NCT #: NCT03014089

Moderna Therapeutics, Inc.

mRNA-1325-P101

A PHASE 1/2, RANDOMIZED, PLACEBO-CONTROLLED, DOSE-RANGING STUDY TO EVALUATE THE SAFETY AND IMMUNOGENICITY OF mRNA-1325 ZIKA VACCINE IN HEALTHY ADULTS IN A NON-ENDEMIC ZIKA REGION

15 March 2017

Statistical Analysis Plan

Version Final 1.1

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Issued by: PPD Date: PPD

PPD

Upon review of this document, including table, listing, and figure shells, the undersigned approves the statistical analysis plan. The analysis methods and data presentation are acceptable, and the table, listing, and figure production can begin.

Approved by: PPD Date: PPD

Valera, LLC

PPD Date: 03/19/2017

PPD to Valera BioBridges, LLC

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Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
BMI	body mass index
CI	confidence interval
eCRF	electronic case report form
ELISPOT	enzyme-linked ImmunoSpot
GMT	geometric mean titer
ICF	informed consent form
IFNγ	interferon gamma
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
mRNA	messenger ribonucleic acid
NCS	not clinically significant
PRNT50	Plaque Reduction Neutralizing Titer EC50
PT	preferred term
SAE	serious adverse event
SAS	Statistical Analysis System
SD	Standard deviation
SMC	safety monitoring committee
SOC	system organ class
TEAE	treatment-emergent adverse event
ULOQ	upper limit of quantification
WHODRUG	World Health Organization Drug Dictionary
ZikaV	Zika Virus

1. Administrative Structure

This study is being conducted under the sponsorship of Moderna Therapeutics, Inc. The safety and immunogenicity statistical analyses are being performed under contract with PPD in collaboration with Moderna Therapeutics, Inc.
This statistical analysis plan has been written according to protocol mRNA-1325-P101, Version 4.0, dated 10 March 2017.

2. Introduction

Zika virus (ZikaV) 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. Currently there is no vaccine to protect against this disease, which has spread rapidly from Asia to most tropical and subtropical regions including the Americas.
Moderna Therapeutics, 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, is non-replicating, 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 is able to induce highly targeted immune responses against infectious pathogens such as ZikaV.
mRNA-1325 is an mRNA-based vaccine candidate being tested for its ability to safely induce an immune response with the intention to be able to prevent ZikaV infection.

3. Objectives

3.1. Primary Objective

The primary objective of this study is to assess the safety of a 2-dose vaccination schedule of mRNA-1325 Zika vaccine, given 28 days apart, across a range of dose levels in flavivirus-seronegative and flavivirus-seropositive subjects compared with placebo.

3.2. Secondary Objectives

The secondary objective of this study is to assess the immunogenicity of a range of doses of mRNA-1325 Zika vaccine and to select a dose to move forward into Phase 3 development, based on changes from Baseline in the following tests:

- ZikaV-specific neutralizing antibody titers measured by a Plaque Reduction Neutralizing Titer EC50 (PRNT50);
- ZikaV antigen-specific stimulation of T cells measured by interferon gamma (IFNγ) enzyme-linked ImmunoSpot (ELISPOT) on subject-derived peripheral blood mononuclear cells (PBMCs).

3.3. Exploratory Objectives

The exploratory objective of this study is to assess the impact of mRNA-1325 Zika vaccine on a range of other functional and diagnostic flavivirus assays.

4. Investigational Plan

4.1. Overall Study Design and Plan

This is a Phase 1/2, double-blind, placebo-controlled, dose-finding study to evaluate the safety and immunogenicity of a range of dose levels of mRNA-1325 Zika vaccine given in a 2-dose vaccination schedule, 28 days apart, and compared with placebo in healthy adult subjects (18 to 49 years of age, inclusive).

The study consists of two parts: Part A includes dose-finding, safety, and immune testing through 28 days following the second vaccination. Once subjects complete the final visit in Part A, they will be entered into Part B. Part B is a blinded follow-up period with assessment of safety through 12 months and immune persistence at 168 (±15) days and 364 (+15) days following the second vaccination in Part A.

Part A

Screening and consent will occur over a 28-day period before randomization (West Nile virus serology screen can occur up to 90 days prior to randomization).

Subjects will be randomly assigned in a blinded fashion in an approximate 4:1 ratio to receive mRNA-1325 or placebo at 1 of 3 dose levels (10 µg, 25 µg, or 50 µg), with each subject receiving 2 vaccinations separated by 28 (+7) days. Dosing will begin with 10 µg (Cohort 2), followed by 25 µg (Cohort 3), and then 50 µg (Cohort 4). There will be no Cohort 1 included in this study. Approximately two-thirds of the enrolled subjects at each dose level will be flavivirus naïve and approximately one-third will be flavivirus seropositive as a result of having received a yellow fever vaccination in the past 20 years

by either: a) military assignment in the previous 20 years that requires a yellow fever vaccination; or 2) yellow fever vaccination documented on an international certificate or other confirmatory documentation of yellow fever vaccination.

