Title: A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Safety, Immunogenicity, and Dose Ranging Study of Purified Inactivated Zika Virus Vaccine (PIZV) Candidate in Flavivirus Naïve and Primed Healthy Adults Aged 18 to 49 Years

NCT Number: NCT03343626

SAP Approve Date: 26 April 2019

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- Other information as needed to protect confidentiality of Takeda or partners, personal information, or to otherwise protect the integrity of the clinical study.

STATISTICAL ANALYSIS PLAN

STUDY NUMBER: ZIK-101

A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Safety, Immunogenicity, and Dose Ranging Study of Purified Inactivated Zika Virus Vaccine (PIZV) Candidate in Flavivirus Naïve and Primed Healthy Adults Aged 18 to 49 Years

Safety, Immunogenicity and Dose Ranging Study of Inactivated Zika Virus Vaccine in Healthy Adults

PHASE 1

Version**:** 2.0

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Prepared by:

PPD

Based on:

Protocol Amendment 3 Version: 7.0
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1.1 Approval Signatures

Study Title: A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Safety, Immunogenicity, and Dose Ranging Study of Purified Inactivated Zika Virus Vaccine (PIZV) Candidate in Flavivirus Naïve and Primed Healthy Adults Aged 18 to 49 Years

Safety, Immunogenicity and Dose Ranging Study of Inactivated Zika Virus Vaccine in Healthy Adults

PHASE 1

PPD

4-26-2019
Date

4-24-2019
Date

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Date

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Date

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3.0 LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
ATC	Anatomical Therapeutic Chemical
CI	Confidence Interval
CRO	Contract Research Organization
CZS	Congenital Zika Syndrome
CSR	Clinical Study Report
DMC	Data Monitoring Committee
eCRF	electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
FAS	Full Analysis Set
FAS	Full Analysis Set
FASI	Full Analysis Set for Immunogenicity
GMT	Geometric Mean Titer
GSD	Geometric Standard Deviation
ICF	Informed Consent Form
ICH	International Council for Harmonization
Ig	Immunoglobulin
IM	Intramuscular
LoD	Limit of Detection
LLoQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
PIZV	Purified Inactivated Zika Virus Vaccine candidate
PPS	Per-Protocol Analysis Set
PPSI	Per-Protocol Analysis Set for Immunogenicity
PRNT	Plaque Reduction Neutralizing Tests
PT	Preferred Term
RS	Randomized Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SCR	Seroconversion Rate
SD	Standard Deviation
SOC	System Organ Class

SPR	Seropositivity Rate
SS	Safety Set
ULoQ	Upper Limit of Quantification
WHO	World Health Organization
WHO-DD	World Health Organization Drug Dictionary
ZIKV	Zika Virus

4.0 OBJECTIVES

4.1 Primary Objectives

To describe the safety of two doses of Purified Inactivated Zika Virus Vaccine candidate (PIZV) given 28 days apart from three different antigen concentrations (2, 5 or 10 µg) in flavivirus naïve and primed healthy adults through 28 days post dose 2.

To select a single vaccine dose level of PIZV for further clinical development.

4.2 Secondary Objectives

To describe the safety of two doses of PIZV given 28 days apart in flavivirus naïve and primed healthy adults through the end of the study.

To describe the immune response to PIZV in flavivirus naïve and primed healthy adults at the following immunogenicity time points 28 days post dose 1, 28 days post dose 2, and 6 months post dose 2.

To describe the persistence of immunity to PIZV at 12 and 24 months post dose 2 in flavivirus naïve and primed healthy adults from the placebo group and the PIZV dosing group that will be selected for further development.

4.3 Study Design

This is a phase 1, randomized, observer-blind, placebo-controlled, safety, immunogenicity, and dose ranging study of PIZV candidate in flavivirus naïve and primed healthy adults aged 18 to 49 years, inclusive, in ZIKV (Zika Virus) endemic and non-endemic regions.

The trial will last approximately 7 or 25 months for each subject, following a screening period prior to Visit 2. The screening period will last for up to 2 weeks (14 days) prior to randomization, with the possibility that some subjects (Flavivirus primed subjects only) will need to repeat some screening procedures if they were not randomized right after the screening period (further clarification is provided in the schedule of trial procedures).

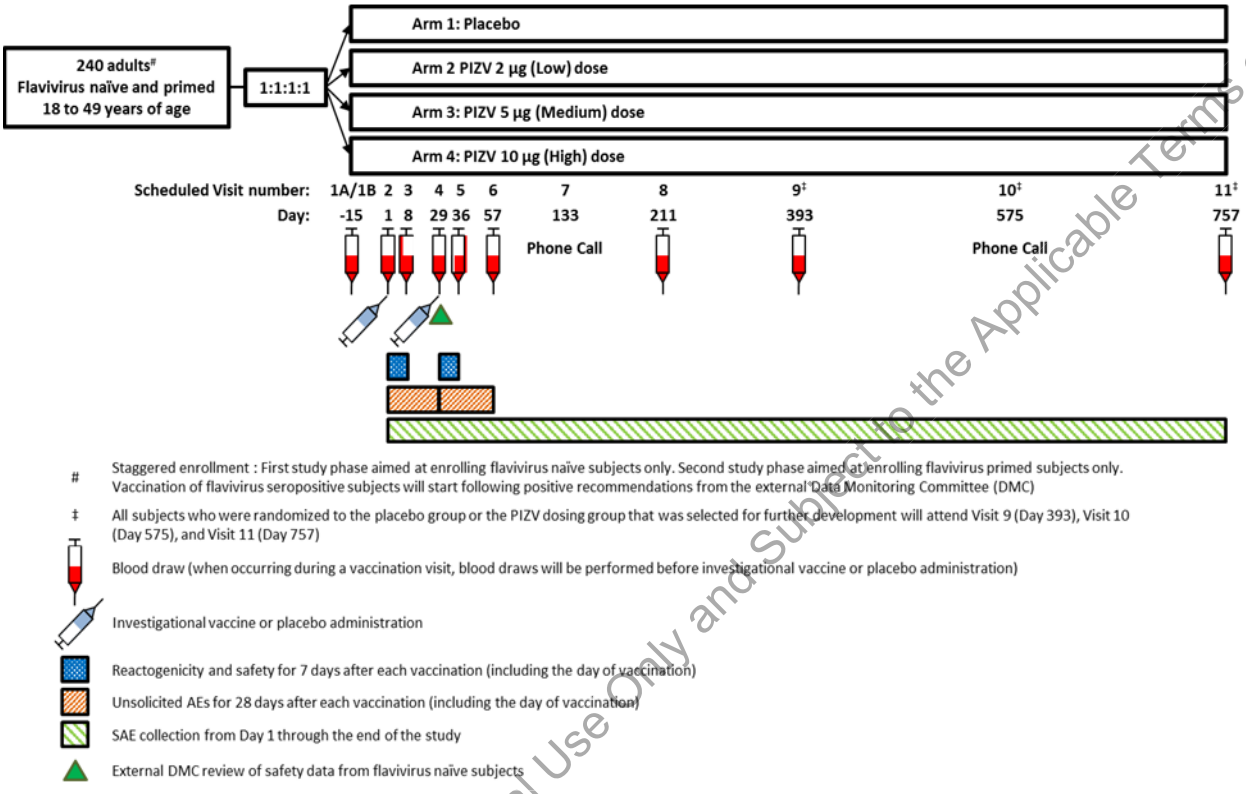
Flavivirus naïve subjects are subjects without detectable serum antibodies against a panel of flaviviruses as measured by a reactive antibody-based assay (Luminex) (refer to Appendix B).

Flavivirus primed subjects are subjects with serum antibodies against a panel of flaviviruses as measured by a reactive antibody-based assay (Luminex) (refer to Appendix B).

Approximately 240 subjects aged 18 to 49 years, inclusive, will be enrolled and equally randomized into four groups (arms) (1:1:1:1) of approximately 60 subjects, each comprised of approximately 30 flavivirus naïve subjects and approximately 30 flavivirus primed subjects. Randomization within each cohort (flavivirus naïve versus flavivirus primed subjects) will be stratified by flavivirus serostatus and age group: 18 to 29 years, inclusive, versus 30 to 49 years, inclusive.

A schematic representation of the trial design is included as Figure 4.a. A schedule of trial procedures is provided in Appendix A.

Figure 4.a Schematic of Trial Design



There will be two study phases as exhibited in Figure 4.b.

The first phase of the study will be aimed at enrolling flavivirus naïve subjects only. All subjects who sign the Informed Consent Form (ICF) and have confirmed eligibility at Screening Visit 1A will be tested for flavivirus serostatus.

Eligible flavivirus naïve subjects enrolled at Screening Visit 1A will be randomized at Visit 2, within two weeks after Screening Visit 1A.

Flavivirus primed subjects will not be enrolled during the first phase of the study (i.e., when identified at Screening Visit 1A) and will be invited to come back for the second phase of the study. They will be contacted after positive recommendations from the external Data Monitoring Committee (DMC) are issued following the review of safety and tolerability data in flavivirus naïve subjects, when the last flavivirus naïve subject has completed study Visit 4 (28 days post dose 1). These flavivirus primed subjects will consequently undergo 2 screening visits: Visit 1A during the first phase of the study and Visit 1B at the outset of the second phase of the study. Flavivirus primed subjects identified at Screening Visit 1A who do not participate in the second phase of the study will be declared screened failure (see Figure 4.b).

Enrollment will be put on hold after all naïve subjects have been enrolled and will resume when positive recommendations from the external DMC are issued, as described above.

The second phase of the study will be aimed at enrolling flavivirus primed subjects only.

