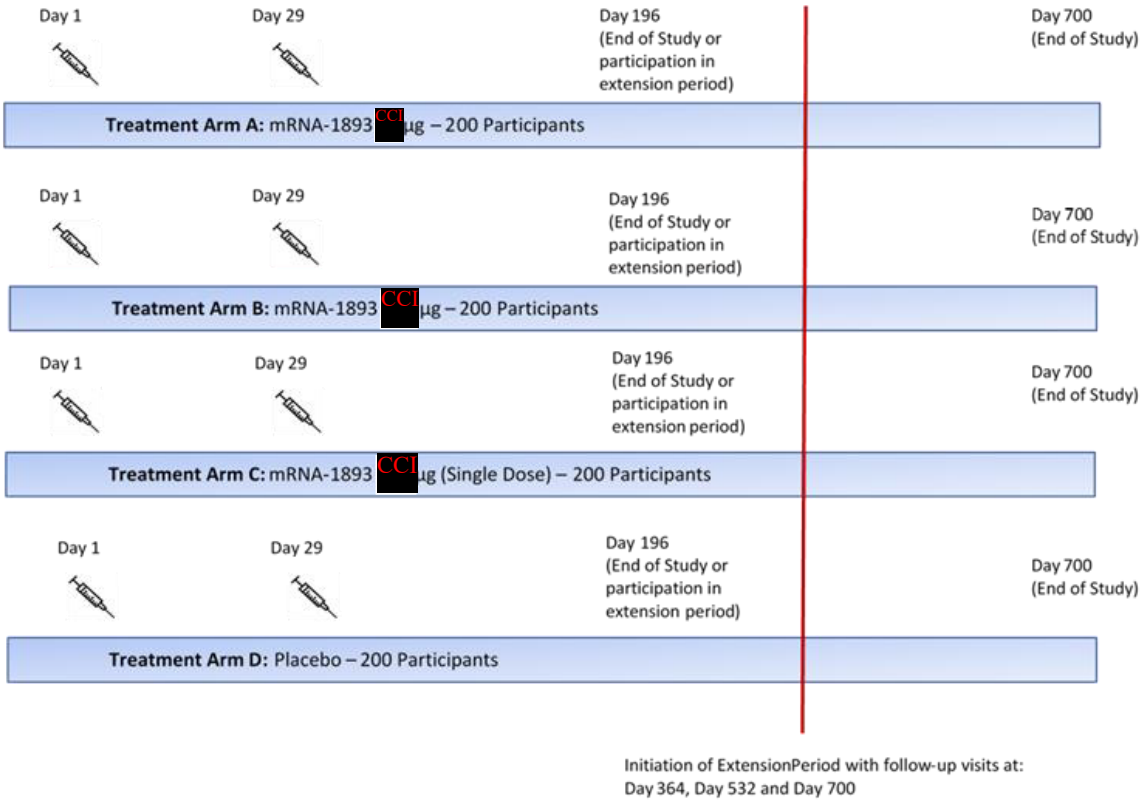
4. Study Design

4.1. Overall Study Design

This is a Phase 2, randomized, observer-blind, placebo-controlled, dose confirmation study.

All participants will be evaluated for their eligibility for enrollment in the Vaccination Period and will be followed for approximately 6 months (Day 196) after their last IP injection. All participants in all treatment arms will have the opportunity to continue to be followed as part of an approximately 24-month Extension Period (through Day 700) after their last IP injection. The Schedule of Assessments (SOA) for all participants through Day 196 is provided in Appendix E and the SOA for participants who continue into the Extension Period through Day 700 is provided in Appendix F. A schematic of the study design is illustrated in Figure 1.

Figure 1: Study Schema



Note: Participants randomly assigned to Treatment Arm C will be administered placebo at Day 1 and mRNA-1893 CCI µg at Day 29.

A total of approximately 800 participants will be randomly assigned to receive either CCI µg or CCI µg mRNA-1893 administered as a 2-dose regimen with at least a 28-day interval between vaccinations (Treatment Arm A or Treatment Arm B), or CCI µg mRNA-1893 administered as a 1-dose regimen (Treatment Arm C), or placebo control administered as a 2-dose regimen with at least a 28-day interval between doses (Treatment Arm D) according to a 1:1:1:1 randomization ratio (ie, 200 participants per treatment arm with approximately 100 participants per baseline flavivirus serostatus in each treatment arm). All treatment arms will be enrolled in parallel. In order to maintain the blinding and to have the same study evaluation time point with both mRNA-1893 dose regimens relative to the last IP injection, participants randomly assigned to Treatment Arm C will be administered placebo at Day 1 and mRNA-1893 at Day 29 (Table 1).

Table 1: Treatment Arms

	Study Day	
	Day 1	**Day 29**
Treatment Arm A	mRNA-1893 CCI µg First vaccination	mRNA-1893 CCI µg Second vaccination
Treatment Arm B	mRNA-1893 CCI µg First vaccination	mRNA-1893 CCI µg Second vaccination
Treatment Arm C	Placebo	mRNA-1893 CCI µg First vaccination
Treatment Arm D	Placebo	Placebo

All participants will have up to 8 clinic visits (Days 0 [Eligibility Visit], 1 (baseline), 8, 29, 36, 57, 85, and 196) and 3 safety telephone calls (Days 112, 140, and 168). Participants who continue into the Extension Period will have 3 additional clinic visits at Days 364, 532, and 700.

Participants who withdraw from further IP injection for any reason after their first IP injection but do not withdraw consent will complete all study procedures through the End of Study (EOS) Visit.

4.2. Statistical Hypotheses

There is no hypothesis testing in this study.

4.3. Sample Size and Power

The sample size for this study is not driven by statistical assumptions for formal hypothesis testing. This sample size is regarded as sufficient to provide an understanding of the safety profile of mRNA-1893 and to select the dose to advance development into future studies.

Approximately 800 participants will be randomly assigned in a 1:1:1:1 ratio to receive 2 doses of CCI µg mRNA-1893 separated by 28 days (Treatment Arm A), 2 doses of CCI µg mRNA-1893 separated by 28 days (Treatment Arm B), a single dose of CCI µg mRNA-1893 on Day 29 following a single dose of placebo on Day 1 (Treatment Arm C), or 2 doses of placebo separated by 28 days (Treatment Arm D). A total of 600 participants will receive mRNA-1893, 200 participants in each dosing arm, with approximately 100 participants in each flavivirus serostatus cohort and dosing arm; approximately 200 participants will receive placebo. Table 2 presents the 95% confidence interval (CI) for 1 participant with an AE and the lowest AE rate detectable with at least 95% probability for each selected sample size. The 2-sided 95% CI was calculated using the Clopper-Pearson

method for one proportion in SAS 9.4 software. A sample size of 200 (or 400) has at least a 95% probability to observe at least 1 participant with an AE at a true 1.49% (or 0.75%) AE rate.

Table 2: 95% Confidence Interval for One Participant with an Adverse Event and the Lowest Detectable Incidence Rate at 95% Probability in Each Selected Sample Size

Sample Size Receiving mRNA-1893	Rate and 95% CI (%) at One Participant with AE			Lowest Detectable Rate (%) with ≥ 95% Probability
	AE Rate	Lower CI	Upper CI	
100	1.00	0.03	5.45	2.95
200	0.50	0.01	2.75	1.49
400	0.25	0.01	1.38	0.75
600	0.17	0.00	0.93	0.5

Abbreviations: AE = adverse event; CI = confidence interval.