Cohort and treatment assignment are shown in Figure 4-1.

Figure 4-1 Cohort and Treatment Assignment

Cohort	Flavivirus Status	Treatment Assignment (µg mRNA-1325 or placebo)	Total (Ratio)
2	+	10	30 (4:1)
	-	10	
	+	placebo	
	-	placebo	
3	+	25	30 (4:1)
	-	25	
	+	placebo	
	-	placebo	
4	+	50	30 (4:1)
	-	50	
	+	placebo	
	-	placebo	

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

For each cohort, a sentinel safety group will occur with 3 flavivirus-seronegative subjects randomized to mRNA-1325 and followed for 7 days after first vaccination, with review of reactogenicity and safety laboratory results, prior to randomizing the remainder of the cohort.

The vaccine will be administered as an intramuscular injection (0.5 mL) into the deltoid muscle as a 2-dose vaccination schedule at Visits 1 and 4 (at least a 28-day interval between dosing). The second dose of study drug will be administered preferably in the same arm used for the first dose. Vaccine accountability, dose preparation, and vaccine administration will be performed by unblinded pharmacy personnel who will not participate in any other aspect of the study. The remainder of the site staff and all subjects will remain blinded to treatment assignment.

Subjects will be instructed on recording reactogenicity, adverse events (AEs), and medications (prescription or over-the-counter) on the memory aid (i.e. diary card), and will be provided measuring tools, and instructed to call or return to the clinic within 24 hours if a reactogenicity score reaches Grade 3 or greater during the first 7 days following vaccination. As standard practice, a reminder call will be made to the subject by the site at least once during the first 7 days following each vaccination to answer any questions and ensure that the memory aid is being completed correctly and consistently. All subjects will

return on 7 (+3) (cellular PBMC testing) and 28 (+7) (humoral neutralization testing) days following each vaccination for safety assessments and blood sampling for immune testing. Part A is concluded for each subject at the time when they return to the clinic for Visit 7 (28 days following the second vaccination). At that time, a subject will be entered into Part B of the study.

Part B – 12 Month Follow-up After Final Vaccination

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

Once entered into Part B, each safety contact will occur by telemedicine (e.g. telephone, text message, internet) every 28 (±7) days, and blood samples for immune persistence will be collected from each subject at 168 (±15) days and 364 (+15) days following the second vaccination. Each safety contact will capture outcomes of any adverse event of special interest (AESI) or serious adverse event (SAE) that remains unresolved since the last visit or is newly identified through scripted query. The telemedicine visits may require additional data through medically attended visits, in addition to medications and vaccination taken by the subject during this time. Subjects will have consented during study enrollment to allow access to additional medical records needed to complete Part B, thereby allowing the blinding of the treatment assignment to be maintained.

4.2. Study Endpoints

The following are the safety (primary) endpoints:

Part A:

- Solicited AEs with toxicity scoring (local and systemic reactogenicity events) collected for 7 days following each vaccination
- Unsolicited AEs collected for 28 days following each vaccination; additional classification if serious, medically attended, leading to study withdrawal, or an AESI
- Safety laboratory test results with toxicity scoring (hematology, serum chemistry, and coagulation) collected at Baseline and 7 (+3) days and 28 (±7) days following each vaccination
- Urinalysis test results with toxicity scoring collected at Baseline and 7 (+3) days following each vaccination

- A subset of safety laboratory test results (as specified in Table 13-1) with toxicity scoring collected additionally at 17 (±3) days following each vaccination and 21 (+3) days (sentinel subjects) following the first vaccination

- Vital sign measurements with toxicity scoring on day of vaccination and 7 and 28 days following each vaccination

Part B:

- AESIs and SAEs through 1 year (or until resolved, whichever occurs first) following the last vaccination

The immunogenicity assessments (cellular and humoral) are as follows:

Part A:

- Neutralizing serum antibody titers (PRNT50) to Zika (Baseline [pre-vaccination at Visit 1] and at 28 days following each vaccination [Visits 4 and 7, respectively])

- Neutralizing serum antibody titers (PRNT50) to yellow fever (Baseline [pre vaccination at Visit 1] and at 28 days following each vaccination [Visits 4 and 7, respectively]) in the flavivirus seropositive group

- T-cell response (cytokine activation to IFNγ or other cytokines) (Baseline [pre-vaccination at Visit 1] and at 7 days following each vaccination [Visits 2 and 5, respectively])

Part B:

- Neutralizing serum antibody titers (PRNT50) to Zika virus at 168 (±15) days and 364 (+15) days (Visits 12 and 19, respectively) following the second vaccination.