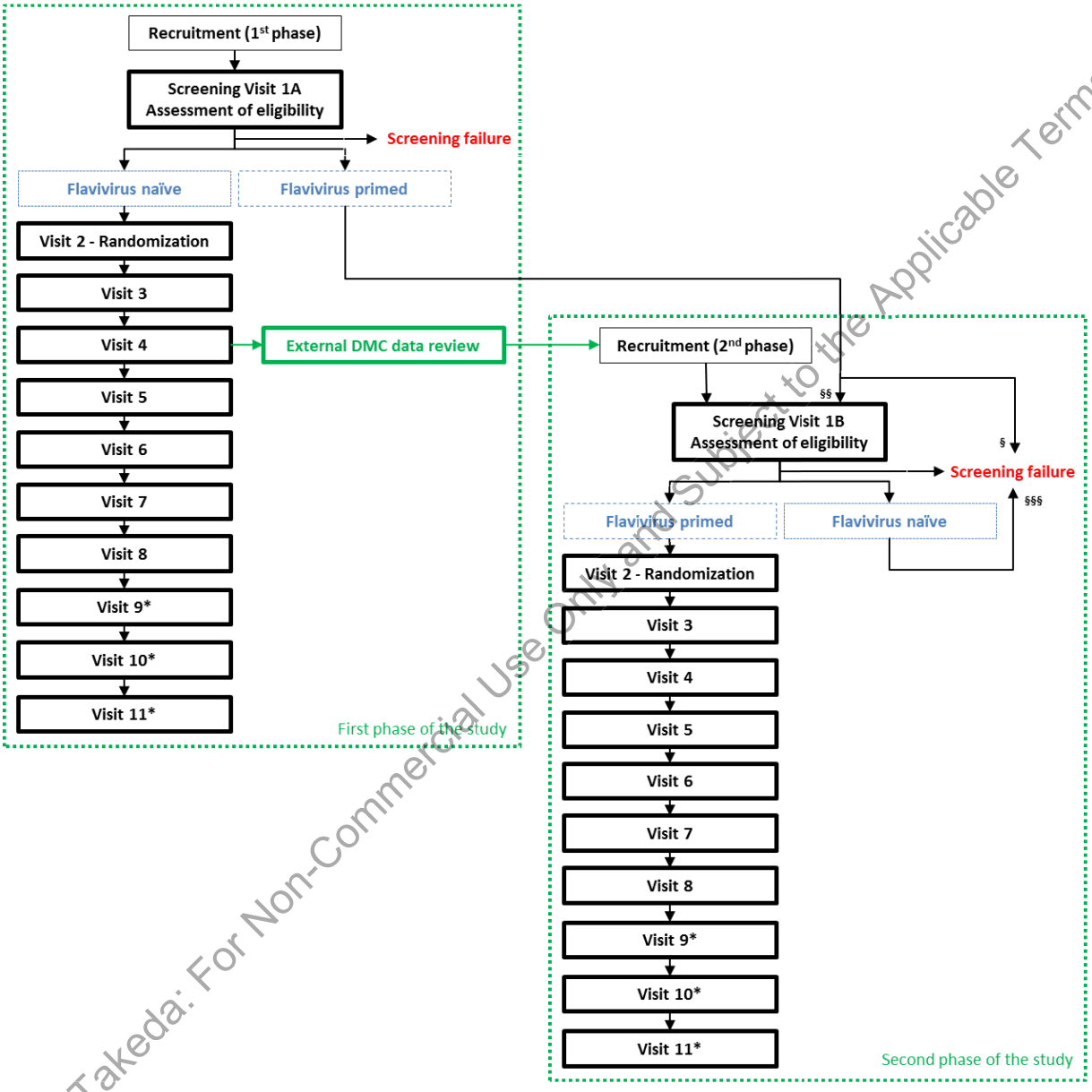
Newly identified eligible flavivirus primed subjects at Screening Visit 1B will be enrolled (randomized) at (second phase) Visit 2, within two weeks after Screening Visit 1B.

Previously identified flavivirus primed subjects (see Figure 4.b) (who were tested at Visit 1A during the first phase and accepted to enter the second phase of the study) will retain their initial subject identification (ID) number and undergo an additional screening visit (Visit 1B) to be tested for eligibility criteria again, (except for the determination of flavivirus serostatus).

Flavivirus naïve subjects identified at Screening Visit 1B (during the second phase of the study) will be declared screened failure (see Figure 4.b).

To avoid the natural exposure of flavivirus naïve subjects to flaviviruses during the study vaccination period, the enrollment into naïve cohort is planned in the sites located in flavivirus non-endemic areas only, and the prohibition of traveling into flavivirus endemic areas until Visit 6. The protocol provides references to flavivirus-endemic areas (refer to Appendix E).

Figure 4.b Staggered Enrollments



* All subjects who were randomized to the placebo group or the PIZV dosing group that was selected for further development will attend Visit 9 at Day 393 (12 months post dose 2 and Visit 11 at Day 757 (24 months post dose 2), and will also be contacted by phone on Day 575 (Visit 10) for safety follow-up.

Each subject will receive two 0.5 mL doses of either PIZV (2, 5 or 10 µg) or placebo intramuscularly (IM) – one dose at Visit 2 (on Day 1) and one dose at Visit 4 (on Day 29) IM into the middle third of the deltoid muscle, preferably in the non-dominant arm.

Each subject will be required to attend 7, 8, 9 or 10 clinical visits and will receive 1 or 2 phone calls depending on their flavivirus serostatus at screening and the vaccine dose level they received:

- Flavivirus naïve subjects identified during the Screening Visit 1A and flavivirus primed subjects identified during the Screening Visit 1B will attend 7 visits and will receive 1 phone call, and possibly 2 additional visits and 1 phone call (thus a maximum of 9 visits) and 2 phone calls if they were randomized to the placebo group or the PIZV dosing group that was selected for further development; and
- Flavivirus primed subjects identified during the Screening Visit 1A will attend 8 visits (including Visit 1A and Visit 1B) and will receive 1 phone call, and possibly 2 additional visits and 1 phone call (thus a maximum of 10 visits and 2 phone calls) if they were randomized to the placebo group or the PIZV dosing group that was selected for further development.

All randomized subjects will thus attend Screening Visit(s) (Visit 1A and/or Visit 1B), Visit 2 at Day 1, Visit 3 at Day 8 (7 days post dose 1), Visit 4 at Day 29 (28 days post dose 1), Visit 5 at Day 36 (7 days post dose 2), Visit 6 at Day 57 (28 days post dose 2), and Visit 8 at Day 211 (6 months post dose 2).

All randomized subjects will also be contacted by phone on Day 133 (Visit 7) for safety follow-up. In addition, all subjects who were randomized to the placebo group or the PIZV dosing group that was selected for further development will attend Visit 9 at Day 393 (12 months post dose 2) and Visit 11 at Day 757 (24 months post dose 2), and will also be contacted by phone on Day 575 (Visit 10) for safety follow-up.

Blood samples will be collected at each site visit: i.e., at Screening Visit(s) (Visit 1A and/or Visit 1B) for flavivirus serostatus determination and eligibility screening tests (including pregnancy testing); at Visits 3 and 5 for routine safety laboratory testing; and at Visits 2, 4, 6, 8, and for subjects who will be randomized to the placebo group or the PIZV dosing group that will be selected for further development) 9 and 11 for immunogenicity, as well as for further development and characterization of assays.

Urine samples will be collected for all subjects at Screening Visit(s) (Visit 1A and/or Visit 1B) for eligibility screening, and at Visits 3 and 5 for routine safety laboratory testing and for women of childbearing potential. Women of childbearing potential will undergo a pregnancy test at Visits 2 (blood/serum pregnancy test) and visit 4 (urine pregnancy test) before each investigational vaccine/placebo administration.

Each subject will receive diary cards to collect solicited AEs for 7 days after each dose (including the days of vaccine/placebo administration), and unsolicited AEs for 28 days after each dose (including the days of vaccine/placebo administration). Additional safety assessments will include new medical conditions (neurological and neuroinflammatory disorders) with onset after the first vaccination, and SAEs collection for the duration of the entire trial. Immunogenicity will be assessed for all subjects 28 days after each vaccine/placebo administration, and 6 months post dose 2, and for subjects who will be randomized to the

placebo group or the PIZV dosing group that will be selected for further development also 12 and 24 months post dose 2.

The schedule of trial procedures is exhibited in Appendix A.

4.4 Investigational Study Vaccine

Takeda's PIZV is a formalin-inactivated ZIKV vaccine candidate with aluminum hydroxide as an adjuvant provided as a liquid in the single-use vials. The investigational vaccine is administered IM as a 2-dose regimen containing 0.5 mL of 2, 5, or 10 µg antigen per dose, 28 days apart.

4.5 Placebo

Sodium chloride 0.9% solution is being used as placebo. It is a sterile, clear, colorless liquid solution of sodium chloride without preservative designed for parenteral use only. The placebo is presented in single-use vials stored at 2°C to 8°C. The placebo is administered IM as a 2-dose regimen of 0.5 mL per dose, 28 days apart.

5.0 ANALYSIS ENDPOINTS

5.1 Primary Endpoints

Safety and Tolerability of PIZV as determined by:

- Percentage of subjects with solicited local reactions (injection site: pain, erythema, swelling, and induration), in each severity category, during the 7-day period after administration of each dose of PIZV or placebo;
- Percentage of subjects experiencing solicited systemic AEs (fever, headache, fatigue, malaise, arthralgia, and myalgia), in each severity category, during the 7-day period after administration of each dose of PIZV or placebo;
- Percentage of subjects experiencing non-serious unsolicited AEs during the 28-day period after administration of each dose of PIZV or placebo; and
- Percentage of subjects experiencing serious AEs (SAEs) during the 28-day period after administration of each dose of PIZV or placebo.

Immunogenicity of PIZV, as determined by:

- Geometric mean titers (GMT) of neutralizing anti-ZIKV antibody levels at 28 days post dose 2.

5.2 Secondary Endpoints

Safety of PIZV, as determined by:

- Percentage of subjects experiencing SAEs throughout the trial.

Immunogenicity of PIZV, as determined by:

- GMT of neutralizing anti-ZIKV antibody levels at 28 days post dose 1, 6, 12 and 24 months post dose 2 in applicable groups;
- Seropositivity rates (SPR) at each immunogenicity time point (28 days post dose 1, 28 days post dose 2, 6, 12 and 24 months post dose 2) in applicable groups; and
- Seroconversion rates (SCR) at 28 days post dose 1 and 28 days post dose 2.

6.0 DETERMINATION OF SAMPLE SIZE

The sample size was not determined based on formal statistical power calculations. Stochastic simulations suggest that the proposed sample size is deemed adequate under a variety of decision-making scenarios.

7.0 METHODS OF ANALYSIS AND PRESENTATION

7.1 General Principles

This Statistical Analysis Plan (SAP) was developed upon the International Conference on Harmonization (ICH) E3 [1] and E9 [2] Guidelines and information provided in the Study Protocol ZIK-101, Version 7.0 dated 12 September 2018 [3]. The Table Shells, Data Listings and Figures will be provided in a separate document.

The specific algorithms and computer code developed for all derivation used to generate the statistical summaries will be provided in the Derived Database and Technical Programming Specifications document.

A blinded data review will be conducted prior to unblinding of subject's vaccination assignment. This review will assess the accuracy and completeness of the trial database, subject evaluability, and appropriateness of the planned statistical methods.

All statistical analyses will be conducted using SAS® Version 9.2, or higher.

Immunogenicity and safety endpoints will be summarized descriptively (frequency and percent for categorical data; and number of subjects with non-missing observations, mean [or geometric mean], standard deviation [SD] [or geometric standard deviation {GSD}], median, minimum, and maximum for continuous data, unless specified otherwise) at all relevant study visits, as appropriate.

All confidence intervals about a parameter estimate will be presented using the same number of decimal places as the parameter estimate.