4.4. Randomization

The randomization will be done in a blinded manner using a centralized Interactive Response Technology (IRT), in accordance with pre-generated randomization schedules. Approximately 800 participants will be randomized in parallel according to a 1:1:1:1 randomization ratio to the 4 treatment arms. Randomization was initially stratified by participants' baseline flavivirus serostatus using a point of care assay under the original protocol (dated 21 February 2021). Under the protocol amendment 1 (16 September 2021), protocol amendment 2 (27 October 2021) and current Amendment 3 (09 December 2021), randomization will be stratified only by participants' region (continental US and Puerto Rico). Only the unblinded pharmacy personnel (Section 4.5) will have controlled access to which arm the participant is randomly assigned.

Blinding and Unblinding This is an observer-blind study. The investigator, study staff, study participants, site monitors, and Sponsor personnel (or its designees) will be blinded to the IP administered until the electronic data capture (EDC) is locked and clinical study report (CSR) has been written, with certain exceptions, please refer to Section 6.3.2 of the protocol for details.

Study site blinded staff will remain blinded to individual treatment assignments until the end of the study.

Except in the case of medical necessity, a participant's treatment assignment should not be unblinded without the approval of the Sponsor. If a participant becomes seriously ill or pregnant during the study, the blind will be broken only if knowledge of the treatment assignment will affect that participant's clinical management. In the event of a medical emergency requiring identification of individual treatment assignment, the investigator will make every attempt to contact the Sponsor medical lead or designee to explain the need for unblinding within 24 hours of opening the code. The investigator will be responsible for documenting the time, date, reason for unblinding, and the names of the personnel involved. The investigator (or designee) will have access to unblind participants within IRT. All unblindings will be tracked via an audit trail in IRT and documented in the final study report.

The Sponsor will remain blinded to IP assignment throughout the treatment phase for each individual treatment arm of the study up until the specified time point for interim analysis (IA), as outlined in Section 6.6.

5. Analysis Populations

The following analysis sets are defined: Randomized Set, Solicited Safety Set, Safety Set, Full Analysis Set (FAS), Per-Protocol (PP) Set.

5.1. Randomized Set

The Randomized Set consists of all participants who are randomized in the study, regardless of the participant's treatment status in the study. Participants will be included in the treatment arm to which they are randomized.

5.2. Solicited Safety Set

The Solicited Safety Set consists of all participants who are randomly assigned and received any IP and contribute any solicited AR data. The Solicited Safety Set will be used for the analyses of solicited ARs and participants will be included in the treatment arm corresponding to the IP they actually received.

5.3. Safety Set

The Safety Set consists of all participants who are randomly assigned and received any IP. The Safety Set will be used for analysis of safety except for the solicited ARs. Participants will be included in the treatment arm corresponding to the IP they actually received.

For Solicited Safety Set and Safety Set, the following rule will be used to determine participant's actual vaccination group:

If a participant only received one injection:

- Participant who received placebo will be summarized under Placebo if the planned treatment arm is also Placebo, otherwise under mRNA-1893 ([CCI] µg) Single Dose.
- Participant who received mRNA-1893 will be summarized under mRNA-1893 as 2-dose regimen which determined by actual dose received according to the dose range mapping rule below.

If a participant received both injections:

- Participant who received placebo for both injections will be summarized under Placebo.
- Participant who received placebo for the first injection and mRNA-1893 for the second injection will be summarized under mRNA-1893 ([CCI] µg) Single Dose.
- Participant who received mRNA-1893 as the first injection will be summarized under the higher dose of vaccination group between two injections (eg, Placebo < mRNA-1893 [CCI] µg < mRNA-1893 [CCI] µg) according to the dose range mapping rule below.

If non-protocol mRNA-1893 dose level occurs, below dosing range mapping rule will be used:

- mRNA-1893 [CCI] µg: If the received dose of mRNA-1893 is > 0 µg and ≤ 65 µg.
- mRNA-1893 [CCI] µg: If the received dose of mRNA-1893 is > 65 µg.

5.4. Full Analysis Set

Full Analysis Set (FAS) for Antibody-Mediated Immunogenicity

The FAS for antibody-mediated immunogenicity consists of all randomized participants who

a) receive any study vaccination,

b) have baseline (Day 1) antibody-mediated immunogenicity data available for those analyses that require baseline data, and

c) have at least one post-vaccination antibody-mediated immunogenicity assessment for the analysis endpoint.

FAS for Cell-Mediated Immunogenicity

The FAS for cell-mediated immunogenicity (CMI) consists of all randomized participants who

a) receive any study vaccination,

b) have baseline (Day 1) CMI data available for those analyses that require baseline data, and

c) have at least one post-vaccination CMI assessment for the analysis endpoint.

Participants will be included in the vaccination group to which they are randomized.

5.5. Per-Protocol Sets

The Per-Protocol (PP) Set for Antibody-Mediated Immunogenicity

PP Set for Antibody-Mediated Immunogenicity consist of all FAS participants who

a) comply with the vaccination schedule,

b) comply with the timings of immunogenicity blood sampling to have post-vaccination results available for at least one assay component corresponding to the immunogenicity analysis objective (antibody-mediated immunogenicity endpoints), and

c) have no major protocol deviations that impact immune response during the period corresponding to the immunogenicity analysis objective (antibody-mediated immunogenicity endpoints).

The PP Set for Antibody-Mediated Immunogenicity will serve as the primary population for the analysis of antibody-mediated immunogenicity data in this study. Participants will be included in the vaccination group to which they are randomized.

PP Set for CMI

PP Set for CMI consist of all FAS for Cell-Mediated participants who

a) comply with the vaccination schedule,

b) comply with the timings of immunogenicity blood sampling to have post-vaccination results available for at least one assay component corresponding to the immunogenicity analysis objective (CMI endpoints), and

c) have no major protocol deviations that impact immune response during the period corresponding to the immunogenicity analysis objective (CMI endpoints).

The PP Set for CMI will serve as the primary population for the analysis of CMI data in this study. Participants will be included in the vaccination group to which they are randomized.

Participants who did not take blood sample for immunogenicity within [21, 42] days after the second injection date will be excluded from the Per-Protocol Set. Participants who only received 1 dose or who received the second dose outside of the window [21, 42] will be excluded from the Per-Protocol Set.

6. Statistical Analysis

6.1. General Considerations

The Schedule of Assessments is provided in Appendix E for the vaccination period and the follow-up period, and Appendix F for the extension period.

Continuous variables will be summarized using the following descriptive summary statistics: the number of subjects (n), mean, standard deviation (SD), median, minimum (min), and maximum (max).

Categorical variables will be summarized using counts and percentages.

Baseline value, unless specified otherwise, is defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the first dose of IP. For immunogenicity tests, the baseline is defined as the most recent non-missing measurement (scheduled or unscheduled) collected before or on the date of first dose of IP.

For the summary statistics of all numerical variables unless otherwise specified, the display precision will follow programming standards. Please see Appendix A for variable display standards.

When count data are presented, the percentage will be suppressed when the count is zero in order to draw attention to the non-zero counts. A row denoted “Missing” will be included in count tabulations where specified on the shells to account for dropouts and missing values. The denominator for all percentages will be the number of subjects in that vaccination group within the analysis set of interest, unless otherwise specified.

Study day relative to the first injection will be calculated as below:

a) study day prior to the first injection will be calculated as: date of assessment/event – date of the first injection;

b) study day on or after the date of the first injection will be calculated as: date of assessment/event – date of the first injection + 1;

Study day relative to the most recent injection will be calculated as below:

a) study day prior to the first injection will be calculated as: date of assessment/event – date of the first injection;

b) study day on or after the date of the first injection but before the second injection (if applicable) will be calculated as: date of assessment/event – date of the first injection + 1;

c) study day on or after the date of the second injection will be calculated as: date of assessment/event – date of the second injection + 1; if study day is on the same day as the second injection, date and time will be compared with the second injection date and time.