5. General Statistical Considerations

All data collected will be presented in listings. Subjects will be identified in the listings by the subject identification number concatenated with the site number.

Data from subjects excluded from an analysis population will be presented in the data listings but not included in the calculation of summary statistics.

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

Unless otherwise specified, the following treatment groups will be used for summary purposes:

- 10 µg mRNA-1325
- 25 µg mRNA-1325
- 50 µg mRNA-1325
- Placebo

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

For the summary statistics of all numerical variables unless otherwise specified, minimum and maximum will be displayed to the same level of precision as reported. Mean, median, and confidence intervals (CIs) will be displayed to one level of precision greater than the data collected. Standard deviation will be displayed to two levels of precision greater than the data collected. P-values will be rounded to three decimal places. If a p-value is less than 0.001 it will be reported as "<0.001." If a p-value is greater than 0.999 it will be reported as ">0.999."

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 treatment within the analysis set of interest, unless otherwise specified.

For the change from Baseline safety summaries, Baseline will be defined as the last non-missing measurement (including repeated and unscheduled assessments) before the first vaccination.

For the immunogenicity analyses, Baseline will be defined as the assessment before the first vaccination.

Study day will be calculated relative to the most recent vaccination. Study day prior to the first vaccination will be calculated as: date of assessment/event – date of first vaccination; study day on or after the date of first vaccination but before the second vaccination (if applicable) will be calculated as: date of assessment/event – date of first vaccination + 1; study day on or after the date of second vaccination will be calculated as: date of assessment/event – date of second vaccination + 1.

For GMT calculation, antibody values reported as below the lower limit of quantification (LLOQ) will be replaced by 0.5 × LLOQ. For fold-rise, values <LLOQ will be replaced by 0.5 × LLOQ for a numerator and by LLOQ for a denominator. If both the numerator and

denominator are <LLOQ, then both will be converted in the same way. Values that are greater than the upper limit of quantification (ULOQ) will be converted to the ULOQ. Missing results will not be imputed.
All analyses will be conducted using SAS Version 9.3 or higher.

5.1. Sample Size

Approximately 90 subjects are planned to receive study treatment. Formal sample size calculations were not performed. The number of subjects was chosen based on feasibility and is considered sufficient to meet the study objectives of identifying a dose and establishing initial safety results in a population of healthy adults in a non-endemic Zika region.

5.2. Randomization and Blinding

The first 3 subjects (sentinel subjects) in each cohort will be randomized to obtain mRNA-1325 of the relevant dose level. The remaining subjects (expansion subjects) in each cohort will be randomly assigned to receive either mRNA-1325 or placebo.
This is an observer-blind study. The investigator, study subjects, site monitors, and study site personnel will be blinded to the study drug administered, with the following exceptions:

- Unblinded pharmacy personnel (of limited number) will be assigned to drug accountability procedures and to prepare and administer mRNA-1325 (or placebo) to all subjects. The designee(s) will have no other study functions than study drug management, documentation, accountability, preparation, and administration. They will not be involved in subject evaluations and will not reveal the study drug identity to either the subject or 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 drug accountability monitor. They will have responsibilities to ensure the site is following all proper drug accountability, preparation, and administration procedures.
- An unblinded statistician will provide a descriptive analysis of safety and immunological endpoints after the completion of each dosing cohort. The interim analyses of immunogenicity data will be performed as outlined in Section 11.

Access to the randomization code will be strictly controlled at the pharmacy.

5.2.1. Breaking the Blind

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

5.3. Analysis Sets

The following analysis sets will be used: Randomized set, Safety set, Per-Protocol set and Intent-to-Treat set.

5.3.1. Randomized Set

The Randomized set will include all subjects who were randomized including sentinel subjects. This set will only be used for descriptive purposes.

5.3.2. Safety Set

The Safety set will include all subjects in Randomized set who receive at least 1 dose of study drug (mRNA-1325 or placebo) and provided any post-vaccination safety data. All subjects in the Safety set will be analyzed according to the study drug actually received.

5.3.3. Per-Protocol Set

The Per-Protocol set will include all subjects in the Randomized set who did not observe a major protocol violation, received the full dose of the assigned study drug within the acceptable vaccination window, had blood collection within accepted visit windows, and had a pre-vaccination and at least 1 serum sample from the post-vaccination immunogenicity testing period available for testing. In the case where there is not a paired sample for the specific time point that data will not be included in the analysis. The process for determining major protocol violations will be provided as a separate study document.