Arithmetic means, geometric means and medians will be presented to 1 more decimal place than the recorded data.

The standard deviations (SDs) will be presented to 2 more decimal places than the recorded data.

Where appropriate, variables will be summarized descriptively by study visit and formulation arm.

For the categorical variables, the count and proportions of each possible value will be tabulated by formulation arm. The denominator for the proportion will be based on the number of subjects who provided non-missing responses to the categorical variable. For continuous variables, the number of subjects with non-missing values, mean, median, SD, minimum, and maximum values will be tabulated.

Unless otherwise specified, all data collected during the trial will be presented in the subject data listings.

7.1.1 Definition of Study Days

Study Day 1 is defined as the date of the first vaccination, as recorded on the eCRF vaccination page. Other study days are defined relative to Study Day 1, with Day 1 being the day prior to Day 1.

Baseline is defined as the last non-missing measurement taken before the first dose of PIZV or placebo. Where time is available, the time of the sample collection must be prior to the first dose of PIZV or placebo. Day 1 observations taken after the vaccination are considered post-baseline values.

7.1.2 Definition of Study Visit Windows

A window convention will be used to determine the analysis value for a given study visit for observed data analysis.

As defined in the schedule of trial procedures (Appendix A) the day of visit is relative to either Day 1 or actual Day of the previous vacation. The analysis windows for immunogenicity assessments are constructed around the target days following the window conventions presented below.

In addition, for visits where PIZV/Placebo is given analysis windows for immunogenicity assessment will exclude days after the actual day of the vaccination, as immunogenicity assessment of post dose 1 must always be prior to administration of dose 2 of PIZV/Placebo scheduled for the same visit.

Based on these rules, Table 7.a exhibits the immunogenicity assessment window for each study visit.

Table 7.a Protocol Defined Visit Windows for Immunogenicity Assessment

Visit	Target Day	Scheduled Vaccination	Visit Window (Study Day / Study Day Relative to Dose) [a]
2	Day 1	Dose 1	Study Day 1 0/+7 [b]
4	Day 29	Dose 2	Study Day 29 -4/+7
6	Day 57		Day 29 relative to Dose 2 -4/+7days
8	Day 211		Day 183 relative to Dose 2 -7/+14 days
9	Day 393		Day 365 relative to Dose 2 -7/+97 days
11	Day 757		Day 729 relative to Dose 2 -14/+14 days

(a) Study days are defined relative to study Day 1. Study days relative to dose are defined relative to the actual date of the specified dose.
(b) Immunogenicity assessments of post dose 1 must be prior to the dose 2 vaccination scheduled for the same visit and where time is available; the time of the sample collection must be prior to the vaccination time. Day 1 observations taken after the first dose are considered post-baseline values.

7.1.3 Conventions for Missing Adverse Event Data

If AE information is missing dates, intensity and/or relationship then the following approaches will be used so that this information can be used in the statistical analysis.

7.1.3.1 Unsolicited Adverse Events Missing or Partial Dates

Missing and partial AE start dates will be imputed only to determine the relationship between the start dates of the event and the date of the most appropriate vaccination of PIZV/Placebo that the

AE is associated with. An AE will be temporally associated with the correct PIZV/Placebo vaccination dose using the following rules:

- If the available start date of AE indicates that the start is between two consecutive PIZV/Placebo vaccination dates, then the AE start date will be imputed such that the AE will be assigned to the earlier dose.
- If the available start date of AE is insufficient to distinguish between one (1) or more PIZV or placebo administration dates, the event end date will be assessed. If possible, the AE will be assigned to the dose in the proximity to the event end date, assuming transient nature of the events.
- If the available date, both start and end dates, information indicates possible association of the AE with multiple doses then the AE start date will be imputed such that the AE is assigned to the first possible dose.
- If both start and end dates are completely missing, then the AE will be assigned to the first PIZV or placebo administration.

7.1.3.2 Adverse Events Missing Intensity (Severity) and Relationship

Missing information regarding "relationship to PIZV or placebo administration", related/not related, for solicited and unsolicited AEs and "severity", mild/moderate/severe, for unsolicited AEs, will be handled using the worst-case approach. That is, unsolicited AEs with missing severity/intensity will be considered as "severe" and solicited and unsolicited AEs with missing relationship will be considered as "related".

Solicited events missing intensity will not be included in the 'any' (count) of the respective solicited event (by grades).

7.1.4 Conventions for Missing Concomitant Medication Dates

Missing and partial medication dates will be assessed only to determine the relationship between the end date of the medication and the dose of PIZV/Placebo. Medication will be considered prior only if partial end date indicates that it was stopped before dose of PIZV/Placebo.

7.2 Analysis Sets

- **Safety Set (SS)**: The safety set will consist of all randomized subjects who received at least one (1) dose of PIZV or placebo.
- **Full Analysis Set (FAS)**: The FAS will include all randomized subjects who have received at least one dose of the investigational vaccine/placebo and provided valid baseline and at least one post-vaccination serology result. Subjects will be included in the FAS analysis 'as randomized'.
- **Per-Protocol Set (PPS)**: The PPS will consist of all subjects in the FAS who have no major protocol violations (relevant for the analysis, primary immunogenicity assessment or persistence assessment) as presented in Table 7.b. These criteria for exclusion of subjects

from the PPS will be reviewed, approved and documented (in a separate document) before or at the same time as SAP, prior to database lock and unblinding as part of the blinded data review. Any changes to these criteria after approval of the SAP will be documented and approved in a separate document. No further changes will be allowed after database lock and unblinding.

- **PPS for the dose selection**: For the dose selection GMT ratio analyses, only subjects with post-vaccination titers at or above Lower Limit of Quantification (LLoQ) (for this study the LLoQ value is set at 26.0) will be included.

All summaries and analyses of safety data will be based on subjects in the **SS**.

The primary immunogenicity analyses will be based on the **PPS**, and additional immunogenicity analyses will be based on the **FAS**.

All analyses for the safety population (**SS**) are to be based on the actual treatment received.

7.2.1 Major Protocol Violations and Evaluability Criteria

All reported protocol deviations, including the ones identified as protocol violations and major protocol violations, will be listed and presented in the final Clinical Study Report (CSR).

All protocol violations will be classified as major or not. Subjects with major protocol violations will be excluded from the PPS to avoid affecting the immunogenicity analysis. The PPS will be reviewed and approved prior to database lock.

Some criterion, but not all, used for the exclusion of subjects from the PPS are exhibited in Table 7.b below.

Table 7.b Criterion for Exclusion from the PPS Analyses

Protocol Deviation Categories	Criteria for Exclusion	Probable Method of Identification
Study vaccination not per protocol	Subject who did not receive both doses of PIZV or placebo	Identified programmatically using dosing data
	Subject who did not receive both doses in the correct interval	Decision will be made on a case by case basis
	Subject who did not receive the assigned IP in accordance with the randomization	Identified by independent statistician to maintain the subject level blinding within study team
	Partial dose administered due to spillage during administration (administration error)	Identified through source documents and provided in blinded fashion to the statistician
	Subject who received expired or IP that was deemed to be affected by the inappropriate storage conditions	Identified through source documents and provided in blinded fashion to the statistician
Procedure not performed per protocol	Blood sampling for immunogenicity analysis outside of the window	Identified through source documents for clinical science review to determine evaluability status for each identified subject
Enrollment criteria not met	Subject who meets any of the exclusion criteria as presented in the study protocol	Identified through source documents for clinical science review to determine evaluability status for each identified subject
	Subject who did not meet any of the inclusion criteria	Identified through source documents for clinical science review to determine evaluability status for each identified subject
Prohibitive concomitant medications/treatments	Subject who used a prohibited medication or was/were administered vaccine(s) not allowed per protocol	Identified through source documents for clinical science review to determine evaluability status for each identified subject
Withdrawal from the study	Subjects who discontinue prior to the end of study. However, subjects who complete Day 57 visit and have post-vaccination serological results will be included in analysis	Identified through source documents for clinical science review to determine evaluability status for each identified subject
Other	Subjects in flavivirus naïve cohort that are seropositive at baseline, as determined by the Plaque Reduction Neutralizing Tests (PRNT) assay [(a)]	Identified through source documents for clinical science review to determine evaluability status for each identified subject

Table 7.b Criterion for Exclusion from the PPS Analyses (continued)

Protocol Deviation Categories	Criteria for Exclusion	Probable Method of Identification
Other	Traveling to flavivirus endemic countries or flavivirus endemic regions of the US/US territories, within 4 weeks prior to screening or planned travel through to Visit 6 (applicable only to subjects to be enrolled into the flavivirus naïve cohort)	Subjects will be identified through source documents or clinical science to determine whether the subject should be evaluated

(a) Subjects will only be excluded from the PPS for immunogenicity analyses for flavivirus naïve cohort (i.e. will not be excluded from the PPS of overall subjects and for flavivirus primed cohort).

7.3 Disposition of Subjects

The reason for screen failure will be summarized based on all screened subjects.

Disposition of all randomized subjects will be summarized by study site for each cohort (Flavivirus priming serostatus) and study arm.

The categories will include:

- Number of subjects screened;
- Number of screen failures;
- Number of subjects lab confirmed;
- Number of subjects randomized (enrolled) by site, dose group and flavivirus serostatus (flavivirus naïve, flavivirus primed and overall);
- Primary reason for ineligibility for randomization;
- Number of subjects who received at least one (1) dose of the PIZV/Placebo;
- Number of subjects who received both doses of PIZV/Placebo;
- Number of subjects who received only one (1) dose of PIZV/Placebo;
- Number of subjects who completed each study visit (by visit);
- Number of subjects that completed all study visits;
- Number of subjects who prematurely discontinued from vaccine regimen (incomplete dosing schedule) with primary reason for discontinuation;
- Number of subjects who prematurely discontinued the study with primary reason for discontinuation.

Major protocol violations leading to exclusion from the **PPS** will be summarized. All protocol deviations will be presented in a subject data listing.

A subject is assumed ongoing unless he/she completed the end of study eCRF, indicating either completion or early termination. The primary reason for study discontinuation will be summarized.

7.4 Demographic and Other Baseline Characteristics

Demographics and baseline characteristics which included age, gender, race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian/Other Pacific Islander, White), body weight, body height and BMI will be summarized descriptively by cohort (flavivirus naïve and primed) and by study arm based on the **SS**, **FAS** and **PPS**.

Summary statistics (number of subjects, mean, and median, SD, minimum and maximum) will be exhibited for continuous variables and number and percentage of subjects within each category will be exhibited for categorical variables.

Inferential analyses of demographic and baseline characteristics will not be performed.

Individual demographic and baseline characteristics will be presented in subject data listings.

For subjects who are screened, but not randomized in the study, these individual subject data as well as the date of informed consent, and reason for screen failure will also be presented in subject data listings.