For calculation regarding antibody titers/antibody concentrations, antibody values reported as below LLOQ will be replaced by 0.5 × LLOQ. Values that are greater than the upper limit of quantification (ULOQ) will be converted to the ULOQ. Missing results will not be imputed.

The following **stages for safety analyses** will be used in this study:

- Up to 28 days after any vaccination: this stage starts at the day of each vaccination and continue through the earliest date of (the day of each vaccination and 27 subsequent days, next vaccination [if applicable]). This analysis period will be used as the primary analysis period for safety analyses including unsolicited AE, except for solicited AR, unless specified otherwise.
- Up to 28 days after the first vaccination.
- Up to 28 days after the second vaccination.
- Overall stage: this analysis period starts at the first injection on Day 1 and continues through the earliest date of (study completion, discontinuation from the study, or death).

Unscheduled visits: Unscheduled visit measurements will be included in analysis as follows:

- In scheduled visit windows per specified visit windowing rules.
- In the derivation of baseline/last on-treatment measurements.
- In the derivation of maximum/minimum on-treatment values and maximum/minimum change from baseline values for safety analyses.
- In individual subject data listings as appropriate.

Visit windowing rules: The analysis visit windows for protocol-defined visits are provided in Appendix B.

Incomplete/missing data:

- Imputation rules for missing prior/concomitant medications, non-study vaccinations and procedures are provided in Appendix C.
- Imputation rules for missing AE dates are provided in Appendix D.
- For laboratory assessments, if majority of results are indefinite, imputation of these values will be considered. If the laboratory results are reported as below the LLOQ (e.g., <0.1), the numeric values will be imputed by 0.5 × LLOQ in the summary. If the laboratory results are reported as greater than the ULOQ (e.g., >3000), the numeric values will be imputed by ULOQ in the summary.
- Other incomplete/missing data will not be imputed, unless specified otherwise.

Treatment groups:

The following vaccination groups will be used for summary purposes:

- mRNA-1893 vaccine: CCI µg (2-dose)
- mRNA-1893 vaccine: CCI µg (2-dose)
- mRNA-1893 vaccine: CCI µg (single-dose)
- mRNA-1893 vaccine: Total
- Placebo

Summary by flavivirus serostatus:

All analyses and data summaries/displays will be provided by flavivirus serostatus (flavivirus-seropositive, flavivirus-seronegative, and/or overall) unless otherwise specified,

please also refer to Section 6.5 Planned Analyses. Baseline flavivirus status is determined by serology testing.

A participant is baseline seronegative if at least three baseline serology testings (dengue IgG, dengue IgM, West Nile IgG and West Nile IgM) are negative, and none of the serology testings is positive. If at least one of the baseline serology testings is positive, the participant is baseline seropositive. If two or more of the serology testings are “missing” or “unknown”, and none of the serology testings is positive, the baseline serostatus will be “unknown” or “missing”. A single testing result of "equivocal" is considered as "unknown", regardless of the numeric values.

All analyses will be conducted using SAS Version 9.4 or higher.

6.2. Background Characteristics

6.2.1. Subject Disposition

The number and percentage of subjects in the following categories will be summarized by flavivirus serostatus and vaccination group as defined in Section 6.1 based on Randomized Set:

- Randomized Set
- Solicited Safety Set
- Safety Set
- Full Analysis Set
- Per-Protocol Set

The percentage will be based on subjects in that vaccination group within the Randomization Set (as randomized), except the Solicited Safety Set and Safety Set for which the percentages will be based on the vaccination group in the Safety Set (as treated).

The number of subjects in the following categories will be summarized based on subjects screened:

- Number of subjects screened
- Number and percentage of screen failure subjects and the reason for screen failure

The percentage of subjects who screen failed will be based on the number of subjects screened. The reason for screen failure will be based on the number of subjects who screen failed.

The number and percentage of subjects in each of the following disposition categories will be summarized by baseline flavivirus serostatus and vaccination group based on the Randomized Set, except otherwise specified:

- Randomized by site
- Received each dose of IP
- Prematurely discontinued before receiving the second dose of IP and the reason for discontinuation
- Completed the main study (c.f. Section 4.1, i.e. the period up to Day 196 visit)
- Prematurely discontinued from the main study and the reason for discontinuation
- Continued into the extension period
- Completed the extension period (percentage based on participants who consent to enter the extension period in the Randomized Set)
- Prematurely discontinued from the extention period and the reason for discontinuation (percentage based on participants who consent to enter the extension period in the Randomized Set)

A subject disposition listing will be provided, including informed consent, subjects who completed the study injection schedule, subjects who completed study, subjects who discontinued from study vaccine or who discontinued from participation in the study, with reasons for discontinuation. A separate listing will be provided for screen failure subjects with reasons for screen failure.

Participants who do not consent to continue into the Extension Period will be considered to have completed the study if they have completed the EOS Visit approximately 6 months (Visit 10 [Day 196]) after the last IP injection. Participants who consent to continue into the Extension Period will be considered to have completed the study if they have completed the EOS Visit approximately 24 months (Day 700) after the last IP injection.

6.2.2. Demographics

Descriptive statistics will be calculated for the following continuous demographic and baseline characteristics: age (years), age group (>=18 and < 50 years, >= 50 and <=65 years) , weight (kg), height (cm), and body mass index (BMI) (kg/m^2). Number and percentage of subjects will be provided for categorical variables such as gender, race, ethnicity, region and flavivirus serostatus at baseline. The summaries will be presented by flavivirus serostatus and vaccination group as defined in Section 6.1 based on the Safety Set, FAS, and PP Set. If the Safety Set differs from the Randomized Set (e.g., subjects randomized but not received any study injection; subjects received study vaccination other than the vaccination group they were randomized to), the analysis will also be conducted using the Randomized Set.

For screened failure subjects, age (years), as well as gender, race, ethnicity will be presented in a listing.

In addition, subjects with any inclusion and exclusion criteria violation will also be provided in a listing.

6.2.3. Medical History

Medical history data will be coded by system organ class (SOC) and preferred term (PT) using the Medical Dictionary for Regulatory Activities (MedDRA).

The number and percentage of participants with any medical history will be summarized by SOC and PT based on the Safety Set. A participant will be counted only once for multiple events within each SOC and PT. SOC will be displayed in internationally agreed order. PT will be displayed in descending order of frequency of mRNA-1893 Total group and then alphabetically within SOC.

Medical history data will be presented in a listing.

6.2.4. Prior and Concomitant Medications

Prior and concomitant medications and non-study vaccination will be coded using the World Health Organization (WHO) drug dictionary (WHODD). The summary of concomitant medications will be based on the Safety Set. Categorization of prior, concomitant, and post medications is summarized in Appendix C Table 4.

The number and percentage of subjects using concomitant medications and non-study vaccination during the 7-day follow-up period (i.e., on the day of injection and the 6

subsequent days) and during the 28 day follow-up period after each injection (i.e., on the day of injection and the 27 subsequent days) will be summarized by flavivirus serostatus and vaccination group as defined in Section 6.1 as follows:

- Any concomitant medications and non-study vaccination within 7 Days Post Injection
- Any concomitant medications and non-study vaccination within 28 Days Post Injection
- Antipyretic or analgesic medication within 28 Days Post Injection

A summary table of concomitant medications and non-study vaccination that continued or newly received at or after the first injection through 28 days after the last injection will be provided by PT in descending frequency in the mRNA-1893 group with all dose level combined.