5.3.4. Intent-to-Treat Set

The Intent-to-Treat set will provide supportive analyses and will include all subjects in the Randomized set regardless of protocol violations, exceeded visit windows, missed vaccination, or missing data. All subjects in the Intent-to-Treat set will be analyzed according to the study drug the subject was randomized to receive and not according to what was actually received, in the event there is a discrepancy.

6. Subject Disposition

6.1. Disposition

Subjects' disposition will be presented in a data listing.

The total number of subjects who receive each vaccination, who complete and discontinue the study, and who are included in each analysis population will be summarized. The number of subjects who discontinue from the study, both overall and according to the reasons for discontinuation, will also be summarized. The reason for discontinuation can be:

- adverse event
- death
- lost to follow-up
- non-compliance with study drug
- physician decision
- pregnancy
- protocol deviation
- site terminated by sponsor
- study terminated by sponsor
- withdrawal by subject
- other

The summaries will be presented by treatment group as defined in Section 5.

6.2. Inclusion and Exclusion Criteria Deviations

Inclusion and exclusion criteria deviations will be presented in a listing.

6.3. Major Protocol Violations

Major protocol violations will be presented in a listing. These data will be provided as a .csv file from agreements made at the Protocol Deviation Meeting at a minimum prior to

database lock. SAS listings will be provided to support this meeting, which is held after the completion of each dose level (or every six months) and just prior to database lock.

7. Demographics and Baseline Characteristics

7.1. Demographics

Demographic information will be listed and summarized. Descriptive statistics will be calculated for the following continuous demographic and baseline characteristics: age (years, calculated as the integer part of [date of informed consent - date of birth]/365.25), weight (kg), height (cm), and body mass index (BMI) (kg/m^2). Frequency counts will be tabulated for the categorical variables gender, race, ethnicity and flavivirus status. The summaries will be presented treatment group as defined in Section 5 for subjects in the Safety set.

7.2. Medical History

Medical history data will be presented in a listing.

8. Treatments and Medications

8.1. Prior and Concomitant Medications

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

In Part A of the study, concomitant medications include all medications (including vaccination outside of trial) taken by the subject from the time of signing the informed consent form (ICF) through 28 days after the second vaccination (Visit 7) and will be recorded in the eCRF. In Part B, receipt of immunomodulators (including vaccines), immunosuppressants, or other concomitant medications that could potentially impact immune response will be collected through Visit 19.

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

To allow accurate assessment of analgesic/antipyretic use during the 7 days after each vaccination, subjects will be inquired directly as to use (absent or present) and if used will be inquired for treatment or prophylaxis.
Chronic administration (defined as more than 14 continuous days) of an immunosuppressant or other immune modifying drug within 6 months prior to vaccine administration or any such products during the active vaccination period (through 4 weeks after their last planned vaccination) is prohibited. An immunosuppressant dose of a glucocorticoid will be defined as a systemic dose of ≥10 mg of prednisone per day or equivalent. The use of topical, inhaled, and nasal glucocorticoids will be permitted.
Prior and concomitant medication will be coded using the WHO Drug Dictionary and be presented in a data listing.

8.2. Medical and Surgical Procedures

Medical or surgical procedures will be presented in a data listing.

8.3. Study Drug Administration

Study drug administration will be presented in a data listing.

9. Safety Analysis

Safety assessments will include monitoring and recording of solicited (local and systemic reactogenicity events) and unsolicited AEs; SAEs, AESIs, clinical laboratory test results including hematology, serum chemistry, and urinalysis; vital sign measurements; and physical examination findings. The Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (CBER 2007) will be used to categorize solicited reactogenicity, safety laboratory test results, and vital sign measurements observed during this study.

9.1. Adverse Events

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.
A treatment-emergent AE (TEAE) is defined as any event not present before exposure to study drug or any event already present that worsens in intensity or frequency after exposure. Adverse events will also be evaluated by the investigator for the coexistence of medically attended AE which is defined as an AE that leads to an unscheduled visit to a healthcare practitioner.

Adverse events will be coded by preferred term (PT) and system organ class (SOC) using Medical Dictionary for Regulatory Activities (MedDRA) and summarized by treatment group as defined in section 5, vaccination (first or second), and overall.
All summary tables (except for the overall summary of AEs) will present SOC and PT, will include counts of subjects, and will be based on TEAEs. System organ class will be displayed in descending order of overall frequency and then alphabetically. Preferred term will be displayed in descending order of overall frequency and then alphabetically within SOC. Percentages will be based upon the number of subjects in the Safety set within each treatment group as defined in Section 5.