7.5 Medical History and Concurrent Medical Conditions

Medical occurrences before administration of the first dose of investigational vaccine/placebo are considered medical history. Medical history and concurrent medical conditions will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA) coding system. The current version of the dictionary will be used. Concurrent medical conditions are conditions that are recorded as ongoing at screening.

A summary table will be provided by system organ class (SOC), preferred term (PT) for each study arm and cohort, based on subjects randomized.

In addition, all medical history and concurrent medical condition data will be listed by study site, dose group and cohort. The subject data listing will contain subject identifier, dose group, SOC, PT and start and stop dates of the medical history and concurrent medications.

7.6 Prior and Concomitant Medications/Vaccinations

Prior and concomitant medications/Vaccinations will be coded using the World Health Organization Drug Dictionary (WHO-DD). The current version of the dictionary will be used.

A prior medication and vaccinations are any medication or vaccination taken before administration of PIZV or placebo. A concomitant medication/vaccination is any medication/vaccination taken on or after administration of PIZV or placebo.

Separate Summary tables for Prior medications and for Concomitant medications, will be provided by Anatomical Therapeutic Chemical (ATC) code and dose group based on subjects randomized. Separate subject data listings for medication history and concomitant medications/vaccinations will be produced by study site and subject number.

These subject data listing will contain subject identifier, dose group and cohort, ATC code, and preferred medication name, dose, frequency, and unit, route of administration, stop date and reason for use.

7.7 Study Vaccine Exposure and Compliance

The investigator records all injections of PIZV or placebo given to the subject in the eCRF. The administration date of PIZV or placebo and time information will be listed for each subject. The compliance rate will be summarized for the SS analysis by dose group presenting the number and percentage of subjects who received both doses and the number and percentage of subjects who received the first dose only.

The duration of follow-up will be summarized based on the SS as a continuous variable and in categories of 6, 12 and 24 months intervals from the second (last) vaccine administration. The categories will be: <6 months follow-up, 6 months post-dose 2 follow-up (study completers if not in groups that go into extension and all who are entering extension), 12 months post-dose 2 follow-up (only applicable to groups in extension) and 24 months post-dose 2 follow-up (completers of the extension).

7.8 Traveling During the Study

For the FV naïve cohort subjects travel is not permitted to any endemic country or region (area) until visit 6 (endemic countries and/or regions are described in Appendix E). If the subject travels to an endemic area within the period defined per protocol, then the subject may be excluded from the PPS, due to the risk of infection with a FV (which would mean the subject would no longer be FV naïve). The chance of the subject being infected, during the subjects' stay in an endemic area, will be evaluated (i.e. epidemiological situation during the time of travel will be evaluated). All travelling collected for visit 1A and the subsequent visits will be summarized.

7.9 Efficacy Analysis

Efficacy was not assessed in this study.

7.10 Pharmacokinetic/Pharmacodynamic Analysis

Not applicable.

7.10.1 Pharmacokinetic Analysis

Not applicable.

7.10.2 Pharmacodynamic Analysis

Not applicable.

7.11 Other Outcomes

The primary and secondary endpoints for safety and immunogenicity as presented in Sections 5.1 and 5.2 will be evaluated using the methods detailed below.

7.11.1 Immunogenicity Analysis

The primary and secondary endpoints for the immunogenicity analyses are presented below and will be evaluated using the methods detailed below. The PRNT test is used for determination of immunogenicity of the investigational vaccine, by assessing the quantity of neutralizing antibodies that bind ZIKA virus in the assay. The assay results are reported as titers (reciprocal value of the dilution of the serum from the vaccinated individual that inhibits for 50% the plaque formation).

The GMT will be calculated as the anti-logarithm of Σ (log transformed titer/n), i.e. as the antilogarithm transformation of the mean of the log-transformed titer, where n is the number of subjects with titer information. The geometric standard deviation (GSD) for GMT will be calculated as the anti-logarithm transformation of the standard deviation of the log-transformed titer. The 95% CI will be calculated as the anti-logarithm transformation of the upper and lower limits for a two-sided CI for the mean of the log-transformed titers.

The fold increase from the baseline antibody titers is calculated as the ratio of the post-vaccination titer level to the pre-vaccination titer level. Considering Q2 PRNT assay's design the minimum of 4-fold increase in titers is considered meaningful.

The primary population for all immunogenicity analyses will be the **PPS.**

Additional immunogenicity analysis will be performed using Zika RVP microneutralization assay, with details documented in a separate SAP Addendum.

7.11.1.1 Definitions for the Immunogenicity Endpoints

The definitions are presented below:

- *Seronegative subjects*: Subjects with no detectable serum antibodies (test results are below LOD) as measured by the neutralization assay.
- *Seropositive subjects*: Subjects with detectable serum antibodies (tested positive at or above limit of detection, LOD) as measured by the neutralization assay.
- *Seroconverted subjects*: Seronegative subjects at baseline with detectable post-vaccination serum antibodies (test results are at or above LOD) and seropositive subjects at baseline (FV primed) with different fold increases in post-vaccination antibodies from baseline, as measured by the neutralization assay.

Considering Q2 PRNT assay's design the minimum of 4-fold increase in titers is considered meaningful. Seronegative subjects (i.e. with titer < LOD) at baseline with detectable post-vaccination serum antibodies (test results are at or above LOD) and seropositive subjects at baseline (i.e. with titer ≥ LOD) with 4-fold increases in post-vaccination antibody titers from baseline are considered as seroconverted subjects.

Subjects tested negative will be assigned the value half of LOD i.e. 5 (10.0/2).

7.11.1.2 Missing and Imputed Immunogenicity Data

Missing data will be addressed in the immunogenicity analyses following the rules specified below:

- **Missing Immunogenicity Data**

  Subjects with missing immunological data will be excluded from the immunological analyses.

- **Titers Measured Below the Lower Limit of Quantitation (LLoQ)**

  A titer value measured equal or above Limit of Detection (LOD) (the LOD value is set to 10.0) and below LLoQ (the LLoQ value is set to 26.0) (≥ LOD and <LLoQ) will be imputed to a value that is half of the LLoQ value i.e., (26.0/2 = 13.0) in summaries and analyses, but both the raw serology data and imputed data will be included in the listing.

- **Titers Measured Below the LLoQ (for the dose selection analysis)**

  For the GMT ratio analyses subjects with imputed post-vaccination titers will not be considered in the analysis to be conservative.

  Subjects negative in the test will be assigned ½*LOD (10.0/2 = 5).

7.11.1.3. Analyses of Primary Immunogenicity Endpoints

The primary immunogenicity endpoint is the GMT, and for dose comparison the ratios of geometric mean titers (GMT) of neutralizing anti-Zika Virus antibody levels at 28 days post dose 2 (Day 57 Visit 6) will be calculated pairwise for the three different PIZV dose levels (2 µg, 5 µg or 10 µg) in flavivirus naïve adults.

The differences in seroconversion rates at each immunogenicity time point (28 days post dose 1, 28 days post dose 2, calculated pairwise for the three different PIZV dose levels (2 µg, 5 µg or 10 µg) in flavivirus naïve adults will also be assessed for the dose comparison.

A sensitivity analysis will be provided using the **FAS**.

All pairwise comparisons will be estimated using the **PPS**, with a sensitivity analysis using the **FAS** without multiplicity adjustment.

7.11.1.4. Analyses of Secondary Immunogenicity Endpoints

The secondary immunogenicity endpoints are:

- The GMT of neutralizing anti-Zika antibody levels at 28 days post dose 1 (Day 29, Visit 4), and Visit 6 (Day 57, Visit 6), 6 months (Day 211, Visit 8), 12 months (Day 393, Visit 9) and 24 months (Day 757, Visit 10) post dose 2 in applicable dose levels (2 µg, 5 µg or 10 µg) or Placebo in flavivirus naïve and primed adults.
- Seropositivity rates (SPR) at each immunogenicity time point (28 days post dose 1, 28 days post dose 2 and 6, 12 and 24 months post dose 2) in applicable dose levels (2 µg, 5 µg or 10 µg) or Placebo in flavivirus naïve and primed adults.
- Seroconversion rates (SCR) at 28 days post dose 1 (Day 29 Visit 4) and 28 days post dose 2 (Day 57, Visit 6) in applicable dose levels (2 µg, 5 µg or 10 µg) or Placebo in flavivirus naïve and primed adults.

Further details related to the dose selection are presented in a separate document, Dose Selection Plan for the Purified Inactivated Zika Virus Vaccine (PIZV); version 1 (07 June 2018).

The details of the immunogenicity analyses are presented as below.

GMT analyses

(1) GMT summary analyses (FV naïve, FV primed, overall)

- Primary analysis: PPS
  - Subjects with post vaccine results ≥ LOD and < LLOQ will be assigned the titer value of 13.0
- Analysis based on the FAS
  - Subjects with post vaccine results ≥ LOD and < LLOQ will be assigned the titer value of 13.0
- Sensitivity analysis for PPS and FAS
  - Subjects with post vaccine results ≥ LOD and < LLOQ will be excluded from this analysis (i.e. data will be considered missing)

(2) Pairwise comparison of GMTs (GMT ratios) (FV naïve only for dose selection)

- Primary analysis: PPS
  - Subjects with Post-vaccination results ≥ LLOQ will be included in the analysis, while the results ≥ LOD and < LLOQ will be considered missing
- Analysis based on the FAS
  - Subjects with Post-vaccination results ≥ LLOQ will be included in the analysis, while the results ≥ LOD and < LLOQ will be considered missing

- Sensitivity analysis for PPS and FAS
  - Subjects with post vaccine results ≥ LOD and < LLOQ will be assigned the titer value of 13.0

SCR analyses

(1) SCR rate summary (FV naïve, FV primed, overall)

FV naïve

- Primary analysis: PPS
  - Initially seronegative Subjects with post vaccination ≥ LOD defined as seroconversion
- Analysis based on FAS
  - Initially seronegative Subjects with post vaccination ≥ LOD defined as seroconversion
- Sensitivity analysis for PPS and FAS
  - Initially seronegative Subjects with post vaccination ≥ LLoQ defined as seroconversion, while the subjects with results ≥ LOD and < LLoQ are excluded from analysis (results in this range are considered 'missing')
  - Initially seronegative subjects and initially seropositive subjects in FV naïve cohort will be included in the sensitivity analysis

FV primed

- Primary analysis: PPS
  - Subjects with baseline results ≥ LOD and <LLOQ will be assigned the value of LLoQ conservatively at baseline, ≥ 4 fold increased (≥ 104) defined as seroconversion
- Analysis based on FAS
  - Subjects with baseline results ≥ LOD and <LLOQ will be assigned the value of LLoQ conservatively at baseline, ≥ 4 fold increased (≥ 104) defined as seroconversion
- Sensitivity analysis for PPS and FAS
  - Subjects with baseline results ≥LOD and <LLOQ will be assigned the value of 13 conservatively at baseline, ≥ 4 fold increased (≥ 52) defined as seroconversion