Medications taken to prevent pain or fever will be collected on eDiary and summaries will be provided based on the Solicited Safety Set by flavivirus serostatus and vaccination group as defined in Section 6.1 for each injection (first or second) and any injection, including within 7 days after injection.

Prior, concomitant and post medications and non-study vaccination will be presented in a listing.

Concomitant Procedures will be presented in a listing.

6.2.5. Study Exposure

Study IP administration data will be presented in a listing.

Study duration will be summarized since randomization, since the first injection, and since the second injection until complete/discontinued the study or the data cutoff date, whichever occurs first, based on the Safety Set.

6.2.6. Major Protocol Deviations

Major protocol deviations are a subset of protocol deviations that may significantly impact the completeness, accuracy, or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being. Major protocol deviations rules will be developed and finalized before database lock.

The number and percentage of the subjects with each major protocol deviation type will be provided by flavivirus serostatus and vaccination group as defined in Section 6.1 based on the Randomized Set.

Major protocol deviations will be presented in a listing.

6.2.7. COVID-19 Impact

A listing will be provided for COVID-19 impact.

6.3. Safety Analysis

Safety and reactogenicity will be assessed by clinical review of all relevant parameters including solicited ARs (local and systemic), unsolicited AEs, SAEs, AESIs, MAAEs, AEs leading to withdrawal from study vaccine and/or study participation, vital signs, and physical examination findings.

All safety analyses will be based on the Safety Set, except summaries of solicited ARs which will be based on the Solicited Safety Set. All safety analyses will be provided by baseline flavivirus serostatus and vaccination group unless otherwise specified. Subgroup analysis based on age group (>=18 and < 50 years, >= 50 and <=65 years) may be performed.

6.3.1. Adverse Events

An AE is defined as any untoward medical occurrence in a subject or clinical investigation subject who is administered a pharmaceutical product at any dose that does not necessarily have to have a causal relationship with the treatment.

A treatment-emergent AE (TEAE) is defined as any event occurring during the study not before exposure to study vaccine or any event already present that worsens after exposure to study vaccine. Adverse events will also be evaluated by the investigator for the coexistence of MAAE which is defined as an AE that leads to an unscheduled visit to a healthcare practitioner.

Unsolicited AEs will be coded by PT and SOC using MedDRA summarized by baseline flavivirus serostatus, vaccination group, and stage (up to 28 days after each vaccination, up to 28 days after any vaccination and overall stage; see Section 6.1 for definitions of vaccination group and stage).

All summary tables (except for the overall summary of AEs) for unsolicited AEs will be presented by SOC and PT for TEAEs with counts of subjects included. SOC will be displayed in internationally agreed order. PT will be displayed in descending order of frequency of total mRNA-1893 and then alphabetically within SOC. When summarizing the number and percentage of subjects with an event, subjects with multiple occurrences of the same AE or a continuing AE will be counted once. Subjects will be presented according to the highest severity (the strongest relationship) in the summaries by severity (of related AEs), if subjects reported multiple events under the same SOC and/or PT.

Percentages will be based upon the number of subjects in the Safety Set within each vaccination group.

6.3.1.1. Incidence of Adverse Events

All summary tables for unsolicited AEs will be provided by baseline flavivirus serostatus (flavivirus-seropositive, flavivirus-seronegative, and/or overall).

An overall summary of unsolicited TEAEs including the number and percentage of subjects who experience the following will be presented:

- Any unsolicited TEAEs
- Any serious TEAEs
- Any fatal TEAEs
- Any unsolicited medically-attended TEAEs
- Any unsolicited TEAEs leading to discontinuation from study vaccine
- Any unsolicited TEAEs leading to discontinuation from participation in the study
- Any unsolicited severe TEAEs
- Any AESI

The table will also include number and percentage of subjects with unsolicited TEAEs that are treatment-related in each of the above categories.

In addition, separate listings containing individual subject adverse event data for all unsolicited TEAEs, unsolicited TEAEs leading to discontinuation from study vaccine, unsolicited TEAEs leading to discontinuation from participation in the study, serious TEAEs, and unsolicited medically-attended TEAEs will be provided separately.

6.3.1.2. TEAEs by System Organ Class and Preferred Term

The following summary tables of TEAEs will be provided by MedDRA SOC and PT using frequency counts and percentages (i.e., number and percentage of subjects with an event):

- All unsolicited TEAEs
- All unsolicited TEAEs that are treatment-related
- All serious TEAEs
- All serious TEAEs that are treatment-related
- All unsolicited TEAEs leading to discontinuation from study vaccine
- All unsolicited TEAEs leading to discontinuation from participation in the study
- All unsolicited severe TEAEs
- All unsolicited severe TEAEs that are treatment-related
- All unsolicited medically-attended TEAEs
- All unsolicited medically-attended TEAEs that are treatment-related
- Any AESI

6.3.1.3.TEAEs by Preferred Term

The following summary tables of TEAEs will be provided by PT using frequency counts and percentages. PTs will be sorted in a descending order according to the frequency in mRNA-1893 Total group.

- All unsolicited TEAEs..
- All unsolicited TEAEs that belongs to selected SMQs (e.g. anaphylaxis, hypersensitivity, cardiomyopathy, cardiac arrhythmia, embolic and thrombotic events, cardiac failure, ischemic heart disease, autoimmune disorder, angioedema, central nervous system haemorrhages and cerebrovascular conditions). SMQs will be analyzed based on narrow and/or broad search, and details will be provided in the TLF shells.

6.3.1.4.TEAEs by System Organ Class, Preferred Term and Severity

The following summary tables of TEAEs will be provided by SOC, PT, and maximum severity (mild < moderate < severe) using frequency counts and percentages:

- All unsolicited TEAEs
- All unsolicited TEAEs that are treatment-related

6.3.1.5.Solicited Adverse Reactions

All summary tables for solicited ARs will be provided by baseline flavivirus serostatus (flavivirus-seropositive, flavivirus-seronegative, and/or overall).

An AR is any AE for which there is a reasonable possibility that the test product caused the AE. The term "Solicited Adverse Reactions" refers to selected signs and symptoms occurring after injection administration during a specified post-injection follow-up period (day of injection and 6 subsequent days). The solicited ARs are recorded by the subject in eDiary. The occurrence and intensity of selected signs and symptoms is actively solicited from the participant during a specified post-injection follow-up period (day of injection and 6 subsequent days), using a pre-defined checklist in the eDiary (i.e., solicited ARs).

The following local ARs will be solicited: pain at injection site, erythema (redness) at injection site, swelling (hardness) at injection site.

The following systemic ARs will be solicited: headache, fatigue, myalgia (muscle aches all over the body), arthralgia (aching in several joints), nausea/vomiting, fever, and chills.

The solicited ARs will be graded based on the grading scales presented in Table 13 in the protocol, modified from the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (DHHS 2007). Investigator will assess Grade 4 events (with exception of fever).

If a solicited local or systemic AR continues beyond 7 days post injection, the participant will be prompted to capture solicited local or systemic AR in the eDiary until resolution.

All solicited ARs (local and systemic) will be considered causally related to vaccination.

All solicited ARs analyses will be based on Solicited Safety Set. All solicited ARs analyses will be provided by flavivirus serostatus and vaccination group as defined in Section 6.1 for each vaccination (first, second), unless otherwise specified.

The number and percentage of subjects who reported any solicited AR, any solicited local AR, and any systemic solicited AR during the 7-day follow-up period after each vaccination will be tabulated by flavivirus serostatus, vaccination group, and vaccination,

with a two-sided 95% exact CI using the Clopper-Pearson method. The same analysis will be conducted for subgroups defined by region (United States and Puerto Rico).