9.1.1. Incidence of Adverse Events

Individual subject listings will be provided for all AEs, AEs leading to withdrawal, AESIs, medically attended AEs.
An overall summary of AEs will be created to include the number and percentage of subjects who experience the following treatment emergent reactions:

- Any AE
- Any treatment-related AE
- Any AE of grade 3 or 4
- Any treatment-related AE of grade 3 or 4
- Any SAE
- Any treatment-related SAE
- Death
- Any AEs leading to withdrawal
- Any AE of special interest
- Any medically-attended AE

9.1.2. Relationship of Adverse Events to Study Drug

The relationship of AEs to the study drug (not related, related) will be captured on the eCRF.
The TEAEs will be summarized by treatment groups as defined in Section 5 and overall, as well as by SOC, PT, and relationship to the study vaccine. If the relationship to study vaccine is missing, then a "missing" relationship category will be included in the summary. A subject with 2 or more AEs within the same SOC or PT level but different relationship will be counted only once in the level using the related incident.

9.1.3. Severity of Adverse Event

The severity of AEs (grade 1, 2, 3, or 4) will be captured on the CRF. The TEAEs will be summarized by treatment groups as defined in Section 5 and overall, as well as by SOC, PT, and severity. If the severity is missing, then a "missing" severity category will be included in the summary. A subject with 2 or more AEs within the same SOC or PT level but different severity will be counted only once in that level using the most severe incident.

9.1.4. Serious Adverse Events

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

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

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

An AE or suspected adverse reaction is considered "life threatening" if, in the view of either the investigator or Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. The investigator must immediately (within 24 hours of awareness) report to the Sponsor any SAE through the agreed upon reporting mechanism.

The SAEs will be presented in a data listing and summarized by treatment groups as defined in Section 5 and overall, as well as by SOC and PT. A subject with 2 or more SAEs within the same level of summarization will be counted only once in that level.

9.1.5. Adverse Events Leading to Withdrawal

The AEs leading to withdrawal will be presented in a data listing.

9.1.6. Adverse Events of Special Interest

Certain AESIs are evaluated after the administration of immunostimulatory agents. All subjects enrolled in the study will be monitored for AESIs from enrollment through the end-of-study (EOS) visit. The occurrence of any of these AEs will be treated as an SAE, meeting the criterion of a "medically important event." The list of AESIs is presented in Section 6.2 of the Protocol. The AESI diagnosis, as well as any medications taken to treat the condition, will be recorded in the subject's eCRF.
All AESIs will be presented in a data listing.

9.1.7. Solicited Adverse Events

The term "reactogenicity" refers to selected signs and symptoms (AEs) occurring after dose administration, to be collected by the subject during the day of each dose administration and for the following 7 days using self-reporting and the memory aid.
The following AEs are included in the memory aid:
Solicited local AEs:

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

Solicited systemic AEs:

- fever
- nausea/vomiting
- diarrhea
- headache
- fatigue/malaise
- generalized myalgia (muscle ache or pain)
- generalized arthralgia

The investigator will later review, confirm, grade, and if applicable attribute AEs including reactogenicity, as absent (Grade 0), mild (Grade 1), moderate (Grade 2), severe (Grade 3), or potentially life threatening (Grade 4) based on the Toxicity Grading Scale for Healthy

Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials (Center for Biologics Evaluation and Research [CBER] 2007; Table6-3 in the protocol).
Any solicited AE that meets any of the following criteria will be entered as an AE in the AE page in the database:

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

The solicited local and systemic AE data will be presented in the data listings, and summarized by treatment as defined in Section 5 and overall, vaccination (first or second), day after vaccination (1-7) and severity. The duration of solicited AEs will be summarized by treatment as defined in Section 5 and overall, and vaccination (first or second).

9.2. Clinical Laboratory Evaluations

The following hematology, serum chemistry, urinalysis, and other laboratory assessments will be performed:

Hematology:	Hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelet count, red blood cell count, and total and differential leukocyte count
Serum chemistry:	Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, amylase, lipase, bilirubin (total and direct), blood urea nitrogen, creatinine, random glucose, potassium, sodium, total protein, albumin, and calcium
Urinalysis:	pH, protein, glucose, ketone, bilirubin, urobilinogen, blood, nitrite, leucocytes, and specific gravity
Other laboratory assessments (coagulation):	Prothrombin time and partial thromboplastin time