(2) Pairwise comparison SCR (dose selection, FV naïve)

FV naïve

- Primary analysis: PPS
  - Initially seronegative Subjects with post vaccination ≥ LOD defined as seroconversion
- Analysis based on FAS
  - Initially seronegative Subjects with post vaccination ≥ LOD defined as seroconversion
- Sensitivity analysis for PPS and FAS
  - Initially seronegative Subjects with post vaccination >=LLoQ defined as seroconversion, while the subjects with results >LOD and < LLOQ are excluded from analysis (results in this range are considered 'missing'
  - Initially seronegative subjects and initially seropositive subjects in FV naïve cohort will be included in the sensitivity analysis

SPR analyses

(1) SPR rate summary (FV naïve, FV primed, overall)

FV naïve

- Primary analysis: PPS
  - Subjects with post vaccination ≥ LOD defined as seropositive
- Analysis based on the FAS
  - Subjects with post vaccination ≥ LOD defined as seropositive
- Sensitivity analysis for PPS and FAS
  - Subjects with post vaccination ≥ LLoQ defined as seropositive while the subjects with results ≥ LOD and < LLoQ are excluded from analysis (results in this range are considered 'missing')

FV primed

- Primary analysis: PPS
  - Subjects with post vaccination ≥ LOD defined as seropositive
- Analysis based on FAS
  - Subjects with post vaccination ≥ LOD defined as seropositive
- Sensitivity analysis for PPS and FAS
  - Subjects with post vaccination ≥ LLoQ defined as seropositive while the subjects with results ≥ LOD and <LLoQ are excluded from analysis (results in this range are considered 'missing')

(2) Pairwise comparison SPR (FV naïve only)

- Primary analysis: PPS
  - Subjects with post vaccination >=LOD defined as seropositive

7.11.1.5 Supportive Immunogenicity Summaries

Supportive immunogenicity summaries will be provided based on the **FAS**. The planned immunogenicity analyses will be based on the **PPS** and are exhibited in Table 7.c below.

Table 7.c Planned Analyses and Populations for Immunogenicity Endpoints

Cohorts	Parameter	Time Point	Descriptive Summaries	Graphical Presentation
Flavivirus Naïve Healthy Adults	GMT	Day 1 (Visit 2)	PPS, FAS	PPS
		Day 29 (Visit 4)	PPS, FAS	PPS
		Day 57 (Visit 6)	PPS, FAS	PPS
		Day 211 (Visit 8)	PPS, FAS	PPS
		Day 393 (Visit 9)	PPS, FAS	PPS
		Day 757 (Visit 11)	PPS, FAS	PPS
	SPR	Day 1 (Visit 2)	PPS, FAS	PPS
		Day 29 (Visit 4)	PPS, FAS	PPS
		Day 57 (Visit 6)	PPS, FAS	PPS
		Day 211 (Visit 8)	PPS, FAS	PPS
		Day 393 (Visit 9)	PPS, FAS	PPS
		Day 757 (Visit 11)	PPS, FAS	PPS
	SCR	Day 29 (Visit 4)	PPS, FAS	PPS
		Day 57 (Visit 6)	PPS, FAS	PPS

7.11.2 Descriptive Summaries

The geometric mean, standard deviation, median, minimum, maximum and 95% confidence intervals will be calculated as the antilogarithm transformation of the mean, standard deviation, median and 95% confidence intervals of the log-transformed titers.

Descriptive statistics for the primary and secondary immunogenicity endpoints, including estimates and 95% confidence intervals (95% CI), calculated using the exact Clopper-Pearson method [4], for GMT, SPR and SCR, will be provided by time point as per Table 7.c (28 days post dose 1 (Visit 2, Day 1), 28 days post dose 2, 6, 12, and 24 months post dose 2 in applicable groups) and by dose level.

For seropositive subjects at baseline, the different fold increases in post-vaccination antibodies from baseline will also be exhibited as percentage of subjects with a ≥ 4-fold rise at each time point by dose level.

Point estimates and 95% CI, calculated using the exact Clopper-Pearson method [4] for ratios in GMT and differences in SCR will be provided for each pair of active study arms (i.e., low, medium and high doses) of FV naïve cohort, to aid a single PIZV dose level selection.

Details related to the pre-specified pairwise comparisons within study arms (2 µg, 5 µg or 10 µg dose level) are presented in Section 7.12.

The analysis for seropositivity rates (SPR) will be based on two immunogenicity time points: 28 days post dose 1 (Day 29 Visit 4) and 28 days post dose 2 (Day 57), for the three PIZV dose levels.

The differences in SPR will be analyzed using Newcombe score method [5] with corresponding 95% CIs, and p-values computed using Fisher's exact test will be exhibited.

Immunogenicity summaries and analyses will be provided by study arm, overall and each cohort (flavivirus naïve and primed cohorts).

Descriptive summaries per dose group will not use baseline titer values (in FV primed subjects) as the covariate in the analyses, thus ANOVA analyses will be conducted.

7.12 Dose Selection Methodology

The dose selection methods detailed in this section are to be used in conjunction with the analyses presented in the dose selection plan. The dose selection analyses are based on the data from FV naïve cohort only. The immunogenicity assessment will therefore be based on the magnitude of the response, as measured by the ratios of geometric mean titer (GMT) of neutralizing anti-ZIKV antibodies, and differences in seroconversion rate between the dosing groups. The distribution of neutralizing titers across the population of each dose group will provide supplemental information for the dose selection.

The primary immunogenicity analysis will be done using PPS.

Safety analyses supporting the dose selection will be performed in the flavivirus naïve, flavivirus primed cohorts and combined.

7.12.1 Geometric Mean Titers Analyses

GMT ratios with corresponding 95% CIs and p-values will be estimated from an analysis of covariance (ANOVA) model including the log-transformed value of the titer as the dependent variable and vaccine dose level (low, medium or high) as independent variables.

The GMT, GMT ratio and 95% CI will be presented on an anti-log scale of the least-square means with associated standard deviations estimated from this ANOVA model.

This analysis will be performed without multiplicity adjustments.

GMTs with or without imputed values for results ≥ LOD and < LLoQ will also be presented as depicted in Table 7.c.

7.12.2 Seroconversion Rate Analyses

Seroconversion in FV naïve subjects is defined as initially seronegative subjects reaching seropositivity post vaccination.

7.13 Other Immunogenicity Analyses

The analyses of GMTs, SCRs and SPRs used in the dose selection on the FV naïve cohort will be repeated for the FV primed cohort and combined cohorts (FV naïve and FV primed cohorts combined). These analyses will also be performed over all relevant time points.

7.14 Graphical Presentations

Graphical presentations for selected immunogenicity parameters will be provided to explore and understand the distributional characteristics for the three dose levels (2, 5 or 10 µg) in the flavivirus naïve cohort and FV primed cohort.

Further details are presented below.

7.14.1 Immunogenicity Figures

The primary immunogenicity parameters presented below will be exhibited graphically using the **PPS**.

7.14.1.1 Geometric Mean Figures

The graphical presentations for the GMT analyses will include:

- Reverse cumulative distribution curves of antibody titer values for baseline, 28 days post vaccination Day 29 (Visit 4), Day 57 (Visit 6), Day 211 (Visit 8), Day 393 (Visit 9) and Day 757 (Visit 11) by dose level (2, 5 or 10 µg) and Placebo overlaid.
- Line plots of the GMTs plotted for baseline (Day 1, Visit 2), 28 days post vaccination Day 29 (Visit 4), Day 57 (Visit 6), Day 211 (Visit 8), Day 393 (Visit 9) and Day 757 (Visit 11) by dose level (2, 5 or 10 µg) and Placebo overlaid.

7.14.1.2 Seroconversion Rate Figures

The graphical presentations for the seroconversion rates will include:

- Bar graphs exhibiting the percentage of subjects with seroconversion including error bars for the 95% CIs plotted for 28 days post vaccination 1 Day 29 (Visit 4) and 28 days post vaccination 2 Day 57 (Visit 6) by dose level (2, 5 or 10 µg).

7.14.1.3 Seropositivity Rate Figures

The graphical presentations for the seropositivity rates will include:

- Bar graphs exhibiting the percentage of seropositive subjects including error bars for the 95% CIs plotted for baseline, 28 days post vaccination Day 29 (Visit 4), Day 57 (Visit 6), Day 211 (Visit 8), Day 393 (Visit 9) and Day 757 (Visit 11) by dose level (2, 5 or 10 µg).

7.14.1.4 Fold-Rise Figures

The graphical presentations for the fold increases will include:

- Bar graphs exhibiting the percentage of subjects with fold increase of 4 and greater including error bars for the 95% CIs plotted for baseline, 28 days post vaccination Day 29 (Visit 4), Day 57 (Visit 6), by dose level (2, 5 or 10 µg).

7.14.2 Primary and Secondary Safety and Tolerability Endpoints

Safety and tolerability of PIZV will be established by the following endpoints.

7.14.2.1 Primary Safety and Tolerability Endpoints

- Percentage of subjects with solicited local reactions (injection site: pain, erythema, swelling, and induration), in each severity category, during the 7-day period after administration of each dose of PIZV or placebo.
- Percentage of subjects experiencing solicited systemic adverse events (AEs) (fever, headache, fatigue, malaise, arthralgia, and myalgia), in each severity category, during the 7-day period after administration of each dose of PIZV or placebo.
- Percentage of subjects experiencing non-serious unsolicited AEs during the 28-day period after administration of each dose of PIZV or placebo.
- Percentage of subjects experiencing serious AEs (SAEs) during the 28-day period after administration of each dose of PIZV or placebo.
- Percentage of events experiencing serious AEs/AEs causing the withdrawal from the study vaccination/study throughout the study.

7.14.2.2 Secondary Safety and Tolerability Endpoints

- Percentage of subjects experiencing SAEs throughout the trial.

All summaries and analyses will be presented by study arm, overall and each cohort (flavivirus naïve and primed cohorts) using **SS**.

Further details related to the safety analysis are presented below.

Number and percentage of subjects and events with solicited local reactions (injection site: pain, erythema, swelling, and induration), in each severity category, during the 7-day period after administration of each dose of PIZV or placebo;

Number and percentage of subjects and events with solicited systemic AEs (fever, headache, fatigue, malaise, arthralgia, and myalgia), in each severity category, during the 7-day period after administration of each dose of PIZV or placebo, or after any PIZV dose;

Number and percentage of subjects and events with non-serious unsolicited AEs during the 28-day period after administration of each dose of PIZV or placebo;

Number and percentage of subjects and events with SAEs during the 28-day period after administration of each dose of PIZV or placebo, and throughout the study;

Number and percentage of subjects and events with serious AEs/AEs causing the withdrawal from the study vaccination/study throughout the study.