The number and percentage of subjects who reported each individual solicited local AR (has a severity grade of Grade 1 or greater) and solicited systemic AR (has a severity grade of Grade 1 or greater) during the 7-day follow-up period after each vaccination will be tabulated by flavivirus serostatus, vaccination group, severity grade and vaccination. The same analysis will be conducted for subgroups defined by region (United States and Puerto Rico).

The number and percentage of subjects who reported each individual solicited AR will also be summarized by flavivirus serostatus, vaccination group, severity grade, day of reporting and vaccination. The number and percentage of subjects experiencing fever (a temperature greater than or equal to 38.0°C/100.4°F by the oral, axillary, or tympanic route) by severity grade and the number and percentage of subjects experiencing a fever of Grade 3 or higher temperature (a temperature greater than or equal to 39.0°C/102.1°F by the oral, axillary, or tympanic route) in cumulative half-degree (°F) increments will be provided.

The onset of individual solicited AR is defined as the time point after each vaccination at which the respective solicited AR first occurred. The number and percentage of subjects with onset of individual solicited AR will be summarized by baseline flavivirus serostatus, vaccination group, study day relative to corresponding vaccination (Day 1 through Day 7) and vaccination.

The duration (days) of each solicited AR will be summarized by baseline flavivirus serostatus, vaccination group and vaccination number (e.g. Dose 1 and Dose 2 and ‘any’). Duration will be calculated as the days of the solicited AR which is reported within the 7 days of vaccination including the day of vaccination, no matter it is intermittent or continued. The duration is calculated as end date – start date +1. If the solicited AR continues beyond 7 days, the consecutive days a solicited AR is reported after 7 days will be included (eg, an event that lasted 5 days in the first 7 days post vaccination and 3 consecutive days beyond 7 days post vaccination, the duration will be reported as 8 (5+3) days.).

All solicited ARs that continue beyond 7 days post vaccination will be presented in separate data listings.

6.3.2. Pregnancy Tests

A point-of-care urine pregnancy test will be performed at the Screening Visit (Day 0) and before each vaccine dose. At any time, a pregnancy test either via blood or point-of-care urine can be performed, at the discretion of the investigator. A by-subject listing will be provided for pregnancy tests.

6.3.3. Vital Sign Measurements

Vital sign measurements, including systolic and diastolic blood pressures, pulse respiratory rate, and body temperature, will be presented in a data listing. The values meeting the toxicity grading criteria will be flagged in the data listing. The abnormalities meeting the toxicity grading criteria (Grade 3 or higher) in any vital sign measurement provided by Table 14 in the protocol will be listed separately. If a subject has a vital sign result with Grade 3 or higher abnormality at any post injection visit, then all results of vital sign measurement for that subject will be presented in the listing. Note that vital signs grading are derived based on the numerical values only.

Observed values and changes from baseline for all vital sign measurements will be summarized at each scheduled visit by flavivirus serostatus and vaccination group as defined in Section 6.1. Shift from baseline in the toxicity grades will also be summarized at each scheduled visit by flavivirus serostatus and vaccination group.

6.4. Immunogenicity Analysis

The analyses of immunogenicity will be based on the PP Set and will be by baseline serostatus group (ie, flavivirus-seropositive, flavivirus-seronegative groups, flavivirus-serostatus missing, and overall) and vaccination group as defined in Section 6.1. Subgroup analysis based on age group (>=18 and < 50 years, >= 50 and <=65 years), gender and region may be performed. If the number of participants in the FAS and PP Set differ (defined as the difference divided by the total number of participants in the PP Set) by more than 10% (for each serostatus group), supportive analyses of antibody-mediated immunogenicity may be conducted using the FAS. The supportive analysis is required if the condition is met at end of study; it is optional for the interim analyses.

The GMT/GMC and geometric mean (GM) level will be calculated using the following formula:

$$10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}(t_i)}{n}\right\}}$$

where $t_1, t_2, \ldots, t_n$ are *n* observed immunogenicity titers or levels.

The geometric mean fold-rise (GMFR) measures the changes in immunogenicity titers or levels within subjects. The GMFR will be calculated using the following formula:

$$10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}\left(v_{ij}/v_{ik}\right)}{n}\right\}} = 10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}(v_{ij})-\log_{10}(v_{ik})}{n}\right\}}$$

where, for *n* subjects, v_{ij} and v_{ik} are observed immunogenicity titers or levels for subject *i* at time points *j* and *k*, $j \neq k$

6.4.1. Immunogenicity Assessments

There will be two types of immunogenicity assessments:

- Antibody-mediated immunogenicity assessments include ZIKV-specific nAbs, as measured by PRNT, MN, and ZIKV-specific bAbs, as measured by ligand binding assay.
- CMI assessments include ZIKV-specific T cells as measured by intracellular cytokine staining assay and sequencing and ZIKV-specific B cells as measured by ELISpot. Blood specimen for CMI will be collected for a subset of participants with seropositive or seronegative status at baseline.

6.4.2. Primary Analysis of Antibody-Mediated Immunogenicity Endpoints

For each group, the following evaluations will be performed at Day 57, or at each time point at which blood samples are collected for antibody-mediated immunogenicity (unless otherwise specified):

- GMT of ZIKV-specific nAbs, in all participants, in initially flavivirus-seronegative, and in initially flavivirus seropositive- participants, as measured by PRNT, will be provided at Day 57 with corresponding 95% CI. The 95% CIs will be calculated based on the t-distribution of the log-transformed values then back transformed to

the original scale for presentation. The following descriptive statistics will also be provided at Day 57: the number of subjects (n), median, minimum and maximum.

- Proportion of participants with seroconversion in ZIKV-specific nAbs from Day 1 to Day 57, as measured by PRNT will be tabulated with 2-sided 95% Clopper-Pearson CIs. The difference of the seroconversion rates between each mRNA-1893 group and placebo group will be provided with 2-sided 95% CIs using the Miettinen-Nurminen method.

- For ZIKV-specific nAb titers, the GMT at Day 57 for each vaccination group, and the ratio of GMT of mRNA-1893 CCI µg (2-dose), mRNA-1893 CCI µg (2-dose), mRNA-1893 CCI µg (single-dose) vs. Placebo respectively, will be estimated based on an analysis of covariance (ANCOVA) model that will be carried out for flavivirus-seropositive groups and flavivirus-seronegative groups separately using the PP Set. The dependent variable will be the nAb at Day 57 with the vaccination group as a factor and the baseline values as covariate (if applicable, ie, for flavivirus-seropositive groups only). The GMT and corresponding 95% CI for each vaccination group, and the ratio of GMT of mRNA-1893 CCI µg (2-dose), mRNA-1893 CCI µg (2-dose), mRNA-1893 CCI µg (single-dose) vs. Placebo respectively, together with corresponding 95% CI will be provided.

6.4.3. Secondary Analysis of Antibody-Mediated Immunogenicity Endpoints

- GMT of ZIKV-specific nAbs, in all participants, in initially flavivirus-seronegative, and in initially flavivirus seropositive- participants, as measured by PRNT and MN, will be provided at Days 1, 8, 29, 36, and 57 (Day 57 only for MN) with corresponding 95% CI. The 95% CIs will be calculated based on the t-distribution of the log-transformed values then back transformed to the original scale for presentation. GMT and corresponding 95% CI will be plotted over time. The following descriptive statistics will also be provided at each time point: the number of subjects (n), median, minimum and maximum.