A pregnancy test (β-human chorionic gonadotropin) will be performed on all female subjects of childbearing potential at Screening and before each dose administration (urine or serum). A follicle-stimulating hormone test will be performed at Screening, as necessary, to confirm post-menopausal status in female subjects, if not documented in the subject's

medical records. Human immunodeficiency virus (types 1 or 2) antibody, hepatitis B surface antigen, and hepatitis C virus antibody will be assessed at Screening.
At Screening, if the subject has no proof of yellow fever vaccination within 10 years, or other documentation of flavivirus status, the West Nile virus screening test will be administered. A West Nile virus serology screen can occur up to 90 days prior to randomization.
A urine screen for drugs of abuse will be performed by the local laboratory at Screening and before dose administration at Visit 1 for opiates, cocaine, phencyclidine, amphetamines, benzodiazepines, and methadone (cannabis excluded).
All safety values that have a toxicity score of Grade 1 or greater will also be evaluated by the investigator and classified as "abnormal clinically significant (CS)", or "abnormal not clinically significant (NCS)." Investigators should use their clinical judgment when considering the clinical significance of any abnormal laboratory findings. All laboratory test values with a toxicity score of Grade 3 or greater will be entered as AEs. Any additional laboratory test value that is determined to be clinically significant will also be recorded as an AE, should that be considered the primary diagnosis. In such instances, the abnormal value and grade will be documented on the AE page of the eCRF. The investigator will continue to monitor the subject with additional assessments until the values have reached the reference range or the values at Screening or until the investigator determines that follow-up is no longer medically necessary. The only exception to this rule would be a laboratory test value that is associated with an identified ongoing AE where that event would be the classifying AE.
All laboratory test results will be presented in the data listings. The results that are outside the reference ranges will be flagged in the data listings. The abnormalities meeting the toxicity grading criteria (grade 2 or higher) in any safety laboratory will be listed separately. If a subject has a laboratory test with grade 2 or higher abnormality at any post vaccination visit, then all results for that subject and laboratory test will be presented in the listing.
For hematology, serum chemistry, coagulation, and numeric urinalysis test results, the observed values and changes from Baseline on Days 7, 17 after each vaccination and for sentinel subjects also on Day 21 post first vaccination will be summarized by treatment groups as defined in Section 5. These lab tests will also be summarized by the toxicity grades.

9.3. Vital Sign Measurements

Vital sign measurements will include systolic and diastolic blood pressures, heart rate, respiratory rate, and body temperature. Vital signs will be measured in Part A at screening, before first vaccination and at 60 (+15) minutes, Day 7, Day 21 (only for sentinel subjects), and Day 28 (before second vaccination) post first vaccination, as well as at 60 (+15) minutes, Day 7 and Day 28 post second vaccination.

If any of the vital sign measurements meet the toxicity grading criteria for clinical abnormalities of Grade 3 or higher, the abnormal value and grade will be documented on the AE page of the eCRF (unless there is another known cause of the abnormality and that would result in an AE classification).

The vital sign measurements will be presented in a data listing. The values meeting the toxicity grading criteria will be flagged in the data listing. Observed values and changes from Baseline will be summarized by treatment groups as defined in Section 5.

9.4. Physical Examination

In Part A, a full physical examination will be performed at Screening and symptom-directed (targeted) physical examinations will be performed before first vaccination and at Day 7, Day 21 (for sentinel subjects only) and Day 28 (before second vaccination) post fist vaccination, as well as at Day 7 and 28 post second vaccination. The full examination will include assessment of skin, head, ears, eyes, nose, throat, neck, thyroid, lungs, heart, cardiovascular, abdomen, lymph nodes, and musculoskeletal system/extremities. Interim physical examinations will be performed at the discretion of the investigator, if necessary, to evaluate AEs or clinical laboratory abnormalities. Prior to vaccination and at 7 days following vaccination, a physical evaluation of the arm that was vaccinated and the associated lymph nodes should be evaluated.

Physical examination data will be presented in a data listing.

Height and weight will be measured and body mass index will be calculated at Screening only and listed together with the demographics data.

10. Immunogenicity Analysis

The following immunogenicity outcome measures (for serum neutralizing antibody titers) and their 95% CIs, where appropriate, will be summarized by treatment group and by visits after each vaccination:

- Geometric mean titer (GMT) of anti-Zika virus neutralizing antibodies (PRNT50 assay)
    - Part A: At Baseline (pre-vaccination at Visit 1) and on 28 days after both first and second vaccination (Visits 4 and 7, respectively)
    - Part B: At 168 (±15) days and 364 (+15) days (Visits 12 and 19, respectively)
- Geometric mean ratio $(GMR)_{Post/Pre}$
    - Part A and Part B: The ratio of post-vaccination GMT to pre-vaccination (Visit 1) GMT of subjects who have a baseline sample (pre-vaccination at Visit 1) and post-vaccination sample at any post-dose time point (Visits 4, 7, 12, or 19)
- Seroconversion
    - Part A: The proportion of subjects with Zika virus PRNT50 titer ≥1:10, ≥1:20, ≥1:40, ≥1:80, ≥1:160, and ≥1:320 at Baseline, Visit 4, and Visit 7
    - Part A: The proportion of subjects with baseline Zika virus PRNT50 titer <1:10 and post-vaccination Zika virus PRNT50 titer of ≥1:20; or with baseline Zika virus PRNT50 titer ≥1:10 and with post-vaccination 4-fold titer increase (Visits 4 or 7)
    - Part B: The proportion of subjects who maintained seroconversion status at specific time points of 168 (±15) days and 364 (+15) days (Visits 12 and 19, respectively)
- Cross-Stimulation with prior flavivirus vaccination (subset of flavivirus-seropositive subjects) (Part A only)
    - Geometric mean titer (GMT) of anti-yellow fever neutralizing antibodies at Baseline, Visit 4, and Visit 7