7.15 Safety Analysis

All summaries and analyses of safety data will be based on subjects in the **SS** data. Unless otherwise specified, safety data will be summarized by dose for each study arm, overall and each cohort (flavivirus naïve and primed cohorts).

Summaries after any PIZV or placebo administration will also be included.

Biologically implausible measurements of body temperature and solicited local symptoms such as body temperature < 35ºC or > 42ºC and erythema and swelling > 50.0 cm will be excluded from the summaries and analyses but included in the data listings.

No inferential analyses of safety data will be performed. Unless otherwise specified, data imputation will not be performed for any missing adverse event.

All new medical conditions (including neurological and neuroinflammatory disorders) with onset after the first vaccination, deaths, related SAEs and SAEs deemed relevant in the context of PIZV safety by the investigator will be summarized.

7.15.1 Solicited Adverse Events Related to Reactogenicity

Reactogenicity will be assessed for 7 days for solicited local injection site following each dose (including day of PIZV or placebo administration) via daily collection of solicited AEs, including local reactions at the injection site (pain, erythema, swelling, and induration) and systemic AEs (headache, fatigue, malaise, arthralgia and myalgia). In addition, body temperature

(preferably measured as oral route) will be collected (with fever defined as body temperature ≥ 38°C/100.4°F.) for 7 days as solicited systemic AEs after each vaccination including the day of vaccination. These solicited AEs and their intensity grades are defined in the study protocol, Table 10.b [3].

For local solicited AEs erythema, induration and swelling, subjects will record the length of the greatest surface diameter. For the systemic solicited AE fever, subjects will record the body temperature in either degrees Fahrenheit (ºF) or degrees Celsius (ºC). Fever data will be displayed in ºC in summaries and data listings. Intensity grades for erythema, induration and swelling will be derived from the recorded diameters and fever will be derived from the recorded body temperature measurements, according to Table 10.b provided in the protocol [3].

For each solicited AE and fever, the number and percentage of subjects reporting AEs and number and percentage of doses followed by an event will be summarized by event severity and by relationship to PIZV/Placebo (only for solicited systemic AEs and local solicited AEs are considered as related vaccination) for each study arm, overall and each cohort (flavivirus naïve and primed cohorts) following each vaccination:

- 30 minutes post vaccination (solicited local and systemic AEs);
- Days 1 through 7 (solicited local and systemic AEs);
- Days 1 through 3 and Days 4 through 7 (solicited local and systemic AEs).

For subjects with more than 1 episode of the same event, the maximum severity will be used for tabulations.

A summary of Time-to-onset and duration of each event will be presented following each administration of PIZV or Placebo. The duration of an event is calculated as the total number of all days the subject reported the event during the solicited period of 7 days post vaccination, regardless of whether the symptom was reported on consecutive days.

Prolonged solicited AEs that continued beyond day 7 will be captured on the Adverse Event eCRF page, indicated by the checkbox “Is this event a continuation of a Solicited event”. These prolonged solicited AEs will be analyzed separately from the unsolicited AEs and presented in a separate subject data listing.

7.15.2 Unsolicited Adverse Event

Any AE captured in the Adverse Event eCRF that is a continuation of a solicited AE will not be included in any unsolicited AE summaries or subject data listings.

Unsolicited AEs will be assessed for 28 days following administration of each dose of PIZV or Placebo (day of administration + 27 days). SAEs and AEs leading to subject withdrawal from the trial will be collected throughout the trial for all subjects.

Unsolicited AEs will be summarized up to 28 days after each vaccination. These summaries will generally be presented in: overall duration of follow-up, up to 28 days after each vaccination, Additionally, AEs from first vaccine dose to 28 days post dose 2 will be summarized.

SAEs and AEs leading to early termination will be assessed throughout the trial duration and presented in 3 ways described for AEs with additional category of events reported throughout the trial duration (from first vaccine dose to: 28 days post dose 2, 6 months post dose 2, 12 months post dose 2 and 24 months post dose 2) and those with the onset after 28 days post each vaccination.

The number and percentages of subjects with an unsolicited AE will be tabulated at each of the following levels:

- Overall (Unsolicited AEs, SAEs and Deaths);
- By System Organ Class (SOC) and Preferred Term (PT);
- Non-serious unsolicited AEs including events with frequency greater than 5% in any study arm up to 28 days after vaccination by SOC and PT (in decreasing frequency);
- Relationship (not related, related) to vaccine by SOC, PT and intensity;
- By SOC, PT and intensity (mild, moderate and severe).

Subjects reporting more than one occurrence for the term (level) being summarized will be counted only once. When relationship or intensity (severity) is concerned the AE with the most closely related occurrence or the highest known intensity will be counted. If the investigator did not provide the relationship then the relationship will be assumed to be related. If the investigator did not provide severity then the worst severity will be assumed.

Unsolicited AEs, SAEs and new medical conditions (neurological and neuroinflammatory disorders) with onset after the first vaccination will be coded using MedDRA and summarized by SOC and PT for each study arm.

Safety summaries will be provided by study arm, overall and each cohort (flavivirus naïve and primed cohorts).

7.15.3 Clinical Laboratory Evaluations

Routine safety laboratory tests that will be performed on blood and urine samples at screening visit for baseline, visit 3 (Day 8, 7 days post dose 1) and Visit 5 (Day 36, 7 days post dose 2) are outlined in Table 9.a in the protocol [3].

Subjects with baseline safety laboratory test results within normal limits or not above Grade 1 as defined in the FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers (refer to Appendix D) are eligible for participation in the study. Toxicity grading scales for laboratory abnormalities will be assigned based on the reference values defined in FDA toxicity grading scales for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials.

For each laboratory parameter with abnormal results at each time point, the number and percentage of subjects and doses will be summarized by grading scale for each study arm, overall and each cohort (flavivirus naïve and primed cohorts) following each vaccination. The change in the grade of the laboratory values as the shift tables.

7.15.4 Vital Signs

The vital signs collected in this study include systolic and diastolic blood pressure, heart rate, and body temperature.

Vital signs will be summarized descriptively by study arm at each applicable visit for overall and each cohort (flavivirus naïve and primed cohorts). Individual subject data will be presented in the subject data listings by dose group, study site, subject number, collection date and time and parameter measured.

Descriptive statistics (number of subjects, mean, median, SD, minimum and maximum), of vital sign parameter (observed and change from previous vaccination) except height, will be summarized by study arm at each applicable visit for overall and each cohort (flavivirus naïve and primed cohorts). The change in the grade of the vital sign values will be summarized in the shift table.

Only vital signs measurements at the scheduled visits will be included in this summary.

7.15.5 12-Lead ECGs

Not applicable.

7.15.6 Other Observations Related to Safety

For dose selection the following safety summaries will be produced:

- Overall rate of fever occurrence after the first and second vaccination by dose;
- Severity of systemic reaction (excluding fever) after the first and second vaccination by dose; and
- Severity of local site reactions after the first and second vaccination by dose.

These summaries will include the occurrence of the event (n/N), probability of the occurrence (%), and 95% Confidence interval of the probability of the occurrence. Newcombe score method [5] with corresponding 95% CIs and p-values computed using Fisher's exact test will be used for this analysis.

7.16 Interim Analysis

A first interim analysis will be performed to include immunogenicity and safety data from all flavivirus naïve subjects up to Visit 6 (on Day 57, 28 days post dose 2); a second interim analysis will be performed for dose selection, and including safety data from all flavivirus primed subjects up to Visit 4 (on Day 29, 28 days post dose 1).

7.17 Analyses for the Final Clinical Study Report

The primary safety and immunogenicity analysis will be performed when all (flavivirus naïve and primed) subjects have completed Visit 6 (Day 57) to provide data to support the planning and execution of other trials in the development plan of PIZV. The analyses will be performed

by a separate set of unblinded statisticians and programmers at a Clinical Research Organization (CRO), who will have access to individual treatment assignments and will not be involved in subsequent trial conduct. The study team at Takeda will have access to the group level unblinded results and will remain blinded to the individual treatment assignment. The remaining personnel (not mentioned before) involved in the conduct of the trial, including those at Takeda, the CRO, and the trial sites, will remain blinded to the individual subject treatment assignment until unblinding after trial completion (database lock for data through- Visit 11).

The final study report will be written for the data up to Visit 6 (Day 57), and two additional addenda will be written: one to report the data from Visit 6 (Day 57) to Visit 9 (Day 393, 12 months post dose 2), and one to report the data from Visit 9 (Day 393) until the end of the study, i.e., Visit 11 (Day 757, 24 months post dose 2).

The timing and the types of analyses with the respective output is provided in Table 7.d.

Table 7.d Timing and the Types of Analyses with the Respective Output

Analysis	Study Time Point	Clinical Study Report/ Data
Interim analysis 1	Day 57 (Visit 6) completed for FV naïve cohort	Analysis of immunogenicity and safety. No study report will be written.
Interim analysis 2	Up to Visit 4 (on Day 29, 28 days post dose 1) for FV primed cohort	Safety assessment and analysis of dose selection based on the immunogenicity data. No study report will be written.
CSR	Day 57 (Visit 6) completed for both FV naïve and primed cohorts	Primary safety and immunogenicity analysis. CSR will be written.
CSR addendum 1	Visit 6 (Day 57) to Visit 9 (Day 393, 12 months post dose 2)	Additional safety and immunogenicity analysis up to visit 9. CSR addendum 1 will be written.
CSR addendum 2	Visit 9 (Day 393) until the end of the study, ie, Visit 11 (Day 757, 24 months post dose 2)	Additional safety and immunogenicity analysis up to visit 11. CSR addendum 2 will be written.

7.18 Changes in the Statistical Analysis Plan

This SAP contains no changes to the planned analyses described in the protocol, except that some additional details are added to some sections for further clarification.

7.18.1 Amendment History

Date	Amendment Number
10 Aug 2018	Initial Analysis Plan
26 Apr 2019	1

7.18.2 Summary of Changes

This section describes changes to the Statistical Analysis Plan Version 1.0, dated 10 Aug 2018.

The main rationale for this amended SAP is the Amendment to Protocol Version 3.0, dated 12 Sept 2018. Minor grammatical and editorial changes are included for clarification purposes only.