- GMFR of ZIKV-specific nAbs, in all participants, in initially flavivirus-seronegative, and in initially flavivirus seropositive- participants, as measured by PRNT and MN at Days 8, 29, 36, and 57 over pre-vaccination (eg, Visit Day 1 [baseline]) will be tabulated with 95% CI. The 95% CIs will be calculated based on the t-distribution of the difference in the log-transformed values then back transformed to the original scale for presentation. GMFR and corresponding 95% CI will be plotted over time.

- The geometric mean ratio (GMR) with 95% CI for treatment difference on the ZIKV-specific nAbs titers between each mRNA-1893 group vs. placebo will be provided at Days 8, 29, 36, and 57. The 95% CI will be calculated using t-distribution for the mean difference in the log scale, and then transformed back to the original scale.
- Proportion of participants with seroconversion in ZIKV-specific nAbs from Day 1 (baseline) to Days 8, 29, 36, and 57 (only for MN), as measured by PRNT and MN will be tabulated with 2-sided 95% Clopper-Pearson CIs. The difference of the seroconversion rates between each mRNA-1893 group and placebo group will be provided with 2-sided 95% CIs using the Miettinen-Nurminen method.
- Proportion of initially flavivirus-seronegative participants with seroresponse in ZIKV-specific nAb titer at Days 8, 29, 36, and 57 (Day 57 only for MN), as measured by PRNT and MN will be tabulated with 2-sided 95% Clopper-Pearson CIs.
- Proportion of initially flavivirus-seropositive participants with a ≥2-fold or 4-fold increase in ZIKV-specific nAb titers from Visit Day 1 (baseline) to Days 8, 29, 36, and 57, as measured by PRNT, MN will be tabulated with 2-sided 95% Clopper-Pearson CIs.

6.4.4. Exploratory Analysis of Antibody-Mediated Immunogenicity Endpoints

- GMT of ZIKV-specific nAbs, in all participants, in initially flavivirus-seronegative, and in initially flavivirus-seropositive participants, as measured by PRNT and MN, will be provided at Days 85, 196, 364, 532, and 700 with corresponding 95% CI. The 95% CIs will be calculated based on the t-distribution of the log-transformed values then back transformed to the original scale for presentation. GMT and corresponding 95% CI will be plotted over time. The following descriptive statistics will also be provided at each time point: the number of subjects (n), median, minimum and maximum.
- GMFR of ZIKV-specific nAbs, in all participants, in initially flavivirus-seronegative, and in initially flavivirus-seropositive participants, as measured by PRNT and MN at Days 85, 196, 364, 532, and 700 over pre-vaccination (eg, Visit Day 1 [baseline]) will be tabulated with 95% CI. The 95% CIs will be calculated based on the t-distribution of the difference in the log-transformed values then back

transformed to the original scale for presentation. GMFR and corresponding 95% CI will be plotted over time.

- Proportion of participants with seroconversion in ZIKV-specific nAbs from Day 1 (baseline) to Days 85, 196, 364, 532, and 700, as measured by PRNT and MN will be tabulated with 2-sided 95% Clopper-Pearson CIs.
- Proportion of initially flavivirus-seronegative participants with seroresponse in ZIKV-specific nAb titer at Days 85, 196, 364, 532, and 700, as measured by PRNT and MN will be tabulated with 2-sided 95% Clopper-Pearson CIs.
- Proportion of initially flavivirus-seropositive participants with a ≥2-fold or 4-fold increase in ZIKV-specific nAb titers from Visit Day 1 (baseline) to Days 85, 196, 364, 532, and 700, as measured by PRNT, MN will be tabulated with 2-sided 95% Clopper-Pearson CIs.
- Proportion of all participants, of initially flavivirus-seronegative participants, and of initially flavivirus-seropositive participants with a ZIKV-specific nAb titer equal or greater than a nAb titer defined as a protective level in an animal model, at baseline, and Days 8, 29, 36, 57, 85, 196, 364, 532, and 700, as measured by PRNT and MN will be tabulated.
- Proportion of participants with positive and negative ZIKV-NS1 specific bAbs at Day 29, 57, 85, 196, 364, 532 and 700, as measured by ELISA will be tabulated with 2-sided 95% Clopper-Pearson CIs.
- Proportion of seroconversion in ZIKV-NS1 specific bAbs at Days 29, 57, 85, 196, 364, 532, and 700, as measured by ELISA will be tabulated with 2-sided 95% Clopper-Pearson CIs. The difference of the seroconversion rates between each mRNA-1893 group and placebo group will be provided with 2-sided 95% CIs using the Miettinen-Nurminen method.

Reverse cumulative curves will be provided for ZIKV-specific nAb titers, as measured by PRNT and MN. Similar analyses will be performed for ZIKV-specific bAbs as measured by ligand binding assay.

6.4.5. Analysis of CMI Endpoints

The CMI assessments will be performed with the additional blood samples collected for peripheral blood mononuclear cells (PBMC) isolation from the CMI subset participants, the analysis details will be provided in a separate document.

6.4.6. Sensitivity Analysis

Sensitivity analysis for selected immunogenicity endpoints may be performed with the same methods described above by baseline flavivirus status determined by serology testing.

6.5. Analysis of occurrence of Flavivirus Infection

Analyses of flavivirus infection will be performed using the FAS. The number and percentage of subjects with at least one positive flavivirus result (Dengue PCR, ZIKV PCR) post-baseline and occurring at any time during the study duration will be provided by baseline flavivirus serostatus and treatment arm. Subjects with at least one positive flavivirus result at post-baseline will be presented in a listing.

6.6. Planned Analyses

The following analyses will be conducted on cleaned data:

1. An IA of safety and immunogenicity data will be performed after all participants have completed Day 57 (28 days after the IP injection). Analysis of nAbs will include baseline, Day 29 and Day 57 visits; Analysis of other immunogenicity data will depend on data availability at the time of analysis. The IA will be performed by a separate team of unblinded programmers and statisticians. Pre-identified Sponsor team members will be unblinded to review treatment level results, as defined in the study Data Blinding Plan. The unblinded statistics team will not be involved in either study design or the regular study conduct. The participants and study sites will remain blinded until the conclusion of the study. The IA will serve as the basis for the progression to the ZIKV Phase 3 studies.
2. The final CSR will include full unblinded analyses of all safety and immunogenicity with individual unblinded listings through Day 196 (all data through approximately 6 months after the last IP injection). Note that safety data will be reported cumulatively up to the cutoff date, and immunogenicity data will be reported for each participant up to their Day 196 visits.

3. An addendum to the CSR will include the safety and immunogenicity data from Month 8 until EOS for all participants enrolled in the Extension Period.

In addition, unblinded data presentation or analysis for DSMB review will be handled by the unblinded team of statisticians and programmers, who are not involved in study design. More details regarding DSMB analysis can be found in the DSMB Charter.

7. Changes from Planned Analyses in Protocol

Not applicable.

8. References

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.List of Appendices

9. List of Appendices

9.1. Appendix A Standards for Safety and Immunogenicity Variable Display in TFLs

Continuous Variables: The precision for continuous variables will be based on the precision of the data itself. The mean and median will be presented to one decimal place more than the original results; the SD will be presented to two decimal places more than the original results; the minimum and maximum will be presented to the same precision as the original results.

Categorical Variables: Percentages will be presented to 1 decimal place.

9.2. Appendix B Analysis Visit Windows for Safety and Immunogenicity Analysis

Safety and Immunogenicity Analysis will be summarized using the following analysis visit window for post injection assessments:

Step 1: If the safety and immunogenicity assessments are collected at scheduled visit, i.e. nominal scheduled visit, the data collected at scheduled visit will be used.