- Geometric mean ratio (Post/Pre) of anti-yellow fever neutralizing antibodies at Visit 4 and Visit 7
- The proportion of subjects with anti-yellow fever antibody titer ≥1:10, ≥1:20, ≥1:40, ≥1:80, ≥1:160, and ≥1:320 at Baseline, Visit 4, and Visit 7
- Additional neutralization assays for other flavivirus may be performed in select subsets

The immunogenicity analyses will be performed separately for the Per-Protocol set and the Intent-to-Treat set.

11. Interim Analysis

Following completion of each cohort in Part A, the database will be locked for that cohort and safety and immune test results will be analyzed through 28 days following the second vaccination. 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 cohort, treatment assignment, and in aggregate for mRNA-1325 exposure. Immunogenicity and safety data, including mean group analyses of change from Baseline, where applicable, will be summarized for each dose cohort. These data are required to inform decisions on dose selection for this and other development programs using the same mRNA platform. Subject-level treatment assignment will not be released to the subjects or to those individuals involved in managing or assessing safety in Part B of the study until that portion of the study is completed.

12. References

Franca RF, Neves MH, Ayres CF, et al. First international workshop on Zika virus held by Oswaldo Cruz Foundation FIOCRUZ in northeast Brazil March 2016 - a meeting report. PLoS Negl Trop Dis. 2016;10(6):e0004760.

Brasil, P, Pereira JP Jr, Raja Gabaglia C, et al. Zika virus infection in pregnant women in Rio de Janeiro - preliminary report. N Engl J Med. 2016 Mar 4 (Epub ahead of print).

13. Schedule of Events

The schedule of events for Part A is presented in Table 13-1 and the schedule of events for Part B is presented in **Error! Reference source not found.**.

Table 13-1 Part A: Schedule of Events

Procedure	**Screening**	**Treatment Period**							
Study visit	**0**	**1**	**2**	**3**	**3a[a]**	**4**	**5**	**6**	**7**
Vaccination Day		**X**				**X**			
Days relative to most recent vaccination	**N/A**	**0**	**7**	**17**	**21**	**28**	**7**	**17**	**28**
Window allowance	**+28**	**0**	**+3**	**±3**	**+3**	**+7**	**+3**	**±3**	**+7**
Informed consent	X								
Inclusion/exclusion criteria	X								
Medical history	X								
Follicle-stimulating hormone (female subjects only)[b]	X								
Serology[c]	X								
West Nile virus screen (ONLY if no proof of yellow fever vaccination or flavivirus status)[d]	X								
Urine drug screen[e]	X	X							
Physical examination[f]	X	X	X		X	X	X		X
Vital sign measurements[g]	X	X/Xc	X		X	X/Xc	X		X
Safety laboratory tests	X[h]	X[h]	X[h]	X[i]	X[i]	X[h]	X[h]	X[i]	X[h]
Urinalysis[j]	X	X	X			X	X		
Pregnancy test (female subjects of childbearing potential)[k]	X	X				X			
Randomization		X							
Immune testing (cellular) (PBMC ELISPOT assay)		X[l]	X				X		
Immune testing (humoral) (PRNT50 neutralization assay)		X[l]				X[l]			X
Vaccination[m]		X				X			
Reactogenicity		X/Xc	X		X	X/Xc	X		
Provision and instruction on memory aid (and tools provided)[n]		X				X			

Procedure	**Screening**	**Treatment Period**							
Study visit	**0**	**1**	**2**	**3**	**3a[a]**	**4**	**5**	**6**	**7**
Vaccination Day		**X**				**X**			
Days relative to most recent vaccination	**N/A**	**0**	**7**	**17**	**21**	**28**	**7**	**17**	**28**
Window allowance	**+28**	**0**	**+3**	**±3**	**+3**	**+7**	**+3**	**±3**	**+7**
Subject memory aid review: solicited local and systemic AEs, oral body temperature, and medications taken			X		X		X		
Subject memory aid review: any unsolicited AEs and related medications			X		X	X	X		X
Collection of memory aid						X			X
All unsolicited AEs (including SAEs, medically attended, leading to study withdrawal, or AESI)[o]		X	X	X	X	X	X	X	X
Concomitant medications[p]	X	X	X	X	X	X	X	X	X

Abbreviations: AE, adverse event; AESI, adverse event of special interest; ELISPOT, enzyme-linked ImmunoSpot; N/A, not applicable; PBMC, peripheral blood mononuclear cell; PRNT50, Plaque Reduction Neutralizing Titer EC50; SAE, serious adverse event.
Childbearing potential defined as any female who has experienced menarche and who is NOT permanently sterile or post-menopausal. Post-menopausal is defined as 12 consecutive months with no menses without an alternative medical cause.