Section	Description of Change
Cover page	Update on author, SAP and protocol version and date
1.1	Update of approval team
7.1.2	Update of study window per protocol amendment
7.2.1 and Table 7.b	Clarification added regarding protocol violation and deviation summary and listing Update of method of PPS identification
7.3	Additional summaries for subject disposition
7.8	Clarification on visits to be summarized
7.15.4	Adding shift table to vital sign, changing "Change from baseline" to "Change from previous vaccination" for descriptive summary
7.16 and Table 7.d	Update on planned analysis for interim and CSR addendum per protocol amendment
Appendix A	Update on Day 133 and Day 393 window per protocol amendment

8.0 REFERENCES

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3. A Phase 1, Randomized, Observer-Blind, Placebo-Controlled, Safety, Immunogenicity and Dose Ranging Study of Purified Inactivated Zika Virus Vaccine (PIZV) Candidate in flavivirus naïve and Primed Healthy Adults Aged 18 to 49 Years. Takeda Vaccines, Inc. Protocol No. ZIK-101 Version 7.0 dated 12 September 2018.
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5. Newcombe, R., 1998. Interval estimation for the difference between independent proportions: comparison of eleven methods. Statist. Med. 17, 873–890.
6. 6. FDA. Guidance for Industry. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. 2007 [22 December 2016]; Available from: http://www.fda.gov/downloads/BiologicsBloodVaccines/ucm091977.

Appendix A Schedule of Trial Procedures

Visit Number		1A	1B [a]	2	3	4	5	6	7	8	9 [u]	10 [u]	11 [u]	In case of early termination
Visit Day		**Day -15 to Day 1**	**Day -15 to Day 1**	**Day 1**	**Day 8**	**Day 29**	**Day 36**	**Day 57**	**Day 133**	**Day 211**	**Day 393**	**Day 575**	**Day 757**	
Clinical Visit		X	X	X	X	X	X	X		X	X		X	X
Phone Contact									X			X		
First and second phase of the study [b]	**Flavivirus naïve [1]**	X		X	X	X	X	X	X	X	X	X	X	X
	Flavivirus primed [2]	X	X	X	X	X	X	X	X	X	X	X	X	X
Second phase of the study [b]	**Flavivirus primed [3]**		X	X	X	X	X	X	X	X	X	X	X	X
Anchored to visit day				**NA**	**Day 1 + 7 days**	**Day 1 + 28 days**	**Day 29 + 7 days**	**Day 29 + 28 days**	**Day 29 + 3 ½ mo**	**Day 29 + 6 mo**	**Day 29 + 12 mo**	**Day 29 + 18 mo**	**Day 29 + 24 mo**	
Acceptable visit window (days before/ days after) [c]		NA	NA	0/+7	-1/+7	-4/+7	-1/+7	-4/+7	-7/+14	-7/+14	-7/+97	-7/+14	-14/+14	
Signed informed consent		X	X [t]											
Remind study procedure to flavivirus primed subjects identified at Visit 1A [2]			X											
Assessment of eligibility criteria [d]		X	X	X										
Demographics [e]		X	X [t]											
Medical history [f]		X	X	X										
Travel history		X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant medications/vaccinations [g]		X	X	X	X	X	X	X		X	X		X	
Physical exam [h]	**Complete**	X	X											
	Symptom directed			(X)	(X)	(X)	(X)	(X)		(X)	(X)		(X)	
Vital signs [i]		X	X	X	X	X	X	X		X	X		X	

Visit Number		**1A**	**1B [a]**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9 [u]**	**10 [u]**	**11 [u]**	**In case of early termination**
Visit Day		**Day -15 to Day 1**	**Day -15 to Day 1**	**Day 1**	**Day 8**	**Day 29**	**Day 36**	**Day 57**	**Day 133**	**Day 211**	**Day 393**	**Day 575**	**Day 757**	
Blood collection [j]	**Flavivirus serostatus**	X	X [t]											
	Eligibility screening and safety laboratory testing	X	X		X		X							
	Serum pregnancy testing [l]	X	X											
	Immunogenicity and assay development			X		X		X		X	X		X	
Urine collection [k]	**Eligibility screening and safety laboratory testing (urinalysis)**	X	X		X		X							
	Urine pregnancy testing [l]			X		X								
Randomization [m]				X										
Check contraindications to vaccination and criteria for delay of vaccination				X		X								
Investigational vaccine or placebo administration [n]				X		X								
Post-vaccination and injection site evaluation [o]				X		X								
Diary card [p]	**Distribution**			Days 1-7	Days 8-28	Days 29-35	Days 36-56							
	Review/collection				Days 1-7	Days 8-28	Days 29-35	Days 36-56						X [q]
Solicited AEs [r]				X		X								
Unsolicited AEs [r]				X	X	X	X	X						
SAEs and AEs leading to withdrawal or discontinuation [s]				X	X	X	X	X	X	X	X	X	X	X

(X)=procedures that are not mandatory but may be performed if deemed necessary; NA=Not applicable.

(a) Applicable only for flavivirus primed subjects
(b) Staggered enrollment: There will be two study phases

- The first phase of the study will be aimed at enrolling flavivirus naïve subjects only [(1)].
  All subjects who sign the ICF and have confirmed eligibility at Screening Visit 1A will be tested for flavivirus serostatus.
  Eligible flavivirus naïve subjects enrolled at Screening Visit 1A [(1)] will be randomized at Visit 2, within two weeks after Screening Visit 1A.
  Flavivirus primed subjects will not be enrolled during the first phase of the study (ie, when identified at Screening Visit 1A [(2)]) and will be invited to come back for the second phase of the study. They will be contacted after positive recommendations from the external DMC are issued following the review of safety and tolerability data in flavivirus naïve subjects, when the last flavivirus naïve subject has completed study Visit 4 (28 days post dose 1). These flavivirus primed subjects will consequently undergo 2 screening visits: Visit 1A during the first phase of the study and Visit 1B at the outset of the second phase of the study. Flavivirus primed subjects identified at Screening Visit 1A who do not participate in the second phase of the study will be declared screened failure.
  Enrollment will be put on hold after all naïve subjects [(1)] have been enrolled and will resume when positive recommendations from the external DMC are issued, as described above.
- The second phase of the study will be aimed at enrolling flavivirus primed subjects only [(2 and 3)].
  Newly identified eligible flavivirus primed subjects at Screening Visit 1B [(3)] will be enrolled (randomized) at (second phase) Visit 2, within two weeks after Screening Visit 1B.
  Previously identified flavivirus primed subjects [(2)] (who were tested at Screening Visit 1A during the first phase and accepted to enter the second phase of the study) will retain their initial subject identification (ID) number and undergo an additional screening visit (Visit 1B) to be tested for eligibility criteria again (except for the determination of flavivirus serostatus).
  Flavivirus naïve subjects identified at Screening Visit 1B (during the second phase of the study) will be declared screened failure.

(c) If the subject has a temporary clinically significant active infection (as assessed by the investigator) or temperature ≥38.0°C/100.4°F, within 3 days of intended investigational vaccine/placebo administration, the investigational vaccine/placebo administration may be delayed as per the assessment of the investigator within the allowed visit window. The visit windows of Visit 3 and Visit 4 should be calculated from the day of the first vaccination. Following the same rule, the visit windows of Visit 5 and Visit 6 should be calculated from the day of the second vaccination.
(d) One single subject identification (ID) number will be assigned to each subject at Screening Visit (1A or 1B). Eligibility by review of relevant inclusion/exclusion criteria will be documented before enrollment.
(e) Demographic information, to be obtained at Screening Visit (1A or 1B), will include age (date of birth), sex, race, and ethnicity as provided by the subject.
(f) Medical history will be collected at Screening Visit(s) (Visit 1A and/or Visit 1B) and at Visit 2 (Day 1) and will include any medical history that may be relevant to subject eligibility for trial participation such as prior vaccinations, concomitant medications, and previous and ongoing illnesses or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an AE that occurs during trial participation, if it represents an exacerbation of an underlying disease/preexisting problem. Adverse occurrences before administration of the first dose of investigational vaccine/placebo are considered medical history.
(g) All medications, vaccines and blood products taken or received by the subjects within 3 months prior to the start of the trial are to be recorded on the source document (patient record) and entered on the Prior and Concomitant Medications eCRF. The use of antipyretics and/or analgesic medications within 24 hours prior to vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be described in the source documents and the eCRF.

(h) Complete physical examination will be performed at Screening Visit(s) (Visit 1A and/or Visit 1B). All subsequent symptom-directed physical examinations, performed if deemed necessary or indicated by review of the subject's medical history, should assess clinically significant changes from the baseline examination. The findings should be documented in the subject's source document; findings consistent with the definition of AEs or SAEs must be transcribed into the electronic Case Report Form (eCRF). For any procedures at the site, the investigator shall follow his/her standard practice.

(i) Vital signs include (however, not limited to) systolic/diastolic blood pressure, pulse rate, respiratory rate, and temperature, and should be documented in the subject's source document and transcribed into the eCRF. Follow standard of care for trial population and operational feasibility.

(j) Blood samples will be collected at each site visit: ie, at Screening Visit(s) (Visit 1A and/or Visit 1B) for flavivirus serostatus determination (approximately 10 mL of blood) and eligibility screening tests (including pregnancy testing) (approximately 10 mL of blood), at Visits 3 and 5 for routine safety laboratory testing (approximately 10 mL of blood), and at Visits 2, 4, 6, 8, and (for subjects who will be randomized to the placebo group or the PIZV dosing group that will be selected for further development) 9 and 11 for immunogenicity, as well as for further development and characterization of assays (approximately 60 mL of blood). Should a blood sampling be performed during a vaccination visit, this sampling must occur before the administration of the investigational vaccine or placebo. The maximum volume of blood taken at any single visit will be between approximately 10 mL and 60 mL, and the approximate maximum total volume of blood for the whole trial will be 280 mL to 410 mL depending on the subject's flavivirus serostatus at screening and the vaccine dose level the subject received. All samples will be collected in accordance with acceptable laboratory procedures. Blood samples will be processed and stored at the trial site as described in the provided Laboratory Manual.

(k) Urine samples will be collected for all subjects at Screening Visit(s) (Visit 1A and/or Visit 1B) and at Visits 3 and 5; and for women of childbearing potential at Visits 2 and 4 before each investigational vaccine/placebo administration for pregnancy testing. Urine samples for eligibility screening and routine safety laboratory testing will be sent to the central laboratory. Urine pregnancy tests will be performed at the study site using kits provided by the sponsor.

(l) Serum pregnancy tests will be conducted in the central laboratory and urine pregnancy tests will be done at the study site using kits provided by the sponsor. Subjects must have a negative urine β-hCG pregnancy test prior to receiving any dose of investigational vaccine/placebo.

(m) If eligible, the subject will be randomized at Visit 2.

(n) The investigational vaccine/placebo will be administered in the deltoid muscle of the non-dominant arm (approximately at the top middle third).

(o) After vaccine/placebo administration at Visit 2 (on Day 1) and at Visit 4 (Day 29), the subject will be observed for at least 30 minutes for observation of solicited AEs, unsolicited AEs, and measurement of body temperature.