Step 2: If the safety and immunogenicity assessments are not collected at the scheduled visit, assessments collected at unscheduled visit will be used using the analysis visit windows described in Table 3 below.

If a subject has multiple assessments within the same analysis visit, the following rule will be used:

- If multiple assessments occur within a given analysis visit, the assessment closest to the target study day will be used.
- If there are 2 or more assessments equal distance to the target study day, the last assessment will be used.

Table 3 Visit Window

Visit	Target Study Day	Visit Window in Study Day
Vital Signs		
Day 1	1 (Date of First Injection)	≤1, Pre-first-dose

Day 1	1 (Date of First Injection)	1, Post-first-dose
Day 8	8	[2, 18]
Day 29	29 (Date of Second Injection)	[19, 29] Pre-second-dose
Day 29	29 (Date of Second Injection)	[29, 32] Post-second-dose
Day 36	36	[33, 46]
Day 57	57	[47, 71]
Day 85	85	[72, 140]
Day 196	196	[141, 280]
Day 364	364	[281, 448]
Day 532	532	[449, 616]
Day 700	700	[617, 760]
Antibody-Mediated Immunogenicity[1]		
Day 1	1 (Date of First Injection)	1, Pre-first-dose
Day 8	8	[2, 18]
Day 29	29	[19, 32]
Day 36	36	[33, 46]
Day 57	57	[47, 71]
Day 85	85	[72, 140]
Day 196	196	[141, 280]
Day 364	364	[281, 448]
Day 532	532	[449, 616]
Day 700	700	[617, 760]
Cell-Mediated Immunogenicity		
Day 1	1 (Date of First Injection)	1, Pre-first-dose
Day 29	29	[19, 32]
Day 36	36	[33, 46]
Day 57	57	[47, 126]
Day 196	196	[127, 280]
Day 364	364	[281, 448]

Day 532	532	[449, 616]
Day 700	700	[617, 760]

1 NS-1 data collected prior to Day 85 will be mapped according to the visit window for available data.

9.3. Appendix A Imputation Rules for Missing Prior/Concomitant Medications and Non-Study Vaccinations

Imputation rules for missing or partial medication start/stop dates are defined below:

1. Missing or partial medication start date:
   - If only Day is missing, use the first day of the month, unless:
     - The medication end date is after the date of first injection or is missing AND the start month and year of the medication coincide with the start month and year of the first injection. In this case, use the date of first injection
   - If Day and Month are both missing, use the first day of the year, unless:
     - The medication end date is after the date of first injection or is missing AND the start year of the medication coincide with the start year of the first injection. In this case, use the date of first injection
   - If Day, Month and Year are all missing, the date will not be imputed, but the medication will be treated as though it began prior to the first injection for purposes of determining if status as prior or concomitant.
2. Missing or partial medication stop date:
   - If only Day is missing, use the earliest date of (last day of the month, study completion, discontinuation from the study, or death).
   - If Day and Month are both missing, use the earliest date of (last day of the year, study completion, discontinuation from the study, or death).
   - If Day, Month and Year are all missing, the date will not be imputed, but the medication will be flagged as a continuing medication.

In summary, the prior, concomitant or post categorization of a medication is described in Table 4 below.

Table 4. Prior, Concomitant, and Post Categorization of Medications and Non-study Vaccinations

	Medication Stop Date		
Medication Start Date	**< First Injection Date of IP**	**≥ First Injection Date and ≤ 28 Days After Last Injection**	**> 28 Days After Last Injection [2]**
< First injection date of IP [1]	P	P, C	P, C, A
≥ First injection date and ≤ 28 days after last injection	-	C	C, A
> 28 days after last injection	-	-	A

A: Post; C: Concomitant; P: Prior
[1] includes medications with completely missing start date
[2] includes medications with completely missing end date

9.4. Appendix B Imputation Rules for Missing AE dates

Imputation rules for missing or partial AE start dates and stop dates are defined below:

1. Missing or partial AE start date:

- If only Day is missing, use the first day of the month, unless:
  - The AE end date is after the date of first injection or is missing AND the start month and year of the AE coincide with the start month and year of the first injection. In this case, use the date and time of first injection, even if time is collected.
- If Day and Month are both missing, use the first day of the year, unless:

- The AE end date is after the date of first injection or is missing AND the start year of the AE coincides with the start year of the first injection. In this case, use the date of first injection

- If Day, Month and Year are all missing, the date will not be imputed. However, if the AE end date is prior to the date of first injection, then the AE will be considered a pre-treatment AE. Otherwise, the AE will be considered treatment-emergent.

2. Missing or partial AE end dates will not be imputed.

9.5. Appendix E Schedule of Assessments for the Vaccination Period and for the Follow-Up Period (Approximately 6 Months)

Procedure	**Eligibility**	**Vaccination Period**						**Follow-Up Period**		**Unscheduled**
Study Day	**D0[1] (Eligibility)**	**D1[1] (Baseline)**	**D8**	**D29**	**D36**	**D57**	**D85**	**D112, D140, D168**	**D196**	
Visit Number	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7-9**	**10 and EOS[2]**	**Unscheduled**
Type of Visit	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**SC**	**C**	**C**
Window Allowance (Days)	**-8**		**+3**	**-3/+7**	**+3**	**-3/+7**	**-3/+7**	**± 15**	**± 15**	
Informed consent, inclusion and exclusion criteria, demography, prior and concomitant medications, and medical history	X									
Physical examination and vital sign measurements[3]	X	X	(X)	X	(X)	X	(X)		(X)	(X)
Blood sample for baseline flavivirus serostatus assessments[4, 5]		X								
Blood sample for antibody-mediated immunogenicity[6]		X	X	X	X	X[7]	X		X	

Procedure	Eligibility	Vaccination Period						Follow-Up Period		Unscheduled
Study Day	**D0[1] (Eligibility)**	**D1[1] (Baseline)**	**D8**	**D29**	**D36**	**D57**	**D85**	**D112, D140, D168**	**D196**	
Visit Number	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7-9**	**10 and EOS[2]**	**Unscheduled**
Type of Visit	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**SC**	**C**	**C**
Window Allowance (Days)	**-8**		**+3**	**-3/+7**	**+3**	**-3/+7**	**-3/+7**	**± 15**	**± 15**	
PBMC collection for cellular immunity (subset of participants from approximately 4 selected sites)		X		X	X	X			X	
Pregnancy test[8]	X	X		X						
Randomization		X								
IP injection (including 30-minute safety observation period after IP injection)[9]		X		X						
Participant eDiary recording of solicited ARs (7 days)		X (Days 1-7)		X (Days 29-35)						
Participant eDiary recording of unresolved solicited ARs (up to 28 days)		X (Days 1-29 and Days 29-57)								
Review of eDiary			X		X					
Follow-up safety telephone call[10]								X		
Recording of MAAEs		X	X	X	X	X	X	X	X	X
Recording of SAEs and AESIs		X	X	X	X	X	X	X	X	X

Procedure	Eligibility	Vaccination Period						Follow-Up Period		Unscheduled
Study Day	**D0[1] (Eligibility)**	**D1[1] (Baseline)**	**D8**	**D29**	**D36**	**D57**	**D85**	**D112, D140, D168**	**D196**	
Visit Number	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7-9**	**10 and EOS[2]**	**Unscheduled**
Type of Visit	**C**	**C**	**C**	**C**	**C**	**C**	**C**	**SC**	**C**	**C**
Window Allowance (Days)	**-8**		**+3**	**-3/+7**	**+3**	**-3/+7**	**-3/+7**	**± 15**	**± 15**	
Recording of concomitant medications and nonstudy vaccinations		X	X	X	X	X	X	X	X	X
Blood sample for asymptomatic flavivirus infection detection[11]							X		X	
Blood sample for symptomatic flavivirus infection detection[11]										X
Informed consent to participate in the Extension Period									X[12]	

Abbreviations: AE = adverse event; AESIs = adverse events of special interest; AR = adverse reaction; C = clinic visit; CMI = cell-mediated immunity; D = day; eDiary = electronic diary; EOS = End of Study; IP = investigational product; MAAE = medically attended adverse event; PBMC = peripheral blood mononuclear cell; RT-PCR = reverse transcription polymerase chain reaction; SAE = serious adverse event; SC = safety (phone) call; US = United States.