Note: X/Xc denotes being performed before and after vaccination.

[a] Visit 3a will comprise of sentinel cohort only.

[b] To confirm post-menopausal status, as needed.

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

[d] At Screening, if the subject has no proof of yellow fever vaccination within 20 years, or other documentation of flavivirus status, the West Nile virus screening test will be administered. West Nile virus serology screen can occur up to 90 days prior to randomization.

[e] Urine drug screen for drugs of abuse will be performed at Screening and before dose administration at Visit 1 for opiates, cocaine, phencyclidine, amphetamines, benzodiazepines, and methadone (cannabis excluded).

[f] Full physical examination at Screening; symptom-directed (targeted) physical examination at all other scheduled time points. Interim physical examinations will be performed at the discretion of the investigator, if necessary. Height and weight will be measured and body mass index calculated at Screening only.

[g] Vital sign measurements (systolic and diastolic blood pressures, heart rate, respiratory rate, and body temperature) at Visit 1 and Visit 4 will be collected once before dose administration and at 60 (+15) minutes after dose administration (before subjects are discharged).

[h] Safety laboratory test values include hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, platelet count, red blood cell count, total and differential white blood cell count, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, amylase, lipase, bilirubin (total and direct), blood urea nitrogen, creatinine, random glucose, potassium, sodium, total protein, calcium, albumin, prothrombin time, partial thromboplastin time.

[i] A subset of safety laboratory test values for albumin, bilirubin (total and direct), prothrombin time, partial thromboplastin time, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase.

[j] Urinalysis parameters include pH, protein, glucose, ketone, bilirubin, urobilinogen, blood, nitrite, leucocytes, and specific gravity.

[k] Urine or serum pregnancy test may be performed.

[l] To occur prior to vaccination.

[m] Vaccination cannot occur if vitals are ≥Grade 2, or if a subject has an intercurrent illness (including a fever). Each subject will be monitored in the clinic for at least 1 hour following vaccination to assess for immediate reactogenicity, with vital sign measurements and local and systemic reactogenicity toxicity scored at 60 (+15) minutes (and earlier if deemed necessary by the investigator).

[n] Subjects will be instructed on recording reactogenicity, AEs, and medications (prescription or over-the-counter) on the memory aid (ie, diary card), and will be provided measuring tools, and instructed to call or return to the clinic within 24 hours if a reactogenicity score reaches Grade 3 or greater during the first 7 days following vaccination. The site will make a reminder call to the subject during the first 7 days (at approximately Day 4) post-vaccination to ensure that the memory aid is being completed correctly and consistently.

[o] In Part A of the study, AEs will be assessed from the time of the first dose administration at Visit 1 through Visit 7. However, for the time period after the informed consent form is signed until before receiving the study drug, AEs will only be recorded when they are defined as one or more of the following: SAEs, AEs of special interest, or AEs leading to study withdrawal.

[p] Concomitant medications include all medications (including vaccination outside of trial) taken by the subject from the time of signing the informed consent form through 28 days after the second vaccination (Visit 7).

Table 13-2 Part B: Schedule of Events

Procedure												
Study visit[a]	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Study day from 1st dose, if 2nd dose not completed	**84**	**112**	**140**	**168**	**196**	**224**	**252**	**280**	**308**	**336**	**364**	**392**
Study day from 2nd dose, if completed	**56**	**84**	**112**	**140**	**168**	**196**	**224**	**252**	**280**	**308**	**336**	**364**
Window allowance	**±7**	**±7**	**±7**	**±7**	**±15**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**+15**
Safety contact	X	X	X	X	X	X	X	X	X	X	X	X
Immune testing (humoral)[b]					X							X
AESIs[c]	X	X	X	X	X	X	X	X	X	X	X	X
SAEs[c]	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medications[d]	X	X	X	X	X	X	X	X	X	X	X	X
EOS visit												X

Abbreviations: AESI, adverse event of special interest; EOS, end of study; SAE, serious adverse event.

[a] Each safety contact in Part B will occur by telemedicine (e.g. telephone, text message, internet) and blood samples will be collected as described in footnote b.

[b] Blood samples for immune persistence will be collected from each subject at 168 (±15) days and 364 (+15) days following the second vaccination.

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

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