(p) Diary cards will be handed out to the subjects at Visits 2, 3, 4 and 5, and reviewed with the subject and collected at the following visit.

(q) In case of early termination during the vaccination period only.

(r) After each dose, solicited AEs (for 7 days including the day of vaccine/placebo administration) and unsolicited AEs (for 28 days including the day of vaccine/placebo administration) will be collected on diary cards by each subject. The investigator will transcribe these data into eCRF.

(s) SAEs and AEs leading to withdrawal from the trial or discontinuation of vaccine/placebo administration will be collected for the duration of the entire trial. SAEs will be reported to the sponsor within 24 hours of the investigator becoming aware of the event. AEs leading to early termination will be recorded by the investigator.

(t) Only for newly screened subjects at Screening Visit 1B (i.e., not for flavivirus primed subjects who were tested at Screening Visit 1A and accepted to enter the second phase of the study).

(u) All subjects who were randomized to the placebo group or the PIZV dosing group that was selected for further development will attend Visit 9 at Day 393 (12 months post dose 2) and Visit 11 at Day 757 (24 months post dose 2), and will also be contacted by phone on Day 575 (Visit 10) for safety follow-up.

Appendix B Serology Plan

TEMPLATE

Template Number:	**FORM-0002496**	**Page:**	**1 of 4**
Version Number:	**2.0**		
This version replaces:	**1.0**		
Parent Document:	**PROC-0002793**		
Template Title:	**Clinical Serology Plan**		

Description of clinical study as per protocol	Study Number: ZIK-101 Short title of study: **Safety, Immunogenicity, and Dose Ranging Study of Inactivated Zika Virus Vaccine in Healthy Adults** IND number: 017673 Eudract #: NA Version: Protocol Amendment 2 - Version 6.0 Date: 11 December 2017
Version of Clinical Serology Plan	Version: 3.0 Replaces version: 2.0 Date: 12 February 2018

Assay name	Supported study endpoint [a]	Assay priority ranking in case of limited sample volume	Required assay validation status [b]	Analytical readout of assay data	Sample type [c]	Sample volume/ time point/ assay [c]	Minimal sample volume reserved as backup [c; d]	Subset of subjects	Time points[e]	Total # of time points	Total # of tests per assay	Contracted bioanalytical vendor	Comments
Flavivirus Screening Assay [1]	Screening	1	FIT	Flavi Serostatus (primed/naïve)	Serum	1 mL	3.6 mL	240	D-15	1	240	Q Squared Lab Solutions Bioanalytical	Screening for Flavi serostatus covering at least: DENV, ZIKV, JEV and WNV
Zika-NT Assay	Primary	1	QUAL	GMT	Serum	1.0 mL	2.0 mL	240	D1,D29, D57,D211 D393[2], D757[2]	4 or 6	1200	Q Squared Lab Solutions Vaccines	Immunogenicity
Immunological Assays Development for future studies	NA	2	NA	unspecified	Serum	20 mL	5.0 mL	240	D1,D29, D57,D211 D393[2], D757[2]	4 or 6	1200	Takeda	For product development

TEMPLATE

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Add rows as needed

Legend:

[a] Primary, secondary and/or exploratory endpoint
[b] Fit-for-purpose (FIT), qualification (QUAL), validation (VAL)
[c] In this table sample means secondary sample (e.g. serum, not blood)
[d] Backup volume shall allow re-measurement of all primary and secondary endpoint assays
[e] Day (D), Month (M), Year (Y)
[1] Safety lab testing are specified in the study protocol and not further detailed in this plan.
[2] Subjects in 'dose selected for further development' and placebo group (Total 120 subjects) will have a D393 and D757 sample collection and total of 6 time points. The other two groups (total 120 subjects) will not have the D393 nor D757 sample collection and therefore a total of 4 time points.

TEMPLATE

Template Number:	**FORM-0002496**	**Page:**	**3 of 4**
Version Number:	**2.0**		
This version replaces:	**1.0**		
Parent Document:	**PROC-0002793**		
Template Title:	**Clinical Serology Plan**		

Primary sample required per time point (e.g. blood draw):

Time point	Primary sample type → secondary sample type	Primary sample volume or amount per time point[a]	Expected secondary sample volume or amount
Screening for flavi serostatus: D-15	Blood→Serum	Approximately 10 mL	Approx. 4.6 mL
Eligibility screening and safety laboratory testing: D-15, D8, D36[c]	Blood	Approximately 10 mL	NA
Immunogenicity and assay development: D1,D29,D57,D211, D393[c], D757[c]	Blood→Serum	Approximately 60 mL	Approx. 28 mL
Total blood drawn in study depends on screening result, requirement start and inclusion into a follow-up period as follows[c]:			
Flavivirus primed subjects **- Start at Screening Visit 1A** **- with 24 months follow-up:** 1x10 mL= 10 mL 4x 10 mL= 40 mL 6x 60 ml= 360 mL Total = approx. 410 mL	**Flavivirus primed subjects** **- Start at Screening Visit 1A** **- without 24 months follow-up:** 1x10 mL= 10 mL 4x 10 mL= 40 mL 4x 60 ml= 240 mL Total = approx. 290 mL	**Flavivirus naïve subjects** **- Start at Screening Visit 1A** **- with 24 months follow-up:** 1x10 mL= 10 mL 3x 10 mL= 30mL 6x 60 ml= 360 mL Total = approx. 400 mL	**Flavivirus naïve subjects** **- Start at Screening Visit 1A** **- without 24 months follow-up:** 1x10 mL= 10 mL 3x 10 mL= 30 mL 4x 60 ml= 240 mL Total = approx. 280 mL
Flavivirus primed subjects **- Start at Screening Visit 1B** **- with 24 months follow-up:** 1x10 mL= 10 mL 3x 10 mL= 30 mL 6x 60 ml= 360 mL Total = approx. 400 mL	**Flavivirus primed subjects** **- Start at Screening Visit 1B** **- without 24 months follow-up:** 1x10 mL= 10 mL 3x 10 mL= 30 mL 4x 60 ml= 240 mL Total = approx. 280 mL	**Flavivirus naïve subjects** **- Start at Screening Visit 1B** **(results in screen failure):** 1x10 mL= 10 mL 1x 10 mL= 10 mL 0x 60 ml= 0 mL Total = approx. 20 mL	

[a] In this table primary sample means e.g. blood, corresponding secondary sample means e.g. serum
[b] Assays for the Eligibility screening and safety laboratory testing are specified in the Study Protocol.
[c] Subjects in 'dose selected for further development' and placebo group (Total 120 subjects) will have an D393 and D757 sample collection and a total of 6 time points. The other two groups (total 120 subjects) will not have the D393 nor D757 sample collection and therefore a total of 4 time points.

TEMPLATE

Template Number:	**FORM-0002496**	**Page:**	**4 of 4**
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This version replaces:	**1.0**		
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Template Title:	**Clinical Serology Plan**		

Reviewed and approved by:

<Clinical Study Physician> Date

<Clinical Project Oversight Manager> Date

<Clinical Serology Project Manager> Date

Appendix C Solicited Local and Systemic Adverse Events and Severity

Table C-1 Solicited Local and Systemic AEs

Local AEs (injection site):	Pain
	Erythema
	Swelling
	Induration
Systemic AEs:	Fever [(a)]
	Headache
	Fatigue
	Malaise
	Arthralgia
	Myalgia

(a) Based on recorded body temperature, fever is defined as body temperature ≥38.0°C/100.4°F regardless of method taken (Brighton Case Definition).

Table C-2 Severity of Solicited Safety Parameters

1. Adverse Event	2. Intensity Grade	3. Severity/Intensity
Pain at injection site	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity with or without treatment
	3	Severe: Prevents daily activity with or without treatment
Erythema at injection site [(a)]	0	<25 mm
	1	Mild: ≥25 – ≤50 mm
	2	Moderate: >50 – ≤100 mm
	3	Severe: >100 mm
Induration at injection site [(a)]	0	<25 mm
	1	Mild: >25 – ≤50 mm
	2	Moderate: >50 – ≤100 mm
	3	Severe: >100 mm
Swelling at injection site [(a)]	0	<25 mm
	1	Mild: ≥25 – ≤50 mm
	2	Moderate: >50 – ≤100 mm
	3	Severe: >100 mm
Fever [(b)]	Record body temperature in °C/°F	
Headache	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity with or without treatment
	3	Severe: Prevents normal activity with or without treatment
Fatigue	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Malaise	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Arthralgia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity
Myalgia	0	None
	1	Mild: No interference with daily activity
	2	Moderate: Interference with daily activity
	3	Severe: Prevents daily activity

(a) Subjects are to record greatest surface diameter in mm in the Diary.
(b) Based on recorded body temperature, fever is defined as body temperature ≥38.0°C/100.4°F regardless of method taken.

Appendix D FDA Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

Toxicity grading scales for laboratory abnormalities should consider the local laboratory reference values when the parameter limit values are defined. The characterization of laboratory parameters among some populations of healthy adults and adolescents may require the exercise of clinical judgment. Source: U.S. Department of Health and Human Services, Food and Drug Administration, and Center for Biologics Evaluation and Research, September 2007 [6].

Table D-1 Table for Laboratory Abnormalities (Serum)

Serum [a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4) [b]
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23-26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN [c]	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal; increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

(a) The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
(b) The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example. a low sodium value that falls within a grade 3 parameter (125-129 mEq/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.
(c) "ULN" is the upper limit of the normal range.

Table D-2 Table for Laboratory Abnormalities (Hematology)

Hematology [(a)]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) – gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) – gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase – cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease – cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease – cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease – cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils – cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased – cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN [(b)]	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase – mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease – mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

(a) The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
(b) "ULN" is the upper limit of the normal range.

Table D-3 Table for Laboratory Abnormalities (Urine)

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

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

Table D-4 Table for Clinical Abnormalities (Vital Signs)

Vital Signs [a]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) [b] (°F) [b]	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia – beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute [c]	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

(a) Subject should be at rest for all vital sign measurements.
(b) Oral temperature; no recent hot or cold beverages or smoking.
(c) When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Source: Appendix E of ZIK-101 Protocol Amendment 2 Version 6.0, dated 11 December 2017.

Appendix E CDC Websites

- World Map of Areas with Risk of Zika: https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika
- Travel & Dengue Outbreaks: https://www.cdc.gov/dengue/traveloutbreaks/index.html
- Yellow Fever Maps: https://www.cdc.gov/yellowfever/maps/index.html
- Geographic Distribution of Japanese Encephalitis Virus: https://www.cdc.gov/japaneseencephalitis/maps/index.html

Source: Appendix D of ZIK-101 Protocol Amendment 2 Version 6.0, dated 11 December 2017.