[1] The Eligibility (Day 0) and Baseline (Day 1) Visits may be combined on the same day.

[2] This visit will be the EOS for all enrolled participants who do not elect to continue into the Extension Period. For participants enrolled in Treatment Arm A (2-dose regimen with CCI µg mRNA-1893), Treatment Arm B (2-dose regimen with CCI µg mRNA-1893), Treatment Arm C (1-dose regimen with CCI µg mRNA-1893), and Treatment Arm D (placebo) who elect to take part in the Extension Period, return visits will occur approximately every 6 months after the last IP injection.

[3] A full physical examination, including weight, and vital sign measurements will be performed at the eligibility evaluation and Days 1, 29, and 57. Height measurements will only be collected at Day 1. Symptom-directed physical examinations may be performed at other scheduled timepoints (X) at the discretion of the investigator. Vital sign measurements (body temperature, systolic and diastolic blood pressure, heart rate, and respiratory rate) will be collected before study procedures and at least 30 minutes after IP injection, prior to discharge of the participant from the study site. Any clinically significant finding identified during a study visit should be reported as an MAAE. Body mass index will be calculated during the eligibility evaluation.

[4] Flavivirus serology testing is to determine if a participant is flavivirus seronegative or flavivirus seropositive at baseline. This is not an enrollment criterion. However, this flavivirus serology testing needs to be performed on a blood sample collected prior to administration of the first vaccination to confirm that the study distribution among subgroups is respected.

[5] Follicle-stimulating hormone level may be measured to confirm menopausal status at the discretion of the investigator.

[6] Blood samples must be collected before injection on vaccination days (Days 1 and 29).

[7] At Visit 5 (Day 57), an additional blood sample (60 mL) will be collected for exploratory research from participants enrolled at 1 US sites. This site will be different from those sites selected for cellular immunity.

[8] Pregnancy testing at the Eligibility Visit (Day 0) and before each IP injection (or at any time at the discretion of the investigator) will be a point-of-care urine test.

[9] Participants will be administered the IP (mRNA-1893 or placebo) according to the randomization. Participant will remain at the study site for safety monitoring for at least 30 minutes after IP injection.

[10] Trained study personnel will call all participants to collect information relating to any MAAEs, SAEs, AESIs, AEs leading to study discontinuation, concomitant medications associated with those events, and any nonstudy vaccinations.

[11] Blood samples collected from all participants at Visit 6 (Day 85) and Visit 10 (Day 196) for detection of asymptomatic flavivirus infections. In case of signs or symptoms suggesting a flavivirus infection (mostly dengue and Zika in the areas where the study will be performed), an additional blood sample will be collected to confirm the diagnosis via specific serology and RT-PCR, and clinical information will be carefully collected to evaluate the severity of the clinical case. Those symptoms are not specific; however, an association of acute fever onset with maculopapular rash, arthralgia, and conjunctivitis during the seasonal circulation of mosquitos must trigger samplings. Also, special attention will be paid to the reporting of neurological signs or symptoms evoking Guillain-Barré syndrome.

[12] At Visit 10 (Day 196), all participants will have the opportunity to provide their consent to participate in the Extension Period.

9.6. Appendix F Schedule of Assessments for the Extension Period

Procedure	Extension Period			
Study Day	**D364**	**D532**	**D700**	
Visit Number	**11**	**12**	**13 and EOS**	**Unscheduled**
Type of Visit	**C**	**C**	**C**	**C**
Window Allowance (Days)	**± 15**	**± 15**	**± 30**	
Physical examination (including weight) and vital sign measurements)[1]	(X)	(X)	(X)	
Blood sample for antibody-mediated immunogenicity	X	X	X	
PBMC collection for cellular immunity (CMI subset participants selected during the Vaccination Period)	X	X	X	
Blood sample for asymptomatic flavivirus infection detection[2]	X	X	X	
Blood sample for symptomatic flavivirus infection detection[2]				X
Recording of MAAEs	X	X	X	X
Recording of SAEs and AESIs	X	X	X	X
Recording of concomitant medications and nonstudy vaccinations	X	X	X	X
Study completion			X	

Abbreviations: AESIs = adverse events of special interest; C = clinic visit; CMI = cell-mediated immunity; D = day; EOS = End of Study; MAAE = medically attended adverse event; RT-PCR = reverse transcription polymerase chain reaction; SAE = serious adverse event.

[1] Symptom-directed physical examinations might be performed at the scheduled timepoints (X) at the discretion of the investigator. Vital sign measurements (body temperature, systolic and diastolic blood pressure, heart rate, and respiratory rate) will be collected before study procedures. Any clinically significant finding identified during a study visit should be reported as an MAAE.

[2] Blood samples collected from all participants at Visits 11, 12, and EOS (Days 364, 532, and 700, respectively) will be used for detection of asymptomatic flavivirus infections. In case of signs or symptoms suggesting a flavivirus infection (mostly dengue and Zika in the areas where the study will be performed), an additional blood sample will be taken to confirm the diagnosis via specific serology and RT-PCR, and clinical information will be carefully collected to evaluate the severity of the clinical case. Special attention will be paid to collect signs or symptoms possibly related to Guillain- Barré syndrome.

PPD	PPD Biostatistics and Programming
	Statistical Analysis Plan (SAP) Client Approval Form

Client:	**ModernaTX, Inc.**
Protocol Number:	**mRNA-1893-P201**

Document Description:	Statistical Analysis Plan
SAP Title:	A Phase 2, Randomized, Observer-Blind, Placebo-Controlled, Dose Confirmation Study to Evaluate the Safety, Tolerability, and Immunogenicity of Zika Vaccine mRNA-1893 in Adults Aged 18 Through 65 Years and Living in Endemic and Non-Endemic Flavivirus Areas
SAP Version Number:	2.0
Effective Date:	20-April-2023

Author(s):

For PPD: PPD

Approved by:

PPD

20-Apr-2023

Date *(DD-MMM-YYYY)*

PPD

20-Apr-2023

Date *(DD-MMM-YYYY)*

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Certificate Of Completion

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Electronic Record and Signature Disclosure: Accepted: 20 April 2023 | 18:31 ID: PPD		
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Electronic Record and Signature Disclosure: Accepted: 20 April 2023 | 18:06 ID: PPD		

In Person Signer Events	Signature	Timestamp

Editor Delivery Events	Status	Timestamp

Agent Delivery Events	Status	Timestamp

Intermediary Delivery Events	Status	Timestamp

Certified Delivery Events	Status	Timestamp

Carbon Copy Events	Status	Timestamp

Witness Events	Signature	Timestamp

Notary Events	Signature	Timestamp

Envelope Summary Events	Status	Timestamps
Envelope Sent	Hashed/Encrypted	20 April 2023 | 17:49
Certified Delivered	Security Checked	20 April 2023 | 18:06
Signing Complete	Security Checked	20 April 2023 | 18:07
Completed	Security Checked	20 April 2023 | 18:32

Payment Events	Status	Timestamps